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Dissociation of Enzyme Oligomers: A Mechanism for Allosteric Regulation

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ABSTRACT: Most enzymes exist as oligomers or polymers, and a significant subset of these (perhaps 15% of all enzymes) can reversibly dissociate and reassociate in response to an effector ligand. Such a change in subunit assembly usually is accompanied by a change in enzyme activity, providing a mechanism for regulation. Two models are described for a physical mechanism, leading to a change in activity: (1) catalytic activity depends on subunit conformation, which is modulated by subunit dissociation; and (2) catalytic or regulatory sites are located at subunit interfaces and are disrupted by subunit dissociation. Examples of such enzymes show that both catalytic sites and regulatory sites occur at the junction of 2 subunits. In addition, for 9 enzymes, kinetic studies supported the existence of a separate regulatory site with significantly different affinity for the binding of either a substrate or a product of that enzyme.

Over 40 dissociating enzymes are described from 3 major metabolic areas: carbohydrate metabolism, nucleotide metabolism, and amino acid metabolism. Important variables that influence enzyme dissociation include: enzyme concentration, ligand concentration, other cellular proteins, pH, and temperature. All these variables can be readily manipulated *in vitro*, but normally only the first two are physiological variables. Seven of these enzymes are most active as the dissociated monomer, the others as oligomers, emphasizing the importance of a regulated equilibrium between 2 or more conformational states. Experiments to test whether enzyme dissociation occurs *in vivo* showed this to be the case in 6 out of 7 studies, with 4 different enzymes.

KEY WORDS: enzyme oligomers, allosteric regulation, subunit dissociation, ligand site between subunits.

I. INTRODUCTION

Most enzymes exist as oligomers, and more than 500 protein oligomers have been tabulated.[1,2] In two separate surveys, oligomeric enzymes constituted 70 to 80% of the total data sets.[3,4] The advantages of a polymeric state have long been understood as the basis for cooperativity and allosteric regulation.[5,6] Where the oligomer form of the enzyme is stable, interaction between subunits has evolved in some enzymes to produce cooperativity by conformational changes whereby binding of a ligand to one subunit alters the degree of binding of the same ligand to other subunits in the same oligomer. The extent of the cooperativity is modified by allosteric effectors, and this type of regulation is now described in all biochemistry textbooks. In such kinetic modeling, it is standardly assumed that the number of subunits per oligomer is invariant.

However, in the last 30 years we have acquired considerable information about a subset of enzymes whose distinctive characteristic is the ability to convert between oligomer and monomer, with a concomitant change in enzyme activity. For such enzymes the equilibrium between monomer and oligomer is appropriately influenced by regulatory metabolite effectors. Various reviews have attempted to quantitate the total number of allosteric enzymes. In a survey of all enzyme kinetic studies published between 1965 to 1976, it was found that 23% of these had non-Michaelis-Menten kinetics, thus providing possible evidence for being allosteric and regulated.[7] In a review of more than 70 different enzymes in nucleotide metabolism, it was found that 32% of these enzymes gave evidence for allosteric behavior.[4] Furthermore, 15% of these enzymes could dissociate under physiological conditions.[4] In the pathways of

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carbohydrate metabolism and nucleotide metabolism, where many enzymes have been reasonably well characterized, it appears that almost half of the allosteric enzymes are also dissociating enzymes when tested *in vitro*.

Because *in vitro* experiments are frequently done at conditions far from physiological, some controversy has arisen as to whether such experimental data have any physiological relevance. While it is desirable to obtain an accurate depiction of how enzymes function *in vivo*, the most important feature about all the studies on dissociating enzymes is that they give information that correlates quaternary structure with catalytic function, as well as with the enzyme's ability to be regulated. Even if a specific enzyme does not dissociate *in vivo*, such data are very helpful in developing an understanding of structure and function. And from the general patterns that are emerging, one or a few simple models will be able to use simple diagnostic data to predict whether an enzyme has this type of regulatory property.

Earlier reviews on this topic tended to focus on a few specific enzymes.[8-11] Important contributions to developing a theoretical framework have come from Frieden[8,12-15] and from Nichol and colleagues,[11,16-18] and a thorough treatment, with extensive mathematical modeling, is in a book by Kurganov.[19] In the present survey, I will focus on over 40 enzymes in 3 major areas of metabolism, with the goal of emphasizing the major patterns.

II. DEFINITIONS AND ASSUMPTIONS

A. Definitions

Any oligomeric protein can be converted to its subunits if sufficiently denaturing conditions are employed. In the present survey the term *dissociating enzyme* will be applied only to enzymes that undergo a shift between oligomer and monomer in response to a physiologically relevant ligand. No generic name has become generally employed, and the terms *associating* or *self-associating* have also been used. The latter term has the disadvantage of excluding heteropolymers, while *dissociating* includes homopolymers and heteropolymers, and because most enzymes are oligomers, it also describes the more common initial event.

Although the great majority of these enzymes contains only 2 or 4 subunits, there also are examples going up to large polymers with more than 100 subunits. The term *native* will designate the quaternary structure that is stable in the absence of any ligands. This is a useful reference state, and should not be confused with whatever form of the enzyme may be favored *in vivo*, where the enzyme will always be in the presence of varying concentrations of substrates and effectors. Figure 1 shows the monomer and oligomer states for four enzymes studied in our laboratory; they will be considered in more detail later. The numbers represent the number of subunits in a particular species. The native form is underlined.

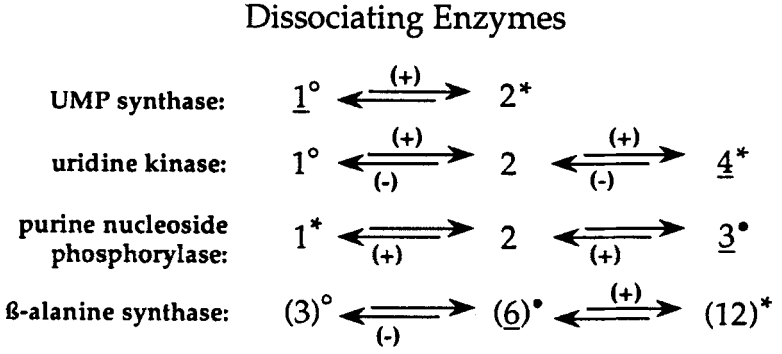


FIGURE 1. Change in activity accompanies dissociation. Enzyme activity: * = most active; • = partly active; ° = inactive. Numbers indicate number of subunits; — = native form of enzyme (no ligands).

Superscript symbols designate the extent of enzyme activity as defined in the figure legend, while the effect of ligands on dissociation or association is also indicated. While the symbols [+] and [–] generally refer to activators and inhibitors, as used throughout the text they will always represent the more active or less active oligomer assembly being stabilized. This distinction is necessary, as there are examples where an inhibitor can stabilize the oligomer species that corresponds to the most active state, even though the inhibitor specifically diminishes catalytic activity. This will be considered in more detail in Section V. An enzyme species is defined as *inactive* when it's measured activity is <5% of the optimum activity.

B. Types of Dissociating Enzymes

Figure 2 shows the most common patterns for oligomerization. Of oligomeric enzymes that have been characterized, slightly more than half form simple dimers, with tetramers being the second most common species.[1–4] This is consistent with each subunit having only a single recognition site for bonding with a like subunit, as shown in Figure 2A. The existence of a second set of bonding sites leads to the formation of a tetramer as a dimer of dimers (D_2 symmetry) as shown in Figure 2B. Alternatively, tetramers could be formed in a cyclic, planar configuration (Figure 2C), although this has yet to be shown for even a single enzyme with a defined crystal structure.[20,21]

Polymers containing an odd number of subunits are fairly rare, and only a limited number of trimers is known.[1,2] Higher-order oligomers are probably all built from trimers and tetramers: a hexamer as a dimer of trimers; oligomers of 8, 16, etc. subunits as dimers, tetramers, etc. of a tetramer. This is consistent with, and possibly the reason for, the fact that most enzymes that polymerize significantly have polymers of increasing size that are integral multiples of 4 subunits. Examples of this latter type include phosphoribosyl pyrophosphate synthase[22] and uridine kinase.[23–25]

C. Assumptions and Cautions

If an enzyme can be shown to dissociate readily *in vitro*, no assumption should be made

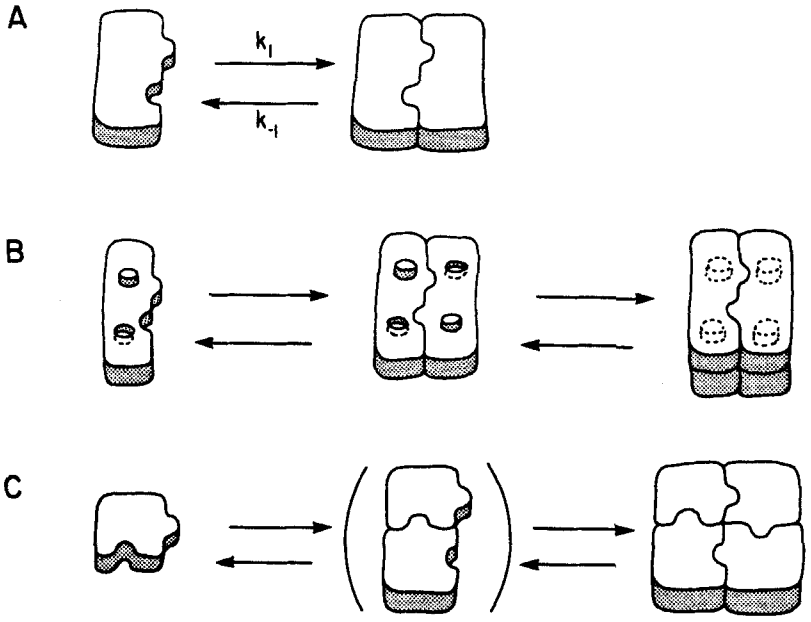


FIGURE 2. Formation of the most common oligomeric structures. In schemes A and B, a twofold rotational symmetry aligns bonding regions between subunits. Scheme C has a fourfold rotational symmetry.

that the same process will occur *in vivo* until appropriate efforts have been made to test the enzyme at concentrations of enzyme and effectors that are physiological. Only when an enzyme has been purified to homogeneity can we accurately quantitate its concentration in the tissue of origin, and by using the specific activity of homogeneous enzyme we can at least estimate its abundance in other cells and tissues. Our knowledge of the concentration *in vivo* of various metabolites is improving, but often is not definitive.[26] A recent review summarizes such values for a subset of these enzymes.[27]

Potential sources for error or discrepant results need to be considered. Especially in the earlier studies to be discussed under Section VI, authors did not systematically define the concentrations of enzyme used in various experiments, although the concentrations of effector ligands are generally defined. This is possibly the basis for some discrepancies between the results of different laboratories on the same enzyme. Preincubation conditions and time periods, whose importance is considered under Section III.E, were not always described and may not have been properly controlled.

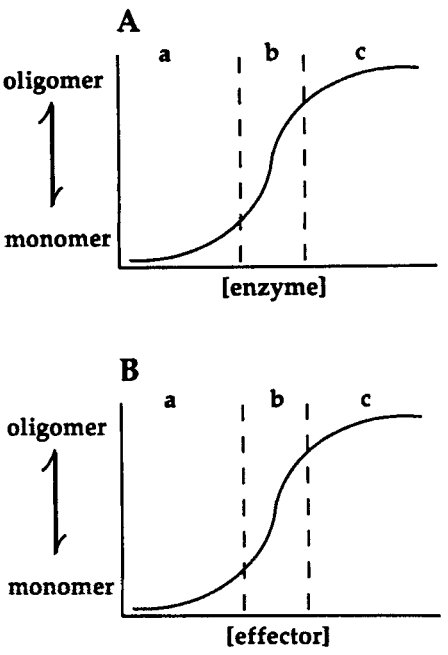


FIGURE 3. Regulation of monomer-oligomer equilibrium. Variation over the range *b* for either enzyme concentration (A) or effector concentration (B) will produce a significant change in catalytic rate if monomer and oligomer have different intrinsic activities.

III. EXPERIMENTAL APPROACHES

A. Diagnostic Features

Isolated enzymes that are studied *in vitro* can be made to change their aggregation state by appropriate changes in pH, temperature, salt concentration, or various denaturants. However, *in vivo* an enzyme would not be subject to such environmental variations. As shown in Figure 3, regulation of an enzyme under physiological conditions is primarily related to (1) enzyme concentration, and (2) effector ligand concentration. Somewhat arbitrary concentration ranges are designated as a–c in Figure 3; the important concentration range for any variable that influences dissociation is in range b, because this is where the major change in enzyme dissociation, and therefore in activity, would occur. If either the concentration of enzyme, or of an appropriate effector ligand, is known to vary over range b *in vivo*, this parameter is likely to be significant for physiological regulation. However, studies done *in vitro* across any concentration range may provide useful information about the enzyme.

Although a change in the synthesis or turnover of cellular proteins occurs in certain physiological states such as critical developmental periods, pregnancy, fasting/starvation, or illness, alterations in their *in vivo* concentration is not likely to be an important regulatory mechanism for most enzymes. Nonetheless, it is useful to determine whether there is an appreciable change in the native molecular weight and in the enzyme's specific activity, as the concentration of enzyme is altered. Important regulatory enzymes tend to respond to specific effectors, whose *in vivo* concentration varies over a broad range. The native molecular weight of many enzymes is often found to be altered in the presence of an appropriate ligand, although the concentration ranges of ligands where this occurs may not be physiologically relevant.

Table 1 summarizes the general methods that have proven useful in identifying dissociating enzymes, and these will be detailed below. Both physical methods and kinetic experiments will be considered; the table emphasizes whether methods can be applied to crude enzyme, or only to pure enzymes. The methods in part A of Table 1 are more useful for diagnostic purposes, because they can be used with crude preparations of enzymes. Even with crude enzyme preparations the first three methods in part A of Table 1 are normally diagnostic for a dissociating enzyme. However, hysteretic kinetics may be produced by conformational changes that do not require dissociation/association.

B. Native Molecular Weight

The process of reversible association/dissociation can be readily measured with isolated enzyme preparations using molecular sieve chromatography or sedimentation. Because enzymes may exist as mixtures of monomers and various oligomers, with the different aggregation states equilibrating either rapidly or slowly, the observed shape of migrating enzyme peaks depends on whether the rate of equilibration between monomers and oligomers is faster or slower than their rate of migration (separation) in the experimental apparatus. The approach consists of obtaining a molecular weight value (or elution absorbance profile) of native enzyme in the absence of any effector ligands, and in the presence of increasing concentrations of effector ligand, as schematically outlined in Figure 4. For a rapidly equilibrating system, one observes a migration profile that represents a statistical average for the entire population during the experiment. This is most obvious for simple monomer-dimer systems, as illustrated at the top of Figure 4. An example of such an enzyme is UMP synthase. In the absence of ligands the enzyme sedimented as a monomer, while in the presence of the saturating substrate, orotidine-5′-phosphate (OMP), the enzyme sedimented as a dimer.[28,29] Very sharp symmetric peaks also could be observed at intermediate ligand concentrations, representative of the average sedimenting position of mixtures rapidly interconverting between monomers and dimers during the time course of the experiment.

When such experiments are initially done in the absence of ligands, the buffer is sometimes not considered. This item may be significant, as even buffer components can be ligands and must therefore be carefully considered. As an example, when the native molecular weight of mammalian UMP synthase was first measured in phosphate buffer, it produced an unusually high M_r of 70,000 for the species assumed to be a monomer;[30] the actual subunit M_r is 52,200. As will be shown later, inorganic phosphate is an allosteric ligand and alters the molecular weight for at least 10 enzymes in the present survey.

For a slowly equilibrating enzyme, one may observe two or more distinctly migrating peaks as shown in Figure 4. About half the enzymes in the present survey can form three or more different aggregation states, and some of these show at least two forms at the same time (Figure 4). In some cases, as with β-alanine synthase (Figure 1), the native species can be shifted in opposite direc-

TABLE 1
Methods for Detecting Regulation of Enzyme Activity by Change in Association of Subunits

With crude or pure enzymes
- Change in molecular weight in response to effector ligands
- Change in specific activity with change in enzyme concentration
- Reactivation of isolated protein peaks (molecular weight species)
- Hysteretic kinetics

With pure enzymes
- Crosslinking subunits to inert supporting resin
- Detection of protein peaks lacking enzyme activity
- Determination of appropriate binding constants

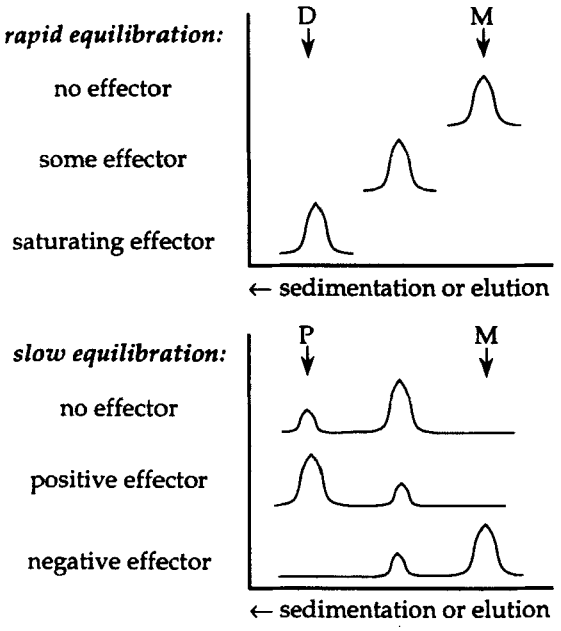


FIGURE 4. Enzymes that readily undergo reversible dissociation exist in an equilibrium of monomers and oligomers or polymers. The position of equilibrium can be shifted by effectors. Only one molecular mass species is observed for systems undergoing rapid equilibration (top), while several different molecular mass species may be separated from one another in systems undergoing slow equilibration (bottom). M, monomer; D, dimer, P, polymer.

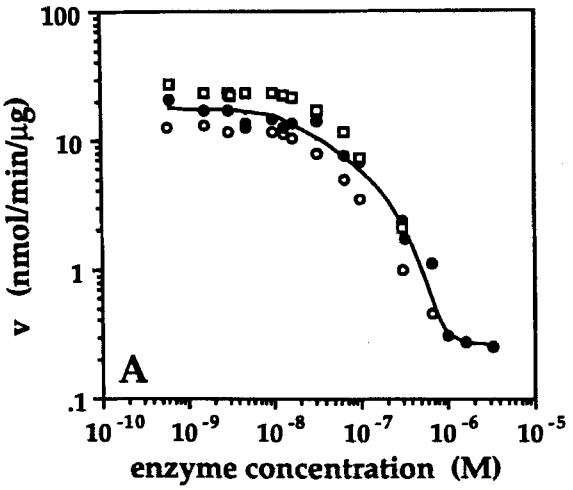
tions as a function of different effectors. For β-alanine synthase, the native enzyme without ligands is a hexamer;[31] in the presence of the substrate (*N*-carbamoyl-β-alanine) the enzyme is converted to the active dodecamer, while in the presence of the product (β-alanine) the enzyme dissociates to the inactive trimer.[32]

It is a significant diagnostic feature of slowly equilibrating enzymes that they produce multiple peaks during sedimentation or molecular sieve chromatography, and sometimes even with ion exchange chromatography, as polymerization changes the proportion of hydrophobic surface to surface with ionic charge. This may also lead to misinterpretation, as in the example of uridine kinase. This enzyme commonly produces chromatographic elution patterns with multiple peaks; many laboratories had interpreted this as signifying two or more isozymes.[33–38] However, it was subsequently shown that homogeneous enzyme, moving as a single species by both isoelectric focusing and denaturing electrophoresis, could still elute as multiple peaks on various types of chromatography.[25]

Several authors have presented a rigorous mathematical analysis for the behavior of macromolecules during mass transport experiments.[39–41] Such modeling and interpretations are useful for the study of dissociating enzymes by sedimentation velocity techniques.

C. Change in Specific Activity with Enzyme Dilution

Because the concentration of enzyme influences the equilibrium between monomers and oligomers, dilution should have an effect on the specific activity of a dissociating enzyme, as it will change the ratio of active and inactive species. Figure 5 illustrates such a dilution experiment with purine nucleoside phosphorylase (see also Figure 1).[42] Enzyme concentration was varied over 4 orders of magnitude, with the specific activity increasing about 50-fold as the enzyme



A

FIGURE 5. Purine nucleoside phosphorylase: change in specific activity as a function of enzyme concentration (A), with enzyme diluted into buffer only (○), or into buffer containing 50 μ*M* inosine (□) or 50 m*M* P_i (●). Change in native M, as a function of substrate concentration (B), with enzyme concentration constant at 3.3×10^{-7} *M*, at 24° (○) or at 4° (●).

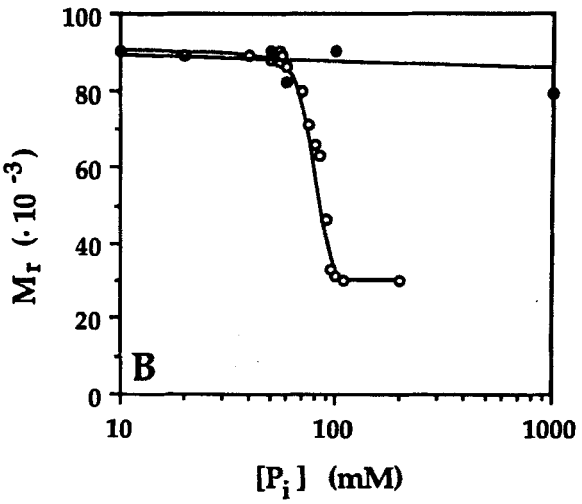


FIGURE 5B

was diluted. Technically, such an experimental curve is limited approximately to the enzyme concentration range shown in the figure: at low enzyme concentration by the sensitivity of product detection, and at high enzyme concentration by the difficulty in maintaining initial rate conditions. Figure 5A shows that as the enzyme concentration is decreased, the addition of a constant concentration of one substrate produces a further increase in the specific activity. This contrasts with the experiment of Figure 5B, where the concentration of enzyme was maintained constant, while the substrate P_i was increased. Below 60 m*M*, P_i had no significant effect on dissociation of this native trimer, while at a concentration of 100 m*M* P_i completely dissociated purine nucleoside phosphorylase to the more active monomeric form. Figure 5B demonstrates that such processes are temperature sensitive, because P_i had no significant effect on dissociation when tested at 4°.

The above example emphasizes the importance of the actual concentration of an enzyme in studies of the native molecular weight. For purine nucleoside phosphorylase (the enzyme in Figure 5), at concentrations most frequently used this enzyme has a native M_r consistent with it being a trimer.[42] However, when the enzyme prepared from chicken liver was diluted up to 4-fold, it migrated by molecular sieve chromatography as a trimer or a dimer and led to a different interpretation.[43] These results are consistent with the data of Figure 5A but emphasize the need to do a number of determinations to assess the native M_r correctly.

The importance of enzyme concentration on enzyme association and concomitant change in activity has not been explored systematically. Other enzymes where this feature is well established include AMP deaminase,[44] Ca^{2+}-ATPase,[45] fumarase,[46] phosphofructokinase,[47–53] isocitrate dehydrogenase,[54] glutamate dehydrogenase,[55] glycogen phosphorylase,[56] phosphorylase kinase,[57] threonine dehydrase,[58] and UMP synthase.[59] This specific aspect has received special attention in an earlier review.[10]

D. Reactivation of Isolated Molecular Weight Species

A final diagnostic tool for reversible dissociation/association is the reactivation of a particular protein peak isolated by chromatography or sedimentation. This is not too common, because the inactive species is likely to go unobserved. Examples where the technique has been used are the enzymes UMP synthase[28] and pyrimidine nucleoside monophosphate kinase.[60] In both cases, inactivation and loss of activity were caused by

oxidation, and incubation of the eluted fractions with either dithiothreitol[28] or with β-mercaptoethanol[60] led to the recovery of some activity and therefore to the detection of the (previously) inactive enzyme peak. Oxidation led to a higher M_r species, presumably because of intersubunit disulfide bonds, as the enzyme was converted back to the monomer by reducing thiols. The inactive oligomers formed in these examples are probably artifacts of the procedure and presumably not physiologically relevant.

E. Hysteretic Kinetics

Implicit in the model of enzyme regulation by reversible dissociation is the concept that the different molecular weight species have different rates of catalytic activity: frequently one species is inactive. This aspect is considerably more difficult to quantitate than changes in molecular size. We know intuitively that the oligomeric species observed in the presence of substrates has catalytic activity, but do the higher or lower aggregation states have activity? When working with impure enzymes, the position of the enzyme after elution or sedimentation is determined by assaying for enzyme activity, to identify peaks as illustrated in Figure 4. Although in such studies a particular molecular weight species is only found by measuring activity, this does *not* mean that the species is intrinsically active, because it is quite possible that one of the substrates in the assay has converted the isolated species into some different but active species in the assay vial. The experimental approach is to do more rapid kinetics in order to assess a particular enzyme species before the substrates can promote a structural transition.

The design and interpretation of proper kinetic experiments have received considerable attention.[12–14,16,17,41,61,62] The two major concerns are to define the oligomer state of the enzyme of the speed of the assay procedure. The first concern can be addressed by doing appropriate physical studies in the presence or absence of ligands, so that the oligomeric state of the enzyme is defined for any condition of preincubation in an assay mixture before activity is elicited by the introduction of the substrate. A source of difficulty is that concentrations of enzyme used in physical studies are frequently much greater than in kinetic assays. Chromatography or centrifugation often require 24 h or more; protein concentration may either be an experimental variable or be kept at a high level in an effort to stabilize the enzyme during such a lengthy experiment. Alternatively, in kinetic experiments the concentration of enzyme is frequently much lower in order to maintain initial velocity rates, even though such enzyme

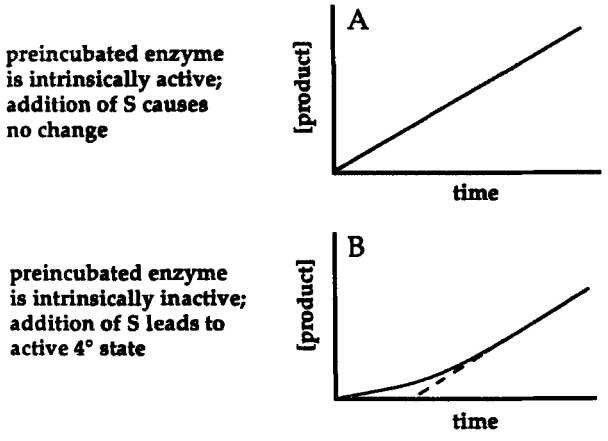


FIGURE 6. Initial progress curves as a kinetic test for substrate-induced association or dissociation. Enzymes are preincubated ± a specific effector to produce a known quaternary state, as determined in Figure 4. Intrinsic catalytic activity is tested by rapidly measuring product formation after the addition of a specific substrate that may also produce a conformational change to the active quaternary state.

concentrations may differ significantly from those used in physical studies. In Figure 6 two patterns for kinetic progress curves under initial rate conditions are shown. If the steady state progress curve intercepts the origin, then the preincubated from of the enzyme is intrinsically active. The lower diagram shows a progress curve for an enzyme that is initially inactive, but that undergoes a conformational change in response to the substrate and is fully active when the progress curve becomes linear at steady state conditions. The term *hysteresis* was introduced by Frieden to describe such nonlinear kinetics,[13,14] and this concept was extended by Neet and Ainslie,[61,62] who also described how to apply it to monomeric enzymes. Such hysteretic kinetics are diagnostic of substrate-induced conformational changes. Extrapolation of the linear progress curve in the lower diagram of Figure 6 to the abscissa yields the time required for the transition from an inactive to an active conformation. This time is usually designated as the transition time, or the lag time. When such a lag time is sufficiently long, it is readily observable in a progress curve, and the earliest enzymes detected for this property had lag times from several seconds to minutes.[8] However, enzymes can have transition times in the millisecond or microsecond range, so that definitive results may require rapid measurement techniques, or may no longer be feasible.

A procedure for estimating a reasonable transition time was made by Frieden[15] for the case where association is diffusion controlled. Because this is a second-order process, the half time for association

$$t_{1/2} = k^{-1}[E]^{-1}$$

where k is the association rate constant, and [E] the concentration of enzyme. Values for k are normally in the range of 10^5 to 10^6 s^{-1} M^{-1}. Assuming an enzyme concentration of 1 μM, the simple association of monomers to form a dimer would require 0.1 to 1 s. If the monomer is inactive and the dimer is active, then the kinetic assay must be sufficiently rapid for such a brief transition time to be detectable. Thus the appropriate selection of enzyme concentration defines the temporal limits for observation. Diluting the enzyme should help to increase the time for such a process and facilitate observation of a lag in the appearance of activity. Many studies are done with enzyme in the range 10^{-8} to 10^{-6} M, but even lower concentrations may be required.

An increase in the observable lag time may also be obtained by lowering the temperature and thereby the rate of oligomerization,[25] or by increasing the preincubation time to assure that the enzyme has completely assumed the desired state of association or dissociation. This was shown by diluting stock preparations of UMP synthase into assay buffer in the absence of ligands, and then measuring the lag time after the addition of substrate, which was known to produce the active dimer.[28] The longer the enzyme was preincubated under dissociating conditions, the longer was the observed lag time after the addition of substrate. Such an observation also underscores the need to maintain preincubation times absolutely constant for all reaction vials when progress curves are obtained with multiple different fixed-time measurements. In this respect, enzymes that can be directly and continuously measured by an on-line spectrophotometric assay are much better candidates for study than the majority of enzymes that require fixed time assays in order that the product can be isolated for quantitation. The use of coupling enzymes to provide spectrophotometric detection needs special consideration for such studies, because the coupling enzymes themselves introduce a lag time in rapid assays.[63–65] In Figure 7 examples of the parameters that affect the observed lag time are shown. With uridine kinase (see also Figure 1) the observed lag time was about 50 s at 0 to 2°C, 15 s at 16°C, and no longer observable at 37°C (Figure 7A).[25] Because the inhibitor CTP also shifts the monomer-tetramer dissociation equilibrium, increasing concentrations of CTP extend the observed transition time with uridine kinase (Figure 7B). With partially purified preparations of UMP synthase (see also Figure 1), a 3-fold dilution in enzyme concentration made the transition time much more apparent (Figure 7C).[59]

F. Crosslinking Subunits to Inert Supporting Resin

This procedure has been used successfully only with pure enzymes.[66–68] The approach

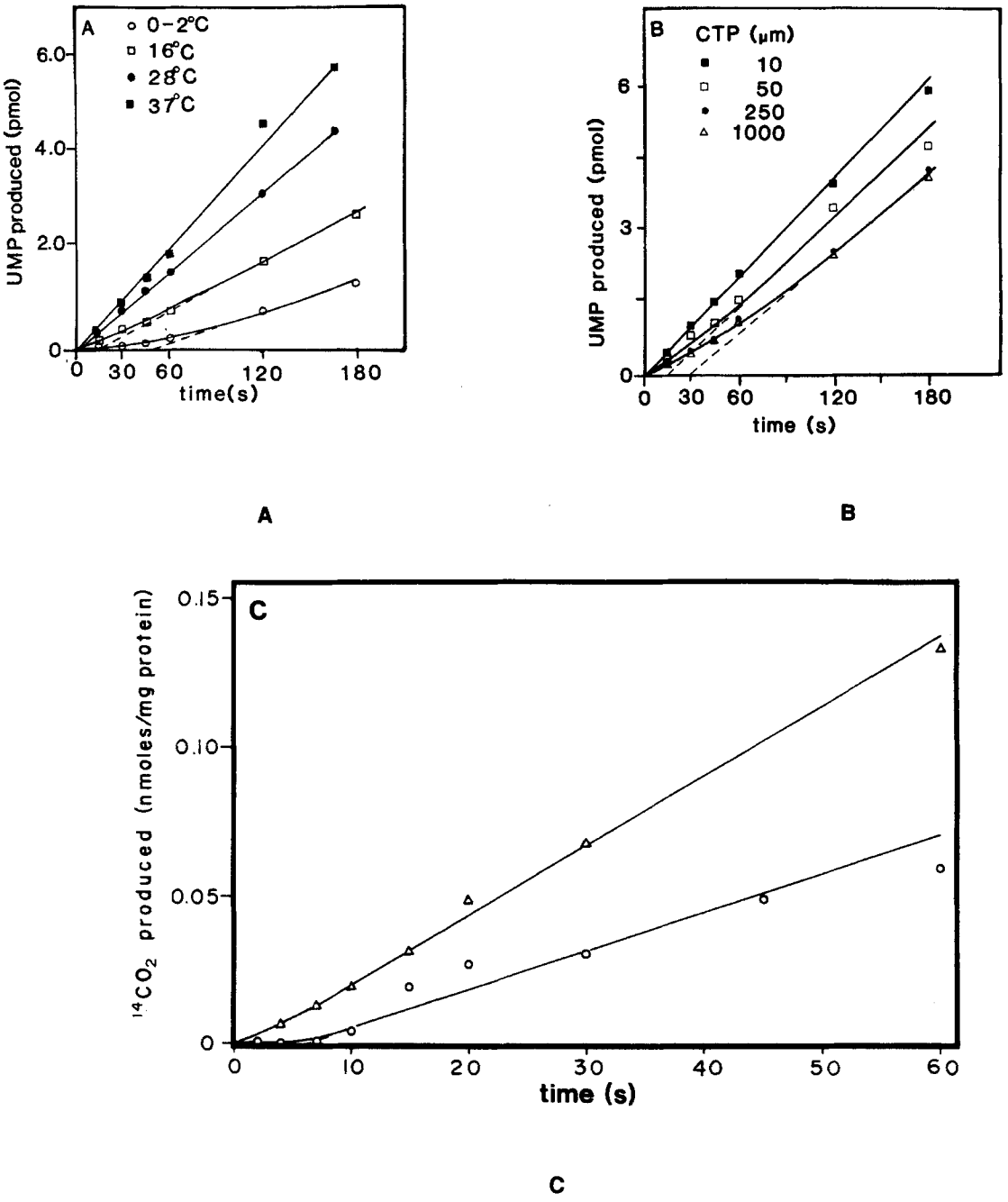


FIGURE 7. Initial velocity studies with uridine kinase and UMP synthase. The appearance of a lag in the progress curve was measured with uridine kinase as a function of temperature (A), or as a function of the dissociating inhibitor CTP (B), and with UMP synthase as a function of enzyme concentration (C) at 1.5 mg protein per milliliter (○) or 4.5 mg protein per milliliter (△).

involves chemical coupling of enzyme to the resin with a procedure that will not significantly alter enzyme activity. Whereas most enzymes are stable oligomers, once coupled to a matrix, they may be treated with a dissociating ligand or a mildly denaturing reagent to produce complete dissocia-

tion. The resin can then be washed free of all uncoupled subunits, and the medium subsequently restored to favor the native conformation of the subunits, which remain dissociated by virtue of being bound to the resin. Samples of such covalently bound enzyme resin can then be tested in kinetic assays.

IV. MODELS FOR ENZYME REGULATION BY DISSOCIATION OF OLIGOMERS

A. Hemoglobin: A Historical Footnote

Because hemoglobin shows positive cooperativity in binding oxygen and because it is so well characterized, hemoglobin has been used traditionally as a model system to explain ligand binding and cooperativity.[5] By 1910 various investigators (cited in Reference 69) had demonstrated cooperativity for oxygen binding with isolated hemoglobin preparations, but the shapes of oxygen binding curves varied considerably. In that year, A. V. Hill proposed that hemoglobin could exist in various states of aggregation, depending on buffer composition, and that oxygen binding curves reflected this variable aggregation state.[69] Hill had no physical measurements on the molecular weight of hemoglobin in these studies, but he found that an equation for a saturation function very exactly modeled the oxygen binding curve:

$$y = 100\frac{Kx^n}{1+Kx^n}$$

where K is the association constant for oxygen binding, x the partial pressure of oxygen, and the exponent n was proposed as representing the average extent of aggregation for human hemoglobin, and therefore the number of O_2 bound per hemoglobin molecule. Testing the equation against published data, Hill found various values of n from 1.67 to 3.19, suggesting differing degrees of oligomerization for hemoglobin as a function of the preparation and its buffer.[69] Although original, this proposal had no impact on studies of enzyme dissociation, and more recent experiments have shown that human hemoglobin remains tetrameric under physiological conditions, and that over most concentrations of O_2 hemoglobin tetramers are either unliganded or fully liganded.[70] Today the exponent n is still used to represent the degree of cooperativity and is commonly designated the Hill coefficient. Although hemoglobin has the potential for dissociation *in vitro*, the concentration of hemoglobin and its effectors do not vary over the appropriate ranges to make this a regulatory mechanism *in vivo*. A possible exception may be lamprey hemoglobin, which normally exists as a dimer in the deoxygenated form and dissociates to the monomer on binding O_2.[71]

B. Models for Correlating Dissociation with Change in Enzyme Activity

1. Models for Allosteric Regulation

Classic models for explaining enzyme regulation by allosteric ligands are based on the assumption of a change between two conformational states, as illustrated in Figure 8a.[5,6] This conformational model assumes an equilibrium between inactive and active forms of the enzyme. The binding of positive effector ligands would stabilize the active form, while inhibitors would stabilize the inactive form. When enzymes are formed as oligomers with two or more subunits, then binding of a ligand to one subunit can communicate a conformational change to an adjoining subunit, leading to an observed change in activity that is designated as cooperativity. Consistent with such a simple conformational model, dissociation/reassociation is a process that can lead to a different conformation: if the intrinsic conformation of the dissociated monomer is inactive, then interaction between subunits in the associated tetramer could serve to stabilize an active conformation (Figure 8a). The standard conformational model is described in all biochemistry textbooks, and therefore will not be described here in greater detail.

An alternative mechanism for utilizing dissociation/association to regulate enzyme activity comes from the ability of some enzymes to form a functional ligand binding site between two subunits. As diagrammed in Figure 8b, in the dissociated subunit the sites for the two substrates, A

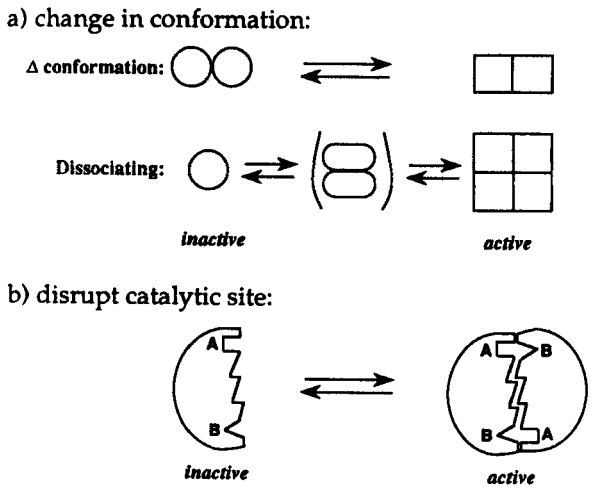


FIGURE 8. Models for conformational change and loss of activity by dissociation.

and B, are spatially too far apart to permit the chemical interaction for catalysis to occur. However, in the dimer the two substrate binding sites are now appropriately positioned to facilitate binding of A next to B. For simplicity, Figure 8b was drawn to emphasize the positions of the A and B sites, but this clearly also involves some conformational changes. Therefore, any process that shifts the equilibrium in Figure 8b directly leads to a change in enzyme activity. The two mechanisms illustrated by Figures 8a and 8b are not mutually exclusive, and some enzymes appear to include aspects of both.

2. Enzymes with Ligands Binding between Subunits

Whereas Figure 8b may appear speculative, there is emerging evidence for the binding of ligands between subunits in a few crystal structures. This feature is slightly more complex than illustrated in Figure 8b, which illustrates a complete catalytic site between subunits, but shows each ligand bound to only one subunit. More realistic would be the binding of a ligand in a pocket between two subunits, with residues from each subunit required for proper binding. Examples of this are summarized in Table 2. For this limited data set, we see two examples of a substrate binding between subunits to form a catalytic site: aspartate carbamoyltransferase and phosphofructokinase. We see one example of a substrate binding at a regulatory site between subunits (hexokinase), and several examples of an allosteric effector binding between subunits.

Evidence that carbamoyl-P, one of the substrates for aspartate carbamoyltransferase (Table 2), binds at the interface of two subunits, came from studies with hybrid oligomers reconstituted from catalytic subunits, each altered at one of two specific sites.[72] The amino acid residues subjected to mutation were tyrosine-165 and lysine-84, which have both been implicated in the binding of carbamoyl-P. The distance between these residues within one subunit is much greater than the distance across the interface between adjoining subunits, suggesting that the active site may be at the junction of two subunits.[73] The catalytic trimers of aspartate carbamoyltransferase from mutants in which either tyrosine-165 or lysine-84 were replaced with a different amino acid were inactive. However, when the inactive oligomers were dissociated, and the 2 types of subunits mixed together and allowed to reassociate, significant catalytic activity was observed. The level of activity regained was about one third of the activity observed in the wild-type trimer and corresponded to the statistically predicted fraction of neighboring subunits having an intact tyrosine-165 —

TABLE 2
Ligand Binding between Subunits, Determined in Crystal Structures

Enzyme	Source	Site (ligand)		Binding between		Ref.
		Catalytic	Regulatory	Subunits	Domains	
Aspartate carbamoyl transferase	*E. coli*	Carbamoyl-P		x		74
Glycogen phosphorylase	Rabbit muscle		AMP	x		80, 81
Hexokinase	Yeast		ATP	x		88
Phosphofructokinase	*B. stearothermophilus*	F-6-P		x		75–78
			ADP; PEP	x		76
	E. coli	F-6-P		x		79
			ADP	x		79
	Rabbit muscle[a]	F-6-P			x	84
			F-2,6-P_2		x	84
			ATP		x	84
			ADP; AMP		x	84

[a] For the enzyme from rabbit muscle, a model was made using the structure for the bacterial enzyme.

lysine-84 pair.[72] This observation suggested that amino acid side chains from neighboring subunits can constitute a functional active site, presumably located at the subunit junction. Also, such results lead to a direct model for allosteric control,[74] as only a modest conformational change is required to alter the size of the catalytic cleft between subunits, leading to decreased binding of carbamoyl-P.

For phosphofructokinase, the data for the bacterial enzymes come from crystal structures for the enzyme isolated from *B. stearothermophilus*[75–77] and from *E. coli*.[78,79] The bacterial enzyme, a tetramer, with one catalytic site and one regulatory site on each subunit is illustrated in Figure 9. Each catalytic site is at the junction of two subunits and the substrate fructose-6-P requires residues from each subunit to bind properly. Thus each subunit participates in forming two complete catalytic sites per dimer. Similarly, the regulatory site is at the junction of two subunits, with positive and negative effectors competing for binding at this site. The activator ADP stabilizes a more open form of the regulatory site, which is physically coupled to a movement that forms the nearby fructose-6-P binding site. In contrast, binding of the inhibitor phosphoenolpyruvate stabilizes a more closed form of the regulatory site, which physically distorts the nearby fructose-6-P binding site. These elegant studies thus show that it is not necessary to invoke global conformational changes, by demonstrating a direct physical linkage whereby modest movements between subunits 1 and 4 (and between 2 and 3) along the x axis of the tetramer lead to equally modest movements between subunits 1 and 2 (and between 3 and 4) along the z axis to either stabilize or to distort the binding site for fructose-6-P, thereby changing the observed K_m for fructose-6-P.[77] If the tetramer of phosphofructokinase depicted in Figure 9 were to dissociate to dimers containing subunits 1 and 4, or subunits 2 and 3, such dimers would have no catalytic activity.

Glycogen phosphorylase is a well-studied enzyme that contains four distinct regulatory sites.[80] For optimal function, the native enzyme, designated phosphorylase *b*, must bind AMP. Alternatively, the enzyme can be covalently modified by phosphorylation to produce the most active enzyme, phosphorylase *a*. Recent studies have now established that the binding of AMP occurs between subunits in the dimer and produces conformational changes very similar to those produced by phosphorylation.[80,81] This enzyme is normally more stable as a less active tetramer,[56,82,83] which is converted to the more active dimer by AMP. The recent crystal structures clearly show how AMP binding stabilizes the active dimer.

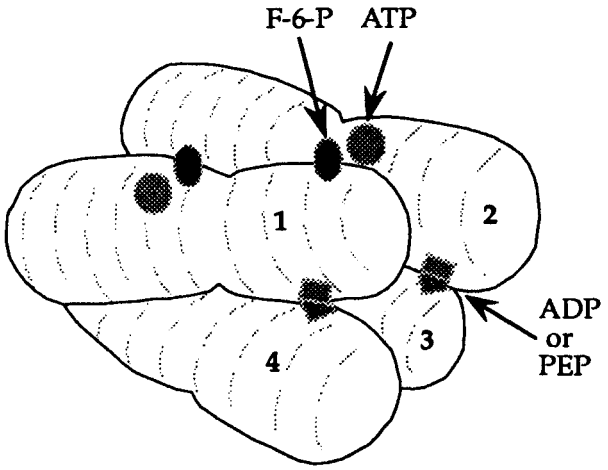


FIGURE 9. Structural model for bacterial phosphofructokinase. Shown are the four subunits of the tetramer, and the ligand binding sites for the two substrates, fructose-6-P (F-6-P) and ATP, as well as the regulatory site for the activator, ADP, or the inhibitor, phosphoenolpyruvate (PEP). (Adapted from Evans and Hudson.[75])

V. BINDING OF SUBSTRATE OR PRODUCT AT AN ADDITIONAL REGULATORY SITE

By definition, substrates and products always bind to the catalytic sites of enzymes. Therefore, the application of Occam's razor leads to an initial assumption that any effects produced by such ligands is via binding at the catalytic site. However, just as enzymes may have evolved complexity by forming binding sites between subunits, they may also have become more responsive to regulation by evolving separate sites for the regulatory action of substrates or products.

An early demonstration of such a structural feature occurred with the mammalian phosphofructokinase by using the structural data for the bacterial phosphofructokinase described above. Poorman et al.[84] determined the sequence for the mammalian phosphofructokinase, which has high homology with the bacterial enzyme, and showed that the mammalian enzyme (twice the size of the bacterial enzyme) is the product of gene duplication plus fusion. Thus each subunit of the mammalian enzyme potentially has two catalytic sites and two regulatory sites, and it was proposed that one of the catalytic sites became altered to a novel regulatory site to facilitate the binding of fructose-2,6-bisphosphate, the most important activator for the mammalian enzyme, which is structurally similar to the product fructose-1,6-bisphosphate.[84] Also, one of the original regulatory sites became mutated, while the bacterial enzyme bound both ADP (activator) and phosphoenolpyruvate (inhibitor) at the same regulatory site (Figure 9), the mammalian enzyme now binds AMP or ADP as an activator at one regulatory site and ATP as an inhibitor at the second of these derived regulatory sites.[84] Thus, the doubling in subunit size for the mammalian phosphofructokinase has produced two extra regulatory sites to provide extra sensitivity for regulating this important enzyme in glycolysis. The high sequence homology made it possible to model the mammalian enzyme by comparison with the bacterial structure, and thus tentatively position all three regulatory sites in a proposed structure.[84] No studies have described whether phosphofructokinase from *B. stearothermophilus* dissociates, but many studies have characterized this feature for the mammalian enzyme, and these will be considered later.

Whereas Poorman et al.[84] used the structure of the bacterial phosphofructokinase to model the enzyme from rabbit muscle and suggest that the regulatory site for ATP lies at the interface of two subunits, additional studies have helped to verify this. In one study the enzyme was briefly treated with protease digestion, which removed only 17 amino acids from the C-terminus.[85] This modified enzyme had normal V_{max} and normal affinity for substrates. However, instead of binding two ATP/subunit, it now bound only one (at the catalytic site). Because the rabbit enzyme has an extra 35-amino acid extension at the C-terminus in comparison to the bacterial enzyme,[84] this more recent study shows that the formation of the new regulatory site for ATP was not due simply to modification of the preexisting regulatory site on the bacterial enzyme, but also required some additional structural element(s) to be added.

Studies with hexokinase show how a substrate can bind at a separate regulatory site. This enzyme from yeast is readily interconverted between monomer, dimer, and tetramer forms,[86–88] with the dimer generally being the most active species. The crystal structure for this enzyme shows a special binding site for ATP between the two subunits, a site that is distinct from the catalytic site.[88] The list in Table 2 is not inclusive for all enzymes, but merely for those enzymes considered in the three metabolic pathways for this review. There are other enzymes that also share this feature, as exemplified by tyrosyl-tRNA synthetase, which binds one tRNA substrate with both subunits of the dimer.[89]

The results with hexokinase provide an interesting demonstration of a substrate functioning as an effector, but at a separate regulatory site. This is a second feature that may be useful in modeling structure with function: the possibility of a separate site, different from the catalytic site, where a substrate or a product may bind as allosteric effector. When a substrate produces an allosteric effect as measured by kinetic cooperativity, it is logical to assume that this conformational response is due to the substrate binding at the catalytic site. However, where the concentration of substrate required to produce a measurable conformational change can be measured by physical studies or by ligand binding studies, the affinity constants so determined are sometimes significantly different from the normal K_m, which measures affinity at the catalytic site. Examples of such enzymes are in Table 3, where affinity at the catalytic site is defined as K_m or K_i, while affinity at a proposed regulatory site is defined as K_d. For hexokinase,[88] phosphofructokinase from *E. coli*,[76–79] and purine nucleoside phosphorylase,[90] there are crystal structures of enzyme plus ligand to support the kinetic

TABLE 3
Enzymes with a Possible Separate Regulatory Site for Substrate or Product

Enzyme	S or (P)	Km or (Ki)	Kd	Ref.
A. β-Alanine synthase (rat liver)	NCβA	8 μ*M*	9 n*M*	31
DNA restriction enzyme *Nae I* (*N. aerocolonigenes*)	DNA	246 n*M*	6 n*M*	91
Hexokinase (rat brain)	(glu-6-P)	(>200 μ*M*)	2.5 μ*M*	92
Phosphofructokinase (*E. coli*)	(ADP)	(200 μ*M*)	25 μ*M*	97
UMP synthase (mouse ascites cells)	OMP	230 n*M*	31 n*M*	99
B. Ca^{2+}-ATPase (rabbit muscle)	ATP	9.5 μ*M*	3.5 m*M*	100
Hexokinase (yeast)	ATP	30 μ*M*	3.0 m*M*	88, 101
Purine nucleoside phosphorylase (calf spleen)	P_i	3.1 m*M*	88 m*M*	42

data; for other enzymes, examples were included in Table 3 only when the difference between the two types of measurements was at least an order of magnitude. For the other enzymes in Table 3, the large difference in affinity as measured by effect on enzyme activity when compared with measurements of some other physical or kinetic change, supports the likelihood of a distinct regulatory site for a ligand that also binds at the catalytic site. For the enzymes in Table 3A, the affinity at the regulatory site is higher than at the catalytic site, while for the enzymes in Table 3B affinity at the regulatory site is lower.

Studies with β-alanine synthase serve as an example of such measurements. This allosteric enzyme hydrolyzes the substrate *N*-carbamoyl-β-alanine (NCβA) to produce β-alanine. Physical studies showed that this substrate converts the enzyme from the native hexamer to a dodecamer.[31,32] Rapid kinetic studies had also shown hysteresis in the progress curve, consistent with a change to a more active conformation following the addition of substrate to the enzyme reaction.[32] However, normal kinetic studies failed to show the expected cooperativity as substrate was increased from low concentrations of about 0.1 K_m (where K_m = 8 μ*M*). Only when kinetics were done at much lower substrate concentrations was cooperativity evident (Figure 10), with the Hill plot showing positive cooperativity at concentrations of NCβA below 15 n*M*, and no cooperativity at concentrations of NCβA above 15 n*M*, where the enzyme had attained the fully active conformation. An interpretation of these results is that the enzyme has a separate regulatory site at which NCβA acts as an obligatory activator.

Kinetic studies with the restriction endonuclease *Nae I* showed that certain oligonucleotide substrates, even though they had the correct nucleotide sequence for binding at the catalytic site, were resistant to hydrolysis.[91] When the enzyme was preincubated with good substrates, it became activated and was now also very efficient with the previously resistant oligonucleotides. Measurement of the K_d for activation produced a value significantly lower than the K_m and suggested a separate site where substrate DNA could bind as an effector.

With rat brain hexokinase, binding studies were done at high and low concentrations of ^{14}C-glucose-6-P in the absence or presence of competing hexoses and hexose-phosphates.[92] It was found that there was a high-affinity K_d for glucose-6-P, almost identical to the normal K_i for this product in standard kinetic studies. A second, low-affinity site was found with a K_d >200 μ*M*. These results suggested that the product binds initially at a high-affinity regulatory site, and that the low-affinity site is the catalytic site. Because

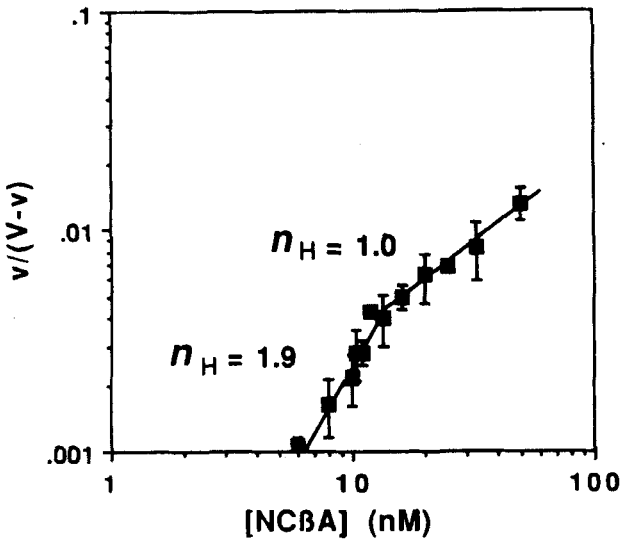


FIGURE 10. Hill plot for β-alanine synthase activity.

mammalian hexokinase has a subunit molecular mass of 100 kDa, whereas the yeast hexokinase has a subunit of 50 kDa, such results are consistent with the evolution of the mammalian enzyme by gene duplication plus fusion. This hypothesis was convincingly validated by a series of experiments by Wilson and colleagues.[93–96] They completely sequenced all three mammalian hexokinases and established that both the N- and C-terminal halves had high identity with the smaller yeast hexokinase and with each other. The sequence identity for the two domains in each mammalian enzyme was 46% for the type I hexokinase,[93] 55% for the type II hexokinase,[95] and 40% for the type III hexokinase.[94] A comparison with the structurally defined yeast enzyme permitted identification of important catalytic residues in the mammalian enzyme; mutational studies indicated that the C-terminal domain retained the functional catalytic site, whereas the N-terminal domain had an altered catalytic site that now functioned as the regulatory site for glucose-6-P.[96] Therefore, one may view the mammalian hexokinase as a dimer that has become covalently joined and then been modified, so that the established linkage between the two previous catalytic sites could now be used for novel allosteric regulation by the product of this enzyme activity. The results with the mammalian hexokinases are thus comparable to the above results for the mammalian phosphofructokinase, where the evidence also strongly supported fusion, after gene duplication of a smaller enzyme, such as found in bacteria, and subsequent modification of one set of catalytic sites to become new regulatory sites.

For bacterial phosphofructokinase, early kinetic studies had established that ADP was an activator, and, as with hexokinase, this product bound preferentially at a high-affinity regulatory site.[97] These kinetic experiments were sufficiently detailed that they clearly showed evidence for ADP binding at two sites. The authors performed a series of initial velocity studies at variable concentrations of fructose-6-P, each experimental set being done at a different concentration of ADP. The resulting velocity curves were sigmoidal in the absence of ADP, thereby demonstrating cooperativity, and increasing ADP concentrations made these activity curves (vs. fructose-6-P) become hyperbolic, demonstrating a conformational change in the enzyme. As ADP was increased, these experiments also demonstrated a resulting decrease in the K_m for the substrate fructose-6-P. However, at the same time V_{max} was clearly decreasing at increasing concentrations of ADP, demonstrating that at lower concentrations ADP activated the enzyme, whereas at higher concentrations ADP inhibited the enzyme.[97] Because the affinity for ADP is higher at the regulatory site, it is normally an activator. For this example, the difference in affinity between the two sites on the enzyme was less than tenfold, but the crystal structure clearly established that the two sites exist.[79]

UMP synthase is an enzyme with two different catalytic activities: orotate phosphoribosyltransferase, which produces orotidine-5′-phosphate (OMP); and OMP decarboxylase, which makes UMP.[98] The substrate OMP, as well as various analogs that are inhibitors of the decarboxylase, can convert the inactive monomer to the active dimer. By comparing the K_d of these compounds needed for dimerization to their K_i or K_m as inhibitors or substrate, it was concluded that dimerization required a separate regulatory site.[29] Kinetic studies also showed that the enzyme has positive cooperativity for OMP at low concentrations of substrate, with a K_{act} of 31 n*M*, in contrast with the apparent K_m of about 230 n*M*.[99] In contrast to the different affinities seen with OMP, comparable studies with the product UMP were interpreted as UMP binding better at the catalytic site, where it produces inhibition, and binding less well at the proposed regulatory site where UMP also acts to stabilize the conformational change leading to the active dimer.[29]

The enzymes in Table 3B all appear to have a second site for binding substrate with a lower affinity. Calcium ATPase has a stable phosphoenzyme intermediate that makes it possible to measure catalytic cycles of ^{32}P-labeled enzyme in the presence of unlabeled nucleotides.[100] When measuring ATP synthesis with unlabeled ADP, the addition of ATP did not inhibit or compete with ADP, but instead produced activation. The high-affinity site was identified as the catalytic site, and activation occurred by binding at the lower-affinity second site.[100] The two ATP-utilizing kinases also give evidence for regulatory sites. For hexokinase, kinetic studies supported the idea of two ATP sites with significant differences in

affinity.[101] These results were confirmed by the crystal structure for the enzyme complexed with ATP.[88] For mammalian phosphofructokinase, many kinetic studies have shown that ATP is both a substrate and an inhibitor. Hexokinase from sheep showed that the high-affinity site binds ATP as a substrate, while at the low-affinity site ATP acts as an inhibitor,[102] consistent with the physiological regulation for this enzyme.

Purine nucleoside phosphorylase responds to the substrate P_i by dissociating from the trimer to the monomer, as shown in Figure 5. While the K_d for the dissociation effect is unphysiologically high, it clearly indicated a separate binding site for phosphate,[42] and this interpretation was supported by the crystal structure for this enzyme complexed with phosphate.[90] Thus whereas two different types of experiments support two distinct binding sites for P_i on purine nucleoside phosphorylase, the weak binding at the second site suggests that P_i is probably an analog for some as yet unidentified regulatory effector that would be expected to have a much higher affinity at this second site.

VI. PROPERTIES OF DISSOCIATING ENZYME IN MAJOR AREAS OF METABOLISM

A. Enzymes in Carbohydrate Metabolism

The enzymes in this branch of metabolism, which were among the first to be characterized as dissociating enzymes, include glycogen phosphorylase *a* (phosphorylase), hexokinase, glyceraldehyde-3-P dehydrogenase, and glucose-6-P dehydrogenase. The enzymes are all listed and summarized in Table 4. For phosphorylase from rabbit muscle it is generally agreed that the native, less active, enzyme is a tetramer and that the most active form is the dimer.[56,82,83] Both the dimer and the tetramer can undergo a $T \rightleftharpoons R$ conformational transition. AMP is an activator that can convert the inactive dimer to the active dimer ($2^\circ \rightarrow 2^*$), as evidenced by converting a kinetic profile that is sigmoidal to one that is hyperbolic.[82] However, AMP alone favors converting the inactive dimer to the tetramer, so that the overall scheme is represented by:

$$2^* \underset{\text{AMP, glycogen}}{\rightleftharpoons} 2^\circ \overset{\text{AMP}}{\rightleftharpoons} 4^\bullet \overset{\text{glycogen}}{\rightleftharpoons} 2^*$$

This scheme is intended to explain why the effects of AMP and glycogen, when added together (no change in M_r), differ from the effects when they are added sequentially. Amyloheptose, a substrate analog, has also been shown to favor dissociation of the native tetramer to the dimer.[83] Enzyme concentration itself affects the dimer-tetramer equilibrium. Association of phosphorylase to the tetramer was observed at an enzyme concentration greater than 0.1 mg/ml, a value below the physiological concentration of phosphorylase, consistent with unliganded enzyme *in vivo* existing as a tetramer.[56] The specific activity of phosphorylase increased directly with dilution of enzyme, consistent with the dimer as the most active form.[56] In this respect, it is noteworthy that dissociation of tetramer to dimer by amyloheptose was observed at an enzyme concentration of 1.5 mg/ml.[83] The enzyme does not readily dissociate to monomers, and the activity of isolated subunits was assessed by crosslinking the enzyme to Sepharose, denaturing the enzyme to cause dissociation, and, after removal of unbound subunits, renaturing the resin-linked enzyme. Subunits were judged to be inactive because the activity remaining was only 3% of that expected for the amount of coupled enzyme.[103] That the procedure itself did not inactivate the enzyme was shown by additional studies where denatured enzyme was stripped of the intrinsic pyridoxal phosphate, which was then replaced by pyridoxal phosphate monomethylester (dephospho-enzyme). Such denatured dephospho-enzyme was mixed with denatured phospho-enzyme and renatured to form hybrid dimers. The hybrid dimers had 50% of the activity of native dimer.[103] However, in a subsequent review it was suggested that this enzyme does not dissociate *in vivo*.[104]

Phosphorylase kinase, the enzyme responsible for the activation of glycogen phosphorylase, may itself be a dissociating enzyme. Like its substrate, phosphorylase kinase is remarkably abundant in rabbit muscle, with a cellular concentration >1 mg/ml.[57] Dilution of enzyme to less than 25 µg/ml led to significant loss in the specific activity.[57] Inasmuch as the enzyme shows a lag

TABLE 4
Dissociating Enzymes in Carbohydrate Metabolism

Enzyme	Source	Oligomeric species	Effectors (+)	Effectors (–)	Ref.
Aldolase	Rabbit muscle	$1^* \rightleftarrows \underline{4}^*$			66, 133, 134
Fumarase	Pig muscle	$1^\circ \rightleftarrows 2 \rightleftarrows \underline{4}^*$	P_i, citrate		46
			malate, ATP		46
Glucokinase	Rat liver	$\underline{1}^\circ \rightleftarrows 2^*$	ATP		110
Glucose-6-P dehydrogenase	Human erythrocytes	$1^\circ \rightleftarrows \underline{2}^\bullet \rightleftarrows 4^*$	$NADP^+$	$NADPH_2$	116
	N. crassa	$\underline{2} \rightleftarrows 4^*$	$NADP^+$		117
Glyceraldehyde-3-P dehydrogenase	Rabbit muscle	$\underline{2}^\circ \rightleftarrows \underline{4}^* \rightleftarrows 8$	$P_i + NAD^+$		113, 114
		$2 \rightleftarrows \underline{4}^*$			115
	Yeast	$1 \rightleftarrows 2^\circ \rightleftarrows \underline{4}^*$	NAD^+	ATP	112
Glycerol kinase	*E. coli*	$1 \rightleftarrows \underline{2}^* \rightleftarrows \underline{4}^* \rightleftarrows 4^\circ$	None	$F\text{-}1,6\text{-}P_2$	130–132
Glycogen phosphorylase *a*	Rabbit muscle	$2^* \rightleftarrows 2^\circ \rightleftarrows \underline{4}^\bullet \rightleftarrows 2^*$	Glycogen; AMP glu-1-P		82
		$\underline{2}^* \rightleftarrows \underline{4}^\bullet$	Amyloheptose		83
		$\underline{2}^* \rightleftarrows \underline{4}^\bullet$	Dilution		56
Hexokinase	Yeast	$1^\circ \rightleftarrows 2^* \rightleftarrows \underline{4}$	P_i; glucose	H^+	86
		$1 \rightleftarrows 2^*$	ATP		88
		$1^\circ \rightleftarrows 2^* \rightleftarrows \underline{2}^\bullet$	Glucose		87
Isocitrate dehydrogenase	*B. emersonii*	$\underline{1}^* \rightleftarrows 2 \rightleftarrows 4^\circ$	Isocitrate citrate	H^+	129
	Bovine heart	$8^\circ \rightleftarrows \underline{16}^* \rightleftarrows 32$	P_i; ADP	NADH	54
Malate dehydrogenase	Pig heart	$1^* \rightleftarrows \underline{2}^*$	Citrate; malate		68, 136
Phosphofructokinase	*E. coli* (pfk-2)	$\underline{2}^* \rightleftarrows 4^*$	F-6-P	ATP	120, 121
	Rabbit muscle	$2^\circ \rightleftarrows \underline{4}^* \rightleftarrows n^*$	P_i; F-6-P	H^+; citrate	47
		$\underline{4}^* \rightleftarrows 8^*$			47
		$1 \rightleftarrows \underline{4}^* \rightleftarrows 16^\bullet$			50
		$1 \rightleftarrows 2 \rightleftarrows \underline{4} \rightleftarrows 4^* \rightleftarrows 16$	ADP; P_i	H^+; citrate	51
			F-6-P; ATP		51
		$1^\circ \rightleftarrows 2^\circ \rightleftarrows 4^\bullet \rightleftarrows \underline{4}^*$	ADP; cAMP	Oxidation	123
	Human erythrocytes	$2^\circ \rightleftarrows 4^* \rightleftarrows 8 \rightleftarrows 16$	F-6-P; low ATP	High ATP	127
	Rat liver	$\underline{4}^* \rightleftarrows 16 \rightleftarrows 64$ etc			185
		$2 \rightleftarrows \underline{4} \rightleftarrows n^*$	$F\text{-}2,6\text{-}P_2$	ATP; dilution	52
Phosphorylase kinase	Rabbit muscle	Protomer$^* \rightleftarrows \underline{n}^\bullet$			57
Pyruvate kinase	Ehrlich ascites	$\underline{2}^\bullet \rightleftarrows \underline{4}^*$	$F\text{-}1,6\text{-}P_2$		127
			PEP; ADP	Alanine	127
	Bovine muscle	$1^\circ \rightleftarrows \underline{4}^*$	DTT		67
	Rabbit muscle	$\underline{4}^* \rightleftarrows 4^\circ$			128, 186

Note: F, fructose; DTT, dithiothreitol; PEP, phosphoenolpyruvate. Enzyme activity: * = most active; • = partly active; ° = inactive; _ = native oligomer.

time in kinetic assays, one of the substrates presumably stabilizes the more active oligomer, although reassociation was not directly demonstrated.

While mammalian glucokinases are often considered to be monomers,[105–109] there is also support for their existing as dimers,[105,110] and this controversial enzyme will be considered in a later section (Section VII.C). The yeast hexokinase is structurally similar and has a very similar reaction. This enzyme clearly forms oligomers, usually in the presence of one substrate. Yeast hexokinase was found to be a native tetramer that dissociated to the active dimer in the presence of glucose or of inorganic phosphate.[86] Alternatively, it was found to sediment as an inactive dimer with a sedimentation value ($S_{20,w}$) of 5.6 S, which adopted a new conformation in the presence of glucose (4.8 S).[87] High salt caused the enzyme to dissociate and sediment as a monomer (3.6 S). A crystal structure for this enzyme showed the dimer to be stabilized by ATP binding at a regulatory site between the two subunits.[88]

Another abundant protein, glyceraldehyde-3-P dehydrogenase, has been reported to be 19% of the total protein in yeast and 10% of total protein in rabbit muscle.[111] The native enzyme is the active tetramer. Incubation of the enzyme with ATP, a feedback inhibitor, led to loss of activity and dissociation into dimers. This activity was not recovered even after dialysis to remove the ATP.[112] Addition of the substrate NAD^+ to the inactive enzyme led to recovery of activity and association back to the tetramer.[112] The same enzyme from rabbit muscle also exists as a tetramer[113,114] when the holoenzyme was tested at an enzyme concentration of 2 mg/ml, although for the apoenzyme there was a mixture of dimers and tetramers at this concentration.[113] *In vitro* this enzyme was also shown to oligomerize further to octamers, although the significance of this was unclear. Acylated enzyme dissociated to dimers, but could be reassociated if NAD^+ and P_i were both added; either one alone was ineffective in restoring activity.[113] Using T-jump relaxation kinetics to follow the dissociation of NADH at different enzyme concentration, negative cooperativity for this ligand was observed.[114] Although the presence of NADH by itself had no effect on association or dissociation (similar to the previous study with NAD^+), the authors found they could model the kinetic cooperativity exactly with a model in which the enzyme dissociated from tetramer to dimer.[114] Such modeling efforts are interesting in light of previous studies showing this enzyme capable of dissociating.[113,115]

In both humans and *Neurospora*, glu-6-P dehydrogenase exists as a native dimer that can associate to the more active tetramer.[116,117] Incubation of the human enzyme with one of the substrates, glucose-6-P, led to the dissociated monomer that is inactive. Reactivation required incubation with the second substrate, $NADP^+$, which also promotes the tetramer form.[116] In similar fashion, the enzyme from *Neurospora* is stabilized as the tetramer in the presence of $NADP^+$.[117] Two laboratories have sought to establish whether dissociation occurs *in vivo* with this enzyme, and these results will be detailed in Section VI.E.[118,119]

The bacterial phosphofructokinase exists in two very distinct isozyme forms.[120] The isozyme phosphofructokinase-1 is the form that is most abundant, and, therefore most studied, and was used for the crystal structures already discussed. This enzyme appears to be a native active tetramer.[120] By comparison, the minor isozyme phosphofructokinase-2 has very different features, binding the 2 substrates in a different temporal sequence, and being responsive to different regulatory effectors than phosphofructokinase-1.[120] It is then interesting that phosphofructokinase-2 exists as a native dimer, which is also the most active form, and that higher concentrations of enzyme, or of ATP (which becomes an inhibitor), lead to the less active tetramer.[120,121] That the effect of ATP occurs at a regulatory site was supported by studies with a form of this enzyme specifically mutated at the regulatory site: the enzyme remained active but sedimented as a dimer, even in the presence of 1 m*M* ATP.[121]

No studies have reported that phosphofructokinase-1 dissociates under physiological conditions. However, the dissociation of the enzyme tetramer, under denaturing conditions, was monitored by the fluorescence of tryptophan-311, a residue at the regulatory site, during increasing concentrations of the chaotropic agent KSCN.[122] Enzyme activity was completely absent at 0.3 *M* KSCN, whereas the fluorescence signal remained unchanged. This loss of enzyme activity was con-

sistent with dissociation of the tetramer to dimers and disruption of the catalytic site, whereas the continued presence of the fluorescence signal showed that the regulatory site remained intact. Considering the four numbered subunits for phosphofructokinase in Figure 9, these results suggested the dissociation of the tetramer to form dimers of subunits 1 + 4 and subunits 2 + 3, so that the initial dissociation step deformed the catalytic site but did not alter the regulatory site. Increasing KSCN produced further dissociation of these dimers to monomers. Fructose-6-P, when added before KSCN, stabilized the tetramer against dissociation; when added after KSCN, it facilitated reassembly of the tetramer and recovery of activity in the presence of KSCN up to 0.5 *M*.[122] These results are consistent with the crystal structure in defining where this enzyme is most susceptible to structural change.

The ability of phosphofructokinase from rabbit muscle to form different M_r species has been studied widely. The earliest studies showed that the enzyme could exist as at least five different oligomeric states:

$$1^\circ \rightleftharpoons 2^\circ \underset{\uparrow H^+}{\rightleftharpoons} \underline{4}^* \overset{\uparrow[E],\downarrow H^+}{\rightleftharpoons} 16^*$$

$$\underline{4}^* \uparrow\downarrow 8^*$$

The octamer was not interpreted as an intermediate to the higher oligomers (16-mer and larger) because the kinetics and conditions for its formation were not consistent with the formation of higher oligomers.[47–53,123,124] Dissociation to the dimer led to inactive enzyme.[47,48,123,124] Increasing enzyme concentration from 0.4 to 20 mg/ml led to higher polymers, as did an increase to >pH 8.[47] Below 200 μg/ml, the enzyme readily dissociated to dimers.[48] When the enzyme was studied at 0.3 to 2 μg/ml by active enzyme centrifugation (presence of both substrates) it remained a tetramer and did not appear to respond to changes in ligand concentration.[49] However, in these last studies there was no proper control for the native (unliganded) state. In the absence of ligands, at enzyme concentration less than 5 μg/ml, the monomer became a significant species. In separate studies, calmodulin was also shown to dissociate phosphofructokinase from rabbit muscle to the inactive dimer.[124]

Because the importance of such dissociation/association for the mammalian enzyme was not seen as relevant by all investigators,[125] a significant study by Lee and colleagues established the importance of proper enzyme preparation.[126] They showed that steps used during purification that could denature the protein, such as protein precipitation by heating or by alcohol (used by earlier investigators), could produce measurable changes in the normal properties of the enzyme. Normal phosphofructokinase was again shown to be a rapidly equilibrating enzyme system by sedimentation studies. But, when phosphofructokinase was purified involving heat or alcohol steps, the enzyme now became a heterogeneous and slowly equilibrating system, with one component that appeared to have native properties as measured by kinetic values or by sedimentation, and a second denatured component that showed poor ability to form tetramers, and that showed altered kinetic values, such as a greater Hill coefficient and a lower K_i for ATP, consistent with this being a less active form of the enzyme.[126] This study again verified the importance of enzyme concentration, both for achieving the tetrameric form, as well as for attaining the active conformation, as shown by Hill coefficients approaching 1.0.

It appears that the native tetramer does not have the same conformation as the active tetramer:

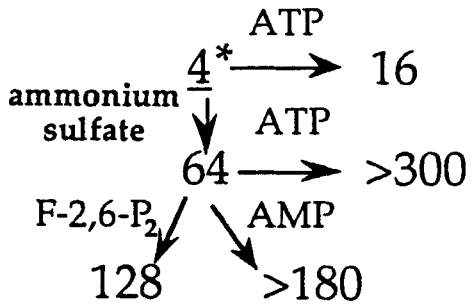
$$\underline{4}^\circ \overset{(+)}{\rightleftharpoons} 4^*$$

Sedimentation of phosphofructokinase in the absence of ligands produced a sedimentation coefficient of 13.5 S, but this decreased to 12.4 S in the presence of substrates.[51] Although both sedimentation constants are consistent with the enzyme being tetrameric, the lower sedimentation value in the presence of substrate was interpreted as the result of a conformational change. Similar observations were made for this enzyme in the presence of either oxidized glutathione or dithiothreitol.[123] The oxidized enzyme (T state) again centrifuged at 13.5 S, while the reduced enzyme (R state) returned to 12.4 S.[123]

Concerned about doing kinetics at a more physiological enzyme concentration of 600 μg/ml, Bosca et al.[53] noted that at high enzyme concen-

tration the K_m for fructose-6-P is significantly decreased; also, the normal cooperativity disappeared, and the enzyme now showed hyperbolic kinetics toward fructose-6-P. Quite similar results were obtained when they diluted the enzyme and then studied its kinetics in the presence of 10% polyethylene glycol, which favored aggregation of the diluted enzyme: now the enzyme was not activated by fructose-2,6-P_2, nor was it inhibited by ATP. To evaluate the effect of activators at high enzyme concentration, these authors chose to study the minor (slower) activities of phosphofructokinase: (1) the fructose-1,6-bisphosphatase, or (2) the ATPase. They observed that these activities were only activated under assay conditions commonly used, where the concentration of enzyme is below physiological levels. The authors concluded that the regulatory behavior of phosphofructokinase is totally dependent on its concentration, which is much higher *in vivo* than it is in most *in vitro* kinetic studies.

With phosphofructokinase from human erythrocytes, it was again observed that the dimer was inactive, and the tetramer active, although forms containing 8 and 16 subunits were also noted.[127] At low concentrations of ATP, this ligand acts principally as a substrate binding at the catalytic site and these authors noted that low [ATP] favored reassociation of inactive dimer to active tetramer. High [ATP] inhibits the enzyme at an allosteric site, and these authors observed that high [ATP] led to dissociation of the active tetramer.[127] The same enzyme from rat liver was shown to polymerize extensively as a function of different ligands:



The results are clearly not representative of the enzyme *in vivo*, inasmuch as ammonium sulfate was required to produce all forms of the enzyme larger than 16 subunits. Nevertheless, under these artificial conditions fairly discrete but different degrees of association were produced by three different ligands. A separate study contrasted the effect of the activator fructose-2,6-P_2 and enzyme concentration.[52] In rat liver the concentration of this enzyme should be greater than 30 μg/ml. The authors used enzyme concentration at 3.2 μg/ml and found that fructose-2,6-P_2 at only 1 μ*M* stabilized polymers much larger than tetramer; because the concentration of this activator in rat liver is in the range 0.1 to 10 μ*M*, the enzyme may well have a large polymeric form *in vivo*. In this study, the larger polymers were readily dissociated to tetramers by ATP.[52]

Pyruvate kinase from mouse, bovine, or rabbit tissues exists as a native tetramer, which is also the most active form.[67,127,128] The mouse enzyme was readily dissociated to the dimer, while ADP or phosphoenolpyruvate stabilized the tetramer.[127] In contrast, with the bovine enzyme, no dissociation was observed under nondenaturing conditions. This enzyme was covalently linked at one subunit to Sepharose and dissociated by denaturation, after which unbound subunits and denaturant were removed. No enzyme activity was measurable with the now monomeric enzyme, suggesting that monomers are inactive.[67] However, such inactivated enzyme could be renatured by adding soluble subunits and permitting them to reassociate with the immobilized subunits, and this also restored enzyme activity. Therefore, it was concluded that the inhibitor phenylalanine produced only a conformational change in the tetramer, and that a simple two-state model fit all the kinetic data.[67]

Isocitrate dehydrogenase provides one of the few examples where significant differences were reported for the enzyme in the two sources examined. The enzyme from *B. emersonii* was shown to be a native monomer, which was also the most active form, and became inactive on forming tetramers.[129] The bovine enzyme was a much larger native 16-mer, which was the most active form, and became inactive on dissociation.[54] The fungal enzyme has the substrate isocitrate as a positive effector,[129] while the bovine enzyme has the product NADH as a negative effector.[54]

Fumarase exists as a native, active tetramer, although the tetramer was unstable at pH <6 or >10, or when the enzyme was diluted.[46] Fumarase

is one of three enzymes where the most active form is stabilized by a product, in this case malate. Citrate, ATP, and P_i also stabilized the tetramer under conditions of enzyme dilution that would otherwise have favored dissociation to the dimer.[46]

Bacterial glycerol kinase was shown to readily interconvert between native dimers and tetramers.[130–132] After dilution the enzyme dissociated to dimers, and these were much less sensitive to inhibition by fructose-1,6-P_2. The inhibitor fructose-1,6-P_2 prevented dissociation and stabilized the T form of the tetramer. While both dimers and tetramers had comparable activity, they did not respond equally to regulatory inhibitors. Kinetic plots of the percent inhibition as a function of inhibitor concentration were sigmoidal at low enzyme concentration and became progressively more hyperbolic at increasing concentrations of the enzyme over a range of 0.011 to 500 μg/ml. The authors concluded that the advantage of the enzyme being in the tetrameric form was that this enzyme species proved more responsive to regulation by the allosteric inhibitor.

Aldolase and malate dehydrogenase form very stable native oligomers and are the two enzymes in Table 4 that are least likely to undergo dissociation *in vivo*. The aldolase monomer was proven to have full activity by crosslinking it to a resin and then removing free subunits after denaturation.[66] When the covalently linked enzyme was renatured, such monomeric enzyme was less stable to heat, changes in pH, or other parameters that lead to denaturation. This was true whether renaturation and reassociation were studied with free subunits[133] or with subunits coupled to an inert resin.[66] Because both monomer and dimer had comparable activity, the probable rationale for the enzyme's normal existence as a dimer was suggested to be its increased stability to denaturation or proteolytic degradation.[134] The study of hybrid aldolase tetramers was also consistent with active monomers. Enzyme was made inactive by succinylation, denatured and dissociated, and then mixed with denatured native enzyme. Hybrid tetramers formed with one to three native subunits, and the activity was proportional to the number of native subunits.[115] An alternative strategy led to formation of hybrids from three different isozymes. The result in this study was that antibodies specific to only one of the aldolase isozymes completely inactivated hetero-tetramers.[135] Explanations for this interesting result include the possibility that binding of antibody to one subunit leads to conformational changes in all subunits, or that some type of subunit interaction is required for activity, although this latter explanation seems to have been ruled out by experiments showing activity in subunits covalently linked to a resin.[66]

Crosslinking to Sepharose has also been used to demonstrate that malate dehydrogenase was active as the dissociated monomer.[68] Additionally, when heterodimers were formed with native enzyme and enzyme inactivated by iodoacetamide, heterodimers had half of the normal activity.[136] Under conditions of denaturation and dissociation, both malate and citrate stabilized the dimer. It was found that the dimer has regulatory features that favor reduction of NAD^+ to NADH, and that citrate specifically activated the potentially reversible reaction in that direction by binding at an allosteric site.[137]

B. Enzymes in Nucleotide Metabolism

In this area of metabolism the earliest enzymes shown to be able to dissociate included deoxythymidine kinase,[138,139] dCMP deaminase,[140,141] and glutamine phosphoribosyl pyrophosphate amidotransferase.[142] All the enzymes in this branch of metabolism are listed and summarized in Table 5. Deoxythymidine kinase from *E. coli* is a native monomer that required association to the dimer for optimum activity.[138,139] Association was produced by the strong activator dCDP, the weak activator dADP, and also by the inhibitor dTTP.[138] The monomer was also less stable, being very temperature sensitive and readily inactivated, while the dimer was more stable.[139] The enzyme from human lymphocytes was also a native monomer and readily associated to the more active dimer in the presence of the substrate ATP.[143] Very similar results were obtained with the enzyme from human fibroblasts. Here again the monomer was heat sensitive but stabilized by both ATP and ADP. Other nucleotides (CTP, GTP, UDP) were unable to protect the enzyme against

TABLE 5
Enzymes in Nucleotide Metabolism

Enzyme	Source	Oligomeric species	Effectors (+)	Effectors (–)	Ref.
Adenine PRTase	Human erythrocytes	2 ⇄ 4			153, 154
AMP deaminase	Rabbit muscle	2 ⇄ 4*	AMP		44
β=Alanine synthase	Rat liver	3° ⇄ 6* ⇄ 12*	NC-β-ala	β-Alanine	31, 32
Carbamoyl phosphate synthase	*E. coli*	1° ⇄ 2* ⇄ 4*	P_i; IMP; ATP	UMP	157
		2* ⇄ 2°		UMP	157
		2° ⇄ 4*	IMP	UMP	158
dCMP deaminase	Chick embryo	2° ⇄ 6*	dCTP	dTTP	140, 141, 145
CTP synthase	*E. coli*	2 ⇄ 4*	UTP, ATP		159–161
	Bovine liver	2 ⇄ 4*	UTP, ATP		162, 187
Deoxythymidine kinase	*E. coli*	1° ⇄ 1* ⇄ 2* ⇄ 2°	dCDP, dADP	dTTP	138, 139
	Human lymphocytes	1 ⇄ 2*	ATP		143
	Human fibroblasts	1° ⇄ 1 ⇄ 2*	ATP		144
Glutamine P-Rib-PP amidotransferase	Pigeon liver	1* ⇄ 2* ⇄ 4*	β-ME		142
	Human placenta	2* ⇄ 4°	P-Rib-PP	AMP, IMP	149, 150
	B. subtilis	2* ⇄ 4*		AMP	148, 188
Hypoxanthine-guanine PRTase	Human fibroblasts	2* ⇄ 4			118
	S. cerevisiae	1* ⇄ 2	P-Rib-PP		155
Orotate PRTase	*S. cerevisiae*	1* ⇄ 2*	Mg^{2+}		155
P-Rib-PP synthase	Human erythrocytes	1° ⇄ 4° ⇄ 8° ⇄ 16* ⇄ 32	ATP, ADP	G-2,3-P_2	155, 165
	S. typhimurium	5 ⇄ 10			163, 164
Purine nucleoside phosphorylase	Bovine spleen	1 ⇄ 2 ⇄ 3*	P_i		42, 168, 169
Ribonucleoside diphosphate reductase: (B1/B2)	*E. coli*	1* ⇄ 2°	ATP	dATP	151
(M1)	Calf thymus	1° ⇄ 2* ⇄ 4* ⇄ 4°	ATP, dTTP		152
UMP/CMP kinase	Rat liver	1* ⇄ 3°	β-ME	Oxidation	60
UMP synthase	Ehrlich ascites	1° ⇄ 2° ⇄ 2*	OMP, UMP		28, 29, 59, 98, 99, 167
Uridine kinase	Ehrlich ascites	1° ⇄ 2 ⇄ 4*	ATP	CTP, UTP	23–25
dUTPase	Human HeLa	2 ⇄ 4*	Mg^{2+}		156

Note: β-ME, β-mercaptoethanol; G-2,3-P_2, 2,3-*bis*-phosphoglycerate; NC-β-ala, *N*-carbamoyl-β-alanine; PRTase, phosphoribosyltransferase. Enzyme activity: * = most active; * = partly active; ° = inactive; — = native oligomer.

heat denaturation. Although not tested in molecular weight studies, these nucleotides presumably do not bind to the enzyme.[144]

Studies on chick embryo dCMP deaminase identified it as one of the earliest dissociating enzymes, where the native enzyme was probably a dimer, which in the presence of the activator dCTP associated into the active hexamer, while the inhibitor dTTP favored the dissociated species.[140] An interesting anomaly is that the original sedimentation data[140,141] and the subunit molecular mass of 20 kDa[145] are most consistent with a

dimer–hexamer system. This would, however, make this the only reported system where hexamers were assembled as trimers of a dimer, rather than the conventional pattern of two trimers forming a hexamer. For the same enzyme from human spleen[146] or from donkey spleen,[147] it was concluded that dissociation was not important.

Also somewhat anomalous is glutamine P-Rib-PP amidotransferase, which, when obtained from different sources, appears to have different properties. The enzymes from pigeon liver[142] and bacteria[148] are native tetramers, while the human enzyme is a native dimer.[149] The inhibitor AMP affects dissociation: with the human enzyme it stabilized the inactive tetramer,[149] and with the bacterial enzyme it dissociated the native tetramer to the less active dimer.[148] This represents an interesting example of how the same enzyme, from evolutionarily widely divergent sources and with different active quaternary states, has evolved the appropriate conformational response to the same ligand: association to the inactive tetramer for the human enzyme and dissociation to the less active dimer for the bacterial enzyme. Also, in each case it is the native oligomer that is most active, and the conformationally altered oligomer, whether by association or by dissociation, that is less active. Kinetics with the human enzyme suggest that the native dimer and the active dimer may not have the same conformation because the enzyme normally showed a lag in rapid kinetics, which disappeared if the enzyme was preincubated with P-Rib-PP.[150] Alternatively, preincubation with the inhibitor AMP increased the lag time.

The important allosteric enzyme ribonucleoside diphosphate reductase was also an early example of dissociation related to change in activity.[151] The bacterial enzyme has a protomer containing two different proteins, B1 and B2. Under native conditions, this protomer remains in the active monomeric form. The regulatory effector dATP induced association to the inactive dimer, while ATP favored the active monomer.[151] The same enzyme in mammals has a somewhat comparable protomer structure, with proteins M1 and M2. However, the two separate proteins appear to independently form homopolymers before joining to form the holoenzyme. In this case, protein M1 exists as a native monomer, which was inactive.[152] Either ATP or dTTP produced association to the dimer that had maximum activity, and either ATP or dATP produced further association to the tetramer, which still had optimum activity. No negative effectors were described for the mammalian enzyme.

Of the various phosphoribosyltransferases, the human enzymes are native dimers (adenine PRTase;[153,154] hypoxanthine-guanine PRTase[118]), while enzymes from bacteria or yeast are native monomers (hypoxanthine-guanine PRTase;[155] orotate PRTase[155]). The original paper on human adenine PRTase stated that this enzyme was trimeric,[153] but subsequent reevaluation of the subunit molecular mass yielded a value of 18 kDa,[154] rather than the earlier and smaller value of 11.1 kDa. Some support for the dissociation of this enzyme *in vivo* came from studies in which human fibroblasts and mouse L cells were fused.[118] Because the two cell lines make enzymes that normally separate readily under isoelectric focusing, the appearance of a hybrid heteropolymer suggested dissociation and mixing of subunits.

Although both hypoxanthine-guanine PRTase and orotate PRTase exist as native monomers in yeast, hypoxanthine-guanine PRTase is also most active as the monomer, while orotate PRTase must associate to the more active dimer.[155] For hypoxanthine-guanine PRTase, the substrate P-Rib-PP promoted the active monomeric form, while orotate PRTase became a dimer and was activated by magnesium.[155] Also responding to magnesium as a positive effector is dUTPase from human HeLa cells.[156] The enzyme exists as a native dimer, while the species with maximum activity was shown to be the tetramer.

AMP deaminase from rabbit skeletal muscle was shown to increase in specific activity as a function of increasing enzyme concentration from 0.1 μg/ml to 1 mg/ml.[44] This suggested that dilution led to a less active dissociated species. Also, the preincubated enzyme showed hysteretic kinetics following the addition of the substrate AMP, consistent with the fact that AMP stabilizes the active tetramer.[44]

β-Alanine synthase is one of a few enzymes where the native species is an intermediate M_r form that can both dissociate and further associate (Figure 1).[31,32] Hysteretic kinetics were consistent with the trimer being inactive.[32] The

substrate *N*-carbamoyl-β-alanine favored association of the native hexamer to the active dodecamer, while the product β-alanine induced dissociation to the trimer. Of the enzymes listed in Tables 4 to 6, this is one of a few examples where the enzyme will not dissociate completely to the monomeric state, except in the presence of denaturing agents. The trimer therefore functions as a stable protomer, and the hexamer and dodecamer may be viewed as a dimer and tetramer of this protomer.

Bacterial carbamoyl phosphate synthase has a protomer composed of two different protein subunits; the protomer in turn could form dimers or tetramers.[157,158] Association to the dimer was favored by P_i,[158] and the active tetramer was promoted by IMP, ATP, or ornithine.[157] UMP promoted dissociation of the active tetramer,[158] or a conformational change that converted a partly active dimer to an inactive dimer.[157] The possibility that separate sites mediated the effects of ligands to promote association or dissociation was explored with analogs of purine nucleotides altered in the pentose at carbons 2 and 3 to the dialdehydes. The dialdehyde of IMP had effects on association and activity exactly like IMP itself. However, while UMP acted as an inhibitor, the dialdehyde of UMP favored the active tetramer of carbamoyl phosphate synthase.[158]

CTP synthase, an important allosteric enzyme, has been shown to have quite comparable properties for enzyme prepared from *E. coli* or calf liver.[159–162] The native enzyme was a dimer, while the most active species was found to be the tetramer. The two nucleotide substrates, UTP and ATP, each favored the most active tetramer species.

In contrast, for P-Rib-PP synthase, enzyme prepared from bacteria and humans gave quite different results. The enzyme from *S. typhimurium* was reported to be a native pentamer, which in turn could associate to decamer and higher polymers.[163] Temperature and acidity were the measured variables: at 4° the enzyme formed large polymers, which at 20° became decamers.[163] The enzyme showed positive cooperativity toward ATP, but this substrate had no effect on dissociation; the substrate ribose-5-P was not tested.[163,164] The report of this enzyme being a pentamer is unusual. No pentamers were reported in the surveys by Klotz et al.,[1,2] and this was one of only two enzymes that were reported as pentamers in a survey of all the enzymes in nucleotide metabolism.[4]

The comparable P-Rib-PP synthase from human erythrocytes was shown to be a native tetramer that polymerized extensively to form active polymers of 16 or 32 subunits.[22,165] Positive effectors included the substrate ATP and purine nucleotide inhibitors such as ADP, while 2,3-*bis*-P glycerate was a negative effector.[22] This same enzyme from *B. subtilis* was purified recently and proven to be a native octamer.[166] Although this enzyme from humans or from *S. typhimurium* can exist in multiple oligomeric species, no experiments were done to test for dissociation or association with P-Rib-PP synthase from *B. subtilis*. The enzyme from human erythrocytes is one of several examples where inhibitors have the effect of inducing the M_r form that is normally most active. This will be specifically considered under "Conclusions".

The enzymes UMP synthase, uridine kinase, and purine nucleoside phosphorylase are all shown in Figure 1. The structure for the bifunctional UMP synthase, containing an orotate PRTase activity and also an OMP decarboxylase activity, was described in Section III.B. The following will apply specifically to the OMP decarboxylase activity. The mouse protein exists as a native, inactive monomer that can associate to an inactive dimer in the presence of P_i, or a variety of small anionic ligands.[29] Association to the active dimer occurred most readily in the presence of OMP, which is the product of the first catalytic center and the substrate for the second catalytic site. Two different conformational forms of the dimer were readily distinguishable: the inactive dimer sedimented at 5.1 S and the active dimer at 5.6 S. It was concluded that association to the inactive 5.1 S dimer was promoted by ligands binding at the catalytic site, while association to the active 5.6 S form was mediated by ligands binding at an allosteric site. The product UMP could also produce the active dimer form but with much poorer affinity for the regulatory site.[29] The 5.1 S dimer was found to be inactive because after preincubation of the enzyme in this form it displayed hysteretic kinetics on

addition of the substrate OMP.[59] In comparable kinetic experiments, the 5.6 S dimer showed no measurable lag time, suggesting it was intrinsically in the active form.

Early experiments that were designed to find a rationale for activation by the substrate, to induce the active dimer, were interpreted as support for channeling of the intermediate, OMP, between different catalytic sites on two subunits in the dimer.[167] More recent work has found no evidence for channeling.[99] However, as would be expected for an enzyme that is converted from an inactive to an active form by its substrate, the OMP decarboxylase activity was shown to have positive cooperativity vs. OMP.[99]

Uridine kinase is an enzyme that can exhibit multiple M_r species that reequilibrate slowly and can therefore be separated. This process is temperature sensitive and is more readily seen at 4°. The most prominent native form at 4° was the tetramer, and this was the only native form at 22°.[25] At 4° polymers containing ≥32 subunits were evident[25] and could be demonstrated by either size exclusion chromatography[23–25] or by ion exchange chromatography.[24,25] The two feedback inhibitors CTP and UTP dissociated the enzyme to the inactive monomer; ATP stabilized the tetramer.[25] The enzyme showed hysteretic kinetics when preincubated in the inactive form, and this was more evident at lower temperature or at higher concentrations of the inhibitor CTP (Figures 7A,B).

Bovine purine nucleoside phosphorylase (PNP) is normally found as a native trimer (Figure 1).[42,168] The enzyme showed a definite and significant increase in specific activity as it was diluted (Figure 5), consistent with enzyme dissociation leading to the more active monomer. The substrate P_i also produced dissociation of trimers to monomers, but only at unphysiologically high concentrations.[42,43] The trimer was at least partly active, and the high concentration of PNP *in vivo* would favor the more stable trimer species.

This enzyme from many sources showed negative cooperativity vs. one or both substrates, suggesting that all the subunits in the native trimer are not equally or fully active, while the dissociated monomer was shown to have maximum activity. Nevertheless, a trimer-monomer equilibrium does not appear sufficient to explain the negative cooperativity, because enzyme that was extremely diluted and presumably in the monomer form still showed negative cooperativity toward phosphate.[169]

Pyrimidine nucleoside monophosphate kinase (UMP/CMP kinase) was shown to be a native monomer, which under oxidizing conditions formed inactive trimers.[60] This was presumably due to disulfide bonds between subunits, as addition of β-mercaptoethanol restored activity and the dissociated species. It is unlikely that this is a physiological form of regulation. UMP kinase is active in the native form, and it is not considered to be a regulated enzyme but is included here to illustrate how manipulation *in vitro* can produce such results.

C. Enzymes in Amino Acid Metabolism

Enzymes for this section are summarized in Table 6. Glutamate dehydrogenase was the first enzyme to receive significant study with respect to dissociation. The enzyme from bovine liver is a native hexamer, which was frequently designated as the protomer in earlier studies.[55,170–172] This protomeric hexamer was judged to be inactive.[55,170,172] The inactive hexamer was stabilized by various negative effectors: GDP, GTP, and ATP.[55,172] The active polymer was formed in response to various positive effectors: NADPH,[55,170,172] ADP, and AMP.[55,171] ADP and GDP were not effectors by themselves, but produced a change in the enzyme's size only when NADH was also present.[55] As the enzyme concentration was increased from 0.12 to 14.7 mg/ml, the change in cooperative kinetics was consistent with the polymeric form being the active form.

In a different approach on the same bovine enzyme, the size of the active form was examined by measuring the target size of the enzyme under a beam of ionizing radiation.[173] The subunit size for bovine glutamate dehydrogenase is 56 kDa, and the target size for a fluorescent signal from enzyme bound-fluoresceine isothiocyanate was 59 kDa, consistent with a single subunit. The target size for enzyme activity, as measured by ionizing radiation, was found to be 257 kDa, which is more consistent with a hexamer or a mixture of hexamers and trimers.[173] Somewhat comparable results were obtained in another study on the

TABLE 6
Enzymes in Amino Acid Metabolism

Enzyme	Source	Oligomeric species	Effectors (+)	Effectors (–)	Ref.
Aspartokinase-homoserine dehydrogenase	*E. coli*	$1 \rightleftharpoons \underline{2}\cdot \rightleftharpoons 4^*$	Threonine		176
β-Alanine synthase	Rat liver	$3^\circ \rightleftharpoons \underline{6}\cdot \rightleftharpoons 12^*$	NC-β-alanine	β-Alanine	31, 32
Carbamoyl phosphate synthase	*E. coli*	$\underline{1} \rightleftharpoons 2 \rightleftharpoons 4^*$	P_i; IMP	UMP	157
		$2^\circ \rightleftharpoons 4^*$	IMP	UMP	158
Carbamoyl phosphate synthase I	Rat liver	$1^* \rightleftharpoons \underline{2}^\circ$	N-AGA		175
Glutaminase	Pig kidney	$\underline{1} \rightleftharpoons 2 \rightleftharpoons n^*$	P_i		189
Glutamate dehydrogenase	Bovine liver	$\underline{6}^\circ \rightleftharpoons 24^*$	NADH, ADP	ATP	55, 170–172
		$3^* \rightleftharpoons \underline{6}^*$	None	None	174
Ornithine aminotransferase	Rat liver	$1^* \rightleftharpoons 3\cdot \rightleftharpoons \underline{6}\cdot$	Ornithine Lysine		178, 179
Threonine deaminase	*E. coli*	$2\cdot \rightleftharpoons 2^\circ \rightleftharpoons \underline{4}^*$	PLP		177
	S. typhimurium	$2^\circ \rightleftharpoons \underline{4}^*$	Threonine		190, 191
Threonine dehydrase	*E. coli*	$\underline{1} \rightleftharpoons 4^*$	AMP	Oxidation	58

Note: NC, *N*-carbamoyl; *N*-AGA, *N*-acetyl glutamic acid; PLP, pyridoxal phosphate. Enzyme activity: * = most active; · = partly active; ° = inactive; — = native oligomer.

purified bovine enzyme by measuring tryptophan phosphorescence of diluted native enzyme (hexameric) or enzyme denatured with 1.5 *M* guanidinium-HCl.[174] Both activity and binding of the coenzyme were measured for the two enzyme forms, and at these dilute concentrations of the enzyme (1 n*M*) substrates or effectors did not appear to affect the extent of association or dissociation.[174] It was concluded that the proper conformation was attained predominantly by intrasubunit bonding.

Bacterial carbamoyl phosphate synthase has already been described (Section VI.B). The comparable mammalian mitochondrial enzyme, carbamoyl phosphate synthase I, exists as a native dimer, which was inactive but dissociated to the active monomeric form in the presence of the obligatory activator *N*-acetyl glutamic acid.[175] Substrates had only a small effect on promoting dissociation, but the activator was effective even at high enzyme concentrations up to 5 mg/ml. The data were interpreted as activation by *N*-acetyl glutamic acid being possible without the enzyme having to dissociate.[175]

Aspartokinase/homoserine dehydrogenase is a bifunctional protein in *E. coli* and exists as a native dimer.[176] The dimer had enzyme activity, but optimum activity occurred with the tetrameric species formed in the presence of threonine. It was considered unlikely that dissociation occurred *in vivo*. The bacterial enzymes threonine dehydrase[58] and threonine deaminase[177] have also shown the ability to dissociate. Threonine dehydrase was a native monomer, and association to the active tetramer was induced by AMP or by increased enzyme concentration. Threonine deaminase was a native dimer, which associated to a less active tetramer in the presence of pyridoxal phosphate.[177] However, the tetrameric species was sensitive to regulation by isoleucine, while the dimer was not.

Ornithine aminotransferase was found to be most active as the monomer, and, at increased concentrations of enzyme, could form trimers and much larger oligomers.[178] When the enzyme was tested *in vitro*, there appeared to be a two-stage association process of monomers to trimers, and the second stage being the

further association of trimers to hexamers. Kinetic studies showed that the K_m, for either the substrate ornithine or α-ketoglutarate, increased in proportion to the association of the monomers. This established that the oligomers had lower activity. The association of monomers to trimers was blocked by the presence of the substrate ornithine, or by lysine.[179] In other words, the substrate stabilized the monomer, which is the most active form. Support for the enzyme being in the higher oligomer form *in vivo* came from studies where rats were fed a high-protein diet. These animals were found to have a much higher concentration of the enzyme in their mitochondria, and also showed a higher K_m for ornithine.[179] It was suggested that such regulation *in vivo* would lead to a less active enzyme and spare ornithine for use in the urea cycle, which would have greater activity because of the high-protein diet.

D. Active Monomers

Table 7 lists seven enzymes for which optimum activity was associated with the monomer. Most of these enzymes readily form oligomers and thus emphasize that the associated oligomer or polymer is not inherently the optimal form. However, because interactions between subunits in an associated oligomer permits more options for structural/functional variations, the association of optimum activity with the oligomeric species is much more commonly observed. Mammalian glucokinase, although considered an active monomer by some authors,[105–109] was omitted from this table and will be considered below.

E. Do Enzymes Dissociate *In Vivo*?

This question is experimentally difficult to test, and researchers have generally worked with purified enzymes *in vitro*. A limited number of enzymes have been tested for this feature (Table 8). Acetyl CoA carboxylase is an enzyme that forms a protomer of four different proteins, and the protomer in turn associates to the active polymer.[180] Clarke and colleagues used the sensitivity of the protomer to avidin (the active polymer does not bind avidin) as a test for measuring the distribution of the enzyme between these two forms. In freshly fed chicks, the isolated liver enzyme was 80% avidin resistant, indicating that it was in the active polymer form.[180] However, food deprivation for 2 or 6 h led to a reduction of the polymer form, because the enzyme was now only 54 or 30% avidin resistant. Comparable studies with rats showed that such changes were readily measured within 30 min of food ingestion.[181] In a different approach, these workers subjected rat hepatocytes to digitonin to make them permeable and then measured the release of soluble acetyl CoA carboxylase. Again they demonstrated that physiologically appropriate variables, such as citrate or cAMP, influenced the release of the soluble protomer.[182]

The enzyme glucose-6-P dehydrogenase (G6PD) had been shown to undergo dissociation in response to NADPH, suggesting that such dissociation might occur *in vivo*.[116] This was tested by the strategy of mixing human skin fibroblasts and mouse L cells under conditions leading to cell fusion, and selection for hybrids.[118] Isolated cell extracts were analyzed by native electrophoresis and gave evidence for a hybrid heterodimer form

TABLE 7
Enzymes More Active as Subunits

Enzyme	Source	Ref.
Carbamoyl-P synthase I	Rat liver	175
Hypoxanthine-guanine PRTase	*S. cerevisiae*	155
Isocitrate dehydrogenase	*B. emersonii*	129
Ornithine aminotransferase	Rat liver	178, 179
Purine nucleoside phosphorylase	Bovine spleen	42
UMP/CMP kinase	Rat liver	60
Ribonucleoside diphosphate reductase: (B1/B2)	*E. coli*	151

TABLE 8
Enzymes Tested for Dissociation *In Vivo*

Enzyme	Source	Experimental test	Δ M_r	Ref.
Acetyl CoA carboxylase	Chicken liver	Alter diet: extract enzyme	Association	180
	Rat liver	Alter diet: extract enzyme	Association	181
	Hepatocytes	Permeabilize → release of enzyme	Association	182
Glu-6-P dehydrogenase	Human fibroblasts + mouse L cells	Cell fusion → heteropolymer	Association	118
	Human erythrocytes	Cell lysis → heteropolymer	No	119
Hypoxanthine-guanine PRTase	Human fibroblasts + mouse L cells	Cell fusion → heteropolymer	Association	118
Ornithine aminotransferase	Rat liver	Alter diet: extract enzyme	Dissociation	178

of G6PD, suggesting dissociation of the parental homodimers and reassociation into a heterodimer. A different strategy was used by Kahler and Kirkman, who used erythrocytes from a woman heterozygous for the A and B isozymes of G6PD.[119] Inasmuch as G6PD is coded on the X chromosome, the process of Lyonization may lead to such overall heterozygosity as being the sum of separate cell types containing only the A isozyme or only the B isozyme. Cells from this heterozygous woman were subjected to lysis and resealing, which would permit exchange and the possible formation of true heterodimers, but no heterodimers were observed. However, this experiment also demonstrated that the procedures for the physical disruption and extraction of enzyme did not produce an artificial hybrid, and therefore gives support to the earlier study with human fibroblasts.[118] That study also found evidence for heteropolymer formation for isozymes of hypoxanthine-guanine PRTase.

VII. CONCLUSIONS

A. Overview

As previously suggested in Figure 1, there is a diversity of dissociating patterns and the corresponding change in activity. While purine nucleoside phosphorylase is most active as a monomer, the other three enzymes are most active at the largest oligomeric state they normally attain. Also, two of these enzymes are most stable as oligomers, one as a monomer and one (β-alanine synthase) at an intermediate state. For two enzymes, only positive effectors have been found, while the other two enzymes respond to both positive and negative effectors. An interesting result from these tabulations is that the great majority of enzymes in Tables 4 to 6 is most stable as tetramers, while surveys of all oligomers by Klotz and colleagues found that dimers were the most frequently observed oligomers for all enzymes.[1,2] Because dissociating enzymes generally respond to allosteric regulation, this difference in the oligomeric assembly implies that tetramers may offer some extra degree of architectural flexibility in interaction between subunits, making tetramers the preferred structures for allosteric enzymes.

Table 9 summarizes this type of information for the entire data set. The monomer is the most active form for only one seventh of the enzymes; for the majority the largest oligomer is also the most active species. Three out of four enzymes are known to respond to positive effectors, and negative effectors are much less common. About half of the enzymes can interconvert between only two different oligomeric species, and one third of them show three M_r forms. For the majority of these enzymes, the native form (in the absence of ligands) is inactive. This would be appropriate for many allosteric enzymes: the native enzyme is more commonly in the T form, and substrates or allosteric ligands are required to produce the active R form.

TABLE 9
Summary on Dissociating Enzymes

Attribute	% of enzymes
Native M_r form is most active ($\underline{x}^*$)	45
Native M_r form is the monomer ($\underline{1}$)	18
Monomer is most active (1^*)	14
Monomer is inactive (1°)	18
Largest oligomer is most active (n^*)	56
Intermediate size is most active (x^*)	11
Substrate → most active form (x^*)	51
Substrate → inactive form (x°)	2
Product/inhibitor → inactive form (x°)	28
Product/inhibitor → most active form (x^*)	8
Responds to positive effectors (+)	74
Responds to negative effectors (–)	33
Responds to (+) and (–)	32
Having two M_r species	47
Having three M_r species	32
Having four M_r species	6
Having more than four M_r species	15

Note: Enzyme activity: * = most active; • = partly active; ° = inactive; — = native oligomer.

However, it is not obligatory that allosteric enzymes be most stable in the T form. The native form, whether T or R, is related to the normal steady-state need of the cell for the enzyme activity, as shown in the comparison of deoxythymidine kinase and uridine kinase. Deoxythymidine kinase leads to the synthesis of dTTP, needed only for DNA synthesis during mitosis, and therefore this enzyme is normally in the inactive T form, until it is activated. Uridine kinase leads to the synthesis of UTP and CTP, which are required continuously for the synthesis of RNA, etc. Therefore, uridine kinase is most stable in the R form, unless it is inactivated by increasing concentrations of UTP and CTP.

It may also not be necessary for an enzyme to attain the most active form *in vivo*. Purine nucleoside phosphorylase is a stable trimer, although this form does not have optimum activity (Figure 5A). Ropp and Traut concluded that although the monomer is the most active form, it may not be a significant species *in vivo*, because the concentration of PNP is normally too great, and because the K_d for phosphate, the substrate that promotes dissociation, is 88 m*M*, a value far above any physiological value for phosphate.[42] However, on examining Figure 5A, it is evident that in the critical concentration range, as the enzyme concentration is varied tenfold from 5×10^{-8} *M* to 5×10^{-7} M, the decrease in the specific activity is also about tenfold, so that the total activity for the more concentrated enzyme is not significantly changed. But, while the concentrated enzyme is less efficient, as measured by catalytic turnover rates, it is much more stable, a feature that may justify maintaining the less active trimer by producing more enzyme. Kinetic studies showed that the more diluted enzyme generally had a biphasic loss of activity over a 60-min time period, even when either of the substrates was present at high concentrations.[42] However, because the crystal structure for this enzyme also shows a second binding site for phosphate,[90] it may be that phosphate is simply a poor analog for an unidentified physiological regulatory ligand.

B. Apparent Contradictions

1. Substrate Inactivates Enzyme

There is one example where a substrate leads to the inactive form. This is phosphofructokinase, which uses ATP as a substrate. However, the

position of this regulatory enzyme in glycolysis makes it sensitive to cellular ATP concentrations, and inhibition by high ATP concentration occurs at a special regulatory site.[76]

2. Product Activates Enzyme

Note that there are four enzymes where the product can also produce the most active conformation (Table 9). This should not be viewed as contradicting physiological regulation. The bacterial phosphofructokinase is one of these enzymes, where an increase in the product ADP also signals energy depletion in the cell, leading to increased glycolysis. For other enzymes, the amount of product used for the *in vitro* experiment always exceeded the physiological concentration of that ligand and suggests that the product is simply a poorly binding analog of the true positive effector. Again, the mammalian phosphofructokinase is a good example. Earlier studies had found that the product, fructose-1,6-bisphosphate, at high and unphysiological concentrations could increase the activity of this enzyme. Such strange results became understandable when the activator, fructose-2,6-bisphosphate, was finally discovered, thereby demonstrating that the product was but a poor analog for the true activator.

In a similar fashion, for UMP synthase the product UMP promotes dimerization of the inactive monomer to the active dimer, but only when UMP is at concentrations >5 m*M*, which are not physiological. Because the substrate, OMP, is a positive effector at concentrations <1 μ*M*, UMP is interpreted as a poor analog of the true effector, and certainly not physiologically important as an activator as inhibition studies show it to have a K_i of 24 μ*M*.[29]

C. Importance for Kinetic Models

An important aspect of dissociating enzymes is that they almost always dissociate, or associate, in response to an appropriate physiological ligand. In the current data set, 18% of the enzymes are monomers in their native state (absence of effector ligands). It is precisely in this state that enzymes are most commonly characterized for their native M_r. But more than half of these monomeric enzymes are most active (or only active) in the oligomeric form, which is produced by substrate. As an example, UMP synthase is a native monomer but shows positive cooperativity for the substrate OMP, which also causes UMP synthase to form a dimer.[28,29,99,167] Thus, binding of OMP at one site on the enzyme can lead to conformational changes, making binding of OMP more favorable at an adjacent site. Because OMP also produces the enzyme dimer, such coupled conformational benefits can be modeled directly.

However, if it is not understood that an enzyme can associate to form an oligomer, then more complex models are necessary to explain how such "monomeric" enzymes show positive cooperativity in kinetic studies. An elegant and clever model proposed a "mnemonic" mechanism for a monomeric enzyme existing in two different conformations (inactive and active), which are claimed not to be in rapid equilibrium, so that the active form retains its conformation after a catalytic cycle and release of ligands. The relaxation time of the active form to the inactive form is suggested to be slow, so that this monomeric enzyme can now display altered affinity (i.e., cooperativity) because it "remembers" the substrate-induced active conformation.[183] This is an interesting proposal, and the available data can sometimes be interpreted with such a model to account for the observed kinetic studies, as in the first example using wheat germ hexokinase.[184]

However, such kinetic modeling is satisfactory only when it is also consistent with physical studies of the enzyme. In the original paper of a mnemonical enzyme, no physical studies were presented to show the possible effects of ligands on the monomeric enzyme.[184] Another good example of such apparent complexity comes from studies of rat liver glucokinase, which was generally considered to be a monomeric enzyme.[105–109] This enzyme also showed positive cooperativity toward glucose.[105,107,110] However, studies measuring the native M_r showed that under some conditions glucokinase can form dimers: as a function of aging or oxidation,[105] or due to the addition of 1 m*M* ATP.[110] Separate studies were un-

able to produce the dimer, even in the presence of substrates, during molecular sieve chromatography.[108] However, in this last study, chromatography was slow (several hours), and the concentration of glucokinase used was enough to completely consume at least one of the substrates, and also to produce products that might have the opposite effect on association. The potential effect of products was not measured. Furthermore, the comparable yeast hexokinase has been shown to form the more active dimer on binding ATP,[88] or on binding glucose.[86,87] Therefore, the actual oligomeric state of rat liver glucokinase when it is active is not resolved, and because it may well dimerize, the reason for its allosteric behavior may be quite comparable to other dissociating enzymes and may not require a mnemonic mechanism.

D. Applications

While all allosteric enzymes do not dissociate or associate, enough of these enzyme so far have shown this feature that the procedure of testing for an appropriate change in subunit association by molecular sieve chromatography or by sedimentation becomes an easy diagnostic test for newly obtained enzymes, especially because the procedure can be performed with impure enzyme samples, as long as an unambiguous assay for the enzyme is available. The same procedure may be useful for verifying whether an enzyme exists as isozymes, where this hypothesis results from the detection of separate peaks, by sedimentation or by chromatography. If such peaks are interconvertible by the addition of appropriate ligands, then a single gene product is usually responsible.

Knowledge of such features for a dissociating enzyme may be employed for the purification of the enzyme. While *affinity chromatography* is well known, for many enzymes no affinity resin is available. An alternative strategy could use *affinity elution* — if a ligand changes the enzyme's oligomeric assembly, it should also alter the enzyme's surface charge, and thereby specifically influence retention of enzyme on adsorption columns. If chromatography is repeated in the presence of the ligand, then the retention of the enzyme should be altered, while contaminating proteins will adsorb as before. An example of using affinity elution is the final step in the purification of β-alanine synthase.[31] The enzyme preparation was at least 30% pure, but the remaining contaminants had very similar adsorption behavior on columns tested. Therefore, the enzyme was purified by gel filtration chromatography in the presence of the substrate, which caused the enzyme to change its native molecular mass from about 240 kDa (hexamer) to almost 500 kDa (dodecamer), thereby easily eluting in pure form ahead of the smaller contaminating proteins.

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REFERENCES

1. **Klotz, I. M., Darnall, D. W., and Langerman, N. R.,** Quaternary structure of proteins, in *The Proteins*, Neurath, H. and Hill, R. L., Eds., Academic Press, New York, 1975, 293–411.
2. **Darnall, D. W. and Klotz, I. M.,** Subunit constitution of proteins: a table, *Arch. Biochem. Biophys.*, 166, 651, 1975.
3. **Hopkinson, D. A., Edwards, Y. H., and Harris, H.,** The distribution of subunit numbers and subunit sizes of enzymes: a study of the products of 100 human gene loci, *Ann. Hum. Genet. (London)*, 39, 383, 1976.
4. **Traut, T. W.,** Enzymes of nucleotide metabolism: the significance of subunit size and polymer size for biological function and regulatory properties, *CRC Crit. Rev. Biochem.*, 23, 121, 1988.
5. **Monod, J., Wyman, J., and Changeux, J.,** On the nature of allosteric transitions: a plausible model, *J. Mol. Biol.*, 12, 88, 1965.
6. **Koshland, D. E., Jr.,** The molecular basis for enzyme regulation, in *The Enzymes*, Vol. 1, Boyer, P. D., Ed., Academic Press, New York, 1970, 342–396.
7. **Hill, C. M., Waight, R. D., and Bardsley, W. G.,** Does any enzyme follow the Michaelis-Menten equation?, *Mol. Cell Biochem.*, 15, 173, 1977.
8. **Frieden, C.,** Protein-protein interaction and enzymatic activity, *Annu. Rev. Biochem.*, 40, 653, 1971.
9. **Phillips, A. T.,** Ligand-induced oligomerization and regulatory mechanism, *CRC Crit. Rev. Biochem.*, 2, 343, 1974.

10. **Dunne, C. P. and Wood, W. A.,** L-Threonine dehydrase as a model of allosteric control involving ligand-induced oligomerization, *Curr. Topics Cell. Regul.*, 9, 65, 1975.
11. **Nichol, L. W.,** Protein interaction patterns, in *Protein-Protein Interactions*, Frieden, C. F. and Nichol, L. W., Eds., John Wiley & Sons, New York, 1981, 1–29.
12. **Frieden, C.,** Treatment of enzyme kinetic data. II. The multisite case: comparison of allosteric models and a possible new mechanism. *J. Biol. Chem.*, 242, 4045, 1967.
13. **Frieden, C.,** Kinetic aspects of regulation of metabolic processes. The hysteretic enzyme concept, *J. Biol. Chem.*, 245, 5788, 1970.
14. **Frieden, C.,** Slow transitions and hysteretic behavior in enzymes, *Annu. Rev. Biochem.*, 48, 471, 1979.
15. **Frieden, C.,** Approaches that can be used with enzymes, in *Protein-Protein Interactions*, Frieden, C. F. and Nichol, L. W., Eds., John Wiley & Sons, New York, 1981, 290–314.
16. **Nichol, L. W., Jackson, W. J. H., and Winzor, D. J.,** A theoretical study of the binding of small molecules to a polymerizing protein system, *Biochemistry*, 6, 2449, 1967.
17. **Nichol, L. W. and Winzor, D. J.,** Ligand-induced polymerization, *Biochemistry*, 15, 3015, 1976.
18. **Nichol, L. W. and Winzor, D. J.,** Binding equations and control effects, in *Protein-Protein Interactions*, Frieden, C. F. and Nichol, L. W., Eds., John Wiley & Sons, New York, 1981, 337–380.
19. **Kurganov, B. I.,** *Allosteric Enzymes*, John Wiley & Sons, New York, 1982.
20. **Matthews, B. W. and Bernhard, S. A.,** Structure and symmetry of oligomeric enzymes, *Annu. Rev. Biophys. Bioeng.*, 2, 257, 1973.
21. **Steitz, T. A., Anderson, C., Bennett, W., McDonald, R., and Stenkamp, R.,** Protomer structure of oligomeric enzymes: symmetry and allosteric interactions in yeast hexokinase, *Biochem. Soc. Trans.*, 5, 620, 1977.
22. **Becker, M. A., Meyer, L. J., Huisman, W. H., Lazar, W., and Adams, W. B.,** Human erythrocyte phosphoribosylpyrophosphate synthetase. Subunit analysis and states of subunit association, *J. Biol. Chem.*, 252, 3911, 1977.
23. **Payne, R. C. and Traut, T. W.,** Regulation of uridine kinase quaternary structure. Dissociation by the inhibitor CTP, *J. Biol. Chem.*, 257, 12485, 1982.
24. **Payne, R. C., Cheng, N., and Traut, T. W.,** Uridine kinase from Ehrlich ascites carcinoma. Purification and properties of homogeneous enzyme, *J. Biol. Chem.*, 260, 10242, 1985.
25. **Cheng, N., Payne, R. C., and Traut, T. W.,** Regulation of uridine kinase. Evidence for a regulatory site, *J. Biol. Chem.*, 261, 13006, 1986.
26. **Traut, T. W.,** Physiological concentrations of purines and pyrimidines (submitted).
27. **Albe, K. R., Butler, M. H., and Wright, B. E.,** Cellular concentrations of enzymes and their substrates, *J. Theor. Biol.*, 143, 163, 1990.
28. **Traut, T. W. and Jones, M. E.,** Interconversion of different molecular weight forms of the orotate phosphoribosyltransferase-orotidine-5′-phosphate decarboxylase enzyme complex from mouse Ehrlich ascites cells, *J. Biol. Chem.*, 254, 1143, 1979.
29. **Traut, T. W., Payne, R. C., and Jones, M. E.,** Dependence of the aggregation and conformation states of uridine 5′-phosphate synthase on pyrimidine nucleotides. Evidence for a regulatory site, *Biochemistry*, 19, 6062, 1980.
30. **Reyes, P. and Guganig, M.,** Studies on a pyrimidine phosphoribosyltransferase from murine leukemia P1534J. Partial purification, substrate specificity, and evidence for its existence as a bifunctional complex with orotidine 5′-phosphate decarboxylase, *J. Biol. Chem.*, 250, 5097, 1975.
31. **Matthews, M. M., Liao, W., Kvalnes-Krick, K. L., and Traut, T. W.,** β-Alanine synthase: purification and allosteric properties, *Arch. Biochem. Biophys.*, 293, 254, 1992.
32. **Matthews, M. M. and Traut, T. W.,** Regulation of *N*-carbamoyl-β-alanine amidohydrolase, the terminal enzyme in pyrimidine catabolism, by ligand-induced change in polymerization, *J. Biol. Chem.*, 262, 7232, 1987.
33. **Krystal, G. and Webb, T. E.,** Multiple forms of uridine kinase in normal and neoplastic rat liver, *Biochem. J.*, 124, 943, 1971.
34. **Keefer, R. C., Morris, H. P., and Webb, T. E.,** 5-Azacytidine-modified patterns of uridine kinase activities in normal and neoplastic tissues, *Cancer Res.*, 34, 2260, 1974.
35. **Greenberg, N., Schumm, D. E., Hurtubise, P. E., and Webb, T. E.,** Uridine kinase activities in normal and neoplastic lymphoid cells, *Cancer Res.*, 37, 1028, 1977.
36. **Ahmed, N. K. and Welch, A. D.,** Some properties of uridine-cytidine kinase from a human malignant lymphoma, *Cancer Res.*, 39, 3102, 1979.
37. **Dubinina, I. G., Shkurko, V. G., and Vornovitskaya, G. I.,** Isolation and properties of uridine kinase from Zajdela ascites hepatoma cells, *Biokhimya*, 46, 1503, 1982.
38. **Ahmed, N. K.,** Multiple forms and inhibitors of uridine-cytidine kinase in neoplastic cells, *Int. J. Biochem.*, 14, 259, 1982.
39. **Gilbert, L. and Gilbert, G. A.,** Sedimentation velocity measurement of protein association, *Methods Enzymol.*, 27, 273, 1973.
40. **Kegeles, G. and Cann, J. R.,** Kinetically controlled mass transport of associating-dissociating macromolecules, *Methods Enzymol.*, 48, 248, 1978.
41. **Steiner, R. E.,** Ligand binding and self-association of proteins, *Mol. Cell. Biochem.*, 31, 5, 1980.

42. **Ropp, P. A. and Traut, T. W.,** Purine nucleoside phosphorylase: allosteric regulation of a dissociating enzyme, *J. Biol. Chem.*, 266, 7682, 1991.
43. **Mora, M. and Bozal, J.,** Phosphorolytic and ribosyl transfer mechanisms of purified chicken liver purine nucleoside phosphorylase, *Comp. Biochem. Physiol.*, 82B, 805, 1985.
44. **Ellis, K. J., Kuntz, K., and Sturtevant, J. M.,** The activation of muscle adenylate deaminase by substrate, *J. Biol. Chem.*, 246, 6631, 1971.
45. **Kosk-Kosicka, D. and Bzdega, T.,** Activation of the erythrocyte Ca^{2+}-ATPase by either self-association or interaction with calmodulin, *J. Biol. Chem.*, 263, 18184, 1988.
46. **Teipel, J. W. and Hill, R. L.,** The subunit interactions of fumarase, *J. Biol. Chem.*, 246, 4859, 1971.
47. **Aaronson, R. P. and Frieden, C.,** Rabbit muscle phosphofructokinase: studies on the polymerization, *J. Biol. Chem.*, 247, 7502, 1972.
48. **Pavelich, M. J. and Hammes, G. G.,** Aggregation of rabbit muscle phosphofructokinase, *Biochemistry*, 12, 1408, 1973.
49. **Hesterberg, L. K. and Lee, J. C.,** Sedimentation study of a catalytically active form of rabbit muscle phosphofructokinase at pH 8.55, *Biochemistry*, 19, 2030, 1980.
50. **Hesterberg, L. K. and Lee, J. C.,** Self-association of rabbit muscle phosphofructokinase at pH 7.0: stoichiometry, *Biochemistry*, 20, 2974, 1981.
51. **Hesterberg, L. K. and Lee, J. C.,** Self-association of rabbit muscle phosphofructokinase: effects of ligands, *Biochemistry*, 21, 216, 1982.
52. **Reinhart, G. D.,** Influence of fructose 2,6-bisphosphate on the aggregation properties of rat liver phosphofructokinase, *J. Biol. Chem.*, 258, 10827, 1983.
53. **Bosca, L., Aragon, J. J., and Sols, A.,** Modulation of muscle phosphofructokinase at physiological concentration of enzyme, *J. Biol. Chem.*, 260, 2100, 1985.
54. **Giorgio, N. A., Jr., Yip, A. T., Fleming, J., and Plaut, G. W. E.,** Diphosphopyridine nucleotide-linked isocitrate dehydrogenase from bovine heart, *J. Biol. Chem.*, 245, 5469, 1970.
55. **Frieden, C. and Coleman, R. F.,** Glutamate dehydrogenase concentration as a determinant in the effect of purine nucleotides on enzymatic activity, *J. Biol. Chem.*, 242, 1705, 1967.
56. **Huang, C. Y. and Graves, D. J.,** Correlation between subunit interactions and enzymatic activity of phosphorylase *a*. Method for determining equilibrium constants from initial rate measurements, *Biochemistry*, 9, 660, 1970.
57. **Kim, G. and Graves, D. J.,** On the hysteretic response of rabbit skeletal muscle phosphorylase kinase, *Biochemistry*, 12, 2090, 1973.
58. **Whanger, P. D., Phillips, A. T., Rabinowitz, K. W., Piperno, J. R., Shada, J. D., and Wood, W. A.,** The mechanism of action of 5′-adenylic acid-activated threonine dehydrase. II. Protomer-oligomer interconversions and related properties, *J. Biol. Chem.*, 243, 167, 1968.
59. **Traut, T. W. and Payne, R. C.,** Dependence of the catalytic activities on the aggregation and conformation states of uridine 5′-phosphate synthase, *Biochemistry*, 19, 6068, 1980.
60. **Maness, P. and Orengo, A.,** Activation of rat liver pyrimidine nucleoside monophosphate kinase, *Biochim. Biophys. Acta*, 429, 182, 1976.
61. **Neet, K. E. and Ainslie, G. R.,** Cooperativity and slow transitions in the regulation of oligomeric and monomeric enzymes, *Trends Biochem. Sci.*, 1, 145, 1976.
62. **Neet, K. E. and Ainslie, G. R.,** Hysteretic enzymes, *Methods Enzymol.*, 64, 192, 1980.
63. **McClure, W. R.,** A kinetic analysis of coupled enzyme assays, *Biochemistry*, 8, 2782, 1969.
64. **Easterby, J. S.,** A generalized theory of the transition time for sequential enzyme reactions, *Biochem. J.*, 199, 155, 1981.
65. **Yang, S. Y. and Schulz, H.,** Kinetics of coupled enzyme reactions, *Biochemistry*, 26, 5579, 1987.
66. **Chan, W. W.-C. and Mawer, H. M.,** Studies on protein subunits. II. Preparation and properties of active subunits of aldolase bound to a matrix, *Arch. Biochem. Biophys.*, 149, 136, 1972.
67. **Porter, D. H. and Cardenas, J. M.,** Single subunits of sepharose-bound pyruvate kinase are inactive, *Biochemistry*, 20, 2532, 1981.
68. **Jurgensen, S. R., Wood, D. C., Mahler, J. C., and Harrison, J. H.,** The immobilization of mitochondrial malate dehydrogenase on Sepharose beads and the demonstration of catalytically active subunits, *J. Biol. Chem.*, 256, 2383, 1981.
69. **Hill, A. V.,** The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves, *J. Physiol. (London)*, 40, iv, 1910.
70. **Chu, A. H., Turner, B. W., and Ackers, G. K.,** Effects of protons on the oxygenation-linked subunit assembly in human hemoglobin, *Biochemistry*, 23, 604, 1984.
71. **Love, W. E., Klock, P. A., Lattman, E. A., Padlan, E. A., and Ward, K. B., Jr.,** The structure of lamprey and bloodworm hemoglobins in relation to their evolution and function, *Cold Spring Harb. Symp. Quant. Biol.*, 36, 349, 1971.
72. **Robey, E. A. and Schachman, H. K.,** Regeneration of active enzyme by formation of hybrids from inactive derivatives: implications for active sites shared between polypeptide chains of aspartate transcarbamoylase, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 361, 1985.
73. **Honzatko, R. B., Crawford, J. L., Monaco, H. L., Ladner, J. E., Edwards, B. P. F., Evans, D. R., Warren, S. G., Wiley, D. C., Ladner, R. C., and Lipscomb, W. N.,** Crystal and molecular structures of native and CTP-linked aspartate carbamoyltransferase from *Escherichia coli*, *J. Mol. Biol.*, 160, 219, 1982.

74. **Kantrowitz, E. and Lipscomb, W. N.,** *Escherichia coli* aspartate transcarbamylase: the relationship between structure and function, *Science*, 241, 669, 1988.
75. **Evans, P. R. and Hudson, P. J.,** Structure and control of phosphofructokinase from *Bacillus stearothermophilus*, *Nature*, 279, 500, 1979.
76. **Evans, P. R., Farrants, G. W., and Hudson, P. J.,** Phosphofructokinase: structure and control, *Phil. Trans. R. Soc. London B*, 293, 53, 1981.
77. **Schirmer, T. and Evans, P. R.,** Structural basis of the allosteric behaviour of phosphofructokinase, *Nature*, 343, 140, 1990.
78. **Evans, P. R., Farrants, G. W., and Lawrence, M. C.,** Crystallographic structure of allosterically inhibited phosphofructokinase at 7 Å resolution, *J. Mol. Biol.*, 191, 713, 1986.
79. **Shirakihara, Y. and Evans, P. R.,** Crystal structure of the complex of phosphofructokinase from *Escherichia coli* with its reaction products, *J. Mol. Biol.*, 204, 973, 1988.
80. **Rath, V. L., Newgard, C. B., Sprang, S. R., Goldsmith, E. J., and Fletterick, R. J.,** Modeling the biochemical differences between rabbit muscle and human liver phosphorylase, *Proteins*, 2, 225, 1987.
81. **Barford, D. and Johnson, L. N.,** The allosteric transition of glycogen phosphorylase, *Nature*, 340, 609, 1989.
82. **Helmreich, E., Michaelides, M. C., and Cori, C. F.,** Effects of substrates and a substrate analog on the binding of 5′-adenylic acid to muscle phosphorylase *a*, *Biochemistry*, 6, 3695, 1967.
83. **Metzger, B., Helmreich, E., and Glaser, L.,** The mechanism of activation of skeletal muscle phosphorylase *a* by glycogen, *Proc. Natl. Acad. Sci. U.S.A.*, 57, 994, 1967.
84. **Poorman, R. A., Randolph, A., Kemp, R. G., and Heinrikson, R. L.,** Evolution of phosphofructokinase — gene duplication and creation of new effector sites, *Nature*, 309, 467, 1984.
85. **Algimantas, P. V., Foe, L. G., and Kemp, R. G.,** Desensitization of muscle phosphofructokinase to ATP inhibition by removal of a carboxyl-terminal heptapeptide, *J. Biol. Chem.*, 262, 5044, 1987.
86. **Gazith, J., Schulze, I. T., Gooding, R. H., Womack, F. C., and Colowick, S. P.,** Multiple forms and subunits of yeast hexokinase, *Ann. N.Y. Acad. Sci.*, 151, 307, 1968.
87. **Shill, J. P., Peters, B. A., and Neet, K. E.,** Monomer-dimer equilibria of yeast hexokinase during reacting enzyme sedimentation, *Biochemistry*, 13, 3864, 1974.
88. **Steitz, T. A., Anderson, W. F., Fletterick, R. J., and Anderson, C. M.,** High-resolution crystal structures of yeast hexokinase complexes with substrates, activators, and inhibitors, *J. Biol. Chem.*, 252, 4494, 1977.
89. **Carter, P., Bedouelle, H., and Winter, G.,** Construction of heterodimer tyrosyl-tRNA synthetase shows $tRNA^{TYR}$ interacts with both subunits, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 1189, 1986.
90. **Ealick, S. E., Rule, S. A., Carter, D. C., Greenhough, T. J., Babu, Y. S., Cook, W. J., Habash, J., Helliwell, J. R., Stoeckler, J. D., Parks, Jr., R. E., Chen, S., and Bugg, C. E.,** Three-dimensional structure of human erythrocyte purine nucleoside phosphorylase at 3.2 Å resolution, *J. Biol. Chem.*, 265, 1812, 1990.
91. **Conrad, M. and Topal, M. D.,** DNA and spermidine provide a switch mechanism to regulate the activity of restriction enzyme *Nae* I, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 9707, 1989.
92. **Lazo, P. A., Sols, A., and Wilson, J. E.,** Brain hexokinase has two spatially discrete sites for binding glucose-6-phosphate, *J. Biol. Chem.*, 255, 7548, 1980.
93. **Schwab, D. A. and Wilson, J. E.,** Complete amino acid sequence of rat brain hexokinase, deduced from the cloned cDNA, and proposed structure of a mammalian hexokinase, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 2563, 1989.
94. **Schwab, D. A. and Wilson, J. E.,** Complete amino acid sequence of the type III isozyme of rat hexokinase, deduced from the cloned cDNA, *Arch. Biochem. Biophys.*, 285, 365, 1991.
95. **Thelen, A. P. and Wilson, J. E.,** Complete amino acid sequence of the type II isozyme of rat hexokinase, deduced from the cloned cDNA: comparison with a hexokinase from Novikoff ascites tumor, *Arch. Biochem. Biophys.*, 286, 645, 1991.
96. **Baijal, M. and Wilson, J. E.,** Functional consequences of mutation of highly conserved serine residues, found at equivalent positions in the N- and C-terminal domains of mammalian hexokinase, *Arch. Biochem. Biophys.*, 298, 271, 1992.
97. **Blangy, D., Buc, H., and Monod, J.,** Kinetics of the allosteric interactions of phosphofructokinase from *Escherichia coli*, *J. Mol. Biol.*, 31, 13, 1968.
98. **McClard, R. W., Black, M. J., Livingstone, L. R., and Jones, M. E.,** Isolation and initial characterization of the single polypeptide that synthesizes uridine 5′-monophosphate from orotate in Ehrlich ascites carcinoma. Purification by tandem affinity chromatography of uridine 5′-phosphate synthase, *Biochemistry*, 19, 4699, 1980.
99. **Traut, T. W.,** Uridine-5′-phosphate synthase: evidence for substrate cycling involving this bifunctional protein, *Arch. Biochem. Biophys.*, 268, 108, 1989.
100. **Coll, R. J. and Murphy, A. J.,** Kinetic evidence for two nucleotide binding sites on the CaATPase of sarcoplasmic reticulum, *Biochemistry*, 30, 1456, 1991.
101. **Shill, J. P. and Neet, K. E.,** Allosteric properties and the slow transition of yeast hexokinase, *J. Biol. Chem.*, 250, 2259, 1975.
102. **Lowry, O. H. and Passonneau, J. V.,** Kinetic evidence for multiple binding sites on phosphofructokinase, *J. Biol. Chem.*, 241, 2268, 1966.
103. **Feldman, K., Zeisel, H., and Helmreich, E.,** Interactions between native and chemically modified subunits of matrix-bound glycogen phosphorylase, *Proc. Natl. Acad. Sci. U.S.A.*, 69, 2278, 1972.

104. **Fletterick, R. J. and Madsen, N. B.,** The structures and related functions of phosphorylase *a*, *Annu. Rev. Biochem.*, 49, 31, 1980.
105. **Niemeyer, H., Cardenas, M. D., Rabajille, E., Ureta, T., Clark-Turri, L., and Peñaranda, J.,** Sigmoidal kinetics of glucokinase, *Enzyme*, 20, 321, 1975.
106. **Holroyde, M. J., Allen, M. B., Storer, A. C., Warshy, A. S., Chesher, J. N. E., Trayer, I. P., Cornish-Bowden, A., and Walker, D. G.,** The purification in high yield and characterization of rat hepatic glucokinase, *Biochem. J.*, 153, 363, 1976.
107. **Storer, A. C. and Cornish-Bowden, A.,** Kinetics of rat liver glucokinase, *Biochem. J.*, 159, 7, 1976.
108. **Cardenas, M. L., Rabajille, E., and Niemeyer, H.,** Maintenance of the monomeric structure of glucokinase under reacting conditions, *Arch. Biochem. Biophys.*, 190, 142, 1978.
109. **Gregoriou, M., Trayer, I. P., and Cornish-Bowden, A.,** Isotope-exchange evidence for an ordered mechanism for rat liver glucokinase, a monomeric cooperative enzyme, *Biochemistry*, 20, 499, 1981.
110. **Berthillier, G. and Got, R.,** Effets de l'ATP et de la photo-oxydation sur l'agrégation de la glucokinase microsomique du foie de rat, *Biochim. Biophys. Acta*, 258, 88, 1972.
111. **Yang, S. T. and Deal, W. C., Jr.,** Metabolic control and structure of glycolytic enzymes. VI. Competitive inhibition of yeast glyceraldehyde-3-phosphate dehydrogenase by cyclic adenosine monophosphate, adenosine triphosphate, and other adenine-containing compounds, *Biochemistry*, 8, 2806, 1969.
112. **Stancel, G. M. and Deal, W. C., Jr.,** Metabolic control and structure of glycolytic enzymes. V. Dissociation of yeast glyceraldehyde-3-phosphate dehydrogenase into subunits by ATP, *Biochem. Biophys. Res. Commun.*, 31, 398, 1968.
113. **Hoagland, V. D., Jr. and Teller, D. C.,** Influence of substrates on the dissociation of rabbit muscle D-glyceraldehyde 3-phosphate dehydrogenase, *Biochemistry*, 8, 594, 1969.
114. **Keleti, T., Batke, J., Ovadi, J., Jancsik, V., and Bartha, F.,** Macromolecular interactions in enzyme regulation, *Adv. Enz. Regul.*, 15, 233, 1977.
115. **Meighen, E. A. and Schachman, H. K.,** Hybridization of native and chemically modified enzymes. I. Development of a general method and its application to the subunit structure of aldolase, *Biochemistry*, 9, 1177, 1970.
116. **Bonsignore, A., Cancedda, R., Nicolini, A., Damiani, G., and DeFlora, A.,** Metabolism of human erythrocyte glucose-6-phosphate dehydrogenase. VI. Interconversion of multiple molecular forms, *Arch. Biochem. Biophys.*, 147, 493, 1971.
117. **Scott, W. A.,** Physical properties of glucose-6-phosphate dehydrogenase from *Neurospora crassa*, *J. Biol. Chem.*, 246, 6353, 1971.
118. **Johnson, G. G., Eisenberg, L. R., and Migeon, B. R.,** Human and mouse hypoxanthine-guanine phosphoribosyltransferase: dimers and tetramers, *Science*, 203, 174, 1979.
119. **Kahler, S. G. and Kirkman, H. N.,** Intracellular glucose-6-phosphate dehydrogenase does not monomerize in human erythrocytes, *J. Biol. Chem.*, 258, 717, 1983.
120. **Kotlarz, D. and Buc, H.,** Regulatory properties of phosphofructokinase from *Escherichia coli*, *Eur. J. Biochem.*, 117, 569, 1981.
121. **Guixe, V. and Babul, J.,** Influence of ligands on the aggregation of the normal and mutant forms of phosphofructokinase 2 of *Escherichia coli*, *Arch. Biochem. Biophys.*, 264, 519, 1988.
122. **Deville-Bonne, D., Le Bras, G., Teschner, W., and Garel, J.-R.,** Ordered disruption of subunit interfaces during the stepwise reversible dissociation of *Escherichia coli* phosphofructokinase with KSCN, *Biochemistry*, 28, 1917, 1989.
123. **Luther, M. A., Gilbert, H. F., and Lee, J. C.,** Self-association of rabbit muscle phosphofructokinase: role of subunit interaction in regulation of enzymatic activity, *Biochemistry*, 22, 5494, 1983.
124. **Mayr, G. W.,** Interaction of calmodulin with muscle phosphofructokinase, *Eur. J. Biochem.*, 143, 513, 1984.
125. **Bock, P. E. and Frieden, C.,** Phosphofructokinase. I. Mechanism of the pH-dependent inactivation and reactivation of the rabbit muscle enzyme, *J. Biol. Chem.*, 251, 5630, 1976.
126. **Luther, M. A., Hesterberg, L. K., and Lee, J. C.,** Subunit interaction of rabbit muscle phosphofructokinase: effects of purification procedures, *Biochemistry*, 24, 2463, 1985.
127. **Hofmann, E., Kurganov, B. I., Schellenberger, W., Schulz, T., Sparmann, G., Wenzel, K.-U., and Zimmermann, G.,** Association-dissociation behavior of erythrocyte phosphofructokinase and tumor pyruvate kinase, *Adv. Enz. Regul.*, 13, 247, 1975.
128. **Oberfelder, R. W., Lee, L. L.-Y., and Lee, J. C.,** Thermodynamic linkage in rabbit muscle pyruvate kinase: kinetic, equilibrium, and structural studies, *Biochemistry*, 23, 3813, 1984.
129. **LéJohn, H. A., McCrea, B. E., Suzuki, I., and Jackson, S.,** Association-dissociation reactions of mitochondrial isocitric dehydrogenase induced by protons and various ligands, *J. Biol. Chem.*, 244, 2484, 1969.
130. **De Riel, J. K. and Paulus, H.,** Subunit dissociation in the allosteric regulation of glycerol kinase from *Escherichia coli*. I. Kinetic evidence, *Biochemistry*, 17, 5134, 1978.
131. **De Riel, J. K. and Paulus, H.,** Subunit dissociation in the allosteric regulation of glycerol kinase from *Escherichia coli*. II. Physical evidence, *Biochemistry*, 17, 5141, 1978.
132. **De Riel, J. K. and Paulus, H.,** Subunit dissociation in the allosteric regulation of glycerol kinase from *Escherichia coli*. III. Role in desensitization, *Biochemistry*, 17, 5146, 1978.

133. **Chan, W. W.-C., Mort, J. S., Chong, S. K. K., and MacDonald, P. D. M.,** Studies on protein subunits. III. Kinetic evidence for the presence of active subunits during the renaturation of muscle aldolase, *J. Biol. Chem.*, 248, 2778, 1973.
134. **Chan, W. W.-C.,** The relationship between quaternary structure and enzyme activity, *Trends Biochem. Sci.*, 1, 258, 1976.
135. **Penhoet, E. C. and Rutter, W. J.,** Catalytic and immunochemical properties of homomeric and heteromeric combinations of aldolase subunits, *J. Biol. Chem.*, 246, 318, 1971.
136. **Jurgensen, S. R. and Harrison, J. H.,** Active subunits in hybrid-modified malate dehydrogenase, *J. Biol. Chem.*, 257, 569, 1982.
137. **Mullinax, T. R., Mock, J. N., McEvily, A. J., and Harrison, J. H.,** Regulation of mitochondrial malate dehydrogenase. Evidence for an allosteric citrate-binding site, *J. Biol. Chem.*, 257, 13233, 1982.
138. **Iwatsuki, N. and Okazaki, R.,** Mechanism of regulation of deoxythymidine kinase of *Escherichia coli*. I. Effect of regulatory deoxynucleotides on the state of aggregation of the enzyme, *J. Mol. Biol.*, 29, 139, 1967.
139. **Iwatsuki, N. and Okazaki, R.,** Mechanism of regulation of deoxythymidine kinase of *Escherichia coli*. II. Effect of temperature on the enzyme activity and kinetics, *J. Mol. Biol.*, 29, 155, 1967.
140. **Maley, G. F. and Maley, F.,** Regulatory properties and subunit structure of chick embryo deoxycytidylate deaminase, *J. Biol. Chem.*, 243, 4506, 1968.
141. **Maley, G. F. and Maley, F.,** Active and inactive sites of deoxycytidylate deaminase and their relation to subunit structure, *J. Biol. Chem.*, 243, 4513, 1968.
142. **Rowe, P. B. and Wyngaarden, J. B.,** Glutamine phosphoribosylpyrophosphate amidotransferase. Purification, substructure, amino acid composition, and absorption spectra, *J. Biol. Chem.*, 243, 6373, 1968.
143. **Munch-Petersen, B.,** Differences in the kinetic properties of thymidine kinase isoenzymes in the unstimulated and phytohemagglutinin-stimulated human lymphocytes, *Mol. Cell Biochem.*, 64, 173, 1984.
144. **Porter, P. M., Bull, D., and Jones, O. W.,** Stabilization of SV_{40} transformed human fibroblast cytoplasmic thymidine kinase by ATP, *Mol. Cell Biochem.*, 35, 59, 1980.
145. **Maley, F., Belfort, M., and Maley, G.,** Probing the infrastructure of thymidylate synthase and deoxycytidylate deaminase, *Adv. Enz. Regul.*, 22, 413, 1984.
146. **Ellims, P. H., Kao, A. Y., and Chabner, B. A.,** Deoxycytidylate deaminase. Purification and some properties of the enzyme isolated from human spleen, *J. Biol. Chem.*, 256, 6335, 1981.
147. **Nucci, R., Raia, C. A., Vaccaro, C., Sepe, S., Scarano, E., and Rossi, M.,** Freezing of dCMP aminohydrolase in the activated conformation by glutaraldehyde, *J. Mol. Biol.*, 124, 133, 1978.
148. **Wong, J. Y., Meyer, E., and Switzer, R. L.,** Glutamine phosphoribosylpyrophosphate amidotransferase from *Bacillus subtilis*, a novel iron-sulfur protein, *J. Biol. Chem.*, 252, 7424, 1977.
149. **Holmes, E. W., Wyngaarden, J. B., and Kelley, W. N.,** Human glutamine phosphoribosylpyrophosphate amidotransferase. Two molecular forms interconvertible by purine ribonucleotides and phosphoribosylpyrophosphate, *J. Biol. Chem.*, 248, 6035, 1973.
150. **Singer, S. C. and Holmes, E. W.,** Human glutamine phosphoribosylpyrophosphate amidotransferase. Hysteretic properties, *J. Biol. Chem.*, 252, 7959, 1977.
151. **Brown, N. C. and Reichards, P.,** Ribonucleoside diphosphate reductase. Formation of active and inactive complexes of proteins B1 and B2, *J. Mol. Biol.*, 46, 25, 1969.
152. **Thelander, L., Eriksson, S., and Akerman, M.,** Ribonucleotide reductase from calf thymus — separation of the enzyme into two nonidentical subunits, proteins M1 and M2, *J. Biol. Chem.*, 255, 7426, 1980.
153. **Thomas, C. B., Arnold, W. J., and Kelley, W. N.,** Human adenine phosphoribosyltransferase. Purification, subunit structure, and substrate specificity, *J. Biol. Chem.*, 248, 2529, 1973.
154. **Holden, J. A., Meredith, G. S., and Kelley, W. N.,** Human adenine phosphoribosyltransferase. Affinity purification, subunit structure, amino acid composition, and peptide mapping, *J. Biol. Chem.*, 254, 6951, 1979.
155. **Sloan, D. L. and Mayer, L.,** Crosslinking studies of the structures of some yeast phosphoribosyltransferases, *Fed. Proc.*, 42, 2192, 1983.
156. **Caradonna, S. J. and Adamkiewicz, D. M.,** Purification and properties of the deoxyuridine triphosphate nucleotidohydrolase enzyme derived from HeLa S3 cells. Comparison to a distinct dUTP nucleotidohydrolase induced in herpes simplex virus-infected HeLa S3 cells, *J. Biol. Chem.*, 259, 5459, 1984.
157. **Powers, S. G., Meister, A., and Haschemeyer, R. H.,** Linkage between self-association and catalytic activity of *Escherichia coli* carbamyl phosphate synthetase, *J. Biol. Chem.*, 255, 1554, 1980.
158. **Boettcher, B. and Meister, A.,** Conversion of UMP, an allosteric inhibitor of carbamyl phosphate synthetase, to an activator by modification of the UMP ribose moiety, *J. Biol. Chem.*, 256, 5977, 1981.
159. **Koshland, D. E., Jr. and Levitzki, A.,** CTP synthetase and related enzymes, in *The Enzymes*, Vol. 10, Boyer, P. D., Ed., Academic Press, New York, 1974, 539.
160. **Long, C. and Koshland, D. E., Jr.,** Cytidine triphosphate synthetase, *Methods Enzymol.*, 51, 79, 1978.
161. **Anderson, P. M.,** CTP synthetase from *Escherichia coli*: an improved purification procedure and characterization of hysteretic and enzyme concentration effects on kinetic properties, *Biochemistry*, 22, 3285, 1983.
162. **McPartland, R. P. and Weinfeld, H.,** Cytidine-5′-triphosphate synthetase of calf liver. Size, polymerization, and reaction stoichiometry, *J. Biol. Chem.*, 251, 4372, 1976.

163. **Schubert, K. R., Switzer, R. L., and Shelton, E.,** Studies on the quaternary structure and the chemical properties of phosphoribosylpyrophosphate synthetase from *Salmonella typhimurium*, *J. Biol. Chem.*, 254, 11394, 1975.
164. **Gibson, K. J., Schubert, R., and Switzer, R. L.,** Binding of the substrates and allosteric inhibitor adenosine 5′-diphosphate to phosphoribosylpyrophosphate synthetase from *Salmonella typhimurium*, *J. Biol. Chem.*, 257, 2391, 1982.
165. **Meyer, L. J. and Becker, M. A.,** Human erythrocyte phosphoribosylpyrophosphate synthetase. Dependence of activity on state of subunit association, *J. Biol. Chem.*, 252, 3919, 1977.
166. **Arnvig, K., Hove-Jensen, B., and Switzer, R. L.,** Purification and properties of phosphoribosyl-diphosphate synthetase from *Bacillus subtilis*, *Eur. J. Biochem.*, 192, 195, 1990.
167. **Traut, T. W. and Jones, M. E.,** Kinetic and conformational studies of the orotate phosphoribosyltransferase: orotidine-5′-phosphate decarboxylase enzyme complex from mouse Ehrlich ascites cells, *J. Biol. Chem.*, 252, 8374, 1977.
168. **Edwards, Y. H., Edwards, P. A., and Hopkinson, D. A.,** A trimeric structure for mammalian purine nucleoside phosphorylase, *FEBS Lett.*, 32, 235, 1973.
169. **Ropp, P. A. and Traut, T. W.,** Allosteric regulation of purine nucleoside phosphorylase, *Arch. Biochem. Biophys.*, 288, 614, 1991.
170. **Frieden, C.,** Glutamic dehydrogenase. I. The effect of coenzyme on the sedimentation velocity and kinetic behavior, *J. Biol. Chem.*, 234, 809, 1959.
171. **Frieden, C.,** Glutamic dehydrogenase. II. The effect of various nucleotides on the association-dissociation and kinetic properties, *J. Biol. Chem.*, 234, 815, 1959.
172. **Frieden, C.,** Different structural forms of reversibly dissociated glutamic dehydrogenase: relation between enzymatic activity and molecular weight, *Biochem. Biophys. Res. Commun.*, 10, 410, 1963.
173. **Kempner, E. S. and Miller, J. H.,** Radiation inactivation of glutamate dehydrogenase hexamer: lack of energy transfer between subunits, *Science*, 222, 586, 1983.
174. **Strambini, G. B., Cioni, P., and Puntoni, A.,** Relationship between the conformation of glutamate dehydrogenase, the state of association of its subunits, and catalytic function, *Biochemistry*, 28, 3808, 1989.
175. **Lusty, C. J.,** Catalytically active monomer and dimer forms of rat liver carbamoyl-phosphate synthetase, *Biochemistry*, 20, 3665, 1981.
176. **Wampler, D. E.,** Threonine-sensitive aspartokinase-homoserine dehydrogenase complex from *Escherichia coli*. Subunit stoichiometry and size of the catalytic subunit, *Biochemistry*, 11, 4428, 1972.
177. **Calhoun, D. H., Rimerman, D. A., and Hatfield, G. W.,** Threonine deaminase from *Escherichia coli*. I. Purification and properties, *J. Biol. Chem.*, 248, 3511, 1973.
178. **Boernke, W. E., Stevens, F. S., and Peraino, C.,** Effects of self-association of ornithine aminotransferase on its physicochemical characteristics, *Biochemistry*, 20, 115, 1981.
179. **Boernke, W. E., Stevens, F. J., Edwards, J. J., and Peraino, C.,** Differential changes in ornithine aminotransferase self-affinity produced by exposure to basic amino acids and increases in the intrinsic electronegativity of the enzyme monomer, *Arch. Biochem. Biophys.*, 216, 152, 1982.
180. **Ashcraft, B. A., Fillers, W. S., Augustine, S. L., and Clarke, S. D.,** Polymer-protomer transition of acetyl CoA carboxylase occurs in vivo and varies with nutritional conditions, *J. Biol. Chem.*, 255, 10033, 1980.
181. **Clarke, B. A. and Clarke, S. D.,** Polymer-protomer transition of acetyl CoA carboxylase as a regulator of lipogenesis in rat liver, *Arch. Biochem. Biophys.*, 218, 92, 1982.
182. **Clarke, S. D. and Salati, L. M.,** Fatty acid-mediated disaggregation of acetyl-CoA carboxylase in isolated liver cells, *Fed. Proc.*, 44, 2458, 1985.
183. **Ricard, J., Meunier, J.-C., and Buc, J.,** Regulatory behavior of monomeric enzymes. I. The mnemonical enzyme concept, *Eur. J. Biochem.*, 49, 195, 1974.
184. **Meunier, J.-C., Buc, J., Navarro, A., and Ricard, J.,** The mnemonical enzyme concept. II. Wheat-germ hexokinase as a mnemonical enzyme, *Eur. J. Biochem.*, 49, 209, 1974.
185. **Pilkis, S. J., El-Maghrabi, M. R., Pilkis, J., and Claus, T. H.,** Studies on the *in vitro* phosphorylation of 6-phosphofructo-1-kinase from rat liver, *Arch. Biochem. Biophys.*, 215, 379, 1982.
186. **Oberfelder, R. W., Barisa, B. G., and Lee, J. C.,** Thermodynamic linkage in rabbit muscle pyruvate kinase: analysis of experimental data by a two-state model, *Biochemistry*, 23, 3822, 1984.
187. **McPartland, R. P. and Weinfeld, H.,** Cooperative effects of CTP on calf liver CTP synthetase, *J. Biol. Chem.*, 254, 11394, 1979.
188. **Meyer, E. and Switzer, R. L.,** Regulation of *Bacillus subtilis* glutamine phosphoribosylpyrophosphate amidotransferase activity by end products, *J. Biol. Chem.*, 254, 5397, 1979.
189. **Olsen, B. R., Svenneby, G., Kvamme, E., Tveit, B., and Eskeland, T.,** Formation and ultrastructure of enzymically active polymers of pig renal glutaminase, *J. Mol. Biol.*, 52, 239, 1970.
190. **Hatfield, G. W. and Burns, R. O.,** Threonine deaminase from *Salmonella typhimurium*. III. The intermediate substructure, *J. Biol. Chem.*, 245, 787, 1970.
191. **Hatfield, G. W. and Burns, R. O.,** Ligand-induced maturation of threonine deaminase, *Science*, 167, 75, 1970.