The finding of large quadrupole splitting and line broadening has been taken as evidence to support this vacancy model. A detailed interpretation of the temperature dependence of the fractions of Fe^{3+} in the light of this model is needed to help put it on a firm footing.

Summary

Changes in the chemical state of ions in solids following nuclear decay processes have been observed by the Mössbauer effect. The mechanisms responsible for these changes are only now being elucidated. In general some modification of the ligand environment appears to be required. These are most often brought about by the radiolytic effects of ionizing radiation. It also appears possible that vacancies, initially present or produced by the Coulomb repulsion of the Auger core, can stabilize new charge states. Other mechanisms which have been invoked are internal pressure effects and the Coulombic fragmentation of molecules by the withdrawal of electrons from the ligands by the Auger core, but there are valid objections to both suggestions. The existence of time-dependent charge relaxation effects has been suggested, but has not been verified experimentally.

Glutamate Dehydrogenase: Anatomy of a Regulatory Enzyme

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In these days of sophistication of scientific research (and I am inclined to use "sophisticated" not only in its modern sense but also in its original connotation), one sometimes encounters doubts as to whether the study of solutions of macromolecules by classical physicochemical means is a meaningful pursuit. We have been caught up in the steamroller of technology. Achievements which provided, a scant two decades ago, in the brilliant minds and hands of the Debyes, the Kirkwoods, the Svedbergs, the Florys, and the Zimms, the tools for a penetrating insight into the world of synthetic and natural polymers have lost much of their glitter for the present day computer-conscious generation of young epigones.

This is not the place to discuss either the considerable achievements in X-ray crystallography or the other, newer, physical techniques (for instance, the unraveling of a large number of protein crystal structures, based on the pioneering and continuing studies of the Paulings, the Kendrews, and the Perutzes) or the deeper philosophical implications of the dehumanizing effect of the computer on science and the human mind. One could call this the "sorcerer's apprentice" effect, in that extrapolation does not necessarily lead to the rewards originally envisaged.

Here I should like to point out that there are fascinating problems related to biomacromolecules, which are clearly important but imperfectly understood, either because they are not yet ripe enough to be dissected by X-rays, nmr, and the newer tools of molecular torture, or because of plain overcrowding in the chambers of inquisition. I should like to show in this Account that, properly coaxed, nature may reveal some of its lesser secrets in the simple, old-fashioned ways. I should like to show that by classical light scattering, sedimentation, viscosity, and related studies we can unravel some of the intricacies of structure and the relation of structure to function of a complex regulatory enzyme, bovine glutamate dehydrogenase.

This is a good place to acknowledge my deep indebtedness to Gordon Tomkins, who in 1966, during my stay at the National Institutes of Health, introduced me to the puzzling aspects of this problem and infected me with the bug of curiosity which has become a steady companion ever since.

The Enzyme: Statement of the Problem

As in all well-organized production schedules, biochemical intermediates in the living cell must be synthesized in closely regulated proportions and suitably linked to the production of related building blocks. The latter are required either for the synthesis of vital macromolecules, in energy cycles, or in processes directed toward storage and utilization of genetic information. In complex biochemical metabolic cycles glutamate dehydrogenase links the carbohydrate Krebs cycle with the pathways of amino acid synthesis. Specifically, it catalyzes the interconversion of glutamic and α-ketoglutaric acids; it performs this function in the mitochondria of the liver. In common with some of the other dehydrogenase enzymes it requires 1 mole of NAD (nicotinamide–adenine dinucleotide) for the reversible stereospecific transfer of 1 mole of hydrogen from its substrate glutamic acid *via* the imino derivative.

This reaction is subject to allosteric regulation and was quoted by Monod, *et al.*,[1] on the basis of the work of Tomkins, *et al.*,[2] in the original exposition of the allosteric hypothesis. A more recent review of allosteric

(1) J. Monod, J. P. Changeux, and F. Jacob, *J. Mol. Biol.*, **6**, 306 (1963).

(2) G. M. Tomkins, K. L. Yielding, N. Talal, and J. F. Curran, *Cold Spring Harbor Symp. Quant. Biol.*, **26**, 331 (1963).

regulation of enzyme activity is that of Stadtman,[3] and detailed discussions on the pyridine nucleotide dependent dehydrogenases and the pyridine nucleotide coenzymes have been given by Sund.[4] The reader is also referred to papers and discussions on glutamate and other dehydrogenases in the proceedings[5] of a symposium on pyridine nucleotide dependent dehydrogenases which was held in Konstanz in the fall of 1969.

In 1952 Olson and Anfinsen[6] made the original observation concerning the reversible association of glutamate dehydrogenase with change in enzyme concentration within the milligram per milliliter concentration range. Ever since the experimental results linked to molecular weight determinations have been confusing because of the variable molecular weight with changing enzyme concentration and experimental conditions. The state and mechanism of aggregation of the enzyme are of great interest in view of the fact that it is believed to exist at concentrations above a few milligrams per milliliter in its active and native form in the mitochondria of the liver, and its biological activity may be related in a complex way to its state of aggregation.[7]

Work by a number of research groups[8,9] disclosed that the state of aggregation of the enzyme in solution, in the milligram per milliliter concentration range, can be drastically altered by the addition of, for instance, small amounts of NADH (reduced form of NAD), in conjunction with an additional regulatory reagent. Out of the large variety of structurally unrelated effectors which have been used, we will mention here only GTP (guanosine triphosphate) which, in conjunction with NADH, may lead to complete dissociation of the enzyme to the oligomeric form. This effect is dramatic and visible to the naked eye. A glutamate dehydrogenase solution in phosphate buffer (0.2 M sodium phosphate buffer, pH 7; 10^{-4} M EDTA) containing 5–10 mg/ml of enzyme is quite turbid by virtue of the light scattered by the high molecular weight material. Addition of GTP and NADH to a concentration of about 10^{-3} M each "instantly" increases the transparency of the solution to about that of pure water.

The readily effected polymerization–depolymerization reaction is related to subtle changes in the substrate specificity of the active enzyme. Thus, all reagents which promote association of the enzyme to a higher molecular weight also activate the glutamate dehydrogenase reaction. On the other hand, reagents which promote dissociation of the enzyme to oligomer units inhibit the glutamate dehydrogenase activity and promote alanine (and other monocarboxylic acid) dehydrogenase reactions.[2,10] Whereas it was originally believed that the two activities were related in a 1:1 correspondence to the state of molecular association, it is now thought that the state of association *per se* does not govern the specificity of the enzymatic reaction. Rather, it appears that the oligomer is capable of existing in at least two active forms with distinct substrate activity[11] but that only the oligomer form exhibiting glutamate dehydrogenase activity is able to polymerize. The loss of the major glutamate dehydrogenase activity and the loss of the ability to polymerize are thus related in the sense that both functions are strongly diminished subsequent to the same allosteric transition.[12] The enzymatic activity toward glutamate is apparently not affected by the state of association of the enzyme. Figure 1 is a schematic presentation of the suspected state of affairs and indicates a partial list of effectors which produce allosteric shifts into either one direction or the other. Advances relating to an understanding of biological function have unfortunately not been paralleled by increased understanding with respect to physical structure. We refer to a recent review[13] on glutamate dehydrogenases of various origins which, together with the appended symposium discussion, presents a detailed description of the complex function of the enzyme; it also highlights the almost complete lack of understanding of molecular structure at the time (1968). Our own efforts constitute an attempt to remedy this situation.

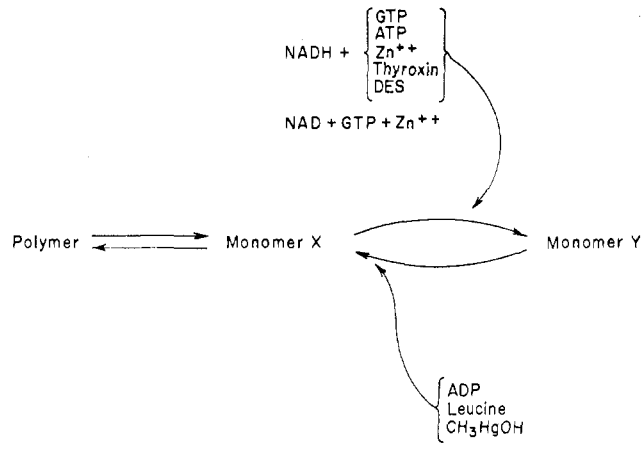


Figure 1. Dissociation of glutamate dehydrogenase. DES refers to diethylstilbestrol. See Stadtman,[3] after Tomkins, *et al.*[10]

(3) E. R. Stadtman, *Advan. Enzymol.*, **28**, 41 (1966).
(4) H. Sund in "Biological Oxidations," T. P. Singer, Ed., Interscience, New York, N. Y., 1968, pp 603, 641.
(5) H. Sund, Ed., "Pyridine Nucleotide Dependent Dehydrogenases," Springer-Verlag, Berlin, 1970.
(6) J. A. Olson and C. B. Anfinsen, *J. Biol. Chem.*, **197**, 67 (1952).
(7) The localization of the enzyme in the mitochondria is not altogether certain—an amazing admission about the limited power, and the necessity of refining, of our basic research tools; compare "Mitochondrial Structure and Compartmentation," E. Quagliariello, S. Papa, E. C. Slater, and J. M. Tager, Ed., Adriatica Editrice, Bari, Italy, 1967, M. Ashwell and T. S. Work, *Annu. Rev. Biochem.*, **39**, 251 (1970), and L. Ernster and B. Kuylenstierna in "Membranes of Mitochondria and Chloroplasts," E. Racker, Ed., Van Nostrand-Reinhold Company, New York, N. Y., 1970, p 172, for discussions on glutamate dehydrogenase and other enzyme *in vivo* localization.
(8) C. Frieden, *J. Biol. Chem.*, **234**, 809 (1959).
(9) K. L. Yielding and G. M. Tomkins, *Proc. Nat. Acad. Sci. U. S.*, **47**, 983 (1961).
(10) G. M. Tomkins, K. L. Yielding, J. F. Curran, M. R. Summers, and M. W. Bitensky, *J. Biol. Chem.*, **240**, 3793 (1965).
(11) The activity is assayed at very low enzyme concentrations, at which oligomeric forms are usually prevalent.
(12) We use allosteric here in the somewhat less than dogmatic sense that binding of small ligands to biologically active macromolecules may produce conformational changes which are responsible for changes in biological activity—sigmoid kinetics which are usually associated with the term allostery have not clearly been observed in the binding of effectors to glutamate dehydrogenase, but the existence of a feedback mechanism is beyond doubt.
(13) C. Frieden in "The Role of Nucleotides for the Function and Conformation of Enzymes," H. M. Kalckar, H. Klenow, A. M. Petersen, M. Ottensen, and J. H. Thaysen, Ed., Academic Press, New York, N. Y., 1969, p 194.

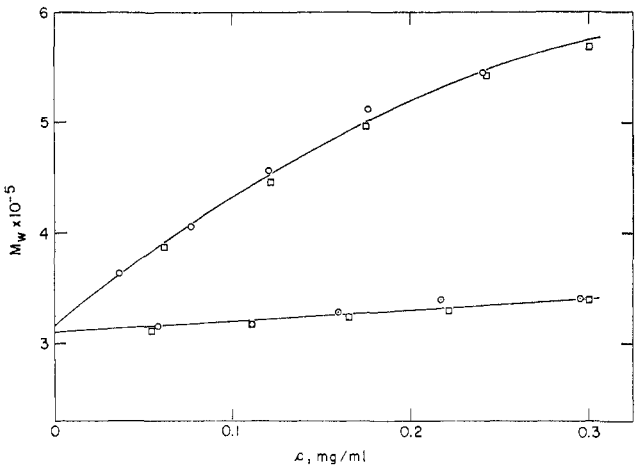


Figure 2. Apparent weight-average molecular weight (M_w) of glutamate dehydrogenase, at low enzyme concentrations. Upper curve, 0.2 M phosphate buffer; lower curve, same as above plus 10^{-3} M GTP, 10^{-3} M NADH. Light scattering experiments at 25°, at 546 mμ. See Eisenberg and Tomkins.[14]

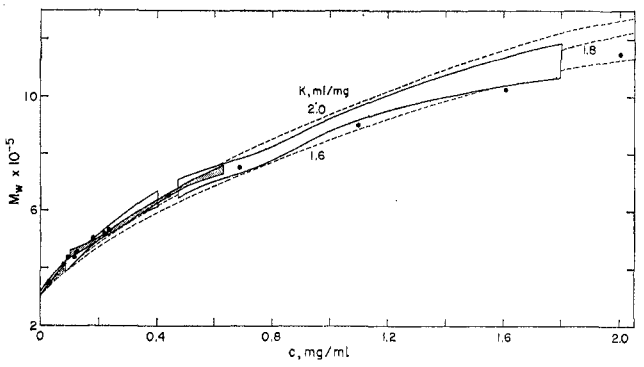


Figure 3. Weight-average molecular weight (M_w) *vs* concentration in 0.2 M phosphate buffer; boxes, computed values from equilibrium sedimentation runs, $t = 20°$; curves, calculated according to reversible infinite linear association (stacking) with a single value of association constant K. See Reisler, *et al.*[16]

The Active Enzyme Oligomer

The values of the molecular weight for the oligomer 250,000–400,000 and for the subunits 44,000–53,000, current in 1966–1967, did not permit a reliable assignment of the number of subunits per glutamate dehydrogenase oligomer; estimates based on the above molecular weight values ranged between four to eight subunits. For the determination of the correct enzyme oligomer molecular weight, careful light scattering measurements were extended[14] to extremely low concentrations in 0.2 M phosphate buffer (Figure 2) to yield 316,000 ± 10,000 daltons for the oligomer molecular weight.

The lower curve in Figure 2 refers to experiments in which GTP and NADH have been added at a concentration of 10^{-3} M. It is seen that these effectors, which bring about loss of biological activity, lead to the almost complete dissociation of the associated enzyme into oligomers (higher concentrations of effectors may be used to achieve complete dissociation). The extrapolated molecular weight (310,000) was found, within experimental error, to be identical with that of the oligomer (316,000), in the absence of these reagents. The binding of the low molecular weight materials thus does not produce a measurable (by our techniques) change in molecular weight. The only change produced may be a conformational transition which, in addition to affecting biological activity, also transforms the enzyme from a sticky structure (but with specific points of attachment) to a nonsticky entity.

We are pleased to note that subsequently published molecular weight values of the oligomer now agree well with our own value. It was also possible to show[15] that light scattering and equilibrium sedimentation (Figure 3) are in excellent agreement; this is important when we consider that the usual equations have been derived for particles of fixed (even if polydisperse) mass, and their applicability at finite concentrations to systems in rapid chemical equilibrium may require justification. Due to uncertainties in extrapolation to zero concentration, problems with absolute calibration, the relation between optical density and enzyme concentration, and the determination of accessory quantities (refractive index and density increments) in light scattering and equilibrium sedimentation, we estimate a possible deviation of about ±3 to ±5% of the true molecular weights from these values. Such an uncertainty, though, is inconsequential for our further considerations.

We also reported[14] 53,500 ± 1500 for the subunit molecular weight from light scattering in 5.7 M guanidine hydrochloride solutions.[16] The ratio of the two numbers is 5.9, which is close enough to establish the fact that the native oligomer is composed of six subunits. A recent, yet tentative, amino acid sequence determination[17] confirms the identity of the subunits and places their molecular weight at the slightly higher value of 56,000 daltons.

Subunits are used by nature as a device toward the solution of complicated regulatory biological process; once the correct number of subunits has been determined, it can be used to establish a physical model of enzyme structure, in which the spatial relationship of these subunits is well defined. This then is only a preliminary step toward the complete description of the location of each individual atom, a goal recently achieved in some selected cases by X-ray crystallography. The physical model which we suggested[18] is

(14) H. Eisenberg and G. M. Tomkins, *J. Mol. Biol.*, **31**, 37 (1968).

(15) E. Reisler, J. Pouyet, and H. Eisenberg, *Biochemistry*, **9**, 3095 (1970).

(16) High concentrations of guanidine hydrochloride denature proteins and cause dissociation into constituent peptide chains which are not covalently bonded to each other; small amounts of β-mercaptoethanol or other reducing agents are added to open SS bridges or to prevent formation of such bridges by oxidation of SH groups which become exposed in the unfolding process. Guanidine hydrochloride is a good solvent for polypeptide chains, and positive deviations from ideality (virial coefficients) are therefore to be expected. Native subunit of oligomer association, on the other hand, is equivalent to negative deviations from ideal behavior.

(17) E. L. Smith, M. Landon, D. Piszkiewicz, W. J. Brattin, T. L. Langley, and M. D. Melamed, *Proc. Nat. Acad. Sci. U. S.*, **67**, 724 (1970).

(18) H. Eisenberg and E. Reisler, *Biopolymers*, **9**, 113 (1970).

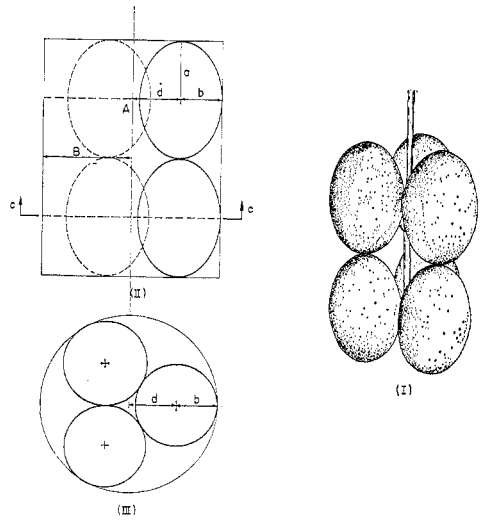
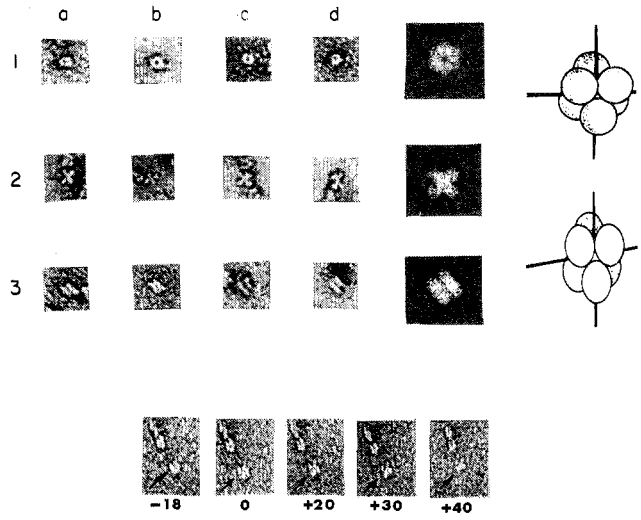


Figure 4. (I) Macroscopic model of glutamate dehydrogenase oligomer. Suspension rod connected for measurement of rotary frictional coefficient about threefold axis of symmetry; (II) logitudinal section of model, where a, b, refer to semiaxes of subunits; (III) cross-section of model at cc. See E. Reisler and H. Eisenberg, *Biopolymers*, **9**, 877 (1970).

illustrated schematically in Figure 4. In the establishment of the model we were strongly inspired by Valentine,[19] who reported some electron micrograph pictures taken of this enzyme, in which he confirms earlier observations that the molecule has a triangular profile.

In our tentative model the oligomer is formed by two layers, each composed of three elongated subunits approximated by prolate ellipsoids of rotation, arranged in triangular fashion.[20] In the individual layers the major axes ($2a$) of the ellipsoids point in the same direction. Two layers, stacked on top of each other, form an elongated oligomer, which can further polymerize, to polymers of indefinite length, L,[14] in the direction of the threefold axis of symmetry of the oligomer. On the basis of studies performed to date it is difficult to distinguish whether the three-subunit layers in the oligomer are arranged in staggered or eclipsed configuration with respect to each other. In Valentine's work[19] only triangular shapes were identified. This seemed to favor the eclipsed form shown above.

Recently Josephs[21] has undertaken an extensive electron microscopy study of glutamate dehydrogenase; in micrographs obtained from his preparation (Figure 5) the staggered conformation is favored. Characteristic projections from different particles can be observed, and also different projections of the same particle, by tilting the angle of observation. Josephs also observes a small amount of triangular shapes which he attributes to cleavage along a twofold axis. Such



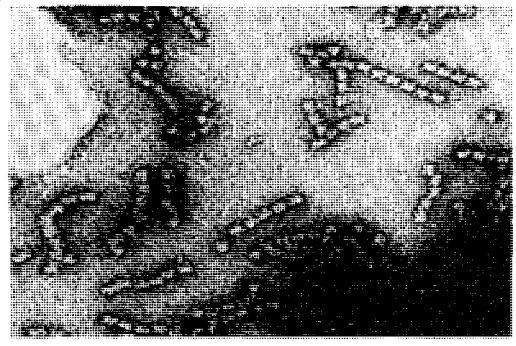


Figure 5. Electron micrographs of glutamate dehydrogenase. Top, isolated oligomers in various orientations, as compared with computed projections of model; center, isolated oligomer at various angles of tilt; bottom, polymer chains of the enzyme. See Josephs.[21]

cleavage with subsequent lateral aggregation may have been prevalent in the preparations used in Valentine's work. So far dissociation in solution into active half-oligomers, containing three subunits each, has not been achieved. The ease with which the enzyme dissociates into oligomeric hexamer, and the reluctance to yield trimers in solution, indicate opposing polarity in the bonding of the two triangular layers.

We have recently[14,18] concluded on the basis of light scattering and viscosity studies that the oligomer associates lengthwise, along the threefold axis of symmetry, to yield long rodlike polymers of indefinite length. In evaluating the quantitative aspects of the proposed model we have been greatly helped by the recent small-angle X-ray scattering study of Sund, *et al.*[22] They found, by careful study over a wide range of concentrations, that the mass per unit length, M/L, of glutamate dehydrogenase is 2340 daltons/Å, and the radius of gyration of the cross section, r_g, equals 30.3 Å, independent of the state of association of the enzyme. Clearly, this confirms the linear association of the enzyme.

From the value M/L = 2340 daltons/Å (now also confirmed by light-scattering studies; see below) and

(19) R. C. Valentine, Fourth European Regional Conference on Electron Microscopy, Rome, 1968, Abstracts, Vol. 2, p 3.

(20) Ellipsoids of rotation are often used to approximate the behavior of globular protein molecules, although to some this idea is objectionable. Still, for multisubunit enzymes the use of ellipsoidal subunits to model some hydrodynamic properties of the enzyme turned out to be quite useful. We shall indicate below that in many cases it is not necessary to use ellipsoids (except for visual satisfaction), and the analysis is more general than indicated by the model.

(21) R. Josephs, *J. Mol. Biol.*, **55**, 147 (1971).

(22) H. Sund, I. Pilz, and M. Herbst, *Eur. J. Biochem.*, **7**, 517 (1969).

M_1 = 312,000 for the molecular weight of the oligomer, we find l = 133 Å for the length of the oligomer; this value is independent of the shape or arrangement of subunits. In terms of the model we calculate $2a$ = 66.5 Å and $2b$ = 43 Å for the major and minor axes of the elliposids in the configuration of Figure 4. (For the staggered model the length increment per oligomer would be the same, but $2a$ and $2b$ would be slightly different.) The value of r_g with respect to the long axis of Figure 4 is calculated to be 28.3 Å. This is rather close to r_g = 30.3 Å reported by Sund, *et al.*,[22] and the slightly lower value given by the model may be due to voids, loose arrangements of the subunits, or hydration. Both Valentine and Josephs report about 80 Å for the triangular "edge" of the oligomer; we find a value $4b$ = 86 Å from the model. The overall agreement may be regarded as satisfactory.

For the radius of gyration, R_g, of the oligomer we calculate 46 Å. We suggested[18] that this can be experimentally verified by small-angle scattering of X-rays in systems in which association does not occur; this study has recently been undertaken by Pilz and Sund (I. Pilz, private communication), and R_g was found to be 47 Å.

Linear Self-Association of Glutamate Dehydrogenase

Following our description of the gross structure of the enzyme hexameric oligomer and of its constituent subunits, we now proceed to discuss the polymerization of the enzyme to more highly associated forms. We have already seen (Figures 2 and 3) that both light scattering and equilibrium sedimentation analysis show that the weight-average molecular weight, M_w, increases with increasing concentration; M_w is an apparent quantity, related to the true value M_w^0 by

$$\frac{1}{M_w^0} = \frac{1}{M_w} - 2A_2c \qquad (1)$$

where A_2 is the second virial coefficient in the expansion of the osmotic pressure in powers of the molecular weight and c is the enzyme concentration in grams per milliliter. For systems in chemical equilibrium, in which association of particles is concentration dependent, M_w^0 can only be evaluated if A_2 is known, or assumed to be known. Since A_2 can be estimated[14,15] to be of the order of 10^{-5} mole ml/g,[2] there exists in 0.2 M phosphate buffer an enzyme concentration range (below 0.4 mg/ml) in which virial effects can be neglected and the nature of the association reaction can be explored without the necessity of taking nonideality effects into account.

The reversible association of glutamate dehydrogenase in phosphate buffer may be represented[15] in the same way as the purine stacking process analyzed by Van Holde and Rossetti,[23] namely in terms of a simple reversible polymerization with one equilibrium constant, K. The mathematics of the stacking process with a single association constant, K, are identical with those

(23) K. E. Van Holde and G. P. Rossetti, *Biochemistry*, **6**, 2189 (1967).

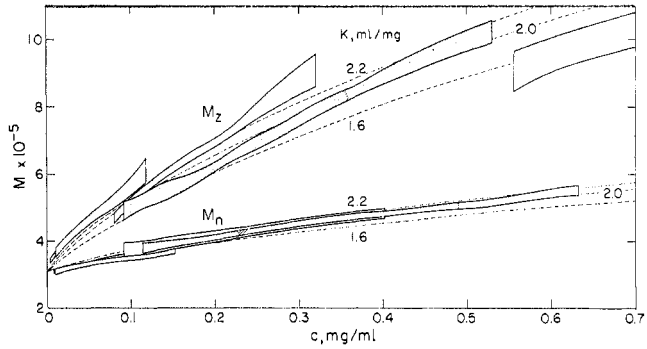


Figure 6. Number-average (M_n) and z-average (M_z) molecular weight from equilibrium sedimentation; curves and symbols as in Figure 3. See Reisler, *et al.*[15]

of the linear condensation polymerization of bifunctional monomers extensively discussed by Flory.[24] We consider the set of reactions

$$P_i + P_1 \rightleftarrows P_{i+1} \qquad (2)$$
$$K_i = c_{i+1}/c_ic_1$$

where P_i is a polymerized species of weight M_i, i runs from unity to n, c_i is the concentration of species P_i in g/ml, and all $K_i = K$ (ml/g) are considered to be equal. As long as $Kc_1 < 1$,[25] a simple calculation shows that

$$X_w^2 = 1 + 4Kc \qquad (3)$$

where $X_w = M_w^0/M_1$ is the weight-average degree of polymerization and c is the sum Σc_i over all species. A plot of $X_w^2 - 1$ *vs.* c is linear at low enzyme concentrations, and from the initial slope K may be determined.[15]

It has been customary to assume that a system, such as this, can be described by one association constant, K, and one constant virial coefficient, A_2, only; therefore values of K and A_2 were fitted[26] from data of M_w *vs.* c. We believe that virial coefficients are usually not constant, and this fitting procedure may not be justified. We discuss this point in detail in a forthcoming publication.[27]

The polymerization scheme described here leads to the so-called "most probable" distribution[24] and the number-average (M_n) and z-average molecular weight (M_z) are related to M_w by $2M_n = M_w + M_1$ and $2M_z = 3M_w - (M_1^2/M_w)$ (in the limit $M_w \gg M_1$ this yields the familiar relationship $M_z:M_w:M_n = 3:2:1$). It is possible to derive both M_z and M_n, in addition to M_w, from equilibrium sedimentation data. We have done this[15] for low concentrations of glutamate dehydrogenase; the results are presented in Figure 6. It is seen that the experimental results agree well with the curve calculated for K close to 2 ml/mg, which was previously derived.

(24) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953.

(25) Comparison of this calculation with the probabilistic approach of Flory[24] identifies Kc_1 with p, the probability that an individual unit is bound to a succeeding unit. The probability must be smaller than unity in a scheme in which unity corresponds to certainty of an event.

(26) P. W. Chun and S. J. Kim, *Biochemistry*, **8**, 1633 (1969).

(27) E. Reisler and H. Eisenberg, *ibid.*, **10**, 2659 (1971).

Enhancement of Linear Self-Association by Binding of Toluene

Recently we have also observed[18] that in aqueous buffered solutions, saturated with toluene, association of glutamate dehydrogenase (even at extremely low protein concentrations) is considerably enhanced. The same type of rodlike associates are formed as in the absence of toluene, and are reversibly dissociated when the toluene is removed. It was shown[28] by light-scattering studies that the association at various temperatures, in the presence of toluene, could still be described by one single constant, about one order of magnitude larger than the value found in the absence of toluene. The association (Figure 7) increases with decreasing temperature; benzene, which is somewhat more soluble in aqueous solutions than toluene, leads to higher degrees of association. A fortunate feature in the studies with toluene is that high molecular weights are achieved at considerably lower enzyme concentrations than in the absence of toluene. Thus, for a given molecular weight, the nonideality term of eq 1 is far less important than in the absence of toluene, for comparable values of A_2. The curves of $X_w^2 - 1$ *vs.* c are linear over a wide range of X_w and K is calculated to be 52, 28.8, and 21.7 ml/mg at 10, 20, and 25°, as compared with 2 ml/mg only at 25° in the absence of toluene.

Solubility studies indicate that in solutions saturated with respect to toluene about 8–12 molecules of toluene are preferentially bound per mole of subunits (mol wt $\sim$ 53,500) to the polymerizing form of the enzyme. Toluene does not bind to the enzyme form which does not associate (in the presence of both 5×10^{-3} *M* GTP and 5×10^{-3} *M* NADH). Addition of GTP and NADH at this concentration to buffered enzyme solutions saturated with toluene resulted in complete dissociation to the oligomeric hexamer. We have also checked enzyme activity in the presence of toluene and found that no change in activity was observed within the error of the experimental determination.

We have seen that with addition of GTP and NADH to buffered enzyme solutions the capacity of the enzyme oligomer to associate as well as its major biological activity are reversibly inhibited. Other effectors, such as ADP (adenosine diphosphate) and NADH, for instance, increase both activity and the capacity to associate. In the present case, though, these two properties are not coupled: if a buffered enzyme solution is carefully saturated with toluene the biological activity is not enhanced, although the molecular weight greatly increases. Addition of GTP and NADH to an enzyme solution saturated with toluene deactivates the enzyme, leads to dissociation to the oligomer form, and inhibits toluene binding; the enzyme to which 1 or 2 moles of coenzyme and GTP is bound per subunit apparently undergoes a reversible structural change which blocks binding of up to 12 molecules of toluene per subunit.

(28) E. Reisler, Ph.D. Thesis, Weizmann Institute of Science, Rehovoth, (1971).

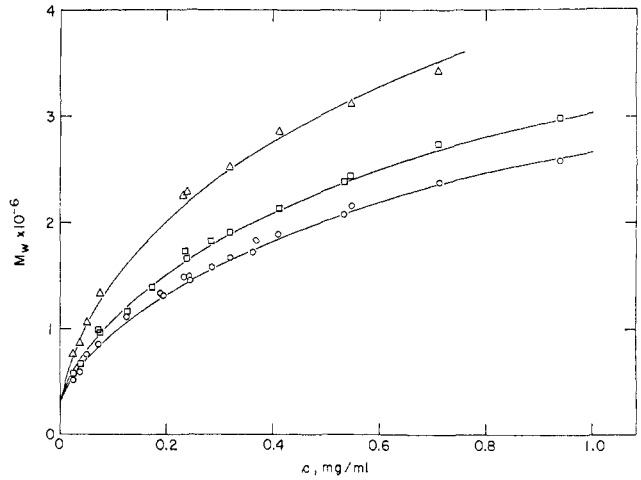


Figure 7. Weight-average molecular weight (M_w) from light-scattering measurements *vs.* concentration, in 0.2 *M* phosphate buffer saturated with toluene: ○, 25°, □, 20°; △, 10°; curves, calculated. See Reisler.[28]

A rather specific binding mechanism may be involved which, at this stage, cannot be defined.

Viscosity and Sedimentation Studies of Glutamate Dehydrogenase and Related Hydrodynamic Models

Intrinsic viscosity is related to the volume swept out by a macromolecule in rotary motion and is thus roughly proportional to the cube of the long linear dimension of the particle, whereas sedimentation viscosity is inversely proportional to the linear dimension itself. Intrinsic viscosity is therefore a quantity more sensitive to changes in dimensions than sedimentation velocity, but unfortunately hard to measure at very low enzyme concentrations; we have attempted to overcome some of these difficulties in a recent study.[29] It is known that, if association proceeds to yield compact, space-filling spherical structures, then the intrinsic viscosity should be independent of the size of the particles. On the other hand, with increasing molecular asymmetry the intrinsic viscosity should increase; exact solutions of the hydrodynamical problem have been given for ellipsoids of rotation and equations for rods can be fairly well estimated. Exact equations for complicated shapes are not available. Experimentally it is found that the intrinsic viscosity of glutamate dehydrogenase markedly increases with increasing concentration. The increase is dramatically steeper in solutions saturated with respect to toluene and greatly reduced with addition of GTP and NADH; the behavior of the sedimentation coefficients follows a similar course.

For the interpretation of the intrinsic viscosity[29] and the sedimentation coefficients of the oligomers,[15] for which exact solutions of the hydrodynamic equations are not available, we performed hydrodynamic model experiments; macroscopic models of the oligomer (the particle in Figure 4 with the attached rod is such a macroscopic model) were cast of suitable materials and either tumbled over various axes or allowed to settle slowly in huge tanks filled with gooey liquids of suitably

(29) E. Reisler and H. Eisenberg, *Biopolymers*, 9, 877 (1970).

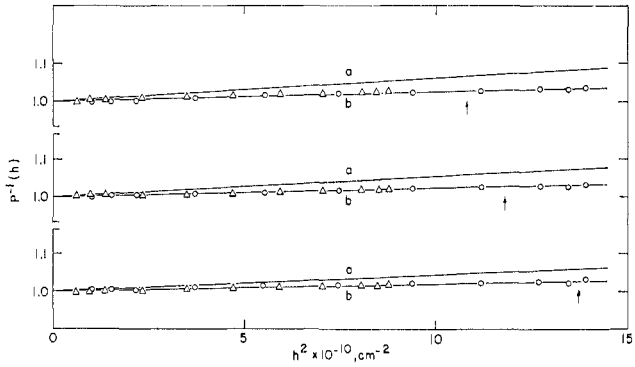


Figure 8. Reciprocal scattering function $P^{-1}(h)$ *vs.* h^2 of solutions of glutamate dehydrogenase in 0.2 *M* phosphate buffer; concentrations in mg/ml (and molecular weights $\times 10^{-5}$ dalton) in ascending order: 0.30 (5.84), 0.39 (6.36), and 0.44 (6.67); $t = 25°$; arrows indicate $h^2\langle R_g^2\rangle_z = 0.08$. See Eisenberg and Reisler.[30]

high viscosity and density (invert sugar or castor oil). Thus the rotational and translational friction coefficients pertaining to intrinsic viscosity and sedimentation could be evaluated, and it was possible to show that the model of the enzyme chosen corresponded closely to the physical quantities evaluated. This represents a useful procedure, not so much to prove a structure, but to eliminate the incompatibility of models proposed, in terms of hydrodynamic performance.

The Angular Dependence of Scattering Dilemma

From the angular dependence of scattering measured at various protein concentrations it is possible to evaluate not only the molecular weight but also the size of the particles by

$$\frac{Hc}{\Delta R(h)} = \frac{1}{M_w{}^0 P_z(h)} + 2A_2c$$

where H is a constant, $\Delta R(h)$ is the reduced scattering intensity (corrected for solvent contribution), $h = (4\pi/\lambda')\sin(\theta/2)$ (λ' is the incident wavelength in the medium and θ is the scattering angle), and $P_z(h)$ is the z average of the particle scattering factor, normalized to $P(0) = 1$. For rodlike particles, in the asymptotic limit of high values of h, a plot of $h\Delta R(h)/\pi Hc$ *vs.* h tends to a constant value equal to the linear mass of the rods, independent of the distribution of rod lengths. The asymptotic behavior of scattering of glutamate dehydrogenase solutions saturated with toluene yields[30] a value of M/L (2250 daltons/Å) in good agreement with the value of 2340 daltons/Å obtained[22] by small-angle X-ray scattering.

From the limiting slope of the extrapolation of the scattering to low values of h it is possible to obtain the z-average of the radius of gyration (R_g) of the particles by the expansion

$$P_z{}^{-1}(h) = 1 + h^2\langle R_g^2\rangle_z/3 + \ldots$$

which is independent of any assumed model for the particles. We can compare this experimental value of $\langle R_g^2\rangle_z$ with calculations based on the experimental value of M/L and the assumed distribution (exact details of the model in terms of ellipsoids are not very important in this calculation). Figure 8 represents[30] experimental limiting slopes of $P_z{}^{-1}(h)$ and two curves calculated (a) for the distribution of lengths corresponding to the kinetic scheme equation (eq 2) and (b) on the assumption that all particles are of equal length. It is seen that the experimental curve is (quite unexpectedly) closer to the latter curve than to the former.

Thus, whereas the molecular weights derived from the intercepts of the scattering curves (or from equilibrium sedimentation) correspond to a broad distribution of molecular weight, the distribution of lengths from the angular dependence of scattering indicates a rather narrow distribution. We are dealing here with a puzzling phenomenon which, although classified as a difficulty, eventually may be related to an optical property which conceivably may disclose additional information with respect to the structure of this enzyme.

The Future

The results of our quest for knowledge concerning glutamate dehydrogenase can now be briefly summarized: we have been able to complement information on function, which has been known for roughly a decade, with information on structure and equilibrium dynamics. With the amino acid sequencing studies of the enzyme in the final stages of completion[17] and the possibility of impending attack by X-ray diffraction, it is reasonable to assume that glutamate dehydrogenase behavior, and variability of behavior from organism to organism, will undergo close scrutiny in the nearest future.

A word of caution is in order: we have discussed above not the totality of the biological machine but just one of its components. If we want our contribution to transcend in significance a simple esthetic intellectual exercise, we must relate the properties of the isolated enzyme to that of the machine itself, the mitochondrion. Is the effect of toluene, for instance, on enzyme association a simple curiosum, or is it in some way related to the fact that glutamate dehydrogenase (in the mitochondrion) may find itself in a related lipid (hydrophobic) environment? Molecular biology, which since its inception has dealt mostly with single macromolecules or macromolecular complexes, is fated to move closer in the future to the study of more complex biological structures. We hope that physicochemical studies will prove as useful in this new departure as they have in the past.

I have already mentioned my indebtedness to Gordon M. Tomkins for introducing me to this problem. I am grateful to Emil Reisler for his stimulating collaboration during the recent years he spent on this subject on his doctoral thesis work. We all enjoyed the year of collaboration with Jean Pouyet in 1966–1967 and value his deep friendship over so many years. Thanks are due to Robert Josephs for his permission to present results of his work in course of publication and to Jamie Godfrey for his very valuable comments.

(30) H. Eisenberg and E. Reisler, *Biopolymers*, in press.