Steady-State Kinetic Study of Rabbit Muscle Phosphofructokinase†

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ABSTRACT: Steady-state kinetic studies of rabbit muscle phosphofructokinase were carried out in 25 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, 50 mM KCl, 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid, 0.1 mM dithiothreitol, 0.33 mM potassium phosphate at 30 °C using a coupled enzyme assay (pyruvate kinase–lactic dehydrogenase) for the product ADP. Steady-state initial velocities were determined under the following conditions: pH 7.0; pH 7.0, 10 μM cAMP; pH 7.0, 10 mM potassium phosphate; and pH 7.43. The substrate MgATP inhibits the reaction at high concentrations, and the initial velocity–fructose 6-phosphate isotherm is sigmoidal at constant MgATP concentration. Under conditions where the enzyme is tetrameric, the data can be fit to a Monod–Wyman–Changeux model in which an active (R) and an inactive (T) conformation are in equilibrium. The binding equilibria are assumed to be adjusted rapidly relative to the rate-determining step. Two binding sites per polypeptide chain are postulated in accordance with available binding data: a catalytic site which binds the two substrates and a regulatory site which binds MgATP in the T state and cAMP in the R state. The results obtained indicate that MgATP binds to the catalytic site of the R and T states with essentially the same affinity. The substrate fructose 6-phosphate shows a marked preference for the catalytic site of the R state at pH 7.0 but displays no preference at pH 7.43. The binding of MgATP at the regulatory site of the T state is much weaker at pH 7.43 than at pH 7.0 but is not markedly changed by the presence of 10 mM potassium phosphate or 10 μM cAMP. The cAMP activates the enzyme by shifting the conformational equilibrium from the T to the R state; phosphate has a similar but smaller effect. The turnover number of the R state is not altered by the presence of 10 μM cAMP and is somewhat decreased by the presence of 10 mM potassium phosphate; it is markedly increased by raising the pH to 7.43. The substrate binding constants vary a factor of 2 to 3 over the range of conditions used with no easily explained pattern. Consideration of available data suggests that the major portion of the regulatory behavior within the tetrameric enzyme can be explained by a concerted conformational change, but additional local conformational changes also must accompany ligand binding.

Rabbit skeletal muscle phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) is of primary importance in the regulation of glycolysis. It is susceptible to activation and inhibition by a number of effectors (Passoneau & Lowry, 1962). The kinetics of the enzyme are complex with respect to variations in substrate and other metabolite concentrations (Lowry & Passoneau, 1966; Hofer & Pette, 1968; Hofer, 1973), to variations in pH (Frieden et al., 1976), and to the aggregation state of the enzyme (Aaronson & Frieden, 1972; Lad et al., 1973). In vivo, phosphofructokinase very likely is regulated by a combination of the above factors.

Despite the vast literature concerned with phosphofructokinase, a coherent kinetic model for the regulation of the enzyme has not yet been presented. The difficulties in obtaining and interpreting steady-state kinetic data have been discussed previously (Hammes & Wu, 1974). However, the equilibrium binding data now available (Kemp & Krebs, 1967; Kemp, 1969; Hill & Hammes, 1975; Wolfman et al., 1978) in conjunction with steady-state kinetic data can be used to develop a working model. In this communication extensive steady-state kinetic data are reported: the substrate concentrations, the phosphate concentration, the cAMP concentration and the pH are varied; the enzyme is maintained in a tetrameric state. The results obtained are interpreted in terms of a simple Monod–Wyman–Changeux model (Monod et al., 1965) in which an active (R) and an inactive (T) conformation exist, and all ligand binding equilibria are adjusted rapidly relative to the rate-determining step. Each polypeptide chain contains a catalytic site which binds MgATP and Fru-6-P[1] and a regulatory site which binds MgATP and cAMP. When binding to the catalytic site, MgATP shows no preference between the R and T states, but it binds only to the regulatory site of the T state thereby inhibiting enzymatic activity. The substrate Fru-6-P binds preferentially to the catalytic site of the R state at pH 7.0 but shows no preference between the R and T states at pH 7.43. The cAMP binds only to the regulatory site of the R state and is, therefore, a strong activator. This model provides a good fit of all of the kinetic data.

Experimental Section

Materials. The ATP, cAMP, Fru-6-P, Fru-1,6-P_2, Tes, dithiothreitol, phosphoenolpyruvate, pyruvate kinase–lactic dehydrogenase enzyme suspension, NADH, triosephosphate isomerase, α-glycerophosphate dehydrogenase, aldolase, serum albumin (bovine), and diphenylamine were from Sigma Chemical Co. All other chemicals were the best available commercial grades, and all solutions were prepared with deionized, distilled water.

Phosphofructokinase. Rabbit skeletal muscle phosphofructokinase was purified by the method of Ling et al. (1966). The final ammonium sulfate precipitate was dissolved in pH 8.0, 0.1 M potassium phosphate, 1 mM EDTA, 0.5 mM dithiothreitol, and dialyzed against the same buffer to give a stock solution of 10–15 mg/mL. The enzyme concentration was determined using an extinction coefficient of 1.02 mL/(mg

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‡ National Institutes of Health Postdoctoral Fellow (GM 05217).

[1] Abbreviations used are: Fru-6-P, fructose 6-phosphate; Fru-1,6-P_2, fructose 1,6-bisphosphate; AMP-PNP, adenylyl imidodiphosphate; Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

0006-2960/78/0417-1818$01.00/0

cm) (Parmeggiani et al., 1966). The specific activity of the enzyme was determined using coupled enzyme reactions as previously described (Ling et al., 1966; Lad et al., 1973). Assays were run under the following conditions: pH 8.0, 33 mM Tris-Cl, 2 mM ATP, 5 mM $MgCl_2$, 2 mM Fru-6-P, 0.1 mM NADH, 2 mM dithiothreitol, 0.20 unit/mL of aldolase, 35 units/mL of triosephosphate isomerase, 3.2 units/mL of α-glycerophosphate dehydrogenase, and 0.03–0.13 μg/mL of phosphofructokinase in a total volume of 3.0 mL. Assays were initiated by the addition of phosphofructokinase, and the reaction velocity was followed by monitoring the change in absorption at 340 nm on a Cary 118 spectrophotometer thermostated at 23 °C. The specific activity of the native enzyme in 0.1 M potassium phosphate, 1 mM EDTA, pH 8.0, was 90–120 units/mg at 23 °C. This apparent specific activity is 20–40 units lower than the actual activity due to the presence of ammonium sulfate in the coupling enzymes (Lad et al., 1973). A unit of enzyme activity is defined as the production of 1 μmol of product per min.

Steady-State Kinetics. The production of ADP by phosphofructokinase was measured utilizing a coupled enzyme system composed of pyruvate kinase and lactic dehydrogenase and monitoring the disappearance of NADH spectrophotometrically. The coupling enzymes were dialyzed overnight against three changes of 100 mL of 50 mM KCl, 25 mM Tes, 5 mM $MgCl_2$, 1 mM EDTA (pH 7.0) and then were filtered through a Whatman GF/C glass fiber filter to remove any precipitate. Assays were carried out in 25 mM Tes, 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.1 mM dithiothreitol, 0.33 mM potassium phosphate, 0.5 mM phosphoenolpyruvate, 7 units/mL of pyruvate kinase, 10 units/mL of lactic dehydrogenase, 0.2 mM NADH. Varied amounts of ATP, Fru-6-P, Fru-1,6-P_2, and cAMP were added from stock solutions. Phosphofructokinase (1.37 μg) was added to initiate the reactions in a total volume of 3 mL. The stock solution of enzyme from which additions were made was at a concentration of 0.14 mg/mL in 0.1 M potassium phosphate, pH 8.0. The velocity of the enzyme reaction was monitored at 340 nm using a Cary 118 or Cary 14 spectrophotometer thermostated at 30 °C. The optimal concentration of coupling enzymes was calculated using the method outlined by Storer & Cornish-Bowden (1974). Concentrations of the nucleotides were measured spectrophotometrically at 260 nm in 25 mM Tes, pH 7.0, using an extinction coefficient of 15 400 M^{-1} cm^{-1} (Dawson et al., 1969). Concentrations of the fructose phosphates were measured using the diphenylamine reaction (Ashwell, 1957).

Results

Steady-state initial velocities were determined in the assay mixture at 30 °C under the following conditions: pH 7.0; pH 7.0, 10 μM cAMP; pH 7.0, 10 mM phosphate; and pH 7.43. These conditions were used to explore the role of phosphate, the activator cAMP, and hydrogen ion on the kinetic behavior. The results obtained are presented in Figure 1 as plots of the initial velocity divided by the concentration of polypeptide chains (mol wt 80 000), $v/(E_0)$, vs. the concentration of MgATP at varying Fru-6-P concentrations. The range of MgATP concentrations used was 6.1 to 65 μM, and the range of Fru-6-P concentrations was 9.2 to 46 μM except for the data at pH 7.0 where the range of Fru-6-P concentration was 15.4 to 77 μM. As is well known, MgATP inhibits the reaction rate at high concentrations, while Fru-6-P and raising the pH activate the enzyme. The steady-state rates were not increased by raising the concentrations of coupling enzymes and substrates, and the rate was linear with time for several minutes. The enzyme activity of the stock solution was measured periodically to check for possible enzyme denaturation; measured rates were corrected for enzyme denaturation when necessary.

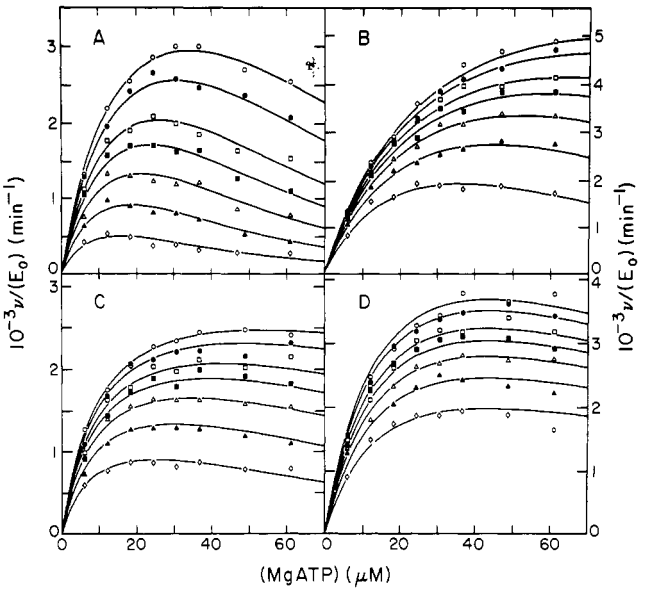

FIGURE 1: Plots of the initial velocity divided by the concentration of polypeptide chains (80 000 mol wt), $v/(E_0)$, for the phosphofructokinase reaction at 30 °C in 3 mL of 25 mM Tes, 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.1 mM dithiothreitol, 0.33 mM potassium phosphate, 500 μM phosphoenolpyruvate, 21 units of pyruvate kinase, 30 units of lactic dehydrogenase, 0.2 mM NADH, 1.37 μg of phosphofructokinase: pH 7.0 (A); pH 7.0, 10 μM cAMP (B); pH 7.0, 10 mM potassium phosphate (C); and pH 7.43 (D). Each curve represents experiments carried out at a fixed Fru-6-P concentration of (reading from the top to the bottom curve) 77.0, 61.6, 46.2, 38.5, 30.8, 23.1, 15.4 μM (A); 46.0, 36.8, 27.6, 23.0, 18.4, 13.8, 9.2 μM (B, C, D). The solid curves were calculated using eq 4 and the parameters in Table I.

Obviously many models can be constructed to fit the kinetic data. We have used a model that contains a relatively small number of adjustable parameters and that is consistent with the known ligand binding stoichiometries (Kemp & Krebs, 1967; Hill & Hammes, 1975; Wolfman et al., 1978). This model is based on the allosteric model of Monod et al. (1965) which postulates two different conformational forms of the enzyme, the R and T states. The R and T states may have different catalytic activities and also may have different binding affinities for substrates and allosteric effectors. Although the original model was postulated for a single ligand, it can be extended readily to multiple ligands if the assumption is made that the binding equilibria for each ligand are independent of the binding equilibria of the other ligands (cf. Pettigrew & Frieden, 1977). Thus for two substrates A and B, the fraction of the enzyme in the R state is

$$f_R = \frac{\alpha\beta(1+\alpha)^{n-1}(1+\beta)^{n-1}}{(1+\alpha)^n(1+\beta)^n + L(1+c\alpha)^n(1+d\beta)^n} \quad (1)$$

where $\alpha = (A)/K_{RA}$, $\beta = (B)/K_{RB}$, $c = K_{RA}/K_{TA}$, $d = K_{RB}/K_{TB}$, K_{RA} is the intrinsic dissociation constant for A binding to the R state, K_{TA} is the intrinsic dissociation constant for A binding to the T state, K_{RB} and K_{TB} are the corresponding constants for B binding to the enzyme, n is the number of ligand binding sites, and L is the ratio of the concentration of T to R states in the absence of ligands A and B. This equation is assumed to be applicable to the binding of MgATP and Fru-6-P at the catalytic site of phosphofructokinase. Binding studies with Fru-6-P and AMP-PNP have suggested the existence of one catalytic site per polypeptide chain (mol wt 80 000) so that n was taken as 4 (Hill &

TABLE I: Steady-State Kinetic Parameters for Phosphofructokinase.[a]

	$10^{-3}\ V_m/(E_0)$[b] (min^{-1})	K_{ATP} (μM)	K_{F6P} (μM)	K_I (μM)	L_0	d
pH 7.0	7.18	17.9	13.7	72.2	12.7	0.15
pH 7.0, 10 μM cAMP	8.88	28.5	5.1	90.6	3.6	0.17
pH 7.0, 10 mM P_i	4.09	9.6	6.1	143	6.1	0.20
pH 7.43	22.3	19.0	12.5	360	1.45	1.0[c]
pH 8.0[d]	18.1	20	21			

[a] 25 mM Tes, 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.1 mM dithiothreitol, 0.33 mM potassium phosphate, 500 μM phosphoenolpyruvate, 7 units/mL pyruvate kinase, 10 units/mL lactic dehydrogenase, 0.2 mM NADH, 0.457 μg/mL phosphofructokinase, 30 °C. [b] Turnover number per polypeptide chain (mol wt 80 000). [c] Assumed value. [d] Hanson et al., 1973; 39.1 mM Tris-Cl, 50 mM KCl, 4 mM $MgCl_2$, 1 mM dithiothreitol, 6 mM $(NH_4)_2SO_4$; a coupled enzyme assay with aldolase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase was used.

Hammes, 1975; Wolfman et al., 1978). The aggregation state of the enzyme under assay conditions is not certain, but the enzyme is tetrameric in the stock solution (Pavelich & Hammes, 1973) and is assumed not to change during the assay.

In treating the kinetic data, only the R state was assumed to be catalytically active so that

$$v = V_m f_R \tag{2}$$

where V_m is the maximum velocity for the enzymatic reaction. The inhibition of the enzymatic reaction by MgATP was assumed to be a result of binding of this ligand to the T state at a regulatory site; MgATP was assumed not to bind to the regulatory site of the R state. This has the effect of altering L such that

$$L = L_0(1 + \gamma)^n \tag{3}$$

where $\gamma = (\text{MgATP})/K_I$, K_I is the dissociation constant for the binding to the regulatory site, and L_0 is now the ratio (T)/(R) in the absence of ligands. Binding measurements have suggested the existence of a binding site for MgATP that is distinct from the catalytic site (Wolfman et al., 1978).

The final equation used to fit the data with A = MgATP and B = Fru-6-P is

$$v = \frac{V_m\alpha\beta(1 + \alpha)^3(1 + \beta)^3}{(1 + \alpha)^4(1 + \beta)^4 + L_0(1 + \gamma)^4(1 + c\alpha)^4(1 + d\beta)^4} \tag{4}$$

In the initial data fitting, it was observed that c was always close to 1; that is, MgATP showed little preference for the R or T state in binding at the catalytic site. Therefore, in the final data fitting c was set equal to 1, thus reducing the number of adjustable parameters to six. The kinetic data were fit to eq 4 using a nonlinear least-squares analysis (Cornish-Bowden & Koshland, 1970; Wharton et al., 1974). The best fit parameters for the four sets of experiments are summarized in Table I. The curves in Figure 1 have been calculated using these parameters and eq 4. In fitting the data at pH 7.43, it was noted that d was approximately 1; therefore, it was set equal to 1.0 to reduce the number of adjustable parameters. This had virtually no effect on the quality of the fit of the data as judged by the sum of squares of the deviations.

The effect of the product Fru-1,6-P_2 on the steady-state initial velocities was also studied. However, Fru-1,6-P_2 is such a strong inhibitor of the reaction that it was not possible to obtain data over a wide range of substrate and Fru-1,6-P_2 concentrations with the coupled enzyme assay used. The general effect of Fru-1,6-P_2 appears to be activation at very low concentrations with product inhibition setting in rapidly during the course of the assay. The extent of linearity depends on both the substrate and Fru-1,6-P_2 concentrations.

Discussion

In studying the steady-state kinetics of phosphofructokinase, a difficult problem is posed in the choice of assay systems. We have chosen an ATP regenerating system because MgATP is both a substrate and inhibitor and it is, therefore, important its concentration not vary during the course of the assay. However, one of the components of the assay system, phosphoenolpyruvate, has been reported to be a weak inhibitor of the enzyme (Colombo et al., 1975). Phosphoenolpyruvate is a competitive inhibitor of citrate binding and presumably would depolymerize the enzyme. The presence of both substrates, however, almost certainly would prevent this depolymerization (Lad et al., 1973). Since the Michaelis constant for the interaction of this substrate with pyruvate kinase is about 70 μM (McQuote & Utter, 1959), we chose a concentration of phosphoenolpyruvate (500 μM) that would saturate this coupling enzyme. In any event, good linearity is observed for the change in absorbance with time, and the concentration of phosphoenolpyruvate does not change appreciably during the course of the reaction. The constant level of phosphoenolpyruvate is very unlikely to change the basic nature of the kinetic mechanism; at worst, it might change the numerical values of the kinetic parameters.

The model used to analyze the data is conceptually simple but contains six adjustable parameters. With such a complex mathematical model, the kinetic parameters should not be interpreted too literally in molecular terms. However, the model not only fits the data well, but the kinetic parameters obtained are reasonable. First it should be noted that this model contains only one catalytic site which binds the two substrates and one regulatory site which binds the adenine nucleotides. In this model, the activating effect of Fru-6-P and Fru-1,6-P_2 is a result of preferential binding to the catalytic site of the R state and is not a consequence of binding to a special regulatory site. As predicted by this model, Fru-1,6-P_2 is an activator at low concentrations and an inhibitor at high concentrations. This aspect of the model also is consistent with the observation that only one binding site per polypeptide chain is found for Fru-6-P and Fru-1,6-P_2. The presence of a regulatory site for adenine nucleotides is in agreement with the finding of one binding site for cAMP per polypeptide chain (Kemp & Krebs, 1967) and two binding sites for MgAMP-PNP, one of which is blocked by cAMP (Wolfman et al., 1978). The kinetic and binding data, however, do not exclude the possibility of separate regulatory sites for cAMP and MgATP.

The results obtained indicate MgATP shows no preference for the R or T state in binding to the catalytic site; this is in accord with the fact that Michaelis–Menten kinetics are observed at low concentrations of MgATP. The substrate Fru-6-P, on the other hand, shows a definite preference for binding to the R state, which gives rise to the well-known sigmoidal

dependence of the initial velocity on the Fru-6-P concentration at constant MgATP. As might be expected, the binding preference at pH 7, as indicated by the parameter d (Table I), is not significantly altered by increased phosphate concentration or the presence of cAMP. At pH 7.43, however, preferential binding of Fru-6-P does not occur. As might be predicted from a simple interpretation of the model, L_0, the ratio of inactive to active states in the absence of substrates, is decreased by raising the phosphate concentration, by the presence of cAMP, and by raising the pH. (Although the parameters in eq 4 are correlated, we have found through computer modeling that changes in L_0 of a factor of two or more are significant.) The dissociation constant characterizing MgATP binding at the regulatory site of the T state is slightly raised by the presence of cAMP and phosphate and is greatly raised by increasing the pH. The fact that cAMP primarily causes a reduction of L_0 with little effect on the inhibition constant for MgATP further supports the idea that MgATP binds primarily to the T state and cAMP to the R state. If this were not the case, both L_0 and K_I would be altered significantly by cAMP. An approximate dissociation constant for cAMP binding to the regulatory site of 27 μM can be calculated if it is assumed cAMP binds only to the R state.[2] Some variations in the dissociation constants characterizing Fru-6-P and MgATP binding at the catalytic sites occur, but these do not seem to be major effects. The turnover number is not significantly altered by cAMP at pH 7.0; the decrease in the turnover number caused by 10 mM phosphate, which appears to be significant, is surprising, and we have no ready explanation for this. As expected, the maximum velocity is appreciably larger at pH 7.43. The steady-state parameters obtained at pH 8.0 (Hanson et al., 1973), which are included in Table I, are quite similar to those obtained at pH 7.43. The molecular basis of the interaction of phosphate ion with phosphofructokinase is not clear. It has a strong stabilizing effect on the enzyme, and obviously has some effect on the dissociation constants for Fru-6-P and MgATP binding (Table I); it also appears to convert the enzyme to the R state, as judged by the decreased value of L_0 in 10 mM potassium phosphate at pH 7.0.

While the proposed kinetic model explains much of the regulatory behavior of phosphofructokinase, some problems remain. The direct binding measurements with Fru-6-P (Hill & Hammes, 1975), MgAMP-PNP (Wolfman et al., 1978), and cAMP (Kemp & Krebs, 1967) all suggest the directly measured dissociation constants are less than obtained from the kinetic analysis. However, this may be attributable to the fact that the kinetic data are obtained at a higher temperature, 30 °C, than the binding data, 4 °C, and to the presence of 500 μM phosphoenolpyruvate in the assay mixture. The binding data also suggest more complex cooperativity with regard to the homotropic interactions of Fru-6-P and MgAMP-PNP. These differences between the kinetic and binding models also may be a result of temperature or of the heterotropic interactions of Fru-6-P and MgATP. Although the concerted conformational change of the proposed model can account for the regulatory processes within the tetrameric enzyme, very likely other local conformational changes also are associated with ligand binding since this model cannot account for negative cooperativity. We are in the process of investigating this matter further with fast reaction kinetic studies.

[2] The dissociation constant for cAMP binding to the regulatory site of the R state is calculated from the relationship $L_0 = L_0'/[1 + (\text{cAMP})/K_{\text{cAMP}}]^4$, where L_0 is determined in the presence of cAMP and L_0' in its absence; K_{cAMP} is the dissociation constant for cAMP binding to the regulatory site of the R state.

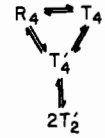

FIGURE 2: A schematic model for the regulation of rabbit muscle phosphofructokinase. R_4 is an active tetrameric species, while T_4, T_4', and T_2' are catalytically inactive. The species T_4' and T_2' are present in significant amounts only in the presence of citrate.

The model we have discussed thus far only considers regulation within the tetrameric enzyme. Inhibition of phosphofructokinase by citrate appears to involve dissociation of the tetramer to an inactive dimer (Lad et al., 1973), although citrate also can inhibit the cross-linked tetramer to some extent (Lad & Hammes, 1974). The effect of citrate can be incorporated into the model we have presented by postulating the presence of a third conformation, T_4', which normally is present in small amounts, binds citrate strongly, and readily dissociates into dimers. This introduces a separate regulatory site for the binding of citrate. This more complete model is schematically depicted in Figure 2. The in vivo modulation of enzyme activity through protein aggregation also may be linked to changes in protein concentration and to changes in pH. A detailed model for the pH dependence of the catalytic activity of the enzyme has been presented (Frieden et al., 1976). This model postulates two rapidly equilibrating forms of the enzyme, protonated and unprotonated, analogous to the R_4 and T_4 forms of the model presented here. The protonated form is assumed to isomerize to an inactive form which subsequently dissociates to a dimer, analogous to the citrate induced isomerization to T_4' and dissociation to T_2'. Although the two models are formally similar, they are based on different types of data, and their direct correspondance remains to be demonstrated.

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Acyl-Coenzyme A Carboxylase of the Free-Living Nematode *Turbatrix aceti*. 1. Its Isolation and Molecular Characteristics†

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ABSTRACT: A biotin containing enzyme which carboxylates acetyl-CoA has been isolated from the nematode *Turbatrix aceti* and purified to homogeneity as judged by the criteria of polyacrylamide gel electrophoresis and ultracentrifugation. The enzyme has a sedimentation coefficient of 18.0 S and a molecular weight of 667 000. It is composed of four protomers having a molecular weight of 140 000 each. Each protomer, in turn, consists of two distinct polypeptide chains (molecular weights 82 000 and 58 000) and one biotinyl prosthetic group which is linked to the 82 000 peptide. The amino acid composition of the nematode carboxylase has also been determined.

The enzymatic details of the conversion of acetyl-CoA to long-chain fatty acids have been studied in a wide range of organisms, including bacteria, yeasts, algae, plants, and higher animals (Vagelos, 1971). The essence of these studies is that all the fatty acid synthesizing systems are similar in the sequence of reactions they catalyze but different in their physical properties and the types of regulatory mechanisms to which they are adapted. A gap in our knowledge exists with regard to the lower invertebrates. Even though they surpass all other animal forms in numbers as well as in anatomical and functional diversity, a full effort has not been made until now to work out the details of their fatty acid synthesizing systems.

The helminths are a group of invertebrates which merit special attention, since previous studies have revealed that they vary greatly in their capacities to synthesize fatty acids de novo. For instance, among the flatworms so far investigated, both the free-living as well as the parasitic forms fail to synthesize fatty acids (Meyer et al., 1966, 1970; Meyer & Meyer, 1972; Ginger & Fairbairn, 1966). Among the nematodes, the free-living organisms such as *Turbatrix aceti* and *Caenorhabditis briggsae* can synthesize fatty acids (Rothstein & Götz, 1968), whereas the parasitic organism *Ascaris lumbricoides* can synthesize these compounds only at a very reduced level, if at all (Beames et al., 1967). Several possibilities have been advanced to account for this metabolic deficiency. They include a repression of enzyme systems at particular stages of the parasite's life cycle (Fairbairn, 1970), an adaptation to a lack of oxygen in the environment, and a general adaptation to a parasitic or symbiotic mode of life (Meyer et al., 1966; Meyer & Meyer, 1972). Clearly, a critical evaluation of these hypotheses requires knowledge of the properties of the enzyme systems associated with fatty acid biosynthesis and the physiological factors which control their activities.

Based on these considerations, we have undertaken the isolation and characterization of acetyl-CoA carboxylase from the free-living nematode *Turbatrix aceti*. We have focused initially on this enzyme, since it catalyzes the first committing step in the reaction sequence leading to long chain fatty acids, and since it plays a critical role as a regulatory enzyme in the control of this process in animals.

In this paper, we describe the purification to homogeneity of this enzyme, its subunit structure, and its amino acid composition.

Experimental Procedure

Materials. Soy peptone was obtained from Humko Sheffield Co.; acyl-CoA esters were from P-L Biochemicals; radioactively labeled chemicals were from New England Nuclear; 4-hydroxyazobenzene-2′-carboxylic acid was from Sigma; β-galactosidase (*E. coli*), alcohol dehydrogenase (horse and yeast), catalase (beef), urease (jack bean), and aldolase (rabbit) were from Worthington Biochemical Corp.; Cellex E and gel electrophoresis reagents were from Bio-Rad; and Sepharose 4B was from Pharmacia Fine Chemicals.

Cellex E (anion-exchange cellulose) was washed for 30 min with 0.25 M NaOH containing 0.25 M NaCl, for another 30 min with 0.25 M HCl, and was then suspended in 10 mM EDTA for several days. Finally, it was washed with 0.3 M phosphate buffer and stored in water at 3 °C.

Cultivation of T. aceti. Turbatrix aceti was obtained from Dr. Morton Rothstein of the State University of New York at

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0006-2960/78/0417-1822$01.00/0