Eigen, M., Hammes, G. G., and Kustin, K. (1960), *J. Amer. Chem. Soc. 82*, 3482.
Fridborg, K., Kannan, K. K., Liljas, A., Lundin, J., Strandberg, B., Strandberg, R., and Wirén, G. (1967), *J. Mol. Biol. 25*, 505.
Gibbons, B. H., and Edsall, J. T. (1963), *Science 140*, 381.
Gibbons, B. H., and Edsall, J. T. (1964), *J. Biol. Chem. 239*, 2539.
Kartha, G., Bello, J., and Harker, D. (1967), *Nature (London) 213*, 862.
Kernohan, J. C. (1964), *Biochim. Biophys. Acta 81*, 346.
King, R. W., and Burgen, A. S. V. (1970), *Biochim. Biophys. Acta 207*, 278.
Kirby, A. H. M., and Neuberger, A. (1938), *Biochem. J. 32*, 1146.
Koltun, W. L., Clark, R. E., Dexter, R. N., Katsoyannis, P. G., and Gurd, S. R. N. (1959), *J. Amer. Chem. Soc. 81*, 295.
Laurent, G., Charrel, M., Castay, M., Nahon, D., Marriq, C., and Derrien, Y. (1962), *C. R. Soc. Biol. 156*, 1461.
Linderström-Lang, K. (1924), *C. R. Trav. Lab. Carlsberg 15*, No. 7.
Maren, T. H. (1967), *Physiol. Rev. 47*, 595.
Meadows, D. (1968), Ph.D. Thesis, Harvard University.
Meadows, D. H., Jardetzky, O., Epand, R. M., Ruterjans, H. H., and Scheraga, H. A. (1968), *Proc. Nat. Acad. Sci. U. S. 60*, 766.
Nozaki, Y., Gurd, F. R. N., Chen, R. F., and Edsall, J. T. (1957), *J. Amer. Chem. Soc. 79*, 2123.
Nyman, P. O. (1961), *Biochim. Biophys. Acta 52*, 1.
Nyman, P. O., and Lindskog, S. (1964), *Biochim. Biophys. Acta 85*, 141.
Pocker, Y., and Dickerson, D. G. (1968), *Biochemistry 7*, 1995.
Pocker, Y., and Meany, J. E. (1965a), *J. Amer. Chem. Soc. 87*, 1809.
Pocker, Y., and Meany, J. E. (1965b), *Biochemistry 4*, 2534.
Rickli, E. E., Ghazanfar, S. A. S., Gibbons, B. H., and Edsall, J. T. (1964), *J. Biol. Chem. 239*, 1065.
Riddiford, L. M. (1964), *J. Biol. Chem. 239*, 1079.
Riddiford, L. M., Stellwagen, R. H., Mehta, S., and Edsall, J. T. (1965), *J. Biol. Chem. 240*, 3305.
Roberts, G. C. K., and Jardetzky, O. (1970), *Advan. Protein Chem. 24*, 447.
Roberts, G. C. K., Meadows, D. H., and Jardetzky, O. (1968), *Biochemistry 7*, 2053.
Schneider, F. (1963), *Z. Physiol. Chem. 334*, 26.
Tashian, R. E., Douglas, D. P., and Yu, Y.-S. L. (1964), *Biochem. Biophys. Res. Commun. 14*, 256.
Taylor, P. W., King, R. W., and Burgen, A. S. V. (1970a), *Biochemistry 9*, 2638.
Taylor, P. W., King, R. W., and Burgen, A. S. V. (1970b), *Biochemistry 9*, 3894.
Verpoorte, J. A., Mehta, S., and Edsall, J. T. (1967), *J. Biol. Chem. 242*, 4221.
Wang, J. H. (1968), $CO_2$: Chemical, Biochemical, and Physiological Aspects, NASA SP-188, p 101.
Whitney, P. L., Fölsch, G., Nyman, P. O., and Malmström, B. G. (1967), *J. Biol. Chem. 242*, 4206.
Wilkinson, G. N. (1961), *Biochem. J. 80*, 324.
Wyckoff, H. W., Hardman, K. D., Allewell, N. M., Inagami, T., Johnson, L. N., and Richards, F. M. (1967), *J. Biol. Chem. 242*, 3984.
Wyckoff, H. W., Tsernoglou, D., Hanson, A. W., Knox, J. R., Lee, B., and Richards, F. M. (1970), *J. Biol. Chem. 242*, 305

# Reversible, pH-Dependent Formation of a Conformer of Rabbit Liver Fructose 1,6-Diphosphatase with Low Catalytic Activity*

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ABSTRACT: The catalytic activity of rabbit liver fructose 1,6-diphosphatase at neutral pH was found to markedly decrease with increasing hydrogen ion concentration. More than 90% of the activity was lost at pH values below 6.7. The presence of certain carboxylic acids such as EDTA, oxaloacetate, and malonate both prevented and reversed the inactivation. Also, recovery of full neutral activity was found upon incubation at pH 7.3. In both instances, the presence of low concentrations of substrate prevented the restoration of activity.

No evidence could be found for any change in molecular size in the two forms of fructose 1,6-diphosphatase. It was therefore concluded that the low-activity form represented a new conformer of the enzyme. A significant consequence of this conformational change was reflected in the increased sensitivity of the enzyme toward its allosteric inhibitor, AMP, and the loss of cooperative interactions among the inhibitor sites.

Our early experiments on rat and rabbit liver fructose 1,6-diphosphatases (D-fructose 1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) indicated the existence of a different form of the enzyme with low catalytic activity at neutral pH (Taketa and Pogell, 1963, 1965). Using a continuous spectrophotometric assay for the enzyme at pH 7.3, reproducible maximal activities were not observed unless the enzyme was

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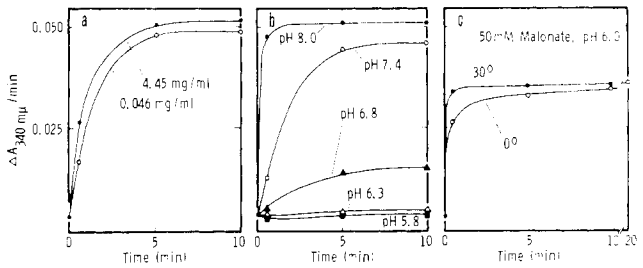


FIGURE 1: Effects of protein concentration, pH, and malonate on reactivation of fructose 1,6-diphosphatase. The final assay volume in these experiments was 1 ml and contained 3.9 units of glucose 6-phosphate isomerase and 0.7 unit of glucose 6-phosphate dehydrogenase. (a) For reactivation at the lower protein concentration (0.046 mg/ml), partially purified fructose 1,6-diphosphatase was incubated at 30° in cuvets for different intervals of time in the presence of 52 mM Tris-HCl, pH 7.3, 10 mM $MgSO_4$, and 21 mM β-mercaptoethanol. Initial reaction rates were determined after adding the remaining components of the complete assay system. At the higher protein concentration (4.5 mg/ml), the enzyme was incubated at 30° in a mixture of 50 mM Tris-HCl, pH 7.3, 10 mM $MgSO_4$, and 20 mM β-mercaptoethanol and aliquots (10 μl) were transferred at various times to the standard assay system (pH 7.3) to measure initial reaction rates. Final protein concentrations in the assay mixture were the same in both experiments. (b) Reaction mixtures containing buffer (50 mM Tris-HCl at pH 8.0 and 50 mM Tris-maleate at the lower pH values), 10 mM $MgSO_4$, 20 mM β-mercaptoethanol, and partially purified enzyme (2.2 mg/ml) were incubated at 30°. Initial reaction rates were determined at timed intervals by transferring aliquots (20 μl) of the incubation mixture to the standard assay system (pH 7.3). (c) Incubation mixtures at 0° and 30° contained 50 mM sodium malonate, pH 6.0, and partially purified enzyme (2.2 mg/ml). Aliquots (20 μl) were assayed at timed intervals in the standard assay system (pH 7.3).

first incubated at neutral pH in the absence of substrate. The extent of inactivation appeared to vary with the nature of the enzyme preparation. Thus, crude undialyzed liver extract and purified enzyme obtained by substrate elution in sodium malonate gave near maximal activities when added directly to the complete assay system, whereas dialyzed liver supernatant and samples purified by ammonium sulfate fractionation had very low activities unless first incubated in the absence of substrate at pH 7.3. Luppis *et al.* (1964) have reported similar kinetic observations. It became evident from our subsequent experiments that this decrease in catalytic activity was pH dependent and occurred in the range below pH 7.3. The enzyme was protected against this inactivation by certain carboxylic acids; also, once the inactivation was produced, it could be reversed either by raising the pH or by incubation in the absence of substrate with the same carboxylic acids.

Detailed kinetic studies were initiated on both crude and homogeneous rabbit liver fructose 1,6-diphosphatase in order to elucidate the mechanisms involved in the interconversion of active and inactive forms of the enzyme and are the subject of the present report. These results show that the fully active and low-activity forms of rabbit liver fructose 1,6-diphosphatase can be reversibly interconverted with no measurable change in molecular weight. Therefore, they may be considered to be conformers (Kitto *et al.*, 1966), differing only in secondary and tertiary structure.

We have previously described in detail the requirement of an activator (EDTA, β-mercaptoethanol, liver pH 6 precipitate) in order to obtain full activity of fructose 1,6-diphosphatase at neutral pH (Pogell *et al.*, 1968). In the present study, the fully reactivated enzyme still required the presence of one of these compounds in the final assay mixture; otherwise, only very low enzyme rates were observed. β-Mercaptoethanol, which had no effect on the acid inactivation, was regularly included in the assay medium. Although this observation clearly distinguishes these two phenomena, it is obvious that they are closely related processes.

## Experimental Section

*Materials.* Chemicals for the enzyme assay were from the sources previously described (Sarngadharan *et al.*, 1970). All other chemicals used were of the highest purity available commercially. All reagents used for enzyme analyses were dissolved in glass-distilled water.

Two different preparations of rabbit liver fructose 1,6-diphosphatase were used in the present studies. A partially purified enzyme was prepared by high-speed centrifugation of a rabbit liver extract to remove particulate material, acid precipitation at pH 4.5 to remove further insoluble protein, and salt fractionation (150 g of solid ammonium sulfate per l. of solution) followed by dialysis and lyophilization of the redissolved precipitate (Pogell, 1964). By this procedure, the specific activity of the enzyme was increased 10- to 15-fold. The above preparation served as an initial source of low-activity fructose 1,6-diphosphatase.

Homogeneous fructose 1,6-diphosphatase was obtained from extracts of fresh rabbit liver by substrate elution from CM-cellulose at pH 6.8 (Sarngadharan *et al.*, 1970). The enzyme wih specific activity of 22 units/mg of protein (22°, pH 9.3) was freed of bound substrate by treating with 20 mM $MgSO_4$ for 20 min at room temperature followed by 1 hr at 3°, and subsequently dialyzing overnight against 5 mM sodium malonate buffer, pH 6.0.

*Assay Methods.* Fructose 1,6-diphosphatase activity at neutral pH was determined spectrophotometrically by following the rate of TPNH formation at 340 mμ in a Gilford multisample absorbance recording system (Taketa and Pogell, 1965). The usual assay system contained: 0.1 mM fructose 1,6-diphosphate; 50 mM Tris-HCl, pH 7.5; 0.15 mM $TPN^+$; 20 mM β-mercaptoethanol; 10 mM $MgSO_4$; excess glucose 6-phosphate isomerase (3.9 units) and glucose 6-phosphate dehydrogenase (0.7 unit); and fructose 1,6-diphosphatase in a final volume of 0.5 ml. For determining initial rates of enzyme activity, the reactions were initiated by adding the enzyme last to the complete assay system. In most other cases, reactions were initiated by addition of substrate.

All pH measurements were made at room temperature. The reported pH values have been corrected to the temperatures of the specific experiments.

## Results

*Reactivation of Enzyme Preparations with Low Activity.* Our first experiments on the nature of the low-activity forms of fructose 1,6-diphosphatase were carried out with a partially purified sample prepared by acid precipitation, ammonium sulfate fractionation, and dialysis (see Experimental Section). This preparation was found to have very low activity without prior incubation at neutral pH in the absence of substrate. The time course of reactivation at pH 7.3 at two different protein concentrations is shown in Figure 1a. In this experiment, the inactive enzyme was incubated with $Mg^{2+}$ and β-mercaptoethanol in the absence of substrate before determination of neutral activity. Maximal activation was observed after 10 min at 30° and was independent of

TABLE I: Reactivation of Inactive Fructose 1,6-Diphosphatase and Prevention of Inactivation of Active Enzyme by Polycarboxylic Acids.[a]

| Compounds (50 mM or as Indicated) | Relative Activity (%) A. Reactivation | | B. Prevention of Inactivation |
|---|---|---|---|
| Tris-maleate + EDTA (10 mM) | 127 | | 111 |
| Oxalate | | | 108 |
| Malonate | 100 | 100[b] | 100 |
| Citrate | 94 | | 100 |
| EDTA (10 mM) | 113 | | 99 |
| Maleate + oxaloacetate (10 mM) | | 84[b] | 96 |
| Malate | 80 | | 97 |
| Isocitrate | 85 | | 91 |
| Orthophosphate | | 55[b] | 75 |
| Methylmalonate | 100 | | 54 |
| Lactate | | 31, 48[b] | 48 |
| Succinate | | 15[b] | 39 |
| Pyruvate | | 83[b] | 34 |
| α-Ketoglutarate | | 73[b] | 29 |
| Tris-maleate | 8 | | 28 |
| *cis*-Aconitate | | 7[b] | 25 |
| NaCl | | | 13 |
| Maleate | 3 | 5, 7[b] | 12 |
| Fumarate | | 6[b] | 6 |
| Acetate | | 8[b] | 5 |
| Glutamate | | | 4 |

[a] Incubation mixture contained partially purified enzyme (inactive, 2.2 mg/ml) (column A) or homogeneous enzyme (active, 16.1 μg/ml) (column B) and the listed compounds at 50 mM concentration unless otherwise specified. After 10 min at 30°, initial rates of enzyme activity were determined by adding aliquots of the incubation mixture last to the complete assay system, pH 7.3. Activities are expressed relative to the activity obtained with the malonate system. The assay conditions for column A were identical with those in Figure 1. [b] $MgSO_4$ (10 mM) and β-mercaptoethanol (20 mM) were also present during reactivation.

protein concentration. Figure 1b shows the effect of varying pH on the reactivation process. A maximum increase in activity occurred within 1 min at pH 8.0; with decreasing pH, both the rate and extent of activation decreased. Very little change was observed below pH 6.3.

Since the enzyme dialyzed against sodium malonate at pH 6.0 or eluted from CM-cellulose with substrate in malonate was found to be fully active, the effect of malonate on reactivation at pH 6.0 was tested. As is shown in Figure 1c, a rapid recovery of full activity occurred which was somewhat faster at 30° than at 0°. The specificity of reactivation by malonate and related compounds at pH 6.0 is summarized in Table I, column A. Malonate, citrate, methylmalonate, isocitrate, malate, and EDTA were all effective in reactivating the enzyme. Succinate, acetate, fumarate, *cis*-aconitate, and maleate had little effect at a concentration of 50 mM. Other

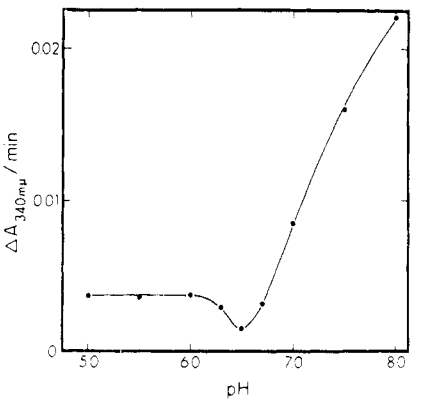


FIGURE 2: Effect of pH on inactivation of fructose 1,6-diphosphatase. Homogeneous enzyme (16.1 μg/ml) was incubated for 10 min at 24° with 50 mM sodium maleate at the pH values indicated. Initial reaction rates were determined after adding 15 μl of incubated enzyme last to the standard assay system at 30° and pH 7.3. These additions did not alter the pH of the final assay system.

compounds such as lactate and phosphate produced a partial restoration of activity. There was no obvious structural relationship among either potent activators or those with no effect. However, all effective activators did contain at least two carboxyl groups and the results clearly showed that this phenomenon was not a simple consequence of increased ionic strength.

*Inactivation of Pure Enzyme.* Purified fructose 1,6-diphosphatase obtained by elution from CM-cellulose with substrate in sodium malonate at pH 6.0 was fully active without further treatment, confirming results previously reported by Luppis *et al.* (1964). Presumably the presence of malonate was responsible for conversion of the enzyme into the active form. More detailed studies of the nature of the inactivation process were carried out with pure rabbit liver fructose 1,6-diphosphatase prepared by elution with substrate in malonate buffer (Sarngadharan *et al.*, 1970). The effect of pH on the extent of inactivation is shown in Figure 2. Enzyme was incubated in sodium maleate buffer of varying pH; the initial rates of remaining activity were determined by adding aliquots of the incubated enzyme last to the complete assay system. The extent of inactivation increased with decreasing pH and maximum inactivation was observed at pH 6.5.

It thus appeared that the varying levels of enzyme activity found in different preparations could easily be accounted for by variations in pH of the different fractions. For example, dialyses were usually carried out against water of pH 6 to 6.5 and should have resulted in preparations of very low activity, as was observed.

Compounds which reactivated enzyme of low activity were equally effective in protecting the fully active enzyme against loss of activity at pH 6. The results shown in Table I, column B were obtained by incubation of the active enzyme with these compounds at pH 6.0 without other additions and were quantitatively very similar to those shown in column A, which represent the reactivation of inactive enzyme. The effects of varying concentrations of EDTA and oxaloacetate in protecting against inactivation are shown in Figure 3. EDTA was the most effective compound in preventing loss of enzyme activity; the approximate concentrations necessary for complete protection from inactivation by different carboxylic acids were as follows: EDTA, 0.1 mM; oxaloacetate, 2 mM; citrate, 5 mM; malonate, 10 mM. Fructose

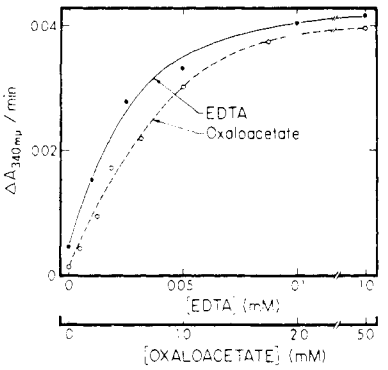


FIGURE 3: Effect of EDTA and oxaloacetate on the inactivation of fructose 1,6-diphosphatase. Homogeneous enzyme (16.1 μg/ml) was incubated for 10 min at 30° in 50 mM sodium maleate buffer at pH 6.0 with varying concentrations of EDTA or at pH 6.5 with the indicated concentrations of oxaloacetate. Aliquots (15 μl) were transferred to the complete assay system (pH 7.3) and the initial reaction rates determined.

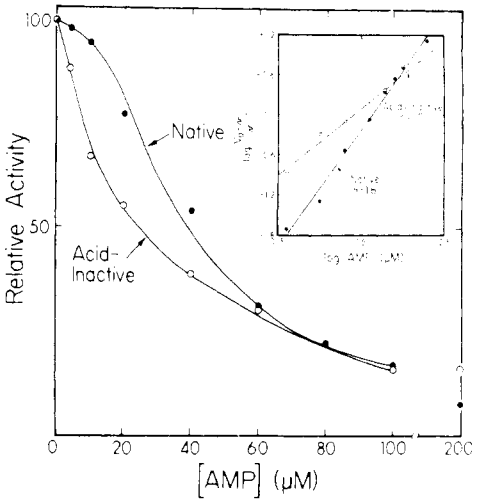


FIGURE 5: AMP inhibition of fully active and low-activity forms of fructose 1,6-diphosphatase. Inactive enzyme (11% of original activity) was prepared by incubating homogeneous enzyme in 50 mM sodium maleate, pH 6.5, for 10 min at 30°. Aliquots of enzyme were added last to the complete assay system, pH 7.3, containing varying concentrations of AMP for the determination of initial reaction rates. Final concentrations of fructose 1,6-diphosphatase were 0.5 μg/ml for active enzyme and 2.6 μg/ml for the inactivated form. Inset: Hill plot of the above data. $V_0$ and $v$ are the uninhibited and inhibited rates, respectively. The lines were drawn using the slopes and AMP concentrations for 50% inhibition obtained by a least-squares fit computational procedure for a straight line. The program for a Hewlett-Packard 9100A calculator was written by Daniel W. Morris.

1,6-diphosphate was not very effective in preventing inactivation at pH 6.5. However, very low levels of substrate prevented reactivation by EDTA at this pH (Figure 4). Only 36% recovery of activity occurred in the presence of 1 μM fructose 1,6-diphosphate. A similar decreased rate of reactivation in the presence of substrate was observed at pH 7.3 in the complete assay system. Thus, binding of substrate to the enzyme (Sarngadharan *et al.*, 1969) prevents the necessary conformational change by EDTA for conversion into the fully active form.

*Molecular Weight of the Low-Activity Form of Fructose 1,6-diphosphatase.* Comparison of the molecular size of the inactive enzyme with active fructose 1,6-diphosphatase (molecular weight of 130,000) (Pontremoli *et al.*, 1965) by both sucrose density gradient centrifugation and Sephadex G-200 gel filtration revealed no measurable difference in molecular weight. The low-activity form of the enzyme gave single symmetrical peaks under the conditions of these experiments. It thus appeared that the differences in catalytic activity of the preparations were caused by changes in conformation of the enzyme rather than by a dissociation or association of polypeptide subunits.

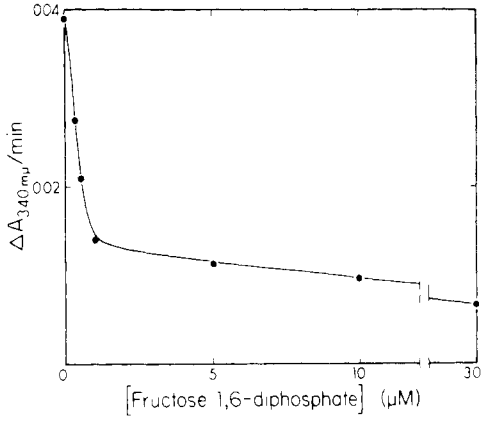


FIGURE 4: Effect of varying concentrations of fructose 1,6-diphosphate on the activation by EDTA at pH 6.5 of inactivated fructose 1,6-diphosphatase. Homogeneous enzyme (22 μg of protein/ml) was incubated for 20 min at 30° in 50 mM sodium maleate, pH 6.5. The resultant enzyme was found to be 92% inactive. Aliquots of the inactive enzyme were diluted with equal volumes of 50 mM sodium maleate, pH 6.5, containing 0.1 mM EDTA and fructose 1,6-diphosphate of the indicated concentrations and incubated for an additional 10 min at 30°. Samples (20 μl) from these tubes were transferred to the complete assay system, pH 7.3, for determination of the initial rates of enzyme activity.

*Difference in Inhibition by AMP of the Two Forms of the Enzyme.* Perhaps the most significant change found upon acid inactivation of fructose 1,6-diphosphatase was the loss of cooperative inhibition by AMP, the allosteric modulator of enzyme activity (Taketa and Pogell, 1965), together with the increased sensitivity of the enzyme to AMP. Figure 5 shows the AMP inhibition curves of the two forms of the enzyme which were obtained when enzyme was added last to the complete reaction mixture. First, it may be noted that while the active enzyme gave the normal sigmoid response curve, the acid-inactivated enzyme showed no indication of interactions among the four AMP sites. The apparent $n$ values from Hill plots (Figure 5, inset) (Taketa and Pogell, 1965) were 1.8 for the active enzyme and 1.0 for the acid-inactivated form. Furthermore, the concentration of AMP needed for half-maximal inhibition of the inactivated form of the enzyme was reduced from 42 to 25 μM. This change in behavior of the enzyme with AMP was reversible and restored to normal sigmoidal behavior upon reactivation.

## Discussion

Our present observations are somewhat similar to those reported for the activation of inactive glutamate dehydrogenase from *Neurospora crassa*. West *et al.* (1967) found a pH-dependent interconversion of active and inactive forms, almost all activity being lost at pH 7 and full activity recovered above pH 8. Furthermore, carboxylic acids such as α-keto-

glutarate, citrate, malate, and EDTA were potent activators at relatively high concentrations (2–10 mM levels). However, one point of difference was that the reactivation of fructose 1,6-diphosphatase had a first-order dependence on activator concentrations, whereas glutamate dehydrogenase showed a sigmoidal response to varying concentrations of α-ketoglutarate and succinate.

The function of EDTA and other carboxylic acids appears the same both in preserving the catalytic activity of the active enzyme and in restoring the activity of inactive preparations at pH 6–6.5. Since we have failed to detect any difference in the molecular size of the fully active and low-activity forms of fructose 1,6-diphosphatase, this inactivation process must involve a conformational alteration in enzyme structure. The simultaneous loss of coopcrative AMP inhibition also favors this view. The carboxylic acids, therefore, are able to convert the inactive conformer into the active form and maintain the enzyme in the active conformation at lower pH values. It is clearly evident that this activation is not a consequence of increased ionic strength. However, no clear structural distinction was obvious between the group of potent activators and those that had no effect. Although all good activators contained at least two carboxyl groups, several dicarboxylic acids had little or no capacity to restore or preserve the enzyme activity. Since fructose 1,6-diphosphate is a potent antagonist of the reactivation process (see Figure 4), a reasonable mechanism for the activation would be to assume that the polycarboxylic acids interact with the enzyme near the substrate site and convert the molecule back to the active conformer. In the presence of substrate, however, reactivation would be prevented because of the low affinity of the carboxylic acids and a possible overlap of the two sites. Or, once fructose 1,6-diphosphate binds to the enzyme, it produces a marked change in conformation (Aoe *et al.*, 1970), thus preventing further proper conformational changes to restore the active form by means of carboxylic acids or by increasing pH.

*Possible Basis of Varying Response to AMP Inhibition in Conformers.* The increased sensitivity to AMP inhibition found with the low-activity form of fructose 1,6-diphosphatase together with the absence of evidence for cooperative interaction among the four AMP binding sites suggests that the emzyme may be in a more unfolded ("relaxed") state under these conditions. In the fully active form, the sigmoidal type response to AMP inhibition would be produced by a folding of the enzyme ("tight form") so that the AMP binding sites were not fully accessible. Each successive binding of an AMP molecule would then partially alter the conformation, making the other binding sites more available. Thus, one would expect the tightest binding of AMP to occur where there was a minimal interaction among the subunits of the enzyme ("relaxed form").

Similar observations of a lowered $K_m$ plus lack of a sigmoidal response curve for substrate have been reported for two other desensitized allosteric proteins, aspartyl transcarbamylase (Gerhart and Pardee, 1963; Weitzman and Wilson, 1966) and threonine deaminase (Freundlich and Umbarger, 1963). This behavior is also, of course, directly analogous to that found with myoglobin and hemoglobin in terms of $O_2$ binding.

Variation in subunit interactions would also explain our observations of a decreased sensitivity of the enzyme to AMP inhibition after purification (Sarngadharan *et al.*, 1969) and that, even after full reactivation of the low-activity form, the sensitivity to AMP is always *decreased* after incubation of fructose 1,6-diphosphatase in the absence of substrate in the standard assay system at neutral pH.

Acknowledgment

We wish to thank Hajime Aoe for excellent technical assistance.

References

Aoe, H., Sarngadharan, M. G., and Pogell, B. M. (1970), *J. Biol. Chem. 245*, 6383.

Freundlich, M., and Umbarger, H. E. (1963), *Cold Spring Harbor Symp. Quant. Biol. 28*, 505.

Gerhart, J. C., and Pardee, A. B. (1963), *Cold Spring Harbor Symp. Quant. Biol. 28*, 491.

Kitto, G. B., Wassarman, P. M., and Kaplan, N. O. (1966), *Proc. Nat. Acad. Sci. U. S. 56*, 578.

Luppis, B., Traniello, S., Wood, W. A., and Pontremoli, S. (1964), *Biochem. Biophys. Res. Commun. 15*, 458.

Pogell, B. M. (1964), *in* Fructose 1,6-Diphosphatase and Its Role in Gluconeogenesis, McGilvery, R. W., and Pogell, B. M., Ed., Washington, D. C., American Institute of Biological Sciences, p 20.

Pogell, B. M., Tanaka, A., and Siddons, R. C. (1968), *J. Biol. Chem. 243*, 1356.

Pontremoli, S., Traniello, S., Luppis, B., and Wood, W. A. (1965), *J. Biol. Chem. 240*, 3459.

Sarngadharan, M. G., Watanabe, A., and Pogell, B. M. (1969), *Biochemistry 8*, 1411.

Sarngadharan, M. G., Watanabe, A., and Pogell, B. M. (1970), *J. Biol. Chem. 245*, 1926.

Taketa, K., and Pogell, B. M. (1963), *Biochem. Biophys. Res. Commun. 12*, 229.

Taketa, K., and Pogell, B. M. (1965), *J. Biol. Chem. 240*, 651.

Weitzman, P. D. J., and Wilson, I. B. (1966), *J. Biol. Chem. 241*, 5481.

West, D. J., Tuveson, R. W., Barratt, R. W., and Fincham, J. R. S. (1967), *J. Biol. Chem. 242*, 2134.