

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/247379002>

Fructose 1,6Diphosphatase from Rabbit Liver. VI. Functional Tyrosyl Residues in the Active Center

ARTICLE *in* BIOCHEMISTRY · SEPTEMBER 1966

Impact Factor: 3.02 · DOI: 10.1021/bi00873a042

CITATIONS

10

READS

4

3 AUTHORS, INCLUDING:



Enrico Grazi

University of Ferrara

171 PUBLICATIONS 1,610 CITATIONS

SEE PROFILE

- Estrada-Parra, S., Rebers, P. A., and Heidelberger, M. (1962), *Biochemistry* 1, 1175.
- Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem.* 66, 375.
- Gardell, S. (1953), *Acta Chem. Scand.* 7, 207.
- Goldstein, I. J., Hollerman, C. E., and Marrick, J. M. (1965), *Biochem. Biophys. Acta* 97, 68.
- Goodman, J. W., and Kabat, E. A. (1960), *J. Immunol.* 84, 347.
- Heidelberger, M., Barker, S. A., and Stacey, M. (1954), *Science* 120, 781.
- Heidelberger, M., Dische, Z., Neely, W. B., and Wolfrom, M. L. (1955), *J. Am. Chem. Soc.* 77, 3511.
- Heidelberger, M., and Kendall, J. E. (1935), *J. Exptl. Med.* 61, 559.
- Hornung, M. O., and Berenson, G. S. (1963), *Proc. Soc. Exptl. Biol. Med.* 114, 31.
- Hough, L., and Jones, J. K. N. (1962), *Methods Carbohydrate Chem.* 1, 400.
- Kuhn, R., Bister, W., and Däfeldecker, W. (1959), *Ann. Chem.* 628, 186.
- Levy, G. A., and McAllan, A. (1959), *Biochem. J.* 73, 127.
- Lin, T.-Y., and Gotschlich, E. C. (1963), *J. Biol. Chem.* 238, 1928.
- Mathews, M. B., and Inouye, M. (1961), *Biochim. Biophys. Acta* 53, 509.
- Perry, M. B. (1964), *Can. J. Biochem.* 42, 451.
- Perry, M. B., and Hulyalkar, R. K. (1965), *Can. J. Biochem.* 43, 573.
- Reissig, J. L., Strominger, J. L., and Leloir, L. F. (1955), *J. Biol. Chem.* 217, 959.
- Roberts, W. K., Buchanan, J. G., and Baddiley, J. (1963), *Biochem. J.* 88, 1.
- Stoffyn, P. J., and Jeanloz, R. W. (1954), *Arch. Biochem. Biophys.* 52, 373.
- Suzuki, H., and Hehre, E. L. (1964), *Arch. Biochem. Biophys.* 104, 305.
- Sweeney, C. C., Bentley, R., Makita, M., and Wells, W. W. (1963), *J. Am. Chem. Soc.* 85, 2497.

Fructose 1,6-Diphosphatase from Rabbit Liver. VI. Functional Tyrosyl Residues in the Active Center*

S. Pontremoli,† E. Grazi, and A. Accorsi

ABSTRACT: When fructose 1,6-diphosphatase from rabbit liver is acetylated with acetylimidazole, the catalytic activity toward fructose 1,6-diphosphate is decreased almost 90%. The changes in catalytic activity are prevented by the presence of the substrate and can be reversed by deacetylation with hydroxylamine. On the

basis of difference spectra and the conditions for hydroxamate formation, it may be concluded that the alterations in enzymatic activity are correlated with acetylation of four tyrosyls. Other groups may be acetylated but only the *O*-acetylation of tyrosine residues can be related to the changes in catalytic activity.

In the course of efforts to identify the functional amino acid residues in fructose 1,6-diphosphatase (FDPase),¹ the authors have previously studied the effects of 2,4-dinitrofluorobenzene on the catalytic activity of the enzyme from rabbit liver (Pontremoli *et al.*, 1965a,b). Marked changes in catalytic activity resulted from the specific dinitrophenylation of a single

sulfhydryl group in the protein molecule; the most significant of these was the large increase in catalytic activity at neutral pH when the enzyme was tested with Mn^{2+} as the metal activator.

In the search for other amino acid residues which may participate in the catalytic activity of FDPase, the authors have now found that treatment with acetyl-imidazole abolishes the hydrolytic activity almost completely. These alterations in catalytic activity correlate closely with the acetylation of four tyrosine residues. The participation of other functional groups, such as ϵ amino, aliphatic hydroxyl, imidazole, and sulfhydryl, in inactivation due to acetylation seems to be excluded.

Materials

Acetylimidazole was prepared by the method of Boyer (1952) and stored *in vacuo* over phosphorus pentoxide. *N,O*-Diacetyltyrosine and *N*-acetyltyrosine

* From the Istituto di Chimica Biologica, University of Ferrara, Ferrara, Italy. Received May 24, 1966. Supported by a grant from the Italian Consiglio Nazionale delle Ricerche, Impresa Enzimologia, by the Grant GM 12291-02 from the National Institutes of Health, and by the Nato Grant No. 218.

† Istituto di Chimica Biologica, Università di Ferrara, Ferrara, Italy.

¹ The following abbreviations are used: FDPase, native fructose diphosphatase; Ac-FDPase, acetylated fructose diphosphatase; Ac-P-FDPase, fructose diphosphatase acetylated in the presence of the substrate fructose 1,6-diphosphate; TPN⁺, triphosphopyridine nucleotide; TPNH, reduced TPN⁺.

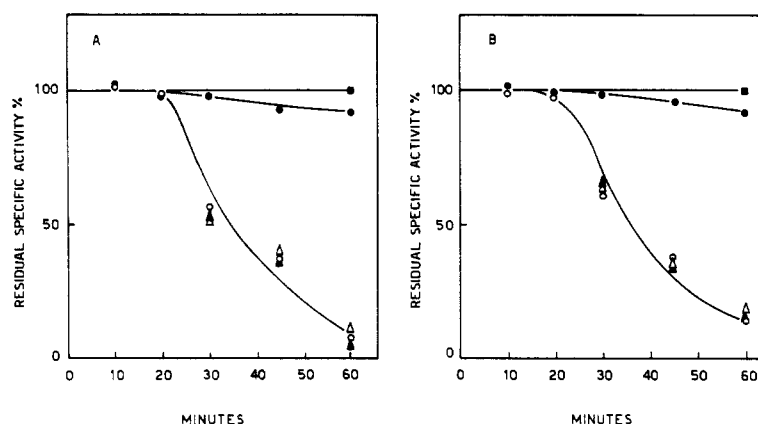


FIGURE 1: Inactivation of FDPase by acetylimidazole and protection by the substrate. Acetylation was carried out at pH 7.5 and 22°. FDPase (3 mg/ml, sp act. 120 units/mg of protein) was dissolved in 50 mM borate buffer, pH 7.5, and solid acetylimidazole (3 mg/ml, 1200-fold molar excess) was added at the beginning of the experiment. At the times indicated aliquots were taken, the protein was precipitated with ammonium sulfate, dissolved in water, dialyzed 90 min at 2° against distilled water, and finally assayed for enzymatic activity. Control without acetylimidazole (●); samples treated with acetylimidazole with or without (○) 1 mM MnCl_2 (Δ) or 10 mM MgCl_2 (▲); sample treated with acetylimidazole in the presence of 1 mM fructose 1,6-diphosphate (●). The catalytic activity was determined at pH 7.5 (A) and at pH 9.1 (B) with Mn^{2+} as activator.

were purchased from Cyclo Chemical Corp., Los Angeles; *p*-hydroxymercuribenzoate was purchased from the California Foundation, Los Angeles. Glucose 6-phosphate dehydrogenase and phosphoglucose isomerase were obtained from Boehringer and Soehne (Germany). D-Fructose 1,6-diphosphate and TPN^+ (sodium salts) were obtained from Sigma Chemical Corp., St. Louis. FDPase was prepared according to the procedure of Pontremoli *et al.* (1965c)

Methods

FDPase activity was measured spectrophotometrically by following the rate of TPNH formation at 340 $\text{m}\mu$ in the presence of excess phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. The usual test system (1 ml) contained 0.1 mM fructose 1,6-diphosphate, 1 mM MnCl_2 (or 10 mM MgCl_2), 40 mM triethanolamine buffer pH 7.5, containing 0.5 mM EDTA (or 40 mM glycine buffer, pH 9.1, without EDTA), 0.15 mM TPN^+ , 2 unit of glucose 6-phosphate isomerase, and 0.3 unit of glucose 6-phosphate dehydrogenase. The temperature was 22°. Unless otherwise indicated the enzymatic analyses have been performed with MnCl_2 in glycine buffer at pH 9.1. One unit of enzyme was defined as the amount which would cause an optical density change of 1.0/min under the above conditions. Specific activity is expressed as units per milligram of protein. Protein concentration was measured with Folin reagent (Lowry *et al.*, 1951) standardized against a known dry weight of dialyzed crystalline FDPase.

Acetylation with acetylimidazole was performed at 22° by incubating the protein (3 mg/ml) in 5 ml of 50 mM sodium borate buffer, pH 7.5. This pH was adopted

since it is optimal for the stability of acetylimidazole. The acetylated protein was precipitated with ammonium sulfate (70% saturation), dissolved in water, and dialyzed against distilled water at 2° for 90 min before analysis. Control samples were treated in the same manner but without addition of acetylimidazole.

Acetylation of tyrosine was estimated with a Zeiss Model PMQII spectrophotometer by the decrease in absorbance at 278 $\text{m}\mu$ (Simpson *et al.*, 1963) in 10 mM Tris buffer, pH 7.5, containing 1 M NaCl. The alkaline and neutral hydroxamate procedures of Hestrin (1949) as modified by Balls and Wood (1956) were employed to measure *O*-acetylation. The degree of modification of the free amino groups was estimated by means of the ninhydrin reaction (Moore and Stein, 1954) using alanine as a standard. Sulfhydryl groups were measured spectrophotometrically by titration with *p*-hydroxymercuribenzoate according to Boyer (1954). Solutions of the reagent were prepared in 0.01 M Tris-HCl buffer, pH 7.5, at approximately 1 mM concentration and standardized against a sample of glutathione of known concentration.

The ionization of the tyrosyl residues of FDPase was measured from the increase with the pH, in absorbance at 295 $\text{m}\mu$ vs. a blank cell containing no enzyme; a molar absorbance coefficient of 2300 for ionized tyrosine was applied (Crammer and Neuberger, 1943). The buffers employed in these experiments were the following: 10 mM Tris-HCl between pH 7.5 and 9.0, 10 mM sodium phosphate between pH 6.7 and 7.0, 10 mM glycine-NaOH between pH 8.2 and 10.0, and 10 mM diethylamine-HCl between pH 9.8 and 10.8. Differences in ionic strength due to differences in buffer composition were minimized by the addition of 0.1 M KCl which was present in every sample.

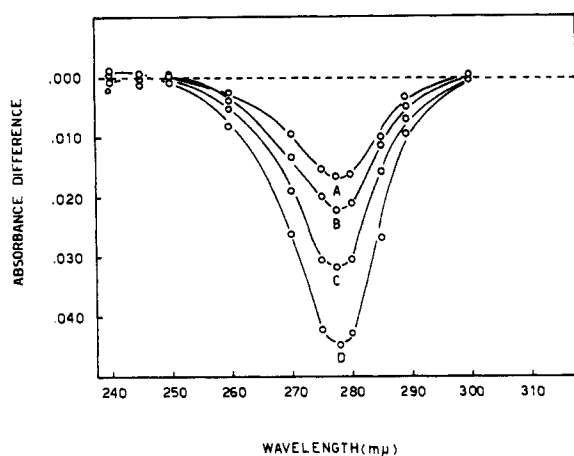


FIGURE 2: Difference spectra of Ac-FDPase *vs.* native FDPase during acetylation. The data are taken from the experiment of Figure 1. Spectra were determined, after removal of excess acetylimidazole by dialysis, in 10 mM Tris buffer, pH 7.5, containing 1 M NaCl with an enzyme concentration of 0.49 mg of protein/ml and a light path of 1 cm. Time of exposure to acetylimidazole: curve A, 10 min; B, 20 min; C, 30 min; D, 60 min. On the ordinate are reported the negative differences in absorbance between Ac-FDPase *vs.* native FDPase used in reference sample.

Results

Inactivation with Acetylimidazole. Treatment of FDPase at pH 7.5 with an excess of acetylimidazole, results in a progressive loss of catalytic activity (Figure 1). At various times after addition of the reagent samples were taken, the protein was precipitated with ammonium sulfate, and the solution was dialyzed against water as described in Methods. If acetylation is carried out in the presence of the substrate fructose 1,6-diphosphate, protection against inactivation is almost complete; on the contrary no protection is obtained by the addition of either Mg^{2+} or Mn^{2+} which are essential for the catalytic reaction. When the enzyme activity was tested with Mg^{2+} instead of Mn^{2+} , similar changes in catalytic activity were observed.

Groups Acetylated. In order to identify the nature of the groups modified during acetylation, the spectral behavior of the acetylated protein, at various times of incubation, was analyzed between 240 and 300 $m\mu$ (Figure 2). The difference spectrum showed maximal changes at 278 $m\mu$, suggesting alteration in the aromatic amino acids residues. The absence of differences in absorption in the range between 240 and 250 $m\mu$ excludes the acetylation of histidine residues. As shown in the figure, the difference in absorbance at 278 $m\mu$ increases during incubation and the difference obtained after 60 min corresponds to a loss of 90% of the catalytic activity.

On the basis of the molar absorbance values for

N,O-diacetyltyrosine and *N*-acetyltyrosine, the number of tyrosyl groups modified can be calculated. Such calculations indicate a total of 10 tyrosine residues *O*-acetylated/mole of enzyme at 90% inactivation (Table I).

TABLE I: Acetylation and Inactivation of FDPase.^a

Time (min)	Sp Act. (units/mg of protein)	Moles of <i>O</i> -Acetyltyrosine Formed/Mole of Enzyme
10	120	3.9
20	120	5.0
30	80	7.3
60	11	10.3

^a The number of tyrosine residues *O*-acetylated was calculated from the decrease in absorbance at 278 $m\mu$ ($\Delta A = 1160$). Data are taken from Figures 1 and 2.

Protection by Substrate. If *O*-acetylation of tyrosine residues is responsible for the loss of catalytic activity on acetylation, then we might expect to find less acetylation in the presence of fructose 1,6-diphosphate which prevented the loss of catalytic activity (see Figure 1). The enzyme was first treated with a 700-fold molar excess of acetylimidazole for 20 min which resulted in the acetylation of 6.0 tyrosyl residues without loss of activity. The enzyme was then precipitated with ammonium sulfate, dialyzed, and aliquots of the dialyzed solution were exposed to a 500-fold molar excess of acetylimidazole in the presence and in the absence of 1 mM fructose 1,6-diphosphate (Figure 3).

In a separate experiment 1 mM fructose 1,6-diphosphate was added at the beginning, together with a 1200-fold molar excess of acetylimidazole (Figure 4). From these results it is apparent that reaction with acetylimidazole involves first the acetylation of six tyrosine residues without loss of catalytic activity, this is followed by inactivation of the enzyme associated with the acetylation of four additional tyrosine residues. The secondary acetylation and the loss of enzyme activity are prevented by the presence of the substrate. The FDPase acetylated in six tyrosine residues and still catalytically active exhibits essentially the same properties of the native form. It retains full catalytic activity, unchanged K_m for fructose 1,6-diphosphate, shows similar pH activity curve, unchanged molecular weight, and the same inhibition by high concentrations of fructose 1,6-diphosphate (Table II). Similar results have been obtained in six different experiments.

Deacetylation with Hydroxylamine. Exposure of acetylated FDPase to hydroxylamine completely reversed the spectral changes and restored the spec-

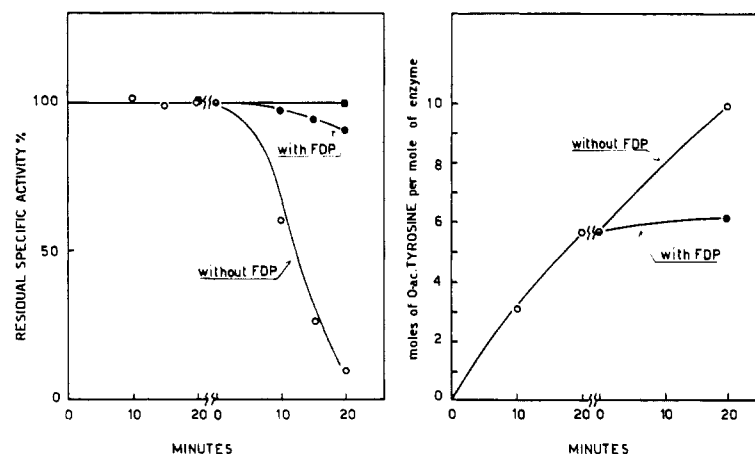


FIGURE 3: Kinetics of inactivation and acetylation in the presence and absence of fructose 1,6-diphosphate. FDPase (3 mg/ml, sp act. = 120 units/mg of protein) was dissolved in 0.05 M borate buffer, pH 7.5, and treated at 22° with 1.7 mg/ml of solid acetylimidazole (700-fold molar excess). After 20 min the protein was precipitated with ammonium sulfate, dissolved in a small amount of water, and dialyzed against distilled water. After dialysis borate buffer, pH 7.5, 0.05 M final concentration, was added, and the solution was diluted with 0.05 M borate buffer to a protein concentration of 1 mM. Aliquots were then taken and to one fructose diphosphate was added to a final concentration of 1 mM. Each aliquot was then treated with 500-molar excess (1.2 mg/ml) of solid acetylimidazole. Control sample without acetylimidazole or fructose diphosphate (■); sample treated with acetylimidazole alone (○); sample treated with acetylimidazole in the presence of fructose diphosphate (●). The number of tyrosyl residues reacting was calculated from the decrease in the absorbance at 278 mμ.

TABLE II: Properties of the Native FDPase and of the Acetylated-Active FDPase.

	FDPase	
	Native	Acetylated-Active
Molecular weight, g	127,000	127,000
Specific activity, units/mg of protein	120	120
pH optimum	9.1	9.1
K_m for fructose diphosphate		
pH 7.5, mM	0.001	0.001
pH 9.1, mM	0.0028	0.003
% inhibition by substrate at pH 7.5		
Fructose diphosphate, 0.1 mM	0	0
Fructose diphosphate, 1.0 mM	24	22
Fructose diphosphate, 3.0 mM	34	36

The K_m for fructose diphosphate and the inhibition by substrate were determined as described under Methods with Mn^{2+} as activator and a fructose diphosphate concentration ranging from 0.005 to 3 mM. The molecular weight was determined in the Spinco Model E ultracentrifuge; the short-column equilibrium method of Yphantis (1960) was used.

trum of native enzyme (Figure 5). Simultaneously the specific activity returned to that of the native enzyme (Table III). The close correlation between deacetylation of *O*-acetylated tyrosines, restoration of the spectrum of the native enzyme, and recovery of full catalytic activity, indicate the participation of tyrosine residues in the catalytic changes observed in FDPase. Lysine amino groups, aliphatic hydroxyls, and free sulfhydryl groups which are susceptible to acetylation are shown not to be related to the reversible alteration of enzymatic activity since these do not follow the changes in catalytic activity (Table III). This is further substantiated by the fact that there is perfect agreement between the number of moles of acetyltyrosine formed when calculated from the changes in the spectra and from the moles of acetylhydroxamate formed after treatment with hydroxylamine at either pH 7.5 and 11.5.

Subtraction of the number of moles formed in the presence of fructose diphosphate from the number formed in the absence of substrate indicates that four tyrosine residues are protected by fructose 1,6-diphosphate. Similar results were obtained in five separate experiments. No alterations in catalytic properties were observed when the enzyme acetylated in the presence of fructose 1,6-diphosphate or the native enzyme were treated with hydroxylamine.

Presence of Buried Tyrosines. The fact that six tyrosyl residues were acetylated before the remaining four tyrosyl residues became available is of interest. It

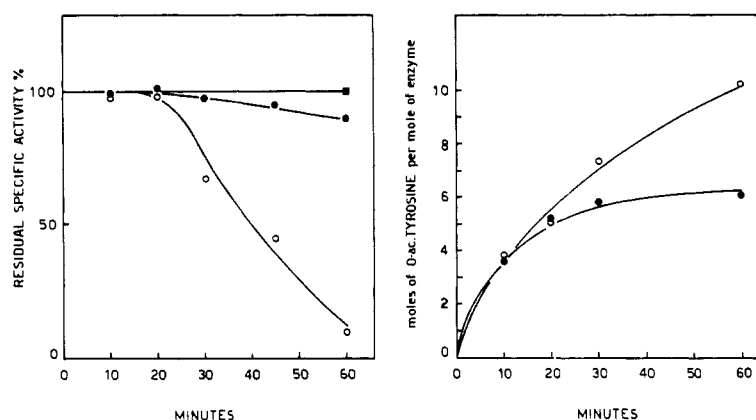


FIGURE 4: Acetylation of FDPase and protection by substrate. The experiment was performed as described in the legend of Figure 1. Control sample (■); sample treated with acetylimidazole (○); sample treated with acetylimidazole in the presence of 1 mM fructose 1,6-diphosphate (●).

TABLE III: Modification of Tyrosyl, Sulfhydryl, and Amino Groups after Treatment of FDPase with Acetylimidazole.^a

	Sp Act.	Acetylated Amino Groups (%)	Free Thiol Groups (mole/mole)	Hydroxamate (mole/mole)		O-Acetyltyrosyl (mole/mole)
				pH 7.5	pH 11.5	
Native FDPase	120	0	20
Acetyl-FDPase	12	8	20	10.3
Acetyl-FDPase prepared in the presence of fructose 1,6-diphosphate	110	7	20	6.1
Acetyl-FDPase treated with NH ₂ OH	120	6	20	10.1	10.2	...
Acetyl-FDPase prepared in the presence of fructose diphosphate and treated with NH ₂ OH	120	8	20	6.2	6.1	...

^a The data are calculated from the experiment described in Figure 4. Determination of sulfhydryl groups, amino groups, hydroxamate, and O-acetyltyrosyl residues was performed as described under Methods. In the titration of sulfhydryl groups with *p*-hydroxymercuribenzoate the rates of titration were equivalent in all cases.

might be postulated that acetylation of the first six residues alters the tertiary structure of the protein sufficiently as to expose buried tyrosines which subsequently react with the reagent. In order to obtain evidence on this point, a titration of the tyrosine which ionized between pH 8.0 and 11.0 was undertaken. The increase in molar extinction coefficient at 295 mμ as a function of the pH is shown in Figure 6. In obtaining the results shown in Figure 6 no differences were detected in the pH regions where two different buffers overlapped, or by increasing the concentration of the buffers from 0.01 to 0.03 M. A few points of the reverse titration curve have also been determined starting from pH 10.5 and they fall

on the forward titration curve. Unfortunately in view of the instability of fructose diphosphatase above pH 10.8, it has not been possible to perform a complete spectrophotometric titration of the enzyme. However, 15 of the 39 tyrosines present in the enzyme protein were titrated in the pH range between 8.0 and 10.8 with an average pK_{app} of 9.8. This indicates that the number of O-acetylated tyrosines observed in our experiments does not exceed the number of tyrosines which dissociate "normally."

Discussion

N-Acetylimidazole has been successfully used by

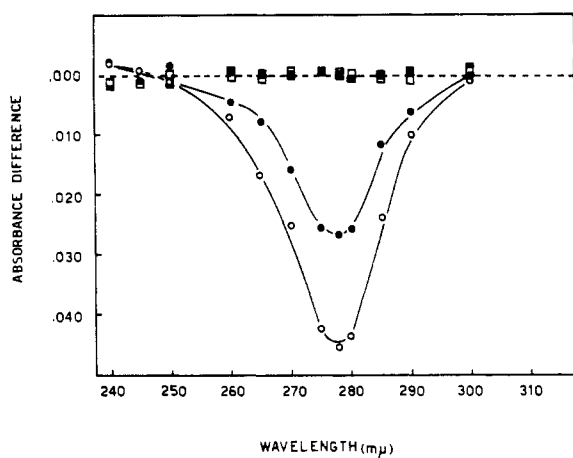


FIGURE 5: Acetylation of FDPase and deacetylation with hydroxylamine. Difference spectra were determined as described in the legend of Figure 2. FDPase acetylated with 1200-fold molar excess of acetylhydrazide (O); as above in the presence of 1 mM fructose 1,6-diphosphate (●); acetylated FDPase treated with hydroxylamine (□); FDPase acetylated in the presence of fructose 1,6-diphosphate treated with hydroxylamine (■). All difference spectra are relative to native FDPase. The treatment with hydroxylamine was performed at pH 7.5 and 22° for 20 min in the presence of 1 M NH_2OH , 1 M NaCl, and 0.01 M Tris buffer.

Simpson *et al.* (1963) to investigate the nature of functional groups associated with the enzymatic activity of carboxypeptidase. More recently Riordan *et al.* (1965) have presented data which indicate the relative specificity of acetylhydrazide for the determination of "free" tyrosyl residues in proteins.

The experimental findings, reported in the present communication, show that treatment of crystalline rabbit liver FDPase with an excess of acetylhydrazide results in almost complete loss of catalytic activity which can be prevented if acetylation is carried out in the presence of substrate. The difference spectrum of native FDPase *vs.* acetyl-FDPase and protected acetyl-FDPase, and the complete restoration of activity which accompanies deacetylation with hydroxylamine at near-neutral pH, suggest that loss of activity is related to acetylation of tyrosine groups. Complete loss of activity is observed when ten tyrosine groups are *O*-acetylated; however, six of these groups are acetylated in the presence of substrate. We may therefore conclude that inactivation is a consequence of acetylation of four tyrosine residues and that these are specifically protected from acetylation by the substrate. These tyrosine residues may participate directly in the catalytic mechanism or in the binding of the substrate, or they may be involved in maintenance of the protein in an active conformation. In the last case it could be necessary to assume that the substrate also induces a conformation change in the enzyme which makes these residues unavailable to acetylation.

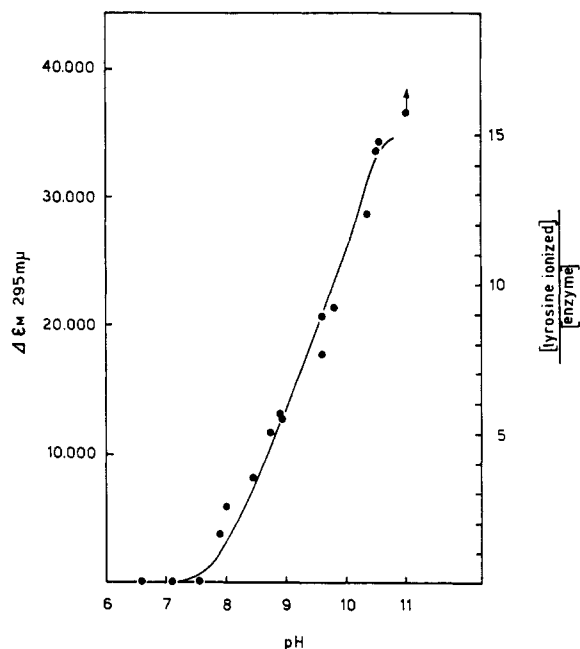


FIGURE 6: Titration of the tyrosine residues in FDPase. Left ordinate: increase in molar absorbance at 295 $\text{m}\mu$ over the value at neutral pH (pH 7.5); right ordinate: number of tyrosyl residues ionized per mole of enzyme the basis of a molar absorbance of 2300; enzyme 0.54 mg/ml, light path 1 cm. The buffers employed are described under Methods. The arrow on the titration point at pH 11 indicates that the titration curve above pH 11 depends on time.

About 15 of the 39 tyrosyl residues present in rabbit liver FDPase have been found free to titrate between pH 8.0 and 10.8 with an average pK_{app} of 9.8. Acetylhydrazide appears to acetylate those residues which dissociate "normally" in FDPase, as has been proposed by Simpson *et al.* (1963).

The acetylation of α - or ϵ -amino groups does not account for the reversible inhibition of enzymatic activity. This is supported by the fact that the decrease in ninhydrin color value as a consequence of acetylation was equal in acetyl-FDPase, in the protected acetyl-FDPase and in the deacetylated, active enzyme after treatment with hydroxylamine. The difference spectra in the range from 240 to 250 $\text{m}\mu$ for acetyl-FDPase as compared with native FDPase did not indicate the formation of acetylhistidyl residues. The lack of difference in acetohydroxamate formation in the reaction with hydroxylamine at neutral pH or at pH 11.5 appears to exclude the acetylation of threonine or serine. The rate of titration and the number of sulfhydryl groups titrated in the acetylated FDPase were not significantly different from those of native FDPase. This excludes the acetylation of sulfhydryl groups.

On the basis of this evidence the authors conclude

that the alteration in enzymatic activity observed by treatment of FDPase with acetylimidazole is associated with the acetylation of four tyrosyl residues. Further work is necessary to define the nature of the changes produced by acetylation of these tyrosine residues and their relation to the catalytic mechanism.

On the basis of the present studies and from results obtained with 2,4-dinitrofluorobenzene the authors can postulate the existence of two regions of the enzyme protein. (1) An active center responsible for the binding and the dephosphorylation of the substrate, which contains specific tyrosine residues or requires a structure provided by these tyrosine residues. (2) A second distinct region which contains a sulfhydryl group capable of regulating the catalytic activity at neutral pH.

This is supported by the results obtained by treatment of FDPase with 2,4-dinitrofluorobenzene (Pontremoli *et al.*, 1965a). Under these conditions where only one cysteine residue is converted to the *S*-dinitrophenyl derivative, the enzyme activity at neutral pH is increased more than twofold over the original. Further work is in progress to investigate the relationship between these two regions.

Acknowledgment

We wish to thank Mr. Lucio Rizzati for his valuable

help in the preparation of the enzyme.

References

- Balls, A. K., and Wood, H. N. (1956), *J. Biol. Chem.* 219, 245.
- Boyer, J. H. (1952), *J. Am. Chem. Soc.* 74, 6274.
- Boyer, P. D. (1954), *J. Am. Chem. Soc.* 76, 4331.
- Crammer, J. L., and Neuberger, A. (1943), *Biochem. J.* 37, 302.
- Hestrin, S. (1949), *J. Biol. Chem.* 180, 249.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem.* 193, 265.
- Moore, S., and Stein, W. H. (1954), *J. Biol. Chem.* 211, 907.
- Pontremoli, S., Luppis, B., Traniello, S., Wood, W. A., and Horecker, B. L. (1965a), *J. Biol. Chem.* 240, 3469.
- Pontremoli, S., Luppis, B., Wood, W. A., Traniello, S., and Horecker, B. L. (1965b), *J. Biol. Chem.* 240, 3464.
- Pontremoli, S., Traniello, S., Luppis, B., and Wood, W. A. (1965c), *J. Biol. Chem.* 240, 3459.
- Riordan, I. F., Warren, E. C., Wacker, W. E. C., and Vallee, B. L. (1965), *Biochemistry* 4, 1758.
- Simpson, R. T., Riordan, J. F., and Vallee, B. L. (1963), *Biochemistry* 2, 616.
- Yphantis, O. A. (1960), *Ann. N. Y. Acad. Sci.* 88, 586.

CORRECTION

In the paper "Biosyntheses of Bacterial Glycogen. IV. Activation and Inhibition of the Adenosine Diphosphate Glucose Pyrophosphorylase of *Escherichia coli* B," by Jack Preiss, Laura Shen, Elaine Greenberg, and Norman Gentner, Volume 5, June 1966, the following correction should be made.

On p 1835 under "Step 2. Protamine Sulfate Fractionation," the paragraph should read (the typographical omission is in italics for emphasis):

"A 1% protamine sulfate solution, 150 ml, was added slowly, with constant stirring, to 500 ml of the crude extract. After 10 min the suspension was centrifuged at 10,000g for 10 min. *This precipitate was discarded. To the supernatant fraction from this centrifugation was added another 150 ml of the 1% protamine sulfate solution. The precipitate from centrifugation of this suspension at 10,000g for 10 min contained the enzymatic activity.*" (The remainder of the paragraph is correct.)