THE EFFECT OF ACETYLATION ON THE ACTIVITY OF RABBIT MUSCLE FRUCTOSE DIPHOSPHATE ALDOLASE

E. L. Pugh and B. L. Horecker

Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York

Received January 6, 1967

Acetyl imidazole has been proposed as a selective reagent for the modification of tyrosine residues in proteins (Riordan et al., 1965). We have now studied the acetylation of rabbit muscle aldolase with this reagent. Under controlled conditions a modified enzyme is produced with kinetic properties which resemble those of the enzyme treated with carboxypeptidase (Drechsler et al., 1959; Rutter et al., 1961; Spolter et al., 1965; Rose et al., 1965). The activity with fructose 1,6-diphosphate falls to about 10% of the initial value, while the rate of cleavage of fructose 1-phosphate remains unchanged. In the presence of an acceptor aldehyde, such as acetaldehyde, as much as 60% of the activity with fructose 1,6-diphosphate is restored. The enzyme can be deacetylated and the changes in catalytic properties reversed by exposure of the acetylated enzyme to neutral hydroxylamine. The carboxy-terminal tyrosine residues are not acetylated. Preliminary evidence indicates that alteration of other tyrosine residues in the protein is responsible for the changes in catalytic properties.

EXPERIMENTAL AND RESULTS

Materials and Methods. Acetyl-1-^{14}C-imidazole was obtained from the New England Nuclear Corp. Aldolase was prepared from rabbit muscle by the method of Taylor et al. (1948) and was recrystallized four times. Aldolase activity was assayed by the method of Racker (1947). Protein concentrations of both native aldolase and the acetylated derivative were estimated by measurement

of absorbance at 280 mμ in 0.1 N sodium hydroxide. The modified enzyme is deacetylated under these conditions and the absorption spectrum is indistinguishable from that of the untreated control.

Inactivation of Rabbit Muscle Aldolase by Acetylation. The rate of cleavage of fructose 1,6-diphosphate (FDP) was observed to fall rapidly on exposure of the enzyme to acetyl imidazole (Fig. 1). The level of inactivation with fructose 1,6-diphosphate as substrate was dependent upon the amount of reagent added. The addition of 5 mM fructose 1,6-diphosphate or 5 mM hexitol diphosphate did not alter the course of the reaction. No loss of activity was observed with fructose 1-phosphate (F-1-P) as substrate. However, if the acetylation reaction was allowed to proceed too far, then activity toward fructose 1-phosphate was also affected and the enzyme was completely inactivated.

Activity of the Acetylated Enzyme in the Presence of Acceptor Aldehydes. Like the enzyme modified by treatment with carboxypeptidase (Spolter et al.,

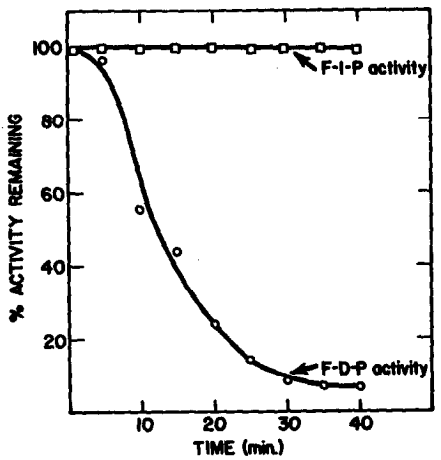

Fig. 1. Effect of acetylation of rabbit muscle aldolase on its activity with fructose 1,6-diphosphate and fructose 1-phosphate. The reaction mixture (1.0 ml) contained aldolase (10 mg) in 50 mM sodium borate, pH 7.6. A fresh solution of acetyl imidazole (0.6 M) in sodium borate, pH 7.6, was prepared and added in 0.02 ml portions at 5 minute intervals. Samples were removed as indicated and assayed for activity with fructose 1,6-diphosphate and fructose 1-phosphate. The initial specific activities (μmoles per min per mg protein) were 14.0 for fructose 1,6-diphosphate and 0.21 for fructose 1-phosphate. At 30 minutes these values had become 1.1 for fructose 1,6-diphosphate and 0.22 for fructose 1-phosphate.

Table I

Stimulation of Activity of Acetylated Aldolase by Various Aldehydes

Aldehyde	Conc.	Relative Activity*
	mM	%
-	-	10
Acetaldehyde	12.5	38
	25.0	59
D,L-Glyceraldehyde	12.5	36
Propionaldehyde	12.5	48
Butyraldehyde	12.5	10
D-Erythrose	12.5	10
Phosphate	12.5	10

* Relative to that of the enzyme before acetylation.

Aldolase was acetylated until the specific activity with fructose 1,6-diphosphate as substrate fell from an initial value of 14.8 to a final value of 1.5. The specific activity with fructose 1-phosphate as substrate remained unchanged at 0.24. Specific activity is expressed as micromoles of substrate cleaved per minute per mg protein. Samples were assayed with fructose 1,6-diphosphate with and without the addition of the aldehydes indicated.

1965), the acetylated aldolase was stimulated by addition of aldehydes to the assay mixture (Table I). Acetaldehyde, glyceraldehyde, and propionaldehyde were effective and in the presence of 25 mM acetaldehyde the activity was restored to about 60% of that of the native enzyme. Erythrose 4-phosphate was a poor acceptor.

Reversal of Acetylation. Exposure of acetylated aldolase to hydroxylamine reversed the changes in catalytic properties (Table II). The activity after treatment with hydroxylamine approached that of the native enzyme exposed to the same conditions.

Amino Acid Residues Modified by Acetylation. Our results support the view that acetyl imidazole can serve as a specific reagent for modification of tyrosine residues in proteins (Riordan et al., 1965). Under the conditions of

Table II

Reversal of the Inhibition with Hydroxylamine

Enzyme	Specific Activity
	μmoles/min/mg
Native	10.5
Native + NH_2OH	9.5
Acetylated	0.7
Acetylated + NH_2OH	7.5

Aldolase was acetylated as described in the legend to Figure 1. Samples of native and acetylated enzyme were treated with 1.1 M hydroxylamine at pH 7.5 and 25° for 10 minutes (Balls and Wood, 1956). The preparations were assayed with fructose 1,6-diphosphate.

acetylation given in Figure 1, 30 moles of radioactive acetate were incorporated per mole of enzyme. This number was found to be quite reproducible for preparations that retained 7-10% of their original activity. Upon treatment of ^{14}C-acetyl enzyme with hydroxylamine at pH 7.5, the radioactivity was completely removed from the protein and converted quantitatively to acetyl hydroxamic acid (Table III). Similar amounts of hydroxamic acid were produced upon treatment with hydroxylamine at neutral and at alkaline pH. The ability to reverse the reaction with neutral hydroxylamine indicates that only sulfhydryl, tyrosyl, or histidyl residues were altered. Since the number of bound acetyl groups was not reduced by exposure of the protein at 24° to pH 2.2 for 2 minutes, we have concluded that acetyl histidine was not present. Acetylation of sulfhydryl groups has not been excluded. However, preliminary studies of the absorption spectrum of the acetylated enzyme indicate that all of the acetyl residues can be accounted for as O-acetyl tyrosine. The acetylated enzyme releases 3 equivalents of free tyrosine upon treatment with carboxypeptidase. It must, therefore, be concluded that the COOH-terminal tyrosine residues are not acetylated. The details of these experiments will be reported elsewhere.

Table III

Amino Acid Residues Modified by Acetylation

Exp	Relative Activity*	Radioactivity Incorporated	Hydroxamic Acid Formation	
			Neutral Hydroxylamine	Alkaline Hydroxylamine
	%	moles per mole of enzyme		
1	6.7	30.0	27.7	28.8
2	10.0	29.5	29.6	30.0

* Tested with fructose 1,6-diphosphate as compared with a control sample of enzyme.

Aldolase was acetylated with acetyl-1-^{14}C-imidazole as described in Figure 1. The acetylated enzyme (1.0 ml) was applied to a 3 x 30 cm column of Sephadex G-25 and eluted with 50 mM sodium borate, pH 7.6. Aliquots of each fraction were taken for determination of radioactivity and protein concentration.

For determination of hydroxamic acid the experiments were repeated on a 3-fold larger scale and after passage through Sephadex a fraction was obtained containing 7.5 mg of protein per ml. Aliquots of this fraction (1 ml) were treated directly with 1 ml of 2 M neutral hydroxylamine and 1 ml of 2 M alkaline hydroxylamine. The amount of hydroxamic acid formed was determined colorimetrically after reaction with acidic ferric chloride (Lipmann and Tuttle, 1945). Similar values were obtained by estimating the amount of radioactivity remaining in solutions after precipitation of the protein.

DISCUSSION

Aldolase which has been modified by acetylation resembles the carboxypeptidase-treated enzyme in several of its properties. The rate of cleavage of fructose 1,6-diphosphate is reduced, while the activity toward fructose 1-phosphate is unchanged, and the enzyme is stimulated by aldehydes. In this respect the modified enzyme resembles transaldolase. We have also shown, in experiments to be reported elsewhere, that the rate of exchange between dihydroxyacetone phosphate and tritiated water is impaired in the acetylated enzyme.

The primary effects of acetylation may be the result of conformation changes which are similar to those produced when the enzyme is treated with carboxypeptidase. The activity of the modified enzyme with fructose diphosphate

decreases, while that with fructose 1-phosphate remains unchanged. That the modification also affects the ability of the Schiff base intermediate to dissociate is suggested by the fact that the cleavage of fructose diphosphate by the acetylated enzyme is stimulated by aldehydes. We have demonstrated that destruction of histidine residues by photooxidation also converts aldolase to an enzyme resembling transaldolase, with accompanying changes in the degree of masking of the COOH-terminal tyrosine residues (Hoffee et al., 1967).

Mehler and Cusic (personal communication) have studied the activity of rabbit muscle aldolase with xylulose diphosphate, fructose diphosphate, and octulose diphosphate and have concluded that the activity of the catalytic site may be affected by the interaction with the second phosphate group. A comparison of the changes in catalytic properties produced by acetylation and photooxidation may provide information as to the nature of these interactions.

REFERENCES

Balls, A. K. and Wood, H. N., J. Biol. Chem. 219, 245 (1956).
Benesch, R. E., Lardy, H. A. and Benesch, R., J. Biol. Chem. 216, 663 (1955).
Drechsler, E. R., Boyer, P. D. and Kowalsky, A. G., J. Biol. Chem. 234, 2627 (1959).
Hoffee, P., Lai, C. Y., Pugh, E. L. and Horecker, B. L., Proc. Natl. Acad. Sci., in press (1967).
Lipmann, F. and Tuttle, L. C., J. Biol. Chem. 161, 415 (1945).
Racker, E., J. Biol. Chem. 167, 843 (1947).
Riordan, J. F., Wacker, W. E. C. and Vallee, B. L., Biochemistry 4, 1758 (1965).
Rose, I. A., O'Connell, E. L. and Mehler, A. H., J. Biol. Chem. 240, 1758 (1965).
Rutter, W. J., Richards, O. C. and Woodfin, B. M., J. Biol. Chem. 236, 3193 (1961).
Spolter, P. D., Adelman, R. C. and Weinhouse, S., J. Biol. Chem. 240, 1327 (1965).
Taylor, J. F., Green, A. A. and Cori, G. T., J. Biol. Chem. 173, 591 (1948).

Acknowledgements

This work was supported by grants from the National Institutes of Health (GM 11301) and the National Science Foundation (GB 1465). This is Communication No. 76 from the Joan and Lester Avnet Institute of Molecular Biology.