ARGININE AS THE C-1 PHOSPHATE BINDING SITE IN RABBIT MUSCLE ALDOLASE

R.R. LOBB, A.M. STOKES, H.A.O. HILL
Inorganic Chemistry Laboratory, South Parks Road, Oxford UK

and

J.F. RIORDAN
Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, Boston, Mass. USA

Received 20 March 1975

1. Introduction

It has previously been suggested that the first step in the reaction of rabbit muscle aldolase, the formation of the enzyme–substrate complex, is stabilized by electrostatic binding of phosphate groups [1]. The presence of positively charged groups in aldolase was further indicated by Castellino and Barker [2], and Ginsberg and Mehler [3] who identified a strong phosphate binding site at the active centre responsible for the interaction of the enzyme with the 1-phosphate group of the substrate. In addition, a relatively weaker binding site was also found. A study of inactivation by pyridoxal phosphate [4] suggests that this latter site, the C-6 phosphate binding site, is a lysine residue, but the nature of the strong C-1 phosphate binding site remained unknown.

In this paper we show that phenylglyoxal, an arginine-selective α-dicarbonyl, is incorporated into rabbit muscle aldolase with concomitant loss of catalytic activity. These results, combined with data from protection experiments, implicate an arginine residue as the strong 1-phosphate binding site in this enzyme.

2. Materials and methods

Rabbit muscle aldolase was from Worthington Enzymes Ltd. Dihydroxyacetonephosphate (DHAP), reduced β-diphosphopyridine nucleotide (DPNH), glycerophosphate dehydrogenase/triosephosphate isomerase mixture (GDH/TIM), were from Boehringer Mannheim Ltd. D-fructose-1, 6-diphosphate (FDP), was from Wessex Biochemicals Ltd. Disodium hydrogen phosphate was Analar grade from B.D.H. Ltd. TRIZMA base was from Sigma Chemical Co. Phenylglyoxal monohydrate was from Aldrich Chemical Co. and twice recrystallised from water. 7-[^{14}C] Phenylglyoxal was prepared from 7-[^{14}C] acetophenone (I.C.N.) according to previously established methods [5].

Radioactivity was determined by standard liquid scintillation techniques using a Packard Tricarb instrument, the sample to be counted being diluted into Bray's solution. Using a Zeiss PMQII spectrometer, protein concentration was determined assuming an absorbance at A_{280} of 0.938 for a 1 mg/ml enzyme solution [6]. Activity was assayed with a Unicam SP800 spectrophotometer by recording the absorbance change at 340 nm after addition of a suitable aliquot of enzyme to a cuvette containing 50 μl 0.1 M FDP, 50 μl 10 mg/ml DPNH, and 10 μl 10 mg/ml GDH/TIM mixture, made up to 3 ml with 50 mM Tris pH 7.5, 25°C.

3. Results

A 0.5 mg/ml solution of rabbit muscle aldolase in 125 mM bicarbonate buffer pH 7.9 is rapidly inactivated by 4 mM phenylglyoxal (fig.1). On gel filtration of the partially inactivated mixture through Sephadex G25 (0.9 X 15cm), the activity remains constant with

 North-Holland Publishing Company – Amsterdam

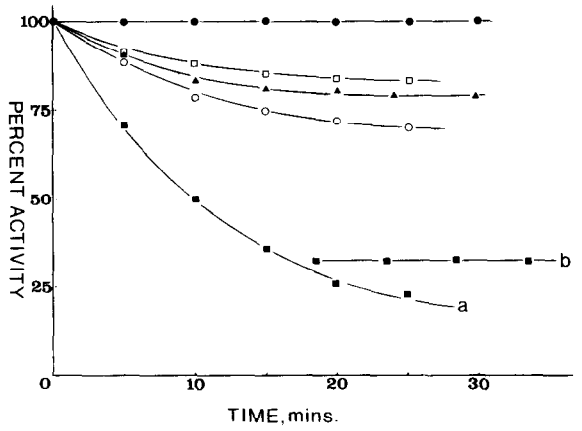

Fig.1. Time course of inactivation of 0.5 mg/ml rabbit muscle aldolase by 4 mM phenylglyoxal in 125 mM bicarbonate pH 7.9, (■a) including activity after desalt through Sephadex G25 (■b), and in the presence of 100 mM phosphate (○), 5 mM FDP (▲), or 5 mM DHAP (□). The control (●) shows no loss of activity during the reaction period.

time. In addition, inactivation is retarded by the presence in the reaction mixture of fructose diphosphate, dihydroxy acetonephosphate, and inorganic phosphate.

The time course of [^{14}C]-phenylglyoxal incorporation into aldolase reveals that loss of activity relates linearly to incorporation of radioactivity over almost the entire reaction course (fig.2). Extrapolation to zero activity correlates with the modification of one arginine per subunit, assuming four equivalent subunits [7], and two phenylglyoxals per arginine, in line with previous observations [8].

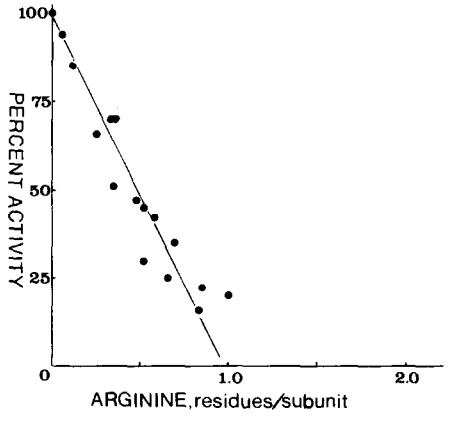

Fig.2. Correlation of residual aldolase activity with arginine modification on incubation with 4 mM [^{14}C]-phenylglyoxal in 125 mM bicarbonate, pH 7.9.

4. Discussion

At least two thirds of all known enzymes act on negatively charged substrates or require anionic cofactors. Interest in the nature of the positively charged groups of these enzymes which bind such substances has recently increased due to the discovery that arginine residues have important binding functions in the alcohol dehydrogenases [9], alkaline phosphatase [10], and lactic and malic dehydrogenases [11,12]. For this reason we are investigating the possibility of arginine as the C-1 phosphate binding site of rabbit muscle aldolase, a typical Class I aldolase.

α-Dicarbonyls are arginine-selective reagents, which react with guanidino groups much more readily than with lysine or histidine residues [13,14]. One of the most useful is phenylglyoxal, which forms derivatives containing two phenylglyoxal moieties per arginine modified. The product is stable on removal of excess reagent, and remains so under mildly acidic conditions thereby allowing isolation of labelled peptides after cleavage of the peptide chain [8].

The irreversible phenylglyoxal modification in conjunction with the [^{14}C] incorporation data, suggest that one arginine per subunit of rabbit muscle aldolase is much more susceptible to attack than the other thirteen; the protection against inactivation afforded by inorganic phosphate and fructose diphosphate further suggests that this arginine is a phosphate binding site in the active site. Dihydroxyacetonephosphate is a competitive inhibitor of aldolases, binding at only one site [15,16], and its phosphate corresponds to the C-1 phosphate of fructose diphosphate. The protection against inactivation afforded by DHAP, therefore, indicates that the arginine being modified is the C-1 phosphate binding site of rabbit muscle aldolase. Whether this is true of class II aldolases is also under investigation.

Acknowledgements

We wish to thank the Science Research Council for a studentship to one of us (RRL) and Oxford Enzyme Group for support.

References

[1] Vehlick, S.F. (1949) J. Phys. Colloid. Chem. 53, 135.
[2] Castellino, F. J. Barker, R. (1966) Biophys. Biochem. Res. Comm. 23, 182.
[3] Ginsberg, A., Mehler, A. H. (1966) Biochemistry 5, 2623.
[4] Shapiro, S., Enser, M., Pugh, E., Horecker, B. L. (1968) Archs. Biochem. Biophys. 128, 554.
[5] Kornblum, N., Frazier, H. W. (1966) J. Amer. Chem. Soc. 88, 865.
[6] Donovan, J. W. (1964) Biochemistry 3, 67.
[7] Lai, C. Y., Horecker, B. L. (1972) in: Essays in Biochemistry (Campbell, P. N. and Dickens, F. eds.) Volume 8, 152–154. Academic Press, London.
[8] Takahashi, K. (1968) J. Biol. Chem. 243, 6171.
[9] Lange, L. G., Riordan, J. F., Vallee, B. L. (1974) Biochemistry 13, 4361.
[10] Daeman, F., Riordan, J. F. (1974) Biochemistry 13, 2865.
[11] Yang, P. C. Schwert, G. W. (1972) Biochemistry 11, 2218.
[12] Foster, M. Harrison, J. H. (1974) Biochem. Biophys. Res. Comm. 58, 263.
[13] Yankeelov, J. A., Mitchell, C. D., Crawford, T. H. (1968) J. Amer. Chem. Soc. 90, 1664.
[14] Riordan, J. F. (1973) Biochemistry 12, 3915.
[15] Rose, I. A., O'Connell, E. L., Mehler, A. H. (1965) J. Biol. Chem. 240, 1758.
[16] Rose, I. A., O'Connell, E. L. (1969) J. Biol. Chem. 244, 126.