Inactivation of chicken muscle enolase by carbodiimide and glycine methyl ester

Gillian A. Russell and Linda A. Fothergill

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland

Received 13 May 1982

Enolase *Carbodiimide* *Active site carboxyl* *Inactivation* *Glycine methyl ester* *Glycolytic enzymes*

1. INTRODUCTION

Enolase (EC 4.2.1.11) catalyses the dehydration of D-2-phosphoglycerate (2-PGA) to 2-phosphoenolpyruvate (PEP) during glycolysis. The enzyme is a dimer with identical subunits ($M_r \sim 45\,000$) and requires divalent metal ions such as Mg^{2+} for catalytic function.

It has been shown that glycidol phosphate, a substrate analogue, inactivates rabbit muscle enolase, and this is interpreted as due to reaction with a carboxyl group at the active site [1]. We report here the chemical modification of chicken muscle enolase with a water soluble carbodiimide and glycine methyl ester. Complete protection was observed in the presence of PEP, and the kinetics of inactivation indicated one molecule of inhibitor was bound per active site.

2. MATERIALS AND METHODS

2.1. *Purification and characterisation of chicken muscle enolase*

Enolase was purified from chicken muscle using a procedure based on the method in [2,3]. The procedure involves extraction, ammonium sulphate fractionation and ion-exchange chromatography with affinity elution by PEP. An additional gel filtration step was sometimes needed to purify the enzyme to homogeneity.

The purified material was characterized by SDS–polyacrylamide gel electrophoresis, amino acid analysis, and enzyme assay. The yield was 6–8 μmol (300 mg) subunit from 350 g muscle, and the purified enolase was shown to have spec. act. ~200 $\mu mol \cdot min^{-1} \cdot mg^{-1}$.

2.2. *Assay of enzyme activity*

Enzyme activity was assayed by monitoring the increase in absorbance at 230 nm due to the formation of PEP from 2-PGA. The method was essentially that in [4]. The assay was done at 25°C in 0.02 M imidazole/HCl buffer (pH 7.0) containing magnesium acetate (10^{-3} M) and EDTA (10^{-5} M). Since 2-PGA is relatively unstable in the assay mixture, it was added (final conc. 10^{-3} M) immediately before the reaction was initiated by addition of enzyme.

2.3. *Chemical modification*

Enolase was inactivated by treatment with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and glycine methyl ester. The reaction was done at 17°C in 10 mM phosphate buffer (pH 6.1). Enolase was 15 μM, and carbodiimide, glycine methyl ester and PEP concentrations were varied as shown in fig.1.

The reaction was started by addition of carbodiimide followed by glycine methyl ester. Samples were removed at timed intervals and diluted 20-fold with cold water before assaying for enzyme activity.

3. RESULTS AND DISCUSSION

The results of inactivation are given in fig.1. Complete inactivation was obtained in 40 min with 100 mM carbodiimide. Complete protection by 5 mM PEP and 5 mM $Mg(NO_3)_2$ occurred at each concentration of carbodiimide. Yeast enolase has also been shown to be sensitive to inactivation by carbodiimides [5], but complete projection by PEP, 2-PGA or 3-PGA was not observed. Yeast enolase,

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

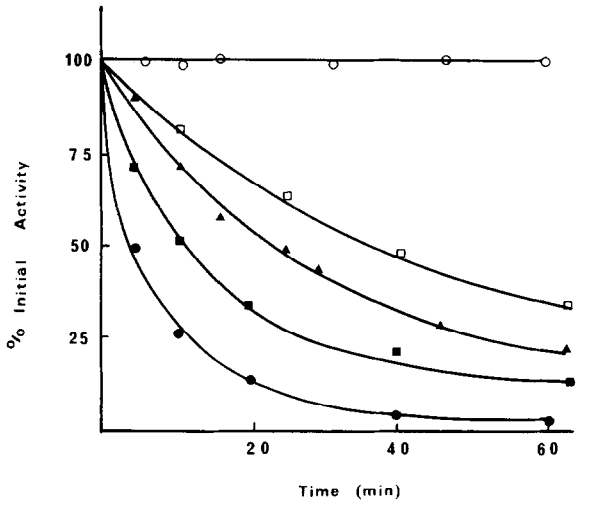

Fig.1. Carbodiimide inactivation of enolase. In each case, enolase was 15 μM in 10 mM phosphate buffer (pH 6.1): (•) EDC 100 mM, glycine methyl ester 30 mM; (▪) EDC 70 mM, glycine methyl ester 21 mM; (▲) EDC 30 mM, glycine methyl ester 9 mM; (□) EDC 10 mM glycine methyl ester 3 mM. Enolase samples incubated with 5 mM PEP and 5 mM $Mg(NO_3)_2$ prior to addition of 10, 30 or 70 mM EDC were also assayed (○).

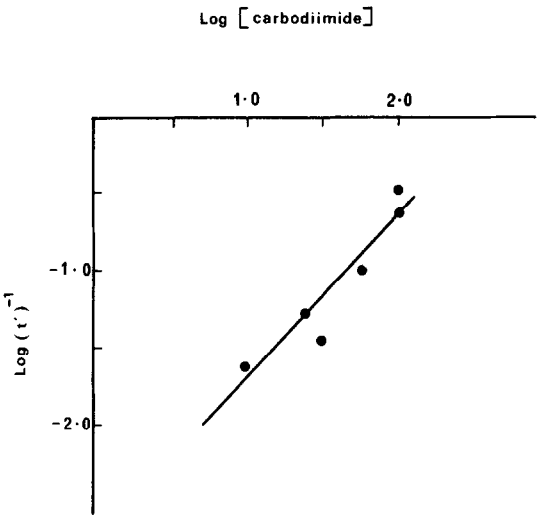

Fig.2. A plot of log [EDC] *vs* log $(t')^{-1}$ where t' is the time to reach 50% inactivation in each case in fig.1. By the method of least squares, it can be shown the slope of the line is 1.0. This indicates one molecule of inhibitor bound per active site.

unlike animal enolase, does not show inactivation with glycidol phosphate [6].

Since inactivation of the enzyme results from its reaction with carbodiimide, by plotting log [EDC] *vs* $\log(t')^{-1}$ (where t' is the time to 50% inactivation in each case) (fig.2), the slope gives the number of EDC molecules reacting per active site. The slope, determined by the method of least squares, is 1.0. This suggests that the modification of one carboxyl group per subunit is sufficient for complete loss of enzyme activity.

The inactivation results presented here show that reaction with carbodiimide and glycine methyl ester may be a useful method for the labelling and isolation of an 'active-site' peptide as part of the determination of the amino acid sequence of chicken muscle enolase in progress in our laboratory.

ACKNOWLEDGEMENTS

We thank Mrs Jean Bathgate and Mr Ian Davidson for skilful technical assistance, and the University of Aberdeen for financial support.

REFERENCES

[1] Rose, I.A. and O'Connell, E.L. (1969) J. Biol. Chem. 244, 6548–6557.
[2] Scopes, R.K. (1977) Biochem. J. 161, 253–263.
[3] Scopes, R.K. (1977) Biochem. J. 161, 265–277.
[4] Westhead, E.W. (1966) Methods Enzymol. 9, 670–677.
[5] George, A.L. and Borders, C.L. (1979) Biochem. Biophys. Res. Commun. 87, 59–65.
[6] Shen, T.Y.S. and Westhead, E.W. (1973) Biochemistry 12, 3333–3337.