The Reactive Serine Residue in Phosphoglucomutase of *Micrococcus lysodeikticus*

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(Received 6 August 1973)

1. Phosphoglucomutase of *Micrococcus lysodeikticus* was labelled at the active site by exchange with ^{32}P -labelled substrates of high specific radioactivity. 2. Partial acid hydrolysis gave rise to radioactive peptides; serine phosphate was identified as one of the derivatives. 3. Comparison of the other ^{32}P -labelled peptides with the peptides obtained from the ^{32}P -labelled rabbit muscle phosphoglucomutase indicates that the sequence around the reactive serine residue is identical in both enzymes.

Phosphoglucomutase (α -D-glucose 1,6-diphosphate- α -D-glucose 1-phosphate phosphotransferase, EC 2.7.5.1) is the enzyme that catalyses the inter-conversion of glucose 1-phosphate and glucose 6-phosphate. The presence of phosphate in this enzyme was demonstrated by Jagannathan & Luck (1949), who also showed that it may readily be exchanged with phosphate in the substrates and thus that it is probably involved in the active centre of the enzyme. Later this phosphate group was shown to be covalently bound to a serine residue (Anderson & Jollès, 1957; Kennedy & Koshland, 1957). Milstein & Sanger (1961) labelled the rabbit muscle enzyme with ^{32}P by allowing it to react with ^{32}P -labelled substrates and showed that the above-mentioned serine residue was present in the sequence Thr-Ala-SerP-His-Asx. The same sequence was reported by Milstein (1961) for yeast and rat liver phosphoglucomutases and by Handler's group for the flounder muscle (Hashimoto *et al.*, 1966) and the *Escherichia coli* (Handler *et al.*, 1965) enzymes. Handler *et al.* (1965) isolated phosphoglucomutases of different species (rabbit muscle and liver, shark and flounder muscle and *E. coli*) containing variable amounts of covalently bound phosphate. On the other hand, the phosphoglucomutases of *Micrococcus lysodeikticus* and *Bacillus cereus* could not be isolated in the phosphorylated form and kinetic studies suggested that their initial-velocity patterns differed from that of the rabbit muscle enzyme (Hanabusa *et al.*, 1966; Alpers, 1969). Britton & Clarke (1972), however, studied the kinetics of phosphoglucomutase of *M. lysodeikticus* and concluded that this enzyme and that of the rabbit muscle share a common phosphoenzyme mechanism. They succeeded in labelling the enzyme with ^{32}P -labelled substrates (Birch *et al.*, 1972).

In the present work a study of the site of labelling of phosphoglucomutase of *M. lysodeikticus* was made by the technique of matching radioactive peptides, with as a comparative control rabbit muscle

phosphoglucomutase. By using this technique we have shown that this site is identical in both enzymes.

Materials and methods

The enzyme from rabbit muscle was a crystalline suspension in 2.5M-(NH₄)₂SO₄ solution, pH 5, from Sigma Chemical Co., St. Louis, Mo., U.S.A. About 1.5 mg of this enzyme was dialysed against water for approx. 3 h and then labelled by following the procedure described by Milstein & Milstein (1968). The enzyme from *M. lysodeikticus* was prepared by the method of Hanabusa *et al.* (1966) and labelled as follows. Approx. 10 units of the enzyme were incubated with 0.19 μCi of glucose 1,6- ^{32}P -diphosphate in a total volume of 0.4 ml of freshly prepared Tris buffer, pH 8.3 (100 mM-Tris-acetate, 25 mM-cysteine, 10 mM-MgCl₂) for 1 min at 30°C. The solution was immediately cooled to 0°C and applied to a Sephadex G-25 column (6 cm $\times$ 0.9 cm) which had been equilibrated with the same buffer at 4°C. The enzyme activity was measured as described by Hanabusa *et al.* (1966). The glucose 1,6- ^{32}P -diphosphate was prepared by incubating 0.2 μmol of purified glucose 1- or 6- ^{32}P -phosphate (Britton & Clarke, 1968), 1 μmol of glucose 1,6-diphosphate and 0.01 ml of a 2 mg/ml solution of rabbit muscle phosphoglucomutase for 1 h at 30°C in a final volume of 0.4 ml of histidine-Tris-MgCl₂ buffer, pH 7.4 (40 mM-histidine, 12 mM-Tris-HCl, 1.1 mM-MgCl₂). The glucose 1,6- ^{32}P -diphosphate was purified by descending chromatography on DEAE-cellulose paper (Whatman DE81) in 0.12M-NH₃, eluted with 5M-NH₃, freeze-dried and stored at -20°C.

Approx. 0.1 mg and 1 mg respectively of *M. lysodeikticus* and rabbit muscle ^{32}P -labelled phosphoglucomutase were partially hydrolysed with acid. The reaction was carried out in small test tubes stoppered with a glass bulb, with 0.5 ml of 5.7M-HCl at

Fig. 1. Electrophoretogram of partially hydrolysed ^{32}P -labelled phosphoglucomutase

The figure shows a tracing of a radioautograph obtained after electrophoresis at pH 3.5 for 1 h on Whatman 3MM paper of partial acid hydrolysates of ^{32}P -labelled phosphoglucomutase of (a) rabbit muscle and (b) *M. lysodeikticus*. Decreasing intensity: ■, ■, ■, ■, ■, ■, □, □.

100°C for 30 min. The contents of the tubes were dried in a desiccator.

The peptides were fractionated by high-voltage paper electrophoresis as described by Michl (1951). The buffer systems and coolants at pH 6.5 and 3.5 were as described by Milstein & Sanger (1961). The voltage used was 53 V/cm.

Results and discussion

The partial acid hydrolysates of rabbit muscle and *M. lysodeikticus* phosphoglucomutases were run side by side on paper electrophoresis at pH 3.5. Both preparations gave identical patterns (Fig. 1). Bands 1 and 2 are serine phosphate and glucose phosphate respectively (Milstein & Sanger, 1961). Bands 3 and 4 from each enzyme were re-run by descending chromatography in butanol-acetic acid-water-pyridine (15:3:12:10, by vol.). Each gave one radioactive spot, which by comparison with the partial acid hydrolysate of the rabbit muscle enzyme appeared to be

Ala-SerP and Thr-Ala-SerP respectively (Milstein & Sanger, 1961). Band 5 in both enzymes appeared as one band at the origin. Milstein & Sanger (1961) obtained with the rabbit muscle enzyme two radioactive bands instead of one, and this was probably due to minor differences in the pH of the electrophoretic buffers. When band 5 was subjected to electrophoresis at pH 6.5, it split into two major and two minor bands. The radioactive patterns obtained were identical in both enzymes. The two major bands were SerP-His-Asx and SerP-His (Milstein & Sanger, 1961). The latter is the strongest radioactive spot from the four spots which had not moved from the origin at pH 3.5 (see Fig. 2). The two minor bands were Thr-Ala-SerP-His-Asx and Thr-Ala-SerP-His (Milstein & Sanger, 1961). 'Fingerprints' of partial acid hydrolysates of both ^{32}P -labelled enzymes were the same and so was the relative intensity of the radioactive spots (Fig. 2).

The matching technique used indicates that the pentapeptide containing the phosphorylated serine

Fig. 2. Two-dimensional electrophoretogram of partially hydrolysed ^{32}P -labelled phosphoglucomutase

The figure shows a tracing of a radioautograph of a two-dimensional electrophoretogram (pH 3.5 and 6.5, 60 min and 50 min respectively) of a partial acid hydrolysate of ^{32}P -labelled phosphoglucomutase of (a) rabbit muscle and (b) *M. lysodeikticus*. Decreasing intensity: ■, ■, ■, □.

residue in *M. lysodeikticus* phosphoglucomutase is identical with that of the rabbit enzyme, since the dipeptides and tripeptides present in bands 3, 4 and 5, closely matching in both enzymes, include five amino acid residues. We conclude that the sequence Thr-Ala-SerP-His-Asx is common to both proteins.

- Alpers, J. B. (1969) *FEBS Symp.* **19**, 241–243
 Anderson, L. & Jollès, G. R. (1957) *Arch. Biochem. Biophys.* **70**, 121–128
 Birch, M., Britton, H. G. & Clarke, J. B. (1972) *Biochem. J.* **130**, 7P–8P
 Britton, H. G. & Clarke, J. B. (1968) *Biochem. J.* **110**, 161–175
 Britton, H. G. & Clarke, J. B. (1972) *Biochem. J.* **128**, 99P

- Hanabusa, K., Dougherty, H. W., del Rio, C., Hashimoto, T. & Handler, P. (1966) *J. Biol. Chem.* **241**, 3930–3939
 Handler, P., Hashimoto, T., Joshi, J. G., Dougherty, H. W., Hanabusa, K. & del Rio, C. (1965) *Isr. J. Med. Sci.* **1**, 1173–1186
 Hashimoto, T., del Rio, C. & Handler, P. (1966) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **25**, 408
 Jagannathan, V. & Luck, J. M. (1949) *J. Biol. Chem.* **179**, 569–575
 Kennedy, E. P. & Koshland, D. E. (1957) *J. Biol. Chem.* **228**, 419–431
 Michl, H. (1951) *Monatsh. Chem.* **82**, 489–493
 Milstein, C. (1961) *Biochem. J.* **79**, 26P
 Milstein, C. & Sanger, F. (1961) *Biochem. J.* **79**, 456–469
 Milstein, C. P. & Milstein, C. (1968) *Biochem. J.* **109**, 93–99