The amino acid sequence around the serine phosphate in phosphoglucomutase

PGM was prepared[1] and labelled with ^{32}P by two methods using the principle of the exchange of phosphate with the substrates[2]. Peptides from [^{32}P]PGM were obtained by partial acid hydrolysis (5.7 *N* HCl for 30 min in a boiling-water bath), subjected to ionophoresis (40 V/cm for 2 h in pyridine–acetate buffer, pH 3.5) and radioautographed. A sample of denatured PGM was subjected to the same labelling and subsequent treatment and used as control. From a tryptic digest of [^{32}P]PGM, a radioactive band was isolated by ionophoresis at pH 6.5. It was also hydrolysed and run in parallel with the hydrolysates described above (Fig. 1). For comparison, the pattern obtained from [^{32}P]DIP-chymotrypsin which had been subjected to exactly the same procedure is shown also. From this pattern and from one obtained by ionophoresis at pH 6.5 it can be concluded that there is no similarity[3] between

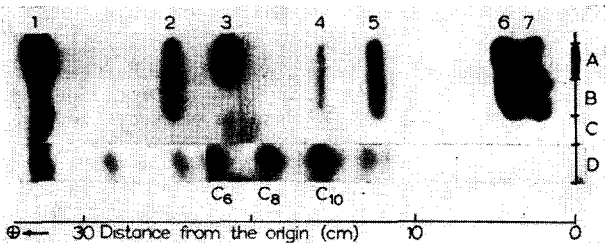

Fig. 1. Radioautograph of ionograms (Whatman No. 52 paper, pyridine–acetate buffer pH 3.5, 40 V/cm, 2 h) of partial acid hydrolysates of ^{32}P derivatives; A, [^{32}P]PGM; B, main radioactive band from a tryptic digest of [^{32}P]PGM (isolated by ionophoresis at pH 6.5); C, denatured PGM subjected to the same labelling and subsequent treatment as A; D, [^{32}P]DIP-chymotrypsin; the Asp·SerP (C6), Asp·SerP·Gly (C8), and SerP·Gly (C10) bands are indicated[5].

the sequence around the serine phosphate in PGM and the reactive serine in chymotrypsin. Both Ser^{32}P·Gly and Asp·Ser^{32}P dipeptides are absent in the partial hydrolysates of [^{32}P]PGM.

Isotopic techniques similar to those used in the studies of the sequence in proteolytic enzymes[4,5] have been used to characterise the radioactive bands present in the hydrolysate. In Fig. 1, band 1 is phosphate and band 3 runs parallel with a glucose 6-phosphate marker. Band 2 parallels a serine phosphate marker in ionophoresis at pH 2.1, 3.5, and 6.5. Finally bands 4, 5, 6 and 7 all yield band 2 after further acid hydrolysis. Bands 6 and 7 are a mixture of at least 2 and 3 components respectively, which were separated and studied further.

When the main component, present in band 7, was rehydrolysed (45 min in 5.7 *N* HCl at 100°) it gave three bands in the positions of the unchanged peptide, serine phosphate and phosphate, respectively. This suggests that it is a dipeptide. The main radioactive product after Edman degradation is phosphate, which is characteristic of peptides with N-terminal serine phosphate[4]. From mobility studies at different pH values it appears to contain two ionizable groups, each with a pK

Abbreviations: PGM, phosphoglucomutase; SerP, serine phosphate; DIP, diisopropyl phosphate residue.

between 5 and 7 (Fig. 2). One is the second acidic group of the phosphate residue and the other corresponds most probably to a basic amino acid residue. After purification of this peptide by ionophoresis at pH 3.5, 6.5 and 2.1, the radioactive spot was found to give a positive Pauly reaction. From these and other considerations to be published, the structure of the peptide is believed to be SerP·His.

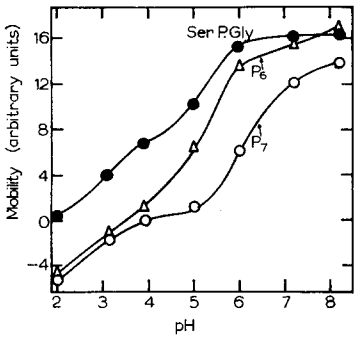

Fig. 2. Relative mobilities at different pH values of some of the main radioactive components of bands 6 and 7. For comparison the mobility of SerP·Gly is also included. Mobilities expressed relative to a standard serine phosphate marker (see ref. 5).

Finally, from partial acid rehydrolysis, Edman degradation, and mobility studies (Fig. 2) of the main component of band 6 it is suggested that it contains the tripeptide built up by addition of an acidic amino acid to the C-terminal histidine residue of the above dipeptide. Two neutral amino acids appear to precede serine phosphate in the peptide chain. The exact nature of these amino acids is under investigation.

The location of one histidine residue next to the active serine is of interest since it has been suggested that histidine is part of the active centre[6], and there are only three such residues present in the molecule[7] (mol. wt., 74,000). It has also been described as part of the active centre in several esterases and reaction mechanisms have been proposed based on the presence of a histidine residue sterically near to the reactive serine[8].

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[1] V. NAJJAR, *J. Biol. Chem.*, 175 (1948) 281.
[2] V. JAGANATHAN AND J. M. LUCK, *J. Biol. Chem.*, 179 (1949) 569.
[3] D. E. KOSHLAND, JR. AND M. J. ERWIN, *J. Am. Chem. Soc.*, 79 (1957) 2657.
[4] B. S. HARTLEY, M. A. NAUGHTON AND F. SANGER, *Biochim. Biophys. Acta*, 34 (1959) 244.
[5] M. NAUGHTON, F. SANGER, B. S. HARTLEY, D. S. SHAW, *Biochem. J.*, in the press.
[6] D. E. KOSHLAND JR., W. J. RAY AND J. M. ERWIN, *Federation Proc.*, 17 (1958) 1147.
[7] L. ANDERSON AND G. R. JOLLÈS, *Arch. Biochem. Biophys.*, 70 (1957) 121.
[8] E. A. BARNARD AND W. D. STEIN, *Advances in Enzymol.*, 20 (1958) 51.

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