Short Communications

Amino Acid Sequences around the Thiol Groups of Myokinase

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Myokinase (ATP-AMP phosphotransferase, EC 2.7.4.3) has been shown to contain two thiol groups (Benesch, Lardy & Benesch, 1955). Reaction of these thiol groups with silver (Noda & Kuby, 1957), dithiobis-(2-nitrobenzoic acid) (Kress, Bono & Noda, 1966) or alkylating reagents (Mahowald, Noltmann & Kuby, 1962) causes a complete loss of enzymic activity, which has led to the suggestion that these groups may be involved in the catalytic mechanism. However, complete reaction with mercurials (Kress *et al.* 1966) will under certain conditions produce a modified myokinase that retains up to 70% of its catalytic activity, and kinetic studies on this modified enzyme showed that the K_m for ADP was little different from that in the native enzyme. Thus the exact function of the thiol groups is obscure, but Kress *et al.* (1966) have suggested that they may be involved in maintaining the optimum active conformation of the enzyme. The present communication describes the determination of the amino acid sequences around the thiol groups of myokinase.

The thiol groups were labelled by disulphide-thiol interchange with [^{35}S]cystamine as described for light-meromyosin fraction 1 by Weeds (1967). A 42 mg. sample of myokinase was treated with 225 μmoles of [^{35}S]cystamine (3.5×10^5 counts/min./ μmole) in 0.5 M-tris-HCl buffer, pH 9.0, containing EDTA (5 mM), at 0°. After 3 min. incubation the protein had been precipitated, and guanidine hydrochloride was added to a final concentration of 5 M to solubilize the myokinase and ensure that all the thiol groups had reacted. After a further 6 hr. incubation at room temperature the solution was acidified and dialysed exhaustively against 0.2 N-acetic acid. The half-cystamine incorporation was determined by measurement of the counts/min./mg. of protein, where the concentration was assayed spectrophotometrically by using $E_{1\text{cm}}^{1\%}$ 5.29 at 279 μm (Noda & Kuby, 1957). The results showed an incorporation of $91.8 \pm 5.5 \mu\text{moles/mg.}$, which is equivalent to 1.96 ± 0.11 moles of half-cystamine/mole of myokinase. Thus the reaction appeared to be quantitative.

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The labelled protein was digested with trypsin in 0.2 M-ammonium acetate at pH 8.0 for 4 hr. at 37°, with a substrate concentration of 3 mg./ml. and a trypsin/substrate weight ratio 1:30. The digest was concentrated by rotary evaporation and then submitted to ionophoresis at pH 6.5. A strip from the ionophoretogram was submitted to further ionophoresis at pH 2 at right angles to the first dimension. Radioautography showed the presence of five bands in the first dimension, each of which gave a single spot at pH 2. These spots were cut out and their radioactivities measured in a scintillation counter. Table 1 shows the radioactivity in each spot expressed as a percentage of the total radioactivity in all the spots. The radioactivity remaining at the origin was negligible.

The paper strips containing the radioactive bands were oxidized with performic acid vapour (Brown & Hartley, 1966) to convert the basic cystamine into cysteic acid, thus increasing the number of negative charges in each peptide by 2. Further ionophoresis at pH 6.5 separated the cysteic acid peptides uniquely from underlying contaminants. The mobilities of the peptides at pH 6.5 before and after oxidation are shown in Table 1 (expressed relative to aspartic acid = 1.0; Offord, 1966). Further ionophoresis of the cysteic acid peptides at pH 3.5 showed that all the peptides were homogeneous except peptide 3, which resolved into at least three components. The amino acid compositions of the pure peptides are also given in Table 1.

There is a marked similarity between the compositions of peptides 1 and 2, and the amino acid sequence determinations show that they are related. This was also observed for peptides 4 and 5. The sequences of peptides 4 and 5 were determined by the 'dansyl'-Edman method (Gray & Hartley, 1963), but further proteolytic degradation was needed to elucidate the sequence of peptides 1 and 2. Peptide 1 was digested with pepsin, and yielded five bands on ionophoresis at pH 6.5. These are listed in Fig. 1 together with the sequence determinations, also obtained by the 'dansyl'-Edman method. Peptides 1P3 and 1P4 were recovered in much lower yields than the other three peptides, showing that the peptic cleavage after glycine was incomplete.

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