# THE AMINO ACID SEQUENCE OF TRYPTIC PEPTIDES OF SHEEP HEART PHOSPHOFRUCTOKINASE.

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## ABBREVIATIONS

| | |
|---|---|
| PFK | Phosphofructokinase |
| PITC | Phenylisothiocyanate |
| PTH | 3-phenyl-2-thiohydantoin |
| PTC | Phenylthiocarbamyl |
| DMSO | Dimethyl sulphoxide |
| TFA | Trifluoroacetic acid |
| Fr | Fraction |
| MW | Molecular weight |
| DNS | Dansyl |
| Ep (6.5) | Electrophoretic mobility at pH 6.5 |
| Ep (2.1) | Electrophoretic mobility at pH 2.1 |

ABSTRACT

Phosphofructokinase was purified from sheep heart. The sedimentation pattern of the purified enzyme was investigated over a protein concentration range 0.7 to 14.5 mg/ml. Two distinct 7 and 30 S boundaries were observed at all concentrations. A minor amount of 19 S material was also present. The 30 S boundary was asymmetric and its concentration dependence was characteristic of a polymerising system in rapid reversible equilibrium.

The dissolved crystalline enzyme usually sedimented as a single trailing 30 S boundary; the molecular weight of this component was estimated at $1.5 \times 10^6$. This value was consistent with x-ray data, which indicated unit cell dimensions of 600 x 250 x 220 Å, implying a protein weight of greater than $10^6$ daltons per asymmetric unit. In one experiment the 30 S component appeared to be undergoing a trimerisation to a 53 S form.

Sodium dodecylsulphate gel electrophoresis indicated a protomer molecular weight of 80,000 to 85,000, which was consistent with a corrected sedimentation coefficient of 3.8 S and a molecular weight of 90,000 for maleyl-phosphofructokinase, and with a corrected sedimentation coefficient of 3.9 S for the urea-dissociated enzyme.

When maleylation was carried out on carboxymethyl-phosphofructokinase in 7.5 M urea, the enzyme was further dissociated to a 40,000 molecular weight subunit. Peptide mapping of tryptic peptides; in which arginine-, histidine-, tryptophan- and tyrosine-containing peptides were located; was consistent with an 85,000 form composed of two identical subunits.

The enzyme was digested with trypsin. Forty-three different peptides were isolated using a combination of: gel filtration, ion exchange chromatography (on Dowex 50 and DE 32 cellulose), paper electrophoresis, and paper chromatography. The complete amino acid sequence was established for 38 of these peptides. The amino acid compositions (and

partial sequences) were established for the other five tryptic peptides. A summary of the amino acid sequence data obtained for the tryptic peptides is shown in Table V.

Seven carboxymethylcysteine-containing peptides were isolated in this investigation, while eight have been isolated from rabbit muscle (Coffee et al. 1973). Six of these peptides had very similar compositions between the two species. The rabbit enzyme contained two carboxymethylcysteine-containing peptides which were not found in sheep heart and the sheep enzyme contained a 20 residue peptide not found in rabbit muscle. This probably reflects genetic variation between the two species.

INDEX

LIST OF FIGURES

LIST OF TABLES