THE CARBOXYL TERMINAL SEQUENCE OF SHEEP HEART PHOSPHOFRUCTOKINASE

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ABSTRACT

The aim of this project was to investigate the carboxyl terminal sequence of sheep heart phosphofructokinase. Existing methods for the preparation of the enzyme from sheep heart proved to be unsuitable because of the insoluble nature of the purified enzyme. Consequently it was necessary to develop a suitable purification scheme before sequencing work could be commenced. Purification strategies involving magnesium ion precipitation of phosphofructokinase, DEAE-cellulose chromatography and agarose chromatography were tried before a suitable method was found.

The carboxyl terminal sequence of phosphofructokinase was investigated by the tritium labelling method of Matsuo, by carboxypeptidase Y digestion and by isolation of the carboxyl terminal peptide generated by tryptic digestion. Digestion of phosphofructokinase by carboxypeptidase Y resulted in the release of leucine, isoleucine and phenylalanine. However attempts to characterise the carboxyl terminal residue by tritium labelling, and to isolate the carboxyl terminal peptide were unsuccessful. The carboxyl terminal sequence suggested by these results and the possible amidation of the carboxyl terminal residue are discussed.

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ABBREVIATIONS

ADP	Adenosine-5$^{'}$-diphosphate
AMP	Adenosine-5$^{'}$-monophosphate
ATP	Adenosine-5$^{'}$-triphosphate
BSA	Bovine serum albumin
cAMP	Cyclic adenosine-3$^{'}$, 5$^{'}$-monophosphate
DCC	Diphenylcarbamyl chloride
DEAE	Diethylaminoethyl
EDTA	Disodium ethylenediaminetetracetate
F-1,6-bisP	Fructose-1,6-bisphosphate
F-6-P	Fructose-6-phosphate
NAD^{+}	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
PFK	Phosphofructokinase
POPOP	1,4-bis-2- (5-phenyloxazolyl) benzene
PPO	2,5-diphenyloxazole
SDS	Sodium dodecyl sulphate
TEMED	N,N,N$^{'}$,N$^{'}$-tetramethylene ethylene diamine
Tris	Tris (hydroxymethyl)amino methane