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SHEEP LIVER PHOSPHOFRUCTOKINASE:

A COMPARISON OF THE PRIMARY STRUCTURE WITH THOSE OF OTHER MAMMALIAN ISOZYMES

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requirements for the degree of Doctor of Philosophy
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ABSTRACT

Phosphofructokinase (PFK) is the key regulatory enzyme of glycolysis, catalysing the synthesis of fructose 1,6-bisphosphate from fructose 6-phosphate and ATP.

Several PFK isozymes have been identified from different tissues, including muscle, liver and platelet. Each isozyme is under the control of a different structural locus in humans the muscle isozyme is carried on chromosome 1, liver on chromosome 21, and platelet on chromosome 10.

The tetramer of PFK is the lowest active form, and there are both species and tissue differences in the composition of the tetramer. Some, such as human muscle PFK consist of four identical subunits (M_4), whereas others, such as human erythrocyte PFK comprise a five membered isozyme system, made up of both muscle and liver subunits.

The activity of PFK is modulated by a variety of effectors such as; ATP, fructose 6-phosphate, ADP, AMP and fructose 2,6-bisphosphate, as well as covalent modification, and hormonal regulation. Each PFK isozyme exhibits its own characteristic kinetic properties in response to changes in effector concentrations. This project aims to compare the primary structures of two PFK isozymes; liver and muscle, from a single species (sheep), in order to explain their different kinetic properties in terms of differences in their primary structures.

A purification procedure for sheep liver PFK was developed, and a 48% yield was obtained, with a final specific activity of 100Units/mg of protein.

Sodium dodecyl sulphate-gel electrophoresis indicated a protomer molecular weight of 84 000 for sheep heart muscle PFK, and 81 000 for sheep liver PFK, indicating that liver PFK is approximately 30 residues shorter than the muscle enzyme.

The comparison of the amino acid compositions showed a significantly lower arginine and lysine content in liver PFK compared to muscle PFK. There was also a lower threonine content in liver PFK, accompanied by an increase in the number of serine residues, compared to muscle PFK. So overall, the number of residues with

hydroxy-alkyl sidechains remained the same. The tyrosine content of liver PFK was also higher than that of muscle PFK.

The comparison of HPLC and FPLC peptide maps of liver and muscle PFK provided by digestion with CNBr and trypsin, showed a number of interesting differences between the two isozymes. Generally, the CNBr peptides of liver PFK appeared to be smaller than those of muscle PFK, while the radioactively labelled cysteine containing peptides from the tryptic digest of muscle PFK, were more hydrophobic than those from liver PFK.

Sequence information was obtained from purified peptides produced by digestion of liver PFK with CNBr, trypsin, and trypsin following maleylation of the sheep liver PFK with maleic anhydride to block the lysine residues. 87% of the amino acid sequence of sheep liver PFK was determined, and aligned with the sequences from rabbit muscle and sheep heart PFK. Approximately 70% sequence identity was observed between the liver and muscle isozymes, particularly in the regions where contacts to substrates and effectors are thought to be made. There are two regions exhibiting major sequence changes between the muscle and liver isozymes, both are thought to lie on the exterior of the molecule, and therefore would not disrupt the tertiary structure. One of these regions however, is thought to contain the ATP inhibitory site; and the large variation in sequence at this site may explain the greater susceptibility of the liver enzyme to ATP inhibition. Other, more subtle changes in the sequence may account for the additional differences in the kinetic properties displayed by muscle and liver PFK.

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LIST OF ABBREVIATIONS

ADP	adenosine 5'-diphosphate
Am Bic	ammonium bicarbonate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
βME	β-mercaptoethanol
Bs	Bacillus stearothermophilus
BSA	bovine serum albumin
Ca^{2+}-CaM	Ca^{2+}-calmodulin complex
CaM	calmodulin
cAMP	cyclic-AMP
DNA	deoxyribonucleic acid
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
DTT	dithiothreitol
Ec	E. coli
EDTA	ethylenediamine tetraacetic acid
F6P	fructose 6-phosphate
F16BP	fructose 1,6-bisphosphate

F26BP	fructose 2,6-bisphosphate
FBPase	fructose bisphosphatase
F16BPase	fructose 1,6-bisphosphatase
F26BPase	fructose 2,6-bisphosphatase
FDNB	1-fluoro-2,4-dinitrobenzene
FPLC	fast protein liquid chromatography
α-GPD	α-glycerophosphate dehydrogenase
G1P	glucose 1-phosphate
G6P	glucose 6-phosphate
G16BP	glucose 1,6-bisphosphate
HPLC	high performance liquid chromatography
MLCK	myosin light chain kinase
NADH	nicotinamide adenine dinucleotide, reduced form
PEG	polyethylene glycol-6000
PEP	phosphoenolpyruvate
PFK	phosphofructokinase
PFK-2	phosphofructokinase-2
Pi	inorganic phosphate
PMSF	phenylmethylsulphonyl fluoride

POPOP	1,4-bis[2(5-phenyloxazolyl)]benzene
PPO	2,5-diphenyloxazole
RM	rabbit muscle
SDS	sodium dodecyl sulphate
SH	sheep heart
SL	sheep liver
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TCA	tricarboxylic acid
TEMED	N,N,N',N'-tetramethylethylenediamine
Tes	N-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid
TPCK	L-1-tosylamide-2-phenylethyl chloromethyl ketone
TPI	triose phosphate isomerase
Tris	tris (hydroxymethyl) aminomethane

The single letter amino acid code is used in accordance with the IUPAC recommendations.

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