DETERMINATION OF THE N-TERMINAL SEQUENCE AND AMINO ACID COMPOSITION OF MM AND BB ISOZYMES OF PORCINE CREATINE PHOSPHOKINASE

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The amino acid sequences of enzymes with a molecular weight of over 18-20 kilodaltons are as a rule relatively independent regions both spatially and functionally. Most frequently the N-terminal region (in some cases, called a domain) consists of 20-40 amino acids, notwithstanding the fundamental structural and functional differences between those enzymes of whose structure they form a part [4]. The principal functional values of the N-terminal regions include the basic manifestation of the properties of the enzyme connected with its localization, its transport during maturation and degradation, and also its signal functions. A no less important characteristic of the N-terminal regions is their property of maintaining constant and variable zones, detectable by interspecific comparison of identical molecular forms of the enzyme, and also by intraspecific comparison of different molecular forms.

The aim of this investigation was to determine the N-terminal sequence and amino acid composition of creatine phosphokinase (CPK), which catalyzes reversible transfer of a phosphate residue from phosphocreatine to ADP and which plays a leading role in the energy metabolism of skeletal and cardiac muscles.

EXPERIMENTAL METHOD

The MM isozyme of CPK was isolated from porcine skeletal muscles by the method in [9]. The BB isozyme of CPK was isolated from porcine brain by double ion-exchange chromatography on DEAE-cellulose followed by chromatography on hydroxyapatite. Hydrolysis of 100-200 μg protein was carried out in 6N HCl for 24 h at 102.5°C in ampuls sealed under nitrogen. Sequencing of 125 nmoles of protein was carried out on a "Beckman-890C" liquid-phase sequencer, using Fast Quadrol program No. 072172.

TABLE 1. Amino Acid Composition of M and B Subunits of CPK

Amino acid	Hog		Rabbit [6]	
	M	B	M	B
Aspartic acid	30	32	28	30
Glutamic acid	27	26	27	27
Serine	20	22	22	21
Glycine	33	36	33	32
Histidine	15	13	17	14
Threonine	18	18	18	18
Alanine	14	20	13	22
Arginine	18	22	18	21
Proline	20	25	19	20
Tyrosine	10	10	10	7
Valine	26	21	28	24
Methionine	10	10	10	11
Isoleucine	14	12	14	14
Leucine	36	40	37	41
Lysine	34	18	34	20
Phenylalanine	16	16	16	18

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Scheme 1

Pro-Phe-Ser-Asn-Thr-His-Asn-Thr-Leu-Lys-Leu-Arg-Phe-Pro-Ala-Glu-Asp-Glu-Phe-Pro-Asp-Leu- (a)
Pro-Phe-Ser-Asn-Ser-His-Asn-Thr-Leu-Lys-Leu-Arg-Phe-Pro-Ala-Glu-Asp-Glu-Phe-Pro-Asp-Leu- (b)
Pro-Phe-Ser-Asn-Thr-His-Asn-(Thr)Leu(Lys)Leu(Arg)Phe(Pro)Ala(Ala-Ala-Ala)Phe-Pro-Asp-Leu- (c)
Pro-Phe-Gly-Asn-Thr-His-Asn-Lys-Tyr-Lys-Leu-Asn-Tyr-Lys-Ser-Glu-Glu-Glu-Tyr-Pro-Asp-Leu- (d)
Pro-Phe-Gly-Asn-Thr-His-Asn-Lys-Val-Lys-Leu-Asn-Tyr-Lys-Pro-Glu-Glu-Glu-Tyr-Pro-Asp-Leu- (e)
Pro-Phe-Gly-Asn-Thr-His-Asn-Lys-Tyr-Lys-Leu-Asn-Phe-Lys-Ala-Glu-Glu-Glu-Tyr-Pro-Asp-Leu- (f)
Pro-Phe-Gly-Asn-Thr-His-Asn-Lys-Phe-Lys-Leu-Asn-Tyr-Lys-Ser-Gln-Glu-Glu-Tyr-Pro-Asp-Leu- (g)
Pro-Phe-Gly-Asn-Thr-His-Asn-Lys-Phe-Lys-Leu-Asn-Tyr-Lys-Pro-Glu-Glu-Glu-Tyr-Pro-Asp-Leu- (h)
Pro-Phe-Gly-Asn-Thr-His-Asn-Lys-Phe-Lys-Leu-Asn-Tyr-Lys-Pro-Glu-Glu-Glu-Tyr-Pro- (i)
Pro-Phe-Gly-Asn-Thr-His-Asn-Lys-Phe-Lys-Leu-Asn-Tyr-Lys-Pro-Glu-Glu-Glu-Tyr-Pro-Asp-Leu- (j)

10 20

N-terminal sequences of M and B subunits of CPK. a) Rabbit B CPK [6]; b) canine B CPK [7]; c) porcine B CPK; d) rabbit M CPK [6]; e) canine M CPK [7]; f) porcine M CPK [8]; g) rat M CPK [2]; h) mouse M CPK [2]; i) monkey M CPK [3]; j) human M CPK [1].

Phenylthiohydantoin derivatives of the amino acids (in the case of sequencing) and phenylthiocarbamyl derivatives (determination of the amino acid composition) were identified quantitatively by reversed-phase high-pressure chromatography on an "Altex-324" programmed chromatograph.

EXPERIMENTAL RESULTS

The N-terminal region of the BB isozyme from porcine brain was sequenced (Table 1) and the amino acid composition of the subunits determined (Table 1) in order to analyze the N-terminal sequence of CPK. The main differences between the composition of the B and M subunits are an increase in the number of nonpolar amino acids (in particular, alanine, proline, and leucine) and a decrease in the number of positively charged amino acids. This fact is the reason for the lower electrophoretic mobility and lower hydrophilicity of the BB isozyme compared with the MM isozyme. Changes of composition of this kind are evidently expressed as the presence of definite amino acid substitutions in the primary structure, leading ultimately to increased lipophilicity of the polypeptide and to reduction of its negative charge. Similar changes have been found by comparing the compositions of M and B subunits of CPK from other sources (for example, from the rabbit [6]; see Table 1), and they characterize differences in structure of the subunits established in the course of evolution.

The results of amino acid analysis show that MM isozymes from different sources are structurally more similar than M and B subunits from the same biological object. Whereas isozymes of MM type are relatively conservative as regards size and cleavage sites, BB CPK exhibit considerable heterogeneity in the manifestation of these properties [5, 7].

Proline is the N-terminal amino acid of porcine MM and BB CPK. This fact does not contradict the data of Takasava, who demonstrated the existence of heterogeneous MM heterodimers with serine or proline as their terminal amino acid [6]. Possibly the enzyme either exhibits definite microheterogeneity within the limits of the same molecular form or it comes into contact with a hydrolase, after which the cells are destroyed in the process of isolation. A no less interesting fact is that some workers were unable to determine the N-terminal residue in rabbit MM CPK, for it was in the blocked state [3]. This can be explained on the grounds that muscle proteins with blocked N-end are normal products of biosynthesis in vivo, and that there are several stages of posttranslational modification [10].

If it is necessary to obtain polyclonal antibodies for use in the identification of one of several highly homologous isozyme sequences, the affinity of the antibodies is increased by the use of the most immunogenic (variable) part of the enzyme as the antigen, and not the whole structure of the native subunit, consisting almost entirely of conservative regions.

A no less essential condition for determination of an essential immunogenic peptide is that such a peptide must not lie in the inner region of the globular enzyme, for the antibodies will have contact chiefly with structures forming the surface of the protein globule. This particular demand is satisfied most fully by a fragment of the N-terminal structure of CPK consisting of a sequence of eight to 15 amino acid residues.

The high degree of homology (80-98%) of subunits of one type among many biological objects leads to the conclusion that this polypeptide performs more than one function. It is unlikely that kinase activity alone is the reason for such a high degree of conservatism consolidated during evolution. Another important fact is that CPK, besides its enzymic function, also plays the role of structural protein, similar in some respects with that of highly conservative molecules such as actin and myosin. Analytical comparison of the N-terminal sequences of the M and B subunits shows that products of the M and B genes are more divergent than M subunits from different species on intraspecific comparison (Scheme).

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