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N-TERMINAL SEQUENCE OF CREATINE KINASE FROM SKELETAL MUSCLE OF RABBIT AND RHESUS MONKEY

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Abstract—1. The first 20 amino acids from the N-terminus of skeletal muscle (MM) creatine kinase from both rabbit and rhesus monkey have been identified and these sequences show considerable homology.
2. Contrary to an earlier report, the N-terminus was not found to be blocked.
3. Both of these sequences show much less homology with the N-terminal sequence of heart muscle (MM) creatine kinase and no homology with that of the heart muscle mitochondrial (MiMi) isozyme.
4. No homology was found between the N-terminal sequence of the mitochondrial isozyme and the URF (unidentified reading frame) proteins of the human mitochondrial genome, indicating that the mitochondrial enzyme is encoded by nuclear genes. This suggests the possibility that an N-terminal peptide may be cleaved from the mitochondrial isozyme on its translocation across the mitochondrial membrane.

INTRODUCTION

Creatine kinase (adenosine-5′-triphosphate creatine phosphotransferase, EC 2.7.3.2) catalyses the reaction:

$$\text{creatine}^{\pm} + \text{MgATP}^{2-} \rightleftharpoons \text{MgADP}^{-} + \text{phosphocreatine}^{2-} + \text{H}^{+}.$$

Present in a wide variety of vertebrate tissues, it is particularly abundant in muscle and nerve and is generally associated with ATP regeneration in contractile and transport processes (Watts, 1973).

The enzyme was first crystallized from rabbit skeletal muscle (Kuby *et al.*, 1954) and shown to comprise two identical sub-units (MM) of total mol. wt 82,600 (Olson and Kuby, 1964; Yue *et al.*, 1967). In addition to this muscle type isozyme (MM), two other electrophoretically distinguishable forms, the brain type (BB) and hybrid (MB), were originally discovered and characterized (Eppenberger *et al.*, 1964). Subsequently, a fourth type of creatine kinase was found in mitochondria (Jacobs *et al.*, 1964; Jacobus and Lehninger, 1973) and this appears to comprise two identical sub-units (MiMi) distinct from the muscle and brain subunit types (Blum *et al.*, 1983).

Surprisingly little information has been reported regarding the primary structure of the enzyme (Watts, 1973; Kenyon and Reed, 1983) and this has largely been directed at a short sequence containing the reactive thiol group. This particular sequence of some 13 amino acids has been determined in creatine kinase from rabbit muscle (Thomson *et al.*, 1964), human and ox muscle (Thomson *et al.*, 1968), normal and dystrophic human muscle (Palmieri *et al.*, 1971), normal and dystrophic chicken breast muscle (Roy, 1974) and rabbit and ox brain (Atherton *et al.*, 1970a, 1970b) and is almost identical in all cases. The comparable sequences in lobster arginine kinase (Der Terrosian *et al.*, 1969) and in earthworm lombricine kinase (Der Terrosian *et al.*, 1971) are also very similar.

The C-terminal dipeptide of the rabbit muscle enzyme has been identified using carboxypeptidase and its removal does not affect catalytic activity (Olson and Kuby, 1964).

Recently Blum *et al.* (1983) have sequenced the first 11 amino acids of the human heart mitochondrial isozyme (MiMi) and the first 15 amino acids of the human heart muscle (MM) isozyme. Otherwise the primary structure has not received further attention. In contrast to Blum *et al.* (1983), Mahowald and Kuby (1960) reported that they were unable to identify the N-terminal amino acid by either the fluorodinitrobenzene or phenylisothiocyanate procedures employing both native and guanidine-denatured enzyme, implying that the N-terminus was modified. In the present study we did not find this to be the case and we now report the sequence of the first 20 amino acids of creatine kinase from both rabbit (*Oxycytolagus cunniculus*) and rhesus macaque monkey (*Macaca mulatta*) skeletal muscle, which we compare with the sequences of Blum *et al.* (1983).

MATERIALS AND METHODS

Creatine kinase from rabbit skeletal muscle was prepared by method B of Kuby *et al.* (1954) using AnalaR ethanol treated as described by Nihei *et al.* (1961).

Creatine kinase from rhesus monkey skeletal muscle was prepared by the method of Chegwidden and Watts (1975) followed by further purification on a Sephadex G-75 column in 0.002 M Tris–Cl buffer, pH 8.3.

Sequence analysis was performed using a Beckman Model 890B automatic sequencer as described by Henriksson *et al.* (1980). The PTH derivatives were identified by HPLC (Kageoka *et al.*, 1981) with the exception of PTH histidine which was identified by Pauly's diazo method (Niall, 1974).

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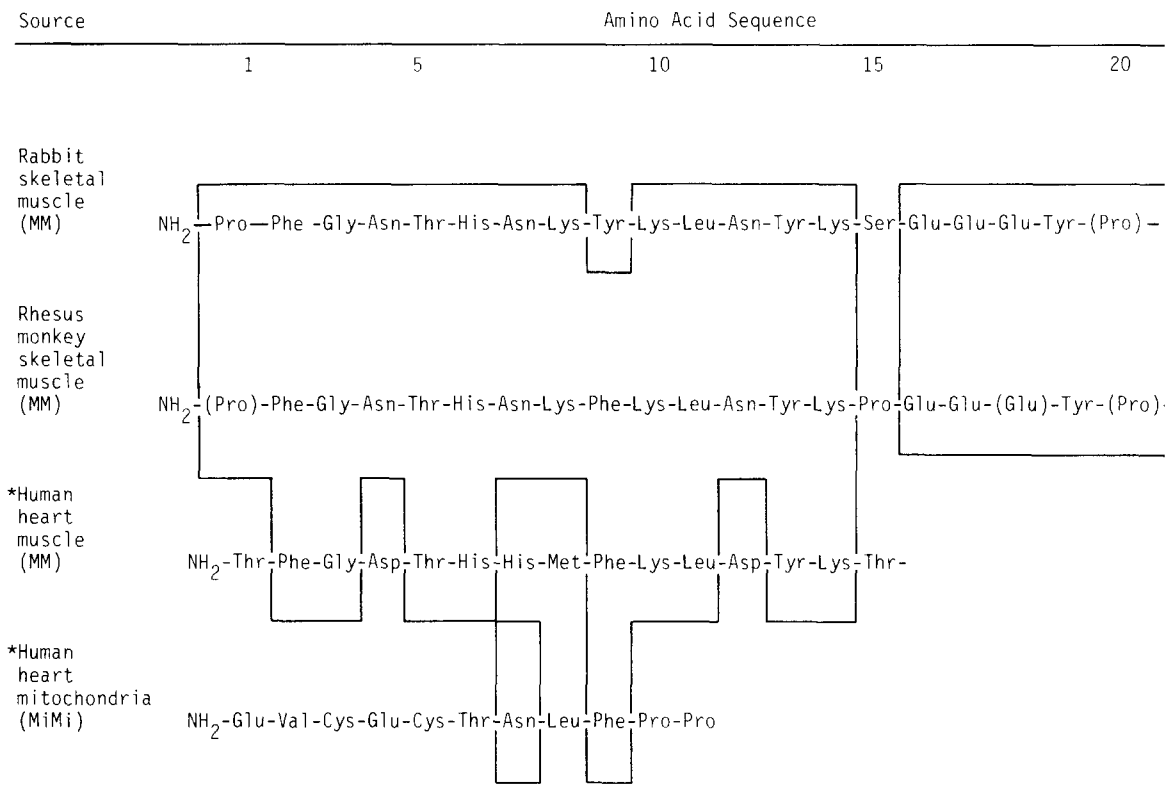

Fig. 1. N-terminal amino acid sequences of rabbit, rhesus monkey and human creatine kinase isozymes. Residues in parentheses are tentative. Identical residues are boxed. *From Blum *et al.* (1983).

RESULTS AND DISCUSSION

The first 20 amino acids of skeletal muscle (MM) creatine kinase from both rabbit and rhesus monkey have been identified. In Fig. 1 these sequences are compared with the N-terminal sequences of the human heart muscle (MM) and human heart mitochondrial (MiMi) isozymes recently reported by Blum *et al.* (1983).

Considerable homology is evident between the rabbit and rhesus monkey sequences, with only two amino acid changes, each requiring only one nucleotide base mutation, among the 20 residues identified. Positions 9 and 15, occupied by tyrosine and serine respectively in the rabbit enzyme, are occupied by phenylalanine and proline in the rhesus monkey enzyme (Fig. 1).

Comparison of our rhesus monkey muscle sequence with the human heart muscle sequence, which may be expected to the more closely related to each other than either is to the rabbit, reveals a surprisingly different picture with six amino acid differences among the 15 residues sequenced in both. Four of these apparent differences are perhaps open to some question. In the present study, positions 4 and 10 of both the rabbit and rhesus monkey enzymes were found to be occupied by asparagine (Fig. 1). The aspartic acid found in both these positions in the human muscle enzyme by Blum *et al.* (1983) may be an artefact due to deamidation (cf. Niall, 1974). Also, no mention is made by Blum *et al.* (1983) of their method of identification of the PTH derivatives. If they employed gas chromatography then discrimination between threonine and proline can be very difficult by this method, which may well explain the apparent differences at positions 1 and 15 (Fig. 1).

The N-terminal sequence of mitochondrial creatine kinase (MiMi) from human heart muscle is completely different from the sequence in the muscle type (MM) isozymes, with the exception of position 9 which is occupied by phenylalanine in all but the rabbit muscle isozyme and position 7 which is occupied by asparagine in all but the human heart muscle isozyme. Furthermore, four of the amino acid differences in the mitochondrial enzyme, among the 11 residues sequenced, would require at least two base changes. Williamson *et al.* (1977) have identified two different components of rabbit muscle creatine kinase of slightly different size, differing in length by about 15 amino acids. It is worth noting, however, that the mitochondrial sequence does not match any part of the first 15 amino acids of the human heart muscle or the first 20 of the rhesus monkey muscle isozyme.

There are several plausible explanations for these differences in sequence. Perhaps through a long period of evolutionary divergence, one isozyme has emerged with a relatively truncated terminus. Alternatively, differential proteolysis of the mitochondrial and muscle isozymes may occur, either as an artefact of isolation or as a normal post-translational modification.

No region of homology was found between the N-terminal sequence of the mitochondrial creatine

kinase and the URF (unidentified reading frame) proteins of the human mitochondrial genome sequenced by Anderson *et al.*, (1981), which indicates that the mitochondrial isozyme is encoded by nuclear genes and post-translationally transported into the mitochondria. In such cases, cleavage of a "signal" peptide commonly occurs on translocation across the mitochondrial membrane (Henning and Neuport, 1983; Suominen and Mäntsälä, 1983), although this has been shown not to be so in the case of cytochrome *c* (Hennig *et al.*, 1983) which enters the mitochondria intact.

The N-terminal residue of rabbit muscle creatine kinase was unambiguously identified as proline (Fig. 1) and although position 1 in the rhesus monkey enzyme, uniquely amongst those identified, showed an elevated level of several PTH derivatives, the most pronounced elevation was also in PTH proline. These findings, however, are not necessarily inconsistent with those of Mahowald and Kuby (1960) who were unable to identify an N-terminal amino acid. Quite possibly the enzyme may be synthesized with a blocked N-terminus, the free N-terminal amino acid observed in the present study being produced by subsequent exposure to a hydrolase after the cells were disrupted in the isolation procedure. It is certainly a tenable hypothesis that muscle proteins with blocked N-termini are the normal *in vivo* products of biosynthesis and several stages of post-translational modification may well occur (Wold, 1984). The identification by Williamson *et al.* (1977) of the two different components of rabbit muscle creatine kinase may possibly be related to this type of process. Trangott and Massaro (1973), using starch gel electrophoresis, have also detected several sub-bands of M-type sub-units, which still persisted following reversible urea denaturation indicating more than conformational differences. Undoubtedly the extended use of nucleotide sequencing techniques, in conjunction with N-terminal amino acid sequencing such as that presented here, will play a major part in resolving this general question (Wold, 1984).

Note added in proof

Subsequent to the acceptance of this paper for publication the cDNA sequence of rabbit muscle creatine kinase, with translated polypeptide sequence, has been published (Putney *et al.*, 1984). These data confirm the results presented here.

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