AMINO-TERMINAL SEQUENCE OF RABBIT MUSCLE PHOSPHORYLASE

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1. Introduction

Rabbit muscle phosphorylase b contains two identical polypeptide chains, each having a mol. wt of 100 000 and a blocked α-amino group [1]. Saari and Fischer [2] isolated 19 fragments by cleaving the enzyme with cyanogen bromide. One of these, an 89-residue fragment designated R-CB-14, had a blocked α-amino group and hence might represent the amino-terminus of the protein. When phosphorylase b is converted to active phosphorylase a, a seryl residue is phosphorylated in a tetradecapeptide sequence [3]. Fragmentation of [^{32}P]phosphorylase a with CNBr revealed that the fragment R-CB-14 contains the site of phosphorylation [2]. The present communication describes both the nature of the amino-terminal blocking group and the amino acid sequence from the amino terminus through the phosphorylation site. This sequence is compared with the corresponding sequence in dogfish phosphorylase [4]

2. Materials and methods

Fragment R-CB-14 was isolated from *S*-carboxymethyl phorylase b as described by Saari and Fischer [2]. The lack of a free α-amino group was confirmed on a Beckman Sequencer, The fragment (5 μmol) was digested with 2 mg of 'TPCK-trypsin' (Worthington Biochemical Corp.) at 37°C in 0.1 M NH_4HCO_3 (pH 8.0) for 15 hr. The lyophilized digest was fractionated on a column (2.0 × 20 cm) of Dowex 50-X8 using a double linear gradient of pyridine–acetate buffers [5]. Further purification was performed on a column (0.9 × 50 cm) of Dowex 1-X2 using a four-chamber gradient of pyridine–acetate buffers [6].

3. Results

Among fifteen peptides isolated, twelve contained free, and three blocked α-amino groups. The composition of one of these blocked peptides (T-II) differed from one of the unblocked peptides (T-VIII-1) by a single lysyl residue (table 1). Since the extra lysyl residue of T-VIII-1 was part of the sequence Lys–Glx–(Ile, Ser, Val, Arg), it is assumed that peptide T-II lacked that lysyl residue and contained an amino terminal pyrrolidone carboxyl residue derived from glutamine. Thus T-II cannot represent the amino-terminus of R-CB-14. The other two blocked peptides (T-IV and T-VIII-2 in table 1) are also related to each other in composition by an extra arginyl residue found in T-VIII-2. Carboxyl-terminal analysis with carboxypeptidase B yielded lysine from T-IV but arginine and lysine from T-VIII-2. Since it is known that trypsin will cleave bonds adjacent to either but not both basic residues in a –Lys–Arg– sequence, it is suggested that these two peptides are related to each other as shown in fig.1.

Hydrazinolysis of each of these two peptides yielded acetyl hydrazide, which was identified by thin-layer chromatography [7]. It appears that T-IV, T-VIII-2 as well as fragment R-CB-14 contain an α-*N*-acetyl group. This conclusion is consistent with the finding that the amino terminus of dogfish phosphorylase also contains this blocking group [4].

The amino acid sequence of peptide T-IV was derived from fragments generated by chymotryptic digestion. Two peptides (T-IV-C-1 and T-IV-C-2) were isolated by chromatography on Dowex 50-X2 using a linear gradient of pyridine–acetate buffers [8]. The sum of their compositions was equal to that of the parent peptide T-IV (table 1). One peptide, T-IV-C-1.

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Table 1
Compositions of peptides isolated from the amino-terminal region of R-CB-14[a]

	Tryptic digest of R-CB-14						Chymotryptic sub-digest of T-IV		Chymotryptic digest of R-CB-14		
Amino acid	T-11	T-IV	T-VIII-1	T-VIII-2	T-XI	T-XVI-1	T-IV-C-1	T-IV-C-2	C-III	C-XIV-1	C-XV-1
Asx		1.13		1.14				1.09	0.14	1.00	0.27
Ser	0.90	1.76	0.84	2.12			0.76	0.92	0.93	0.88	1.09
Glx	1.21	1.99	1.15	2.28				2.13	0.17	2.96	0.95
Pro	0.12	1.14		0.88			1.12		0.90		0.10
Gly	0.24										1.18
Ala									0.12		
Val	1.00		1.00								1.00
Ile	0.92		1.08								0.98
Leu		1.00		1.00			086		1.00		1.18
Lys	0.24	0.99	1.02	1.24		1.00		1.00		2.12	0.48
Arg	1.01	0.97	1.08	2.00	1.00		0.76		1.05	0.98	1.36
No. of residues	5	9	6	10	1	1	4	5	4	8	6
% Yield	38	68	4	3	45	58	41	23	55	10	15

[a]Values are expressed as molar ratio on the basis of one mole of the residue underlined. Values smaller than 0.1 are omitted.

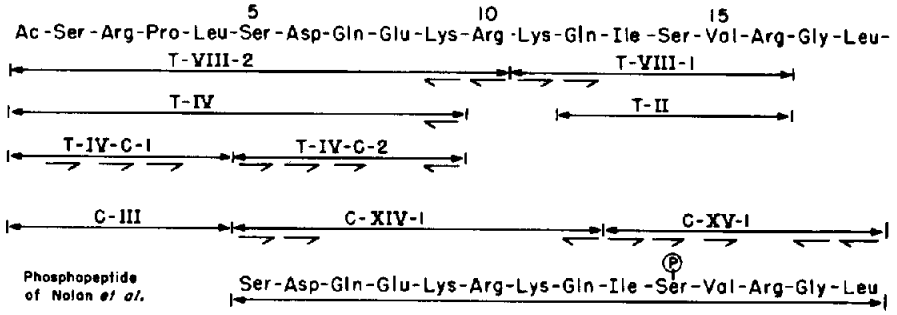

Fig.1. Amino terminal sequence of rabbit muscle phosphorylase. Arrows under tryptic (T) or chymotryptic (C) peptides (table 1) denote subtractive Edman degradation [9] (——›) or carboxypeptidase digestion (‹——). The amide distribution among residues 6–8 is taken from Nolan et al. [3]. Peptide T-IV-C-1 was deacylated before Edman degradation (see text). Free lysine and free arginine from residues 10 and 11 were obtained in 58 and 45% yield respectively (table 1). Peptide IV-A of Nolan et al. [3] was *O*-phosphorylated as indicated (P).

had a blocked α-amino group which was removed by brief treatment with 6 N HCl (5 min, 100°C). The sequences of T-IV-C-1 and T-IV-C-2 are also given in fig.1.

Comparison of the sequence of T-IV (Ac–Ser–Arg–Pro–Leu–Ser–Asx–Glx–Glx–Lys) with that of the phosphorylated peptide of Nolan et al. [3] reveals a 5 residue overlap, to support the alignment illustrated in fig.1. Thus the phosphorylation site is identified as Serine-14.

4. Discussion

The relationship between the amino terminal sequence of phophorylase and the sequence of the phosphorylated peptide was confirmed by the characterization of three peptides derived from a chymotryptic digest of R-CB-14 (fig.1). One peptide (C-III) was identical to T-IV-C-1; another (C-XIV-1) extended the sequence of T-IV-C-2 to include residues 10–12 (fig.1); the third (C-XV-1) was identical in composition and sequence with the carboxyl terminus of the phosphorylated peptide.

The derived sequence is compared in fig.2 with the amino terminal sequence of dogfish phosphorylase. Although neither sequence can be considered to be rigorously proven until the complete sequences of the two molecules are known, the alignments are consistent with all data at present available. The apparent similarity of the two structures not only places the site of phosphorylation at residue 14 in the 865 amino acid sequence of the polypeptide chain of rabbit phosphorylase, but also indicates a striking similarity in the amino-terminal sequences of the rabbit and dogfish enzymes.

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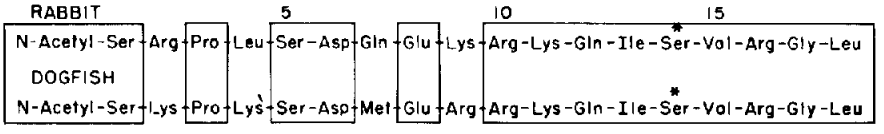

Fig.2. Comparison of the proposed amino terminal sequences of phosphorylases from rabbit and dogfish [4] muscle. The sites of phosphorylation [3,4] are indicated by asterisks. The dogfish sequence was constructed by aligning the amino-terminal heptapeptide D-CB-A, of Cohen et al. [4] and the phosphopeptide (residues 8–18) derived from their fragment D-CB-14.

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