N-TERMINAL SEQUENCES OF *ESCHERICHIA COLI* AND POTATO PHOSPHORYLASE

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1. Introduction

All α-glucan phosphorylases (EC 2.4.1.1) isolated from bacteria, yeast, plants and different animals are active as dimers and contain one pyridoxal-5′-phosphate group/subunit [1]. The cofactor essential for activity is bound to a sequence, which was conserved in the course of evolution from bacteria to mammals [2]. The subunit molecular weight was reported as 81 000 for *Escherichia coli* [2], 97 412 for rabbit muscle [3] and 103 500 for potato tuber phosphorylases [4]. Despite structural similarities phosphorylases are subject to different modes of regulation. In bacteria, phosphorylase activity is controlled by induction [5–8], the yeast enzyme is stimulated by phosphorylation [9] and animal phosphorylases are regulated allosterically by binding specific effectors or by covalent modification by means of phosphorylation near the N-terminus [1]. Only in the case of plant phosphorylases no regulation has as yet been found [4].

The availability of homogenous N-terminally unblocked *E. coli* and potato phosphorylases permitted a direct comparison of the N-terminal sequence of these post-translationally non-regulated enzymes with published sequences of the enzymes from animal sources. This work therefore reports to what extent prokaryotic and plant phosphorylases differ in the first 15 and 19 amino acids, respectively, including serine 14, whose phosphorylation is known to modulate the activity of animal phosphorylases.

2. Materials and methods

Escherichia coli maltodextrin phosphorylase was isolated as in [2]. Potato phosphorylase was prepared as in [10].

Sequence analysis was performed by automatic solid-phase Edman degradation [11] of protein attached to isothiocyanate–glass beads [12]. Phosphorylase (8 mg) was desalted over Sephadex G-25 in 10 mM sodium-phosphate buffer (pH 7.5) to remove any remaining ammonium salts. The protein fractions were kept at 40°C and concentrated under a stream of N_2 to ~1 ml. Isothiocyanate–glass beads (75 Å pore size, 125 mg) were added, the pH was raised to 9.6 by addition of triethylamine and the suspension was rotated for 1 h at 40°C. The glass beads were washed by centrifugation with 6 M guanidine–HCl until A_{280} of the supernatant was <0.4. The yield of the protein attached was ~64%. Washing was repeated 3 times with water, 2 times with methanol and 2 times with diethylether. The carrier was diluted in the column (200 × 3 mm) of the sequencer with ~500 mg inert glass beads. Higher concentrations of phosphorylase blocked the column during cleavage with trifluoroacetic acid. An automatic solid-phase sequencer was used (Sequemat Inc., Watertown, MA, model 12 S). Amino acid >PhNCS were identified by thin-layer and high performance liquid chromatography as in [13]. Arg and His >PhNCS were determined directly after conversion in the liquid chromatograph.

3. Results and discussion

A comparison of the N-terminal sequences of *E. coli* maltodextrin phosphorylase and potato phosphorylase with the sequences of phosphorylase from rabbit muscle [3], rat muscle [14], human muscle [15], rabbit liver [16], dog fish [17] and yeast [9] is presented in table 1. In contrast to the well conserved

Table 1
Comparison of the N-terminal sequence of phosphorylases

	Sequence (positions 1, 5, 10, 15)
E.coli	H -Ser-Gln-Pro-Ile-Phe-Asn-Asp-Lys-Gln-Phe-Gln-Glu-Ala-Leu-Ser-Arg-Gln-Trp-Gln-
Potato	H -Thr-Leu-Ser-Glu-Lys-Ile-His-His-Pro-Ile-Thr-Glu-Gln- X -Gly-
Rabbit muscle [3]	(P) over Ser: Ac-Ser-Arg-Pro-Leu-Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu-Ala-
Rat muscle [14]	(P) over Ser: - Ser-Asp-Gln-Asp-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu-
Human muscle [15]	(P) over Ser: - Lys-Gln-Ile-Ser-Val-Arg-
Rabbit liver [16]	(P) over Ser: - Arg-Gln-Ile-Ser-Ile-Arg-
Dogfish muscle [17]	(P) over Ser: Ac-Ser-Lys-Pro-Lys-Ser-Asp-Met-Glu-Arg-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu–
Yeast [9]	(P) over Thr: – Leu-Thr-Gly-Phe-Leu-Pro-Gln-

pyridoxal-5′-phosphate binding region, the N-terminus of the phosphorylases studied is much more variable.

(i) The non-regulated phosphorylases have a free α-amino group in contrast to all the other phosphorylases studied so far, which have a blocked N-terminus.

(ii) The first amino acid is a hydroxy-amino acid: Thr in potato phosphorylase and Ser in all other cases.

(iii) The non-regulated enzymes show considerable differences with animal and probably also with yeast phosphorylases (assuming that the phosphorylated yeast sequence is at the N-terminus). Not considering the N-terminal Thr, the potato phosphorylase sequence is completely different. A plausible explanation would be that the potato enzyme which has the largest M_r has an elongated N-terminal region.

(iv) *Escherichia coli* maltodextrin phosphorylase shows by direct comparison identity with animal phosphorylases only in position 1,3 and 16. Moreover, we have searched for a more general homology allowing for insertions, deletions and conservative exchanges using the relative substitution frequency factors [18]. Table 2 gives a matrix for the N-terminal sequence of *E. coli* and rabbit muscle phosphorylase. Scores for every possible pair of amino acids are listed according to [18].

The score for identity is 8, 6 indicates a pair of amino acids which are frequently exchanged in homologeous proteins, 0 is the value for a rare exchange, 3 is the average exchange frequency and 5,4,2,1 occupy intermediate scores of the scale. Based on these criteria for the first 4 residues the homology is unequivocal. A score of 26 is reached, compared with 32 for 4 identical residues, i.e., a ratio of 0.81. Scoring all possible pairs of the matrix of these 4 N-terminal amino acids as a measure for random sequences of the same composition yields 56/128 = 0.44 or 44% of the maximal score.

Extending the direct comparison from position 5–19 a score of 51/123 = 0.41 is reached. This is nearly the score for random sequences of the same composition 724/1845 = 0.39. Deletion of position 5 in the rabbit muscle enzyme yields a similar ratio of

Table 2
Comparison of the N-terminal sequence of *E. coli* maltodextrin phosphorylase and rabbit muscle phosphorylase according to [18]

	1 Ser	Gln	Pro	Ile	5 Phe	Asn	Asp	Lys	Gln	10 Phe	Gln	Glu	Ala	Leu	15 Ser	Arg	Gln	Trp	Gln (E.coli)
1 Ser	8	4	3	2	2	5	3	3	4	2	4	4	4	2	8	4	4	3	4
Arg	4	5	3	1	1	3	1	5	5	1	5	3	2	2	4	8	5	3	5
Pro	3	3	8	1	1	1	3	3	3	1	3	4	4	1	3	3	3	0	3
Leu	2	3	1	5	5	1	1	2	3	5	3	1	2	8	2	2	3	3	3
5 Ser	8	4	3	2	2	5	3	3	4	2	4	4	4	2	8	4	4	3	4
Asp	3	4	3	0	1	5	8	3	4	1	4	5	3	1	3	1	4	0	4
Gln	4	8	3	0	0	4	4	4	8	0	8	5	3	3	4	5	8	2	8
Glu	4	5	4	1	0	4	5	4	5	0	5	8	4	1	4	3	5	1	5
Lys	3	4	3	1	0	4	3	8	4	0	4	4	3	2	3	5	4	1	4
10 Arg	4	5	3	1	1	3	1	5	5	1	5	3	2	2	4	8	5	3	5
Lys	3	4	3	1	0	4	3	8	4	0	4	4	3	2	3	5	4	1	4
Gln	4	8	3	0	0	4	4	4	8	0	8	5	3	3	4	5	8	2	8
Ile	2	0	1	8	3	1	0	1	0	3	0	1	2	5	2	1	0	3	0
Ser	8	4	3	2	2	5	3	3	4	2	4	4	4	2	8	4	4	3	4
15 Val	2	2	2	5	3	1	1	2	2	3	2	2	3	5	2	2	2	2	2
Arg	4	5	3	1	1	3	1	5	5	1	5	3	2	2	4	8	5	3	5
Gly	3	2	3	1	0	3	3	3	2	0	2	3	3	1	3	3	2	1	2
Leu	2	3	1	5	5	1	1	2	3	5	3	1	2	8	2	2	3	3	3
Ala	4	3	4	2	1	3	3	3	[illegible]	1	3	4	8	2	4	2	3	1	3

52/123 = 0.42. In this comparison the similarity of Asn–Asp–Lys–Gln–X–Gln in the prokaryotic and Gln–Glu–Lys–Arg–X–Gln in the eukaryotic enzyme becomes apparent with a value of 30/40 = 0.75. The highest score in the sequence comparison from position 5 onwards to 19 is obtained when position 5 of the *E. coli* enzyme is deleted: 58/114 = 0.51. Realignment after deletion matches the phosphorylated Ile–Ser–Val–Arg sequence of the rabbit muscle phosphorylase with Leu–Ser–Arg of the *E. coli* enzyme, which is not a substrate for mammalian phosphorylase kinase [19]. Differences in the neighbourhood of Ser 15 are large enough to prevent phosphorylation by phosphorylase kinase. As shown in [20] substitution of any of the residues surrounding Ser 14 in rabbit muscle phosphorylase strongly decreases phosphorylase kinase activity. Phosphorylation of *E. coli* phosphorylase with endogenous [21] or other protein kinases have not been performed so far.

In conclusion, these results indicate that by gradual mutations a regulatory function can be added to a catalytically active protein in the course of evolution. This makes the suggestion that the regulatory properties located in the N-terminal region of the mammalian phosphorylases result from fusion of a regula-

tory and a catalytic ancestral gene [22] less likely.

The differences in size of prokaryotic and mammalian phosphorylases can not be explained by an additional N-terminal sequence. These differences may reflect insertions and deletions in other parts of the molecule, e.g., the binding site for glycogen, which is not documented for the prokaryotic enzyme. We hope, that the N-terminal sequence of *E. coli* phosphorylase reported here might help to locate the start of the *malP* gene, the structural gene for phosphorylase in the *E. coli* maltose regulon [6].

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