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DEPHOSPHORYLATION OF ^{32}P-TETRADECAPEPTIDE DERIVED FROM PHOSPHORYLASE a SHOWS LIGAND SENSITIVITY WITH PHOSPHORYLASE b'

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SUMMARY : The dephosphorylation of ^{32}P-tetradecapeptide derived from phosphorylase a is described. The rate of enzymatic dephosphorylation by phosphatase is inhibited in the presence of phosphorylase b. Phosphorylase b', a modified form of phosphorylase a in which the phosphorylated site has been removed by tryptic attack, can increase the liberation of ^{32}P from the tetradecapeptide. The dephosphorylation also shows ligand sensitivity in the presence of phosphorylase b'. AMP is inhibitory, glucose and glucose 6-P can abolish the AMP inhibition. The complex of phosphorylase b' and tetradecapeptide could form also a tetramer supported by dephosphorylation studies at 15°.

INTRODUCTION

Phosphoprotein phosphatase catalyzes the dephosphorylation of various phosphoproteins. The dephosphorylation reactions are important in the regulation of glycogen metabolism. The activity of phosphorylase (EC 2.4.1.1), key enzyme of glycogen degradation, is dependent whether Ser-14 is phosphorylated (a form) or dephosphorylated (b form). The dephosphorylation of phosphorylase a depends upon a variety of mechanisms controlled by ligands and proteins (for review, see 1). They can influence the rate of dephosphorylation with the modification of phosphorylase a or phosphatase. A phosphotetradecapeptide derived from phosphorylase a and containing the phosphorylated Ser-14 proved to be a useful tool in determining the exact mechanism of control. It has been demonstrated that the peptide could be dephosphorylated by phos-

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phatase (2) and lost the ligand sensitivity with AMP, glucose and glucose 6-P (2-4). Thus the ligands are unable to modify the peptide.

In this communication we report results which show that the phosphorylated tetradecapeptide can gain ligand sensitivity in the presence of phosphorylase b'.

MATERIALS AND METHODS

Crystalline phosphorylase b was prepared from rabbit skeletal muscle according to the procedure of Fischer and Krebs (5). ^{32}P-labelled phosphorylase a was prepared using ^{32}P-ATP (Isotope Institute, Hungary) with purified phosphorylase kinase (6). Phosphorylase b' was prepared from AMP-free phosphorylase a (7). The specific activity of the preparate was 38-40 U/mg. Phosphorylase phosphatase was prepared from rabbit liver (8) and stored in 50 % glycerol at -20°. Glycerol was removed by dialysis prior use. ^{32}P-tetradecapeptide was isolated after digestion of ^{32}P-phosphorylase a by chymotrypsin (2,3). The specific radioactivity of the purified peptide was 160 μCi/mole. Amino acid composition of the peptide was also checked by amino acid analysis.

Phosphatase reactions were carried out at 30° or 15° in 40 mM MES (4-morpholino-ethanesulfonic acid) buffer (pH 7.0) containing 1 mM EDTA, 0.5 mM dithiothreitol, 0.8 mM tetradecapeptide, 0.03 mM phosphorylase a, b or b' and phosphatase of various concentrations. The reaction was initiated by addition of phosphatase. Aliquots of the reaction mixture were withdrawn, stopped and assayed for radioactivity released from ^{32}P-phosphorylase a as described previously (6). The dephosphorylation of ^{32}P-tetradecapeptide was followed as described by Nolan et al (2) with the modification of Tessmer and Graves (9). The ^{32}P radioactivity of the aliquots was detected by scintillation counting in Bray's solution.

RESULTS

At 30°, the dephosphorylation of ^{32}P-tetradecapeptide by phosphatase was inhibited in the presence of phosphorylase b, whereas phosphorylase a did not alter the reaction rate. Phosphorylase a was inhibitory only after its dephosphorylation, i.e. when phosphorylase b was formed (Fig. 1A). The presence of peptide did not influence the inactivation of phosphorylase a although both are substrates of phosphatase (Fig. 1B). Since the tetradecapeptide derived from the NH_2-terminal region of phosphorylase a, we also checked the effect of phosphorylase b', a form of

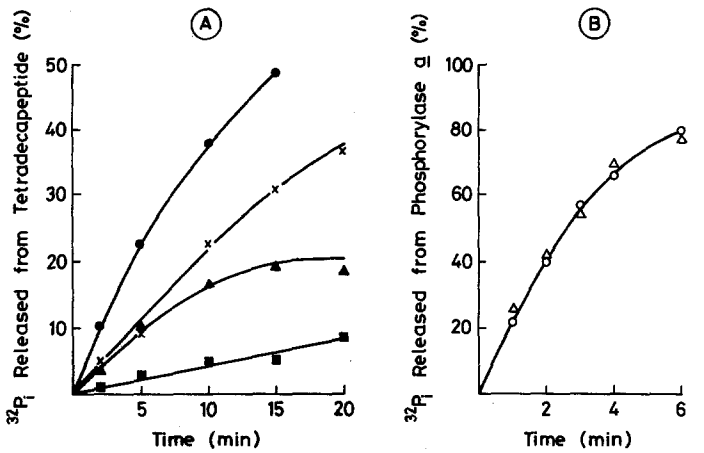

Figure 1. Effect of phosphorylase a, b and b' on the dephosphorylation of ^{32}P-tetradecapeptide at 30°. Phosphatase reaction was carried out with 3.10^{-8} M phosphatase. Dephosphorylation of phosphopeptide (A) in the absence (x) and presence of phosphorylase a (▲); b (■) and b' (●); dephosphorylation of phosphorylase a (B) in the absence (c) and presence (△) of phosphopeptide.

the enzyme in which this region has been removed. As shown, addition of phosphorylase b' to phosphopeptide increased the rate of dephosphorylation (Fig. 1A).

Influence of ligands on the dephosphorylation of peptide was also investigated. AMP, a potent inhibitor of phosphorylase a inactivation (2,3), was ineffective even in 1 mM concentration. Neither glucose nor glucose 6-P did change the reaction rate. However, these ligands could influence the dephosphorylation of peptide in the presence of phosphorylase b' (Table 1). Addition of AMP caused a significant inhibition and glucose or glucose 6-P suspended AMP inhibition.

When the phosphatase reaction was run at 15°, the dephosphorylation of peptide decreased in the presence of phosphorylase b' (Fig. 2). The effect of glucose was more apparent at 15°. Glucose accelerated the process in such a great extent to outrun considerably the reaction rate observed in the absence of phosphorylase b'.

DISCUSSION

The dephosphorylation of ^{32}P-tetradecapeptide by phosphatase was stimulated by phosphorylase b' at 30° and inhibited at 15°.

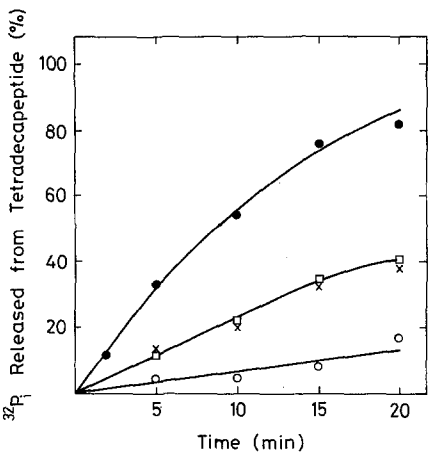

Figure 2. Dephosphorylation of ^{32}P-tetradecapeptide at 15°. Dephosphorylation of phosphopeptide was carried out with 5.10^{-7} M phosphatase in the absence of effectors (x) and in the presence of 30 mM glucose (□), phosphorylase b' (o), phosphorylase b' + 30 mM glucose (●).

The dual effect of phosphorylase b' could be explained as follows. Phosphorylase b' lacks that peptide region surrounding Ser-14 (7). The phosphopeptide can bind to this region of phosphorylase b' resulting in a pseudo-phosphorylase a (10,11). The aforementioned complex promotes the dephosphorylation of peptide at 30° (Fig. 1). This complex also exists in an equilibrium between dimeric and tetrameric forms characteristic of phosphorylase a. This was supported with the experiments carried out at 15°. It is known that phosphorylase a forms a tetramer at lower temperature and phosphatase can dephosphorylate only the dimeric form (12,13). At 15° the tetradecapeptide and phosphorylase b' occur as a tetramer decreasing the rate of dephosphorylation. Glucose can stimulate the inactivation of phosphorylase a at lower temperature causing the dissociation of tetrameric a (14). Thus the stimulatory effect of glucose (Fig. 2) could be attributed to the dissociation of tetrameric peptide-phosphorylase b' complex into dimeric ones.

The inhibitory effect of phosphorylase b on the dephosphorylation of peptide is connected with the motility of NH_2-terminal part of phosphorylases. The NH_2-terminal part (18-20 amino acids containing Ser-14) of phosphorylase b is motile as judged by X-ray crystallography. The phosphorylation of Ser-14, i.e. the formation of phosphorylase a, fixes the NH_2-terminal tail (15,16). This structural difference between phosphorylase b and a has also been supported by crosslinking (17). Our results indicate that tetradecapeptide is embedded in that region of phosphorylase b which is not occupied with the motile NH_2-terminal tail. The motile tail prevents the dephosphorylation of bound peptide hiding Ser-phosphate group. The same surface of phosphorylase a is occupied with the covalently attached phosphorylated tail, therefore the phosphopeptide cannot bind to phosphorylase a.

Table 1

Effect of phosphorylase b' and ligands on the dephosphorylation of ^{32}P-tetradecapeptide.

The reactions were carried out at 30° in the presence of 5.10^{-8} M phosphatase. The reaction rates are expressed as per cent of the rates measured with phosphopeptide or phosphopeptide + phosphorylase b'.

Ligands	Rate of Dephosphorylation (%)	
	^{32}P-peptide	^{32}P-peptide + phosphorylase b'
none	100	100
10^{-5} M AMP	100	48
10^{-4} M AMP	95	0
5.10^{-3} M Glc 6-P	102	108
10^{-2} M Glucose	103	117
10^{-5} M AMP + 5.10^{-3} M Glc 6-P	98	114
10^{-5} M AMP + 10^{-2} M Glucose	101	105

Tetradecapeptide in the presence of phosphorylase b' gains ligand sensitivity as shown in Table 1. The phosphorylated Ser-14 in phosphorylase a is hydrogen bonded to specific arginine residues (18). AMP binding site is quite close to Ser-14 (18) thus the two sites are easily communicated modifying the phosphatase reaction. The complex of phosphopeptide and phosphorylase b' suggests that the Ser-phosphate group of peptide may interact with the arginine residues of b'. In consequence of this conformational changes also occur in the peptide-phosphorylase b' complex on the effect of AMP or glucose and glucose 6-P, hiding or exposing the Ser-phosphate group. Phosphorylase b' also increases the rate of dephosphorylation of phosphopeptide. It may be supposed that the complex of phosphopeptide and b' contains more phosphatase recognizing sites (positively charged amino acid residues, 18) than the peptide alone increasing the affinity of phosphatase.

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