Phosphorylase Kinase Specificity

A COMPARATIVE STUDY WITH cAMP-DEPENDENT PROTEIN KINASE ON SYNTHETIC PEPTIDES AND PEPTIDE ANALOGS OF GLYCOGEN SYNTHASE AND PHOSPHORYLASE* 

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K.-F. Jesse Chan, Michael O. Hurst, and Donald J. Graves‡

From the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50011

A synthetic pentadecapeptide, 1

Thr-Leu-Ser-Val-Ser-Ser-Leu-Pro-Gly-Leu-Glu,

corresponding to the phosphorylatable site at the NH2 terminus of glycogen synthase, could be phosphorylated stoichiometrically at seryl residue 7 by both phosphorylase kinase and cAMP-dependent protein kinase. Phosphorylation of seryl residue 3 also occurred after prolonged incubation with cAMP-dependent protein kinase. Kinetic studies show that the pentadecapeptide is a better substrate for phosphorylase kinase. Peptide consisting of residues 1-11 was not as good a substrate and substitution of Arg-4 by Lys and Ser-9 by Arg in the undecapeptide decreased and increased phosphorylase kinase reaction rates, respectively. Higher rates of phosphorylation were obtained with peptides of the phosphorylatable site of phosphorylase kinase.

A peptide with the sequence, Leu-Ser-Tyr-Arg-Arg-

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Tyr-Ser-Leu was phosphorylated initially by phosphorylase kinase and cAMP-dependent protein kinase at Ser-2 and Ser-7, respectively. Upon longer incubation, second site phosphorylation occurred with both kinases. A peptide of the same sequence with D-amino acids could not be phosphorylated but was a competitive inhibitor of both enzymes. The results suggest that optimal interaction of the two kinases depends on various factors including the orientation of arginyl groups with respect to the phosphorylatable serine.

Protein phosphorylation is well recognized as an important control mechanism in biological systems (1-4). At least two aspects are essential for protein phosphorylation. First, the phosphorylatable site must be accessible to the enzyme, and second, the phosphorylatable region must contain structural and chemical elements for the formation and reaction of the enzyme-substrate complex. The molecular basis of the substrate specificity for phosphorylase kinase (EC 2.7.1.38) and cAMP-dependent protein kinase (EC 2.7.1.37) has been studied by the use of synthetic peptide analogs corresponding to the phosphorylatable regions of the native proteins (5-9). It is believed that particular residues in these fragments act as specific determinants (primary specificity) for phosphorylation. The results indicate that phosphorylase kinase prefers an arginyl residue on the COOH-terminal side of the phosphorylatable hydroxyamino acid whereas cAMP-dependent protein kinase prefers multiple arginyl residues on the NH2-terminal side (6, 7). However, other studies have demonstrated that several residues surrounding the phosphorylatable seryl residue are also important for phosphorylase kinase recognition and that the presence of multiple arginines per se is insufficient to ensure optimal rates of phosphorylation by cAMP-dependent protein kinase (9). On the basis of theoretical proposals (10), Graves et al. (11) suggested that differences in specificity between these two kinases might depend on the recognition of different sides of a β-turn. NMR studies also indicated that cAMP-dependent protein kinase prefers peptide substrates with either β-turn or β-coil conformation (12).

It has been found that skeletal muscle glycogen synthase (EC 2.4.1.11) can be phosphorylated readily by phosphorylase kinase (13-16) and up to 0.6 mol of 32P/synthesize subunit can be incorporated. As with phosphorylase kinase (EC 2.4.1.11), the phosphorylatable site is at the NH2 terminus of the protein, and sequence analysis shows that seryl residue 7 is the site of phosphorylation (17, 18) but no arginyl residue is found on the COOH-terminal side of the phosphorylatable serine. Glycogen synthase also is known to be a good substrate for CAMP-dependent protein kinase (1, 2, 4). Yet no report has demonstrated that seryl residue 7 is phosphorylated by this enzyme, although the possibility has been suggested (15). Similarly, seryl residue 14, the site of phosphorylation in phosphorylase, has been shown to be a substrate for phosphorylase kinase. Synthetic peptide analgs of the phosphorylatable site of phosphorylase, however, can be phosphorylated by both kinases (6, 7). Thus, it appears that the microenvironment and the three-dimensional structure of the phosphorylatable region (secondary specificity) may also play an important role.

To gain further insight into the functional role of phosphorylase kinase and its substrate specificity, synthetic peptides and substrates, in particular, those corresponding to the phosphorylatable regions of glycogen synthase and phosphorylase, were made and kinetic studies were carried out to compare with those of cAMP-dependent protein kinase, wherever possible.

EXPERIMENTAL PROCEDURES

Materials

[γ-32P]ATP was prepared essentially according to Ref. 19. Boc amino acids were purchased from Peninsula. Trypsin (treated with L-1-tosylamido-2-phenylethylchloromethyl ketone), thermolysin, and chymotrypsin were products of Sigma. Other materials were of reagent grade and available commercially.

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‡ To whom reprint requests should be addressed.

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Substrate Specificity of Phosphorylase Kinase

Methods

Protein Preparations—Phosphorylase kinase (20), phosphorylase (21), and the catalytic subunit of CAMP-dependent protein kinase (22) were prepared as described previously.

Peptide Synthesis and Purification—A pentadecapeptide (residues 1–15) of the glycogen synthase sequence was synthesized on a Beckman Model 990 automated peptide synthesizer by using the solid phase method of Merrifield (23) and was cleaved from resin with HBr/trifluoroacetic acid to avoid side reactions (24) due to glutamic acid being the COOH-terminal residue of the peptide (25). The pentadecapeptide was then deprotected with boron-Tris (trifluoroacetic acid) and purified by using gel filtration and high pressure liquid chromatography. The purity of the peptide was determined by high voltage paper electrophoresis at pH 6.8 and autoradiography. The radioactive peptide mixture was characterized by a linear gradient of acetic acid buffer and by amino acid analysis on a Durrum D-400 amino acid analyzer. Other peptide analogs were synthesized and purified essentially as described above. The tetracdecapeptide (residues 5–18) and N-acetylated octadecapeptide (residues 1–18) of the phosphatase sequence were synthesized and purified previously in our laboratory (7, 27).

Assays of Enzyme Activity—Standard assays of phosphorylase kinase activity comprised 50 mM Tris, 50 mM β-glycerophosphate (pH 8.2 or 6.8), 8 mM MgAc2, 3 mM [γ-32P]ATP, 0.1 mM CaCl2, various concentrations of peptide substrates, and enzyme (vide infra). With the catalytic subunit of CAMP-dependent protein kinase, reactions were carried out at pH 6.8 in reaction mixtures just described except that 0.5 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid was included. Concentrations of peptides were determined by amino acid analysis.

The rates of 32P incorporation into peptides were assayed either by using phosphocellulose P81 paper, or Whatman ET31 paper, or by using AG 1 × 8 anion-exchange chromatography (7, 28).

Determination of Site of Phosphorylation of the Pentadecapeptide—The phosphorylated 32P-pentadecapeptide was isolated by using AG 1 × 8 anion-exchange chromatography, lyophilized, and de-salted on a Bio-Gel P-2 column. Thermolysin digestion of this peptide was carried out in 0.2 M pyridine-0.1 M CaCl2 at an enzyme-to-substrate ratio of 1:27 (w/w). The 32P-fragment was then purified by DE52 cellulose chromatography using a linear gradient of 10 to 200 mM NH4HCO3. The purity of the 32P-fragment was determined by two-dimensional thin layer chromatography using polyamide sheets in solvent 1:1.5% formic acid, and solvent 2:10% acetic acid in benzene (v/v).

RESULTS

Phosphorylation of the Pentadecapeptide from Glycogen Synthetase—When the synthetic pentadecapeptide of skeletal muscle glycogen synthase, Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ser-Leu-Pro-Gly-Leu-Glu, was tested as substrate for phosphorylase kinase, reaction characteristics similar to those found with phosphorylase or a tetracdecapeptide of the phosphorylatable site of phosphorylase, Ser-Asp-Glu-Glu-Ser-Lys-Gln-Ile-Ser-Val-Ar-Gly-Leu (30), were obtained. These include a lag in product formation at pH 6.8 which is abolished by preincubation with the pentadecapeptide, an activity ratio (pH 6.8/8.2) of 0.05 for the initial phase of the reaction, and an absolute requirement of Ca2+ for activity. Addition of exogenous δ subunit (calmodulin) of phosphorylase kinase into the reaction mixture stimulated the rate of phosphorylation of this peptide about 1.6-fold, similar to the findings for the phosphorylation of phosphorylase and synthase, as previously proposed (14, 15).

Unlike native glycogen synthase (14), the synthetic pentadecapeptide could be phosphorylated stoichiometrically by both phosphorylase kinase and CAMP-dependent protein kinase (Fig. 1). Upon prolonged incubation (12 h), 1.2 to 1.6 mol of 32P could be incorporated into the pentadecapeptide by CAMP-dependent protein kinase, whereas the extent of phosphorylation remained unchanged for the phosphorylase kinase reaction.

Site of Phosphorylation of the Pentadecapeptide—The site of phosphorylation of the pentadecapeptide was determined by thermolysin digestion of the phosphorylated peptide. Peptide mapping and autoradiography of the purified 32P-peptide digest indicated that both kinases phosphorylated the same site (Fig. 2). Amino acid analysis showed that the common 32P-thermolysin peptide contained only leucine and serine. Subsequent dansylation of this fragment and thin layer chromatography (29) showed that leucine is at the NH2 terminus. According to the specificity of thermolysin cleavage, we conclude that the seryl residue in the pentadecapeptide is the site of phosphorylation, which is identical with that found in the native protein (17, 18). Amino acid analysis of the second phosphorylated peptide obtained only with CAMP-dependent protein kinase showed equivalent molar amounts of proline, leucine, serine, threonine, and arginine. To determine whether seryl residue 3 or threonyl residue 5 was the site of phosphorylation, the 32P-pentadecapeptide was treated with trypsin, and the digest was analyzed by high voltage paper electrophoresis at pH 6.5 and autoradiography. The radioactive peptide migrated as a slightly positively charged species and reacted with the arginine reagent, phenanthrenequinone (32). Because of these findings, we suggest that seryl residue 3 is the second site of phosphorylation for CAMP-dependent protein kinase.

Kinetic Studies with Synthetic Peptide Substrates—Kinetic parameters of phosphorylase kinase and CAMP-dependent protein kinase for the pentadecapeptide were determined at pH 8.2 and 6.8, respectively. Although a single arginyl residue is present only on the NH2-terminal side of the phosphorylatable serine, the pentadecapeptide is a better substrate for phosphorylase kinase, as shown by an approximately 2-fold lower apparent Km and a 2-fold higher Vm (Table I). It should be noted that when the molar activities are compared between the two kinases, much bigger differences are ob-
served. Per monomer of phosphorylase kinase (αβγδ), M₀ = 330,000, and for the catalytic subunit of cAMP-dependent protein kinase, M₀ = 41,000; the molar activities for the pentadecapeptide are ~4.7 and 0.29 molecules s⁻¹, respectively.

Other synthetic peptides also were tested: an N-acetylated octadecapeptide (Ac-Ser-Arg-Pro-Leu-Ser-Asp-Glu-Lys-Gly-Pro-Leu-Ser-Val-Gly-Arg-Gly-Leu; Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu) and a tetradecapeptide (Ser-Asp-Glu-Lys-Arg-Lys-Gly-Pro-Leu-Ser-Val-Arg-Ser-Asp-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu; Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ser-Ser-Leu-Pro-Gly-Leu-Glu; Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Arg-Gly-Leu), both corresponding to the phosphorylatable NH₂ terminus of phosphorylase. Earlier, we found that with synthetic peptides, whereas the apparent Kₚ values were always higher and Vₚ values lower than with native phosphorylase (7, 8). These peptides did not contain the sequence, Pro-Leu, found in the NH₂ terminus of both phosphorylase and glycogen synthase. Because the possibility existed that the Pro-Leu sequence might influence the binding and reaction of the two kinases, kinetic studies also were undertaken with these two peptides. With the phosphorylase (5 → 18) peptide, the apparent kinetic parameters obtained for phosphorylase kinase (Table I) agreed very well with those reported previously (7). The phosphorylase (1 → 18) peptide, which includes the Pro-Leu sequence, did not affect the apparent Kₚ of phosphorylase kinase, although the apparent Vₚ value was slightly increased. Comparison of the kinetic parameters for the peptides glycogen synthetase (1 → 15), phosphorylase (1 → 18), and phosphorylase (5 → 18) shows that the apparent Vₚ values are 3- to 4-fold higher for the latter two peptides, whereas the apparent Kₚ values differ by less than 2-fold. Similar differences in kinetic parameters were obtained when native glycogen synthase and phosphorylase were used as substrates for phosphorylase kinase (14, 15, 18). Both the phosphorylase (1 → 18) and phosphorylase (5 → 18) peptides also served as substrates for cAMP-dependent protein kinase (Table I). No significant differences in the apparent kinetic parameters were found between these two peptides, suggesting that the extra NH₂-terminal residues are not important for enzyme recognition.

A region with high tendency of forming β-structure, Pro-Gly-Leu (33), is located at the COOH-terminal segment of the pentadecapeptide glycogen synthase (1 → 15). As shown in Table II, omission of this sequence in the undecapeptide, glycogen synthase (1 → 11) peptide, resulted in ~2-fold decrease in the apparent Vₚ value and ~1.8-fold increase in the apparent Kₚ, suggesting that this structural feature in the pentadecapeptide may be important for recognition by phosphorylase kinase. The requirement of basic residues around the phosphorylatable site was also investigated by using the glycogen synthase (1 → 11) peptide analogs. Substitution of Ser-9 by Arg-9 increased the apparent Vₚ value about 7-fold, but only slightly increased the apparent affinity for this glycogen synthase (1 → 11)Arg⁹ peptide. Substitution of Arg⁹ by Lys⁹, however, changed the glycogen synthase (1 → 11)Lys⁹ peptide into a poorer substrate with higher apparent Kₚ and lower apparent Vₚ values. In addition, substrate inhibition was observed at concentrations above 1.5 mM. In terms of primary specificity, these results indicate that phosphorylase kinase prefers arginyl residue(s), particularly on the COOH-terminal side of the phosphorylatable seryl residue, in agreement with the previous suggestion (7).

To examine further the specificity of phosphorylation of peptide substrates, the action of phosphorylase kinase and

![High-voltage electrophoresis diagram](image)

**FIG. 2. Identification of sites of phosphorylation in the pentadecapeptide by autoradiography.** The phosphorylated pentadecapeptides were cleaved by thermolysin, and the ³²P-fragments were isolated as described under "Experimental Procedures." These ³²P-fragments were then subjected to two-dimensional peptide mapings and autoradiography. Autoradiograms for phosphorylase kinase (A) and cAMP-dependent protein kinase (B) reactions are shown.

**TABLE I**

*Summary of kinetic parameters for phosphorylase kinase and cAMP-dependent protein kinase with synthetic peptides as substrates*

<table>
<thead>
<tr>
<th>Peptide substrates*</th>
<th>Phosphorylase kinase</th>
<th>cAMP-dependent protein kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kₚ (μM)</td>
<td>Vₚ (μmol min⁻¹ mg⁻¹)</td>
</tr>
<tr>
<td>Glycogen synthase (1 → 15)</td>
<td>0.70</td>
<td>0.88</td>
</tr>
<tr>
<td>Phosphorylase (5 → 18)</td>
<td>1.20</td>
<td>2.50</td>
</tr>
<tr>
<td>Phosphorylase (1 → 18)</td>
<td>1.20</td>
<td>3.90</td>
</tr>
<tr>
<td>Glycogen synthase (1 → 11)</td>
<td>1.02</td>
<td>0.32</td>
</tr>
<tr>
<td>Glycogen synthase (1 → 11) Arg⁹</td>
<td>0.81</td>
<td>2.25</td>
</tr>
<tr>
<td>Glycogen synthase (1 → 11) Lys⁹</td>
<td>3.50</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Peptide substrates: glycogen synthase (1 → 15); Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ser-Ser-Leu-Pro-Gly-Leu-Glu; phosphorylase (5 → 18); Ser-Asp-Glu-Lys-Arg-Lys-Gly-Pro-Leu-Ser-Val-Arg-Gly-Leu; glycogen synthase (1 → 11); Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ser-Ser-Leu; glycogen synthase (1 → 11)Lys⁹; phosphorylase (5 → 18). The numbers indicate the residue numbers found in the native proteins.

*Substrate inhibition.
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Figure 3. SP-Sephadex chromatography of the Leu-Ser-Tyr-Arg-Arg-Tyr-Ser-Leu peptide phosphorylated by cAMP-dependent protein kinase. The peptide was phosphorylated in a reaction mixture of 0.5 mM peptide, 1.0 mM \([\gamma^{32}P]ATP\), 12.5 mM MgCl\(_2\), 25 mM 4-morpholineethanesulfonic acid buffer, 50 \(\mu\)g/ml cAMP-dependent protein kinase, pH 6.5, at 30 \(^\circ\)C for 28 h. Excess \([\gamma^{32}P]ATP\) was removed by AG 1 × 8 chromatography, and the products were lyophilized, taken up in 1.5 ml H\(_2\)O, and applied to an SP-Sephadex column (1.2 × 9 cm) equilibrated with 0.05 M pyridine-acetate, pH 2.6. The column was eluted with a 400-ml gradient of 0.05-0.5 M pyridine-acetate, pH 2.6-3.6, and 3-ml fractions were collected.

cAMP-dependent protein kinase was tested on the peptide

Leu-Ser-Tyr-Arg-Arg-Tyr-Ser-Leu. Earlier, it was reported by Graves et al. (11) that with a peptide of similar sequence, Leu-Ser-Tyr-Arg-Gly-Tyr-Ser-Leu, phosphorylation by phosphorylase kinase phosphorylated Ser-2 and cAMP-dependent protein kinase Ser-7. Similar results were seen with the symmetrical peptide and \(K_m\) values of 2.9 and 0.12 mM were obtained for phosphorylase kinase and cAMP-dependent protein kinase. Upon prolonged incubation with both kinases, second site phosphorylation was found to occur. Fig. 3 shows a sulfopropyl (SP)-Sephadex column profile of products obtained after 28 h of incubation with cAMP-dependent protein kinase. Three radioactive peaks were obtained. The first was shown to be ATP. The second was digested with trypsin, followed by high voltage paper electrophoresis and autoradiography. Two spots were found, both containing radioactivity, showing the peak to be a peptide phosphorylated on both serines. Similar treatment of the third peak produced two fluorescamine-positive spots, one of which was radioactive and negatively charged. Thus, the third peak is a monophosphopeptide, phosphorylated on the COOH-terminal serine. Similar experiments were done with phosphorylase kinase similar results, the monophosphopeptide in this case being phosphorylated on the NH\(_2\)-terminal serine. A peptide with the sequence Leu-Ser-Tyr-Arg-Arg-Tyr-Ser-Leu was synthesized with n-amino acids to determine if phosphorylation could occur with both kinases but with the opposite initial preferences observed with the L-peptide. No phosphorylation was found to occur but both enzymes were inhibited competitively by the D-peptide with \(K_i\) values of 0.8 and 1.2 mM for phosphorylase kinase and cAMP-dependent protein kinase, respectively.

**DISCUSSION**

The phosphorylation sites of skeletal muscle glycogen synthase (17, 18) and glycogen phosphorylase (34) possess significant degrees of sequence homology. Both sites are located on the NH\(_2\)-terminal end of their respective proteins and comparison of their structures (Table II) with results obtained with the action of kinases on synthetic peptides can give some insight into the specificity of phosphorylation.

Peptides of the glycogen synthase sequence are not as good substrates for phosphorylase kinase as peptides of the phosphorylase sequence, but the differences aren't large. A gap exists in the sequence between synthase and phosphorylase (Table II) consisting of residues Asp-Gln-Glu-Lys-Arg, but this change probably does not contribute to the differences in rates because earlier studies showed that these residues in peptide segments of phosphorylase were not important for phosphorylation (7). The substitution of two basic residues in the phosphorylase sequence, Lys-11 and Arg-16, by Arg and Ser, respectively, likely is important. In a monodecapeptide of the glycogen synthase sequence (Table I), substitution of Arg-4 by Lys decreased the maximal velocity about 2-fold and increased the \(K_m\) value 3.5 times. Substitution of Ser-9 by Arg in the glycogen synthase (1 → 11) peptide had the opposite effect. In this case the \(V_m\) value went up 8-fold with a slight reduction in the \(K_m\) value. We were not surprised that arginine on the COOH-terminal side had a positive effect on phosphorylation because substitution of this residue in peptides of the phosphorylase sequence always greatly reduced the rate of phosphorylation. Thus, the two changes of the basic residues could cancel each other and might account for why the rate differences between phosphorylase and synthase peptides aren't large. Kemp and John (35) have also shown that Arg-4 in the glycogen synthase sequence is important, because a peptide where this residue is substituted by Leu cannot be phosphorylated by phosphorylase kinase. Multiple binding is no doubt important for phosphorylase kinase recognition and earlier studies showed that all residues in the immediate region of the phosphorylatable serine are important for phosphorylase kinase action (8). Because a conserved sequence exists around the phosphorylatable site of the two proteins, the structure may be sufficient to give good alignment even though the COOH-terminal arginyl residue is missing.

The importance of arginine residues and their orientation is illustrated further by studies with the symmetrical peptide Leu-Ser-Tyr-Arg-Arg-Tyr-Ser-Leu. The preference of phosphorylase kinase for the NH\(_2\)-terminal serine, and of cAMP-dependent protein kinase for the COOH-terminal serine, shows clearly that both kinases prefer arginine residues in particular orientations relative to the phosphorylatable serine.

**TABLE II**

<table>
<thead>
<tr>
<th>Phosphorylase Synthase</th>
<th>Ac-Ser-Arg-Pro-Leu-Ser-Asp-Gln-Glu-Lys-Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- - Pro-Leu-Ser</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>Lys-Gln-ile-Ser(P)-Val-Arg-Gly-Leu-Asp-Glu-Val-Glu</td>
</tr>
<tr>
<td>Synthase</td>
<td>Arg-Thr-Leu-Ser(P)-Val-Ser-Ser-Leu-Pro-Gly-Leu-Glu</td>
</tr>
</tbody>
</table>

**FIG. 3**
Other interactions are possible because second site phosphorylation can occur with both enzymes. Because the peptide has a palindromic-like sequence, a nearly equivalent arrangement of side chain groups (e.g. arginine to serine) could occur in the active site for second site phosphorylation if binding of the peptide chain could occur with opposite polarity. Alternatively, the kinases may simply interact with the peptide in the same direction but with different residues for first and second site phosphorylation. The ability of the enzymes to catalyze second site phosphorylation gave rise to the experiments with the $\alpha$-analog of the symmetrical peptide. There was, however, no phosphorylation, showing that the stereochemistry of the substrate is important. The peptide was a competitive inhibitor, showing that the enzymes do recognize it enough for binding, even if there is no catalysis. That this is not merely a simple recognition of guanidino groups is shown by the fact that the inhibition pattern of the $\alpha$-peptide analogs varied with the relative position of the arginines in other synthetic peptides.¹²

It is not understood why CAMP-dependent protein kinase can phosphorylate the tetradecapeptide but not phosphorylase itself, nor why phosphorylase kinase phosphorylates peptides less efficiently than it does phosphorylase. These facts indicate that the tertiary structure of the substrate is important in specificity. For example, it has been suggested earlier in our laboratory that the lack of reaction of CAMP-dependent protein kinase with phosphorylase might be caused by a conformation that shields Arg-10 and exposes Arg-16 (7). However, there is no arginyl residue on the COOH side of Ser-7 in glycogen synthase, although a sequence with high tendency of forming $\beta$-structure, Pro-Gly-Leu (33), is present. A similar structural feature also occurs in phosphorylase, at least with the $\alpha$ form (36), around residues 18 $\rightarrow$ 20 (Table II). Because deletion of the last four amino acids from the COOH end of the pentadecapeptide increased the apparent $K_m$ 1.4-fold and decreased the apparent $V_m$ ~3-fold (Table I), it is tempting to suggest that this structural element may act as a secondary specificity to prevent the binding of CAMP-dependent protein kinase to the substrate. Studies on the possible influence of the structure elements are in progress by using longer peptide substrates including an NH$_2$-terminal CNBr fragment derived from phosphorylase, which contains considerable tertiary structure as observed through circular dichroism. Preliminary results indicate that CAMP-dependent protein kinase still could not phosphorylate the CNBr fragment whereas, with phosphorylase kinase, the reaction proceeded readily.²

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¹ M. O. Hurst and D. J. Graves, unpublished results.


Phosphorylase kinase specificity. A comparative study with cAMP-dependent protein kinase on synthetic peptides and peptide analogs of glycogen synthase and phosphorylase.

K F Chan, M O Hurst and D J Graves


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