Role of Phosphorylated Aminoacyl Residues in Generating Atypical Consensus Sequences Which Are Recognized by Casein Kinase-2 but Not by Casein Kinase-1†

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ABSTRACT: Casein kinase-2 (CK-2) is a ubiquitous Ser/Thr specific protein kinase that recognizes phosphorylatable residues located upstream of acidic determinants, its consensus sequence being Ser(Thr)-Xaa-Xaa-Acidic. Here we show that the phosphotetrapeptide AcSer(P)-Ser(P)-Ser-Ser(P), which is devoid of the canonical consensus sequence, is nevertheless phosphorylated by CK-2 with rates comparable to that of typical peptide substrates Ser-Glu-Glu-Glu-Glu-Glu and Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu routinely employed for assaying CK-2 activity. The phosphopeptide AcSer(P)-Ser-Ser(P) [but not Ac-Ser-Ser(P)-Ser(P) or AcSer(P)-Ser(P)-Ser] is also phosphorylated albeit less efficiently than AcSer(P)-Ser(P)-Ser-Ser(P). Further N-terminal elongation with additional phosphoseryl residues to give the peptides AcSer(P)-Ser(P)-Ser(P)-Ser-Ser(P) and AcSer(P)-Ser(P)-Ser(P)-Ser(P)-Ser-Ser(P) does not improve but rather slightly decreases the phosphorylation efficiency by CK-2. These two peptides are conversely excellent substrates for CK-1, which does not appreciably phosphorylate either AcSer(P)-Ser-Ser(P) or AcSer-(P)-Ser(P)-Ser-Ser(P). Either individual or multiple replacement of the phosphorylated residues with glutamic acid in the peptide AcSer(P)-Ser(P)-Ser-Ser(P) drastically reduces the phosphorylation efficiency by CK-2, the phosphoseryl residue at position −2 playing an especially crucial role which cannot be surrogated by glutamyl residues. Phosphoserine is also more effective than glutamic acid at positions +1 and +2 of peptides that fulfill the consensus sequence Ser-Xaa-Xaa-Glu, the K_{cat} value of Ser-Ser(P)-Ser(P)-Glu-Glu being 3-fold higher than that of Ser-Glu-Glu-Glu-Glu which, in turn, is a better substrate than Ser-Ala-Ala-Glu-Glu. Such a superiority of phosphoserine over glutamic acid is conversely not so evident at the canonical +3 position, Ser-Ala-Ala-Glu-Glu and Ser-Ala-Ala-Ser(P)-Ser(P) being comparable substrates. These data suggest that partial phosphorylation of clustered hydroxylic residues might generate phosphoacceptor sites for CK-2 which are not predictable on the basis of its canonical consensus sequence. In particular, a very effective atypical consensus sequence is Ser(P)-Ser(P)-Ser-Ser(P). Such a sequence is not recognized by casein kinase-1 (CK-1) whose specificity is determined by phosphorylated residue(s) located at more remote positions on the N-terminal side, notably −3 and −4.

While most Ser/Thr-specific protein kinases are basophilic in that they recognize sites specified by positively charged residues, only a few kinases require acidic residues as specificity determinants, a feature that is typical of Tyr-specific protein kinases [reviewed by Kemp and Pearson (1990) and Pinna (1991)]. Such acidophilic Ser/Thr protein kinases are collectively termed casein kinases after their preference for casein over histones as an artificial substrate. However, apart from the tissue-specific enzymes responsible for the phosphorylation of casein within the lactating mammary gland [whose consensus sequence is the triplet Ser-Xaa-Glu(Asp) (Mercier, 1981; Meggio et al., 1988b)], they also include two classes of ubiquitous and pleiotropic protein kinases involved in the phosphorylation of a variety of metabolically relevant proteins and conventionally termed casein kinase-1 and -2 (CK-1 and CK-2)[1] [reviewed by Pinna (1990) and Tuazon and Traugh (1991)].

CK-1 displays the character of a phosphate-directed protein kinase since its site recognition is determined by prior phosphorylation of a serine located upstream of the target residue at position −3 or less effectively at position −4 (Flotow et al., 1990; Meggio et al., 1991a). Glutamic and aspartic acids are almost incapable of substituting for phosphoserine in this respect.

On the contrary, CK-2 readily phosphorylates target seryl residues which are located on the N-terminal side of multiple carboxylic residues (Meggio et al., 1984; Marin et al., 1986; Kuenzel et al., 1987), a seryl residue at position +3 being especially needed (Marchiori et al., 1988). Consequently its consensus sequence which was established with the aid of peptide substrates was found to be Ser(Thr)-Xaa-Xaa-Glu-(Asp). Although phosphoserine (Pinna, 1990; Litchfield et al., 1990) and phosphotyrosine (Meggio et al., 1991b) can substitute for carboxylic residues as specificity determinants, CK-2 does not display any marked preference for a phosphate

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[1] Abbreviations: Ser(P), phosphoserine; Thr(P), phosphothreonine; Tyr(P), phosphotyrosine; CK-1, casein kinase-1; CK-2, casein kinase-2.

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group over a carboxylic group.

In view of the above findings, it was rather surprising that the triphosphopeptide AcSer(P)-Ser(P)-Ser(P) was found to be readily phosphorylated by CK-2 after previous partial dephosphorylation with acid phosphatase (Meggio et al., 1988a). The only possible interpretation of this finding was that phosphoserine can act as a specificity determinant at a position(s) other than the canonical one (+3), which is obviously lacking in a tripeptide. Also, a second unexpected finding was the slow yet significant phosphorylation of Thr-4 in the src phosphopentapeptide Asn-Glu-Tyr(P)-Thr-Ala, despite the absence of any C-terminal acidic residue (Perich et al., 1990). In order to assess whether, and to what extent, phosphorylated residues could actually generate atypical phosphoacceptor sites for CK-2, a number of derivatives of the phosphopeptide AcSer(P)-Ser(P)-Ser(P) were synthesized and assayed for their capability to serve as substrates for CK-2. The results of this study are described in the present report.

Materials and Methods

The synthesis of peptides Glu-Ser-Glu, Glu-Glu-Ser-Glu, and Glu-Ala-Ser-Glu has been accomplished manually by the continuousflow solid-phase procedure on the Kieselguhr-supported poly(dimethylacrylamide) resin functionalized with 4-(hydroxymethyl)phenoxy-acetic acid (NovaSyn KA). Cleavage from the resin was achieved by treatment of the peptide–resin with TFA containing 5% anisole. The other nonphosphorylated peptides were prepared by the Boc mode of solution-phase peptide synthesis followed by catalytic hydrogenation (palladium) of the protected peptides in 50% TFA/AcOH. The Ser(P)- and Thr(P)-containing peptides were prepared by the use of Boc-Ser(PO_3Ph_2)-OH or Boc-Thr(PO_3Ph_2)-OH in the Boc mode of solution-phase peptide synthesis followed by hydrogenation (platinum) of the protected Ser(PO_3Ph_2)- or Thr(PO_3Ph_2)-containing peptides in 50% TFA/AcOH (Perich et al., 1991; Perich & Johns, 1991a,b). The structure of all peptides was confirmed by ^{13}C NMR spectroscopy and FAB mass spectrometry.

Rat liver casein kinase-2 was purified to near homogeneity as previously described (Meggio et al., 1981) with a subsequent FPLC Mono Q chromatographic step. Casein kinase-1 was partially purified as previously described (Meggio et al., 1981). The specific activity was determined to be approximately 120 and 19 units/mg for CK-2 and CK-1 respectively, one unit being defined as the amount of enzyme transferring 1 nmol of P/min to casein.

Reaction conditions for peptide phosphorylation experiments were the following: 50 mM Tris-HCl buffer, pH 7.5, containing 12 mM $MgCl_2$, 100 mM NaCl, 1 mM peptide substrate (unless differently indicated), and 0.01-0.02 unit of enzyme. The incubation started with addition of 25 μM [γ-^{32}P]ATP and was terminated by cooling in ice. Since neither the phosphocellulose (Glass et al., 1978) nor the ion-exchange (Kemp et al., 1976) procedures were suitable for most of the acidic peptide substrates studied here, the incorporated ^{32}P was determined by partial acid hydrolysis of the peptide (6 N HCl, 4 h at 105 °C) as previously described (Meggio et al., 1984) followed by isolation and quantitation of the radioactive phosphoserine. The general applicability of the partial acid hydrolysis procedure was previously established by showing that (i) identical results are obtained by assaying the ^{32}P incorporated by suitable kinases into a series of peptides with two or more basic residues but otherwise dissimilar structures (RRREEESEEE, RRRDDDSDDD, NFKSPVKTIR, RRASVA, RKMKDTDSEEEIR, RRRGSESSEE) either by the traditional phosphocellulose paper method (Glass et al., 1978) or by the partial acid hydrolysis procedure (Meggio et al., 1984) and (ii) a number of different ^{32}P radiolabeled phosphoseryl peptides were found to undergo comparable losses of inorganic phosphate upon digestion with 6 N HCl at 105 °C for 4 h, Ser(^{32}P) recovery being 45 ± 5%.

Kinetic constants of peptide substrates were determined by regression analysis of double-reciprocal plots constructed from initial rate measurements.

Table I: Phosphorylation Rate of Phosphopeptides by Casein Kinase-2 and Casein Kinase-1[a]

peptide	phosphorylation rate[b] by CK-2	CK-1
AcSer-Ser(P)-Ser(P)	4.9	<0.5
AcSer(P)-Ser-Ser(P)	40.9	<0.5
AcSer(P)-Ser(P)-Ser	5.3	<0.5
Ser-Ser-Ser(P)	19.8	<0.5
AcSer-Thr(P)-Thr(P)	0.5	<0.5
Ala-Ala-Ser-Ser(P)-Ser(P)	0.7	<0.5
Ser-Ser(P)-Ser(P)-Ala-Ala	<0.5	<0.5
AcSer(P)-Ser(P)-Ser-Ser(P)	127.3	1.7
AcSer(P)-Ser(P)-Ser(P)-Ser	5.3	<0.5
AcSer(P)-Ser(P)-Ser(P)-Ser-Ser(P)	73.6	18.3
AcSer(P)-Ser(P)-Ser(P)-Ser(P)-Ser-Ser(P)	63.9	21.5
Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr	<0.5	100.0
Ser-Glu-Glu-Glu-Glu-Glu	100.0	<0.5
Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu	74.6	<0.5

[a] The concentration of peptides was either 1 mM or 0.25 mM for CK-2 and CK-1, respectively. The phosphorylatable residue in each peptide is underlined. [b] Expressed as percent of the phosphorylation rate of reference peptides Ser-Glu-Glu-Glu-Glu-Glu and Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr for CK-2 and CK-1, respectively.

Results

The three singly unphosphorylated derivatives of the triphosphopeptide AcSer(P)-Ser(P)-Ser(P), bearing the unoccupied serine at positions 1, 2, and 3, respectively, were synthesized and assayed as substrates for CK-2. As shown in Table I, only AcSer(P)-Ser-Ser(P) was appreciably phosphorylated by CK-2 relative to the standard peptide substrate SEEEEE, and its two isomers, AcSer-Ser(P)-Ser(P) and AcSer(P)-Ser(P)-Ser [as well as its phosphothreonyl derivative AcSer-Thr(P)-Thr(P)] were nearly unaffected. This indicates that the suitable substrate generated by partial dephosphorylation of AcSer(P)-Ser(P)-Ser(P) (Meggio et al., 1988a) was AcSer(P)-Ser-Ser(P) and not AcSer-Ser(P)-Ser(P) as expected. The incapability of the two phosphorylated serines adjacent to the C-terminal side of serine to serve solely as effective specificity determinants is confirmed by the negligible phosphorylation rate of Ala-Ala-Ser-Ser(P)-Ser(P) and Ser-Ser(P)-Ser(P)-Ala-Ala (Table I).

On the other hand, the relevance of the N-terminal phosphorylated residues which was previously outlined by the superiority of Asn-Glu-Tyr(P)-Thr-Ala over its dephosphorylated derivative (Perich et al., 1990) was corroborated by two significant findings: First, there was a marked decrease in the phosphorylation rate caused by a change of the N-terminal Ser(P) [compare Ser(P)-Ser-Ser(P) with Ser-Ser-Ser(P)] and second, there was a remarkable increase in the phosphorylation rate with the addition of a second N-terminal Ser(P) residue, the tetrapeptide AcSer(P)-Ser(P)-Ser-Ser(P) being a substrate as good as the best peptide substrates that fulfill the canonical consensus sequence and are routinely employed for assaying CK-2 activity, namely Ser-Glu-Glu-Glu-Glu-Glu and Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (see Table I). However, neither AcSer(P)-Ser(P)-Ser(P)-Ser nor Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr is apprecibly phosphory-

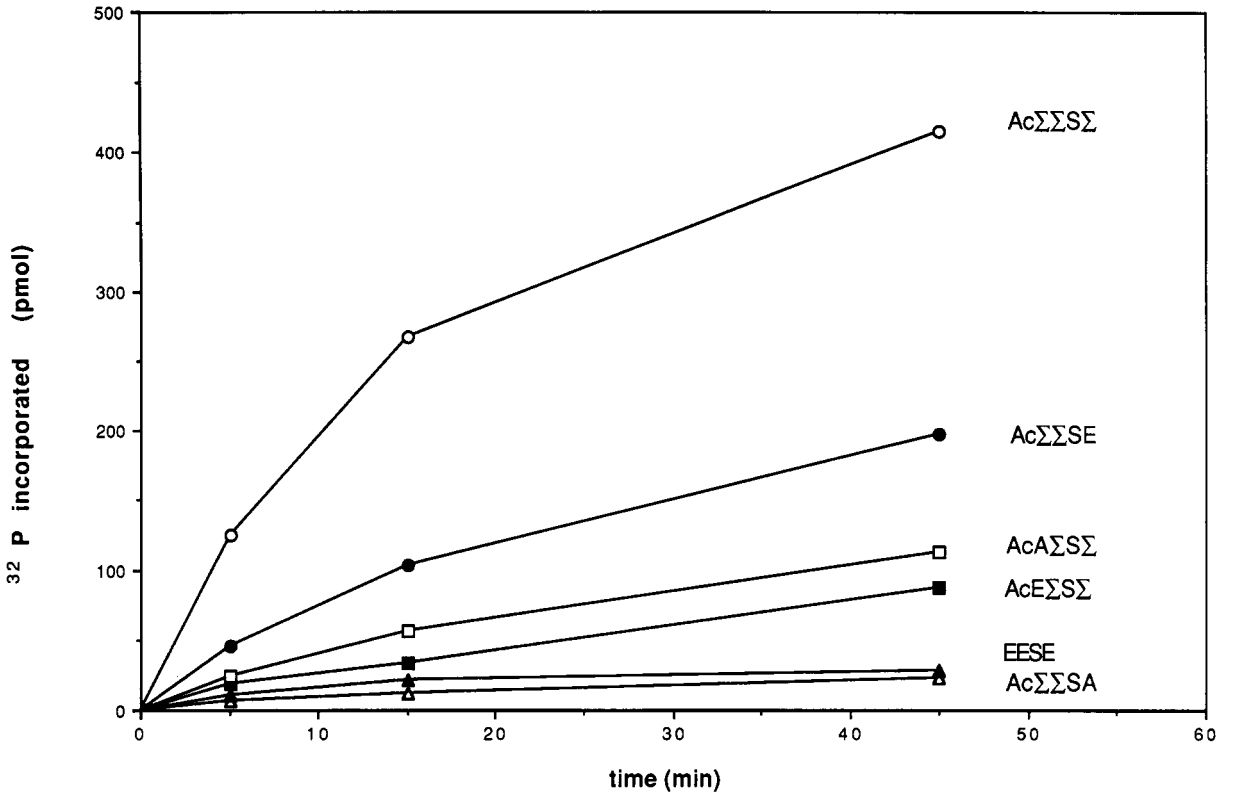

FIGURE 1: Time course of phosphopeptides phosphorylation by CK-2: Effect of Glu for Ser(P) substitutions. The concentration of the peptides was 1 mM. Phosphorylation conditions and evaluation of phosphate incorporated in peptide substrates are described in the Materials and Methods section. The one-letter symbols for amino acids are adopted, and the greek letter Σ denotes *phospho*serine.

lated by CK-2 despite the presence of three N-terminal phosphoseryl residues. The failure of the former peptide to be phosphorylated by CK-2 could be related to the location of its phosphorylatable serine at the C-terminal end. The failure of the latter peptide, which is an excellent substrate for CK-1 (Meggio et al., 1991a), would indicate that phosphoseryl residues must be located at positions −1 and −2 in order to serve as specificity determinants for CK-2, the more remote N-terminal positions being ineffective, or even detrimental. Such a conclusion is corroborated by the behavior of the tetra- and pentaphosphorylated peptides AcSer(P)-Ser(P)-Ser(P)-Ser-Ser(P) and AcSer(P)-Ser(P)-Ser(P)-Ser-(P)-Ser-Ser(P) which are phosphorylated by CK-2 actually less readily than AcSer(P)-Ser(P)-Ser-Ser(P). This differentiates CK-2 from CK-1 whose phosphorylation sites are specified by N-terminal phosphoseryl residues located at either position −3 or, less efficiently, −4. Consequently, neither AcSer(P)-Ser-Ser(P) nor AcSer(P)-Ser(P)-Ser-Ser(P) is readily phosphorylated by CK-1, while both AcSer(P)-Ser-(P)-Ser(P)-Ser-Ser(P) and AcSer(P)-Ser(P)-Ser(P)-Ser-(P)-Ser-Ser(P) are excellent substrates (see Table I). The superiority of latter over the former, in terms of both phosphorylation rate (Table I) and kinetic parameters (Table II) would indicate that the favorable effects of phosphoseryl residues at positions −3 and −4 on the K_m value are additive if not synergistic.

To establish whether phosphorylated residues are strictly required in order to generate atypical phosphorylation sites for CK-2 or can be successfully replaced by carboxylic amino acids, a number of glutamyl derivatives of AcSer(P)-Ser-(P)-Ser-Ser(P) were compared with the parent peptide for their susceptibility to CK-2 phosphorylation. As shown in Figure 1, the Glu for Ser(P) substitution at position −2 is especially detrimental indicating that in this N-terminal position a phosphorylated side chain plays an unique role which cannot

Table II: Kinetic Constants of Phosphopeptides for CK-1[a]

peptide[b]	V_{max} [nmol/(min·mg)]	K_m (mM)	V_{max}/K_m
AcSer(P)-Ser(P)-Ser-Ser(P)	nd[c]	nd[c]	
AcSer(P)-Ser(P)-Ser(P)-Ser-Ser(P)	8.0	0.30	26.6
AcSer(P)-Ser(P)-Ser(P)-Ser(P)-Ser-Ser(P)	6.8	0.09	75.5

[a] The average values from three or more experiments are shown. The standard error was ≤15%. [b] The phosphorylatable residue is underlined. [c] Not determined due to the negligible phosphorylation of the peptide up to a 0.25 mM concentration.

be even partially surrogated by glutamic acid, the peptide AcGlu-Ser(P)-Ser-Ser(P) being actually a substrate as poor as AcAla-Ser(P)-Ser-Ser(P), if not worse. The same substitution at position +1 is less detrimental as shown by the still fairly fast phosphorylation of AcSer(P)-Ser(P)-Ser-Glu, whereas the phosphorylation of its alanyl derivative is negligible. The phosphorylation of the triply substituted glutamyl derivative Glu-Glu-Ser-Glu is also negligible compared to that of the parent triply phosphorylated peptide. These data in conjunction with previous data (Perich et al., 1990) support the view that the position(s) where phosphorylated residues are crucially required in order to generate atypical phosphorylation sites for CK-2 is(are) those adjacent to the N-terminal side of serine, −2 and −1. This concept is corroborated by the kinetic constants of the peptides listed in Table III, roughly falling into three categories: (a) peptides fulfilling the canonical consensus sequence for having either glutamic acid or phosphoserine at position +3 (peptides 1 and 2); (b) peptides fulfilling the consensus sequence for having glutamic acid at position +3 but varying for having either two additional glutamyl residues or two phosphorylated residues at positions +1 and +2 where acidic groups are known to increase the phosphorylation efficiency of CK-2 (Marchiori et al., 1988) (peptides 3, 4, 6, 8, 9); (c) peptides lacking the canonical

consensus sequence whose phosphorylation is specified by phosphorylated residues at atypical positions, namely −1, −2, and +1 (peptides 11–18).

From the data of Table III, it is clear that the effectiveness of phosphorylated vs carboxylic substitution increases in passing from the first group of peptide substrates to the second group and the third group. As previously reported (Pinna, 1990; Litchfield et al., 1990; Meggio et al, 1991b), phosphoserine is only moderately more effective than glutamic acid at the canonical position (compare Ser-Ala-Ala-Ser(P)-Ser(P) with Ser-Ala-Ala-Glu-Glu). Phosphorylation becomes however more effective when the Ser(P) residues are located at the ancillary positions +1 and +2 (compare Ser-Ser(P)-Ser(P)-Glu-Glu with Ser-Glu-Glu-Glu-Glu), and the Thr(P) residue is less effective than the Ser(P) residue at these positions. Such an auxiliary effect of both Ser(P) and Thr(P) is due to their phosphorylated side chain since it is lost in the unphosphorylated derivatives, Ser-Ser-Ser-Glu-Glu and Ser-Thr-Thr-Glu-Glu, respectively.

The peptides in which phosphorylated residues appear to play the most crucial role as specificity determinants are those not fulfilling the canonical consensus sequence for CK-2 and which are nevertheless phosphorylated, AcSer(P)-Ser(P)-Ser-Ser(P) representing the prototype of these unorthodox peptide substrates. Its phosphorylation is dramatically impaired upon substitution of glutamic acid for phosphoserine, Glu-Glu-Ser-Glu exhibiting a 9-fold lower V_{max} value. It is clear however by comparing the kinetic constants of peptides 13 and 14 that the most detrimental substitution is the one upstream of the target serine.

Discussion

A first indication that N-terminally located phosphorylated residues could at least weakly substitute for the canonical consensus sequence for CK-2 (Ser-X-X-Acidic) was provided by the phosphorylation of Thr-4 in the src phosphopeptide Asn-Glu-Tyr(P)-Thr-Ala and by its dependence on previous phosphorylation of Tyr-3 (Perich et al, 1990). In this case, however, the phosphorylation rate is very low compared to those of typical peptide substrates of CK-2 including acidic residues at the crucial +3 position. Here we show that, conversely, the phosphotetrapeptide AcSer(P)-Ser(P)-Ser-Ser(P) is a good substrate whose phosphorylation rate by CK-2 is comparable to those of the best known peptide substrates routinely used for assaying the activity of this protein kinase. While the phosphotripeptide AcSer(P)-Ser-Ser(P) is still a fairly good substrate, a number of derivatives bearing two or three phosphorylated side chains at different positions, including AcSer-Ser(P)-Ser(P), AcSer(P)-Ser(P)-Ser, Ala-Ala-Ser-Ser(P)-Ser(P), Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr, and AcSer(P)-Ser(P)-Ser(P)-Ser are nearly unaffected by CK-2. Therefore, the motif Ser(P)-Ser(P)-Ser-Ser(P) is especially suited for undergoing phosphorylation by CK-2; all three phosphoserines do not seem to be strictly required as indicated by the slower but still appreciable phosphorylation of AcSer(P)-Ser-Ser(P), AcSer(P)-Ser(P)-Ser-Glu, and AcGlu-Ser(P)-Ser-Ser(P). Clearly, however, all three phosphorylated residues at positions −2, −1, and +1 are required in order to achieve optimal phosphorylation efficiencies, comparable to those of the best canonical peptide substrates. The unique role of a phosphate group at position −2 is outlined by the finding that its substitution with a glutamyl residue generates a substrate as poor as the alanyl derivative, suggesting that a carboxylic side chain cannot replace a phosphate group at position −2. At position +1, however, a glutamyl residue is capable of partially substituting for phosphoserine since AcSer(P)-Ser(P)-Ser-Glu is still a substrate, while AcSer(P)-Ser(P)-Ser-Ala is not. The same applies to position −1, considering that the low phosphorylation efficiency of Glu-Glu-Ser-Glu was reduced by replacement of Glu-2 with an alanyl residue (Table III). These observations are of special interest considering that the acidic residue of the peptide binding site of PKA (Glu-170), responsible for the binding of the arginine at position −2 in the peptide substrate of this enzyme (Knighton et al., 1991), is replaced in CK-2 by a histidine (His-158) whereas the PKA hydrophobic residues that interact with the C-terminal side of the peptide substrate (Leu-198, Pro-202, Leu-205) (Knighton et al., 1991) are replaced in CK-2 by either arginyl or lysyl residues (Hanks & Quinn, 1991). It is conceivable therefore that by coming in contact with a phosphate side chain, whose second dissociation is incomplete, His-158 may become fully protonated ultimately giving rise to an electrostatic interaction with the phosphate group. This could provide an explanation for the finding that a carboxylic side chain, which is fully deprotonated at neutral pH, cannot substitute for the phosphoserine at position −2. The additional observation that the PKA glutamic acid interacting with Arg at position −3 in the peptide substrate (Glu-127) (Knighton et al., 1991) is conservatively replaced by Asp (Asp-118) in CK-2 (Hanks & Quinn, 1991) may account for the fact that an additional phosphate at position −3, instead of further improving the phosphorylation efficiency, represents a detrimental feature, AcSer(P)-Ser(P)-Ser(P)-Ser-Ser(P) actually being a substrate not as good as AcSer-(P)-Ser(P)-Ser-Ser(P). A remarkable feature of these atypical sites is that they are generated by phosphorylated residues. Such a behavior differentiates the atypical consensus sequence X(P)-X(P)-Ser-X(P) from the canonical sequence Ser-X-X-Acidic where the phosphoserine and carboxylic amino acids are almost equally effective as specificity determinants. A somewhat intermediate effect of Ser(P) for Glu substitution is observed whenever it occurs at position +1 and +2 in the canonical consensus sequence Ser-Xaa-Xaa-Glu: carboxylic residues in these positions substantially improve the phosphorylation efficiency but this effect is more pronounced if they are replaced by phosphoseryl residues. It is expected therefore that prior phosphorylation of a few residues in a cluster of adjacent serines will either trigger or potentiate the phosphorylation of an additional seryl residue(s) by CK-2. Whether this mechanism is physiologically relevant is presently unknown. In any event, CK-2 appears to be an ideal candidate for maximally phosphorylating seryl clusters which are found in variably phosphorylated forms in proteins like RBP (Vaughn et al., 1987) where they seem to play a role for the transport across the oocyte membrane (Miller et al., 1982). The growing number of proteins that include multiply phosphorylated sites, reviewed by Roach (1991), provides a wide range of new potential targets for CK-2; seryl and/or threonyl residues which are flanked by phosphorylated side chains have been elucidated in a wide variety of proteins which include several protein tyrosine kinases, phosphorylase kinase α subunit, rhodopsin, and ribosomal protein S6. On the basis of the findings reported here, it is conceivable that CK-2 could be involved in the phosphorylation of such proteins, despite their lack of canonical consensus sequences. This therefore, suggests that the search of future targets should be broadened so as to not overlook potential phosphorylation sites.

Our present observation also provides a rationale for the excellent phosphorylation of phosvitin, a widely used substrate for CK-2 since the pioneering studies of Rabinowitz and Lipmann (1960). Phosvitin lacks any canonical consensus

Table III: Kinetic Constants of Peptide Substrates for CK-2[a]

	peptide[b]	K_{cat}[c]	K_m (mM)	K_{cat}/K_m
(1)	Ser-Ala-Ala-Ser(P)-Ser(P)	10.0	8.3	1.2
(2)	Ser-Ala-Ala-Glu-Glu	9.8	18.1	0.5
(3)	Ser-Glu-Glu-Glu-Glu	42.7	3.2	13.3
(4)	Ser-Ser(P)-Ser(P)-Glu-Glu	108.1	2.5	43.2
(5)	Ser-Ser-Ser-Glu-Glu	9.8	2.4	4.0
(6)	Ser-Thr(P)-Thr(P)-Glu-Glu	54.0	7.1	7.6
(7)	Ser-Thr-Thr-Glu-Glu	6.9	1.8	3.8
(8)	AcSer-Ser(P)-Ser(P)-Glu-Glu	105.9	0.4	264.7
(9)	AcSer-Thr(P)-Thr(P)-Glu-Glu	54.1	1.0	54.1
(10)	AcSer-Ser-Ser-Glu-Glu	27.0	1.3	20.7
(11)	AcSer(P)-Ser-Ser(P)	61.4	2.0	30.7
(12)	AcSer(P)-Ser(P)-Ser-Ser(P)	442.0	1.3	176.8
(13)	AcGlu-Ser(P)-Ser-Ser(P)	74.2	2.1	35.3
(14)	AcSer(P)-Ser(P)-Ser-Glu	326.6	4.0	81.6
(15)	Glu-Glu-Ser-Glu	49.7	1.3	38.2
(16)	Glu-Ala-Ser-Glu	27.5	5.0	5.5
(17)	AcSer(P)-Ser(P)-Ser(P)-Ser-Ser(P)	442.0	4.8	92.0
(18)	AcSer(P)-Ser(P)-Ser(P)-Ser(P)-Ser-Ser(P)	264.8	2.6	101.8

[a] The average values from three or more experiments are shown. The standard error was ≤10%. [b] The phosphorylatable residue is underlined. [c] K_{cat} is expressed as nanomoles of P incorporated per minute per nanomole of CK-2, assuming 130 000 as the M_r of CK-2.

sequence for CK-2 in its molecule (Bryne et al., 1984) and is characterized by huge clusters of phosphoseryl residues creating atypical consensus sequences around the rare unoccupied seryl residues embebbed between phosphoserines. Such phosphate-directed atypical phosphoacceptor sites may also account for the early observation that Thr-130 of α_s2 casein is an excellent target for CK-2 (Meggio et al., 1978) despite the fact that threonine is an intrinsically poor substrate, as compared to serine (Marin et al., 1986). α_s2-Casein Thr-130 is actually included into the sequence Ser(P)-Thr-Ser(P)-Glu-Glu which fulfills both the consensus sequences of CK-2, namely, the canonical one, for having a glutamic acid at position +3, and the phosphate-directed one, for being flanked by two phosphoseryl residues.

It should be finally noted that although the atypical consensus sequences for CK-2 are partially determined by N-terminally located phosphoseryl residues, as occurs with CK-1 (Flotow et al., 1990; Meggio et al., 1991a), these two kinases nevertheless differentiate for the positions of the phosphorylated determinants, which are −1/−2 for CK-2 and −3/−4 for CK-1. Consequently, the β-casein-derived peptide, Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr, which is an excellent substrate for CK-1, is not affected by CK-2. Conversely, the phosphotetrapeptide AcSer(P)-Ser(P)-Ser-Ser(P), shown here to be a good substrate for CK-2, is very poorly phosphorylated by CK-1, which readily phosphorylates its N-terminally extended derivatives AcSer(P)-Ser(P)-Ser(P)-Ser-Ser(P) and AcSer(P)-Ser(P)-Ser(P)-Ser(P)-Ser-Ser(P).

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