Inactivation of Nuclear Inhibitory Polypeptides of Protein Phosphatase-1 (NIPP-1) by Protein Kinase A*

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We have recently purified two potent and specific inhibitory polypeptides of protein phosphatase-1 from the particulate fraction of bovine thymus nuclei (Beullens, M., Van Eynde, A., Stalmans, W., and Bollen, M. (1992) J. Biol. Chem. 267, 16538-16544). Here it is reported that these inhibitors, termed NIPP-1a (18 kDa) and NIPP-1b (16 kDa), are excellent substrates (K_m = 0.1 μM) for phosphorylation by protein kinase A on both Ser and Thr residues. Phosphorylation was temporally closely related with an inactivation of NIPP-1. Maximal phosphorylation by protein kinase A (1.5 mol of phosphate/mol of NIPP-1) caused an 8-fold increase in the concentration of NIPP-1 required for half-complete inhibition of the catalytic subunit of protein phosphatase-1, irrespective of the concentration of the phosphatase. Phosphorylation decreased the binding of NIPP-1 to immobilized protein phosphatase-1. NIPP-1 could be efficiently and completely reactivated by incubation with the catalytic subunit of protein phosphatase-2A. The type-1 catalytic subunit was much less effective, however, even when present in a molar excess to NIPP-1.

Chromatography of a salt extract of the particulate nuclear fraction on Mono Q revealed three species of PP-1. One of these species, termed PP-1Na, contained NIPP-1 as a subunit and could be activated 6-fold by incubation with protein kinase A under phosphorylating conditions. This activation of PP-1Na is opposite to the known inhibition of cytoplasmic species of protein phosphatase-1 by protein kinase A.

Reversible protein phosphorylation on serine/threonine residues represents the most ubiquitous mechanism for the control of nuclear processes like mitosis, transcription, DNA replication, and nucleocytoplasmic transport (1-3). Many structural and regulatory polypeptides in the nucleus are even slightly modified. First, NIPP-1 was extracted from the particulate fraction of bovine thymus nuclei (9). Two of these inhibitors (NIPP-la and NIPP-lb) have been identified as a positive regulator of PP-1 (11). We report here that NIPP-1 behaves like an inhibitory subunit of one species of the particulate nuclear PP-1 (PP-1Na), and that NIPP-1a and NIPP-1b lose their inhibitory potency after phosphorylation by PKA. This contrasts with the known regulation of cytoplasmic inhibitors of PP-1 (inhibitor-1 and DARPP-32), which are activated through phosphorylation by PKA (8).

**Experimental Procedures**

Materials—PP-1c (12) and PP-2Ac (13) were purified from rabbit skeletal muscle. PP-1c-Sepharose was prepared according to the recommendations of Pharmacia; 20 μg of PP-1c were coupled to 0.5 g of CNBr-activated Sepharose 4B at pH 5.3. Control Sepharose was prepared in the same way except for the absence of PP-1c. NIPP-1a and NIPP-1b were purified from bovine thymus nuclei until after reversed phase chromatography as described previously (9), with two slight modifications. First, NIPP-1 was extracted from the particulate fraction of bovine thymus nuclei. The abbreviations used are: PKA, catalytic subunit of cAMP-dependent protein kinase (protein kinase A); NIPP-1, nuclear inhibitors of PP-1; PP-1, protein phosphatase-1; PP-1c, catalytic subunit of PP-1; PP-1N, nuclear PP-1; PP-2A, protein phosphatase-2A; PP-2Ac, catalytic subunit of PP-2A; CREB, cAMP response element-binding protein; PAGE, polyacrylamide gel electrophoresis; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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‡ The abbreviations used are: PKA, catalytic subunit of cAMP-dependent protein kinase (protein kinase A); NIPP-1, nuclear inhibitors of PP-1; PP-1, protein phosphatase-1; PP-1c, catalytic subunit of PP-1; PP-1N, nuclear PP-1; PP-2A, protein phosphatase-2A; PP-2Ac, catalytic subunit of PP-2A; CREB, cAMP response element-binding protein; PAGE, polyacrylamide gel electrophoresis; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.
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An Improved Procedure for the Extraction of NIPP-1—We have previously solubilized NIPP-1 by incubation of the particulate nuclear fraction from bovine thymus with 0.3 M NaCl during 30 min at 0 °C (9). More recently we have noted, however, that about 4 times more heat-stable inhibitor of PP-1 is extracted during incubation at 30 °C (Table 1). The inhibitors solubilized at 30 °C could not be differentiated from the previously described NIPP-1a and NIPP-1b, with respect to molecular mass on SDS-PAGE, inhibitory potency, and specificity (not shown). Interestingly, the better extraction of NIPP-1 at 30 °C was not associated with a similar increase in solubilized phosphorylase phosphatase activity (plus 70%) or total protein (plus 25%). Moreover, the degree of latency of the phosphorylase phosphatase released (ratio of spontaneous/trypsin-revealed activity) was the same at 0 °C (0.41) and 30 °C (0.43). This means that the previously (9) calculated ratio between the particulate amounts of NIPP-1 and of PP-1 has been underestimated about 2-fold. Indeed, comparison of the trypsin-revealed phosphorylase phosphatase activity (which reflects the concentration of PP-1c) with the NIPP-1 activity suggests now a 2-fold molar excess of PP-1c (Table 1), rather than the previous values of 4-5 (9).

Phosphorylation by PKA—Both NIPP-1a (18 kDa) and NIPP-1b (16 kDa) were excellent substrates for phosphorylation by PKA (Fig. 1A). Actually, NIPP-1 was already well phosphorylated by protein kinase C, although it was insufficient to catalyze a measurable phosphorylation of model substrates like Kemptide or histone IIA (not shown). The phosphorylation of NIPP-1 was completely blocked by addition of 5 μM of a specific inhibitory peptide of PKA (not illustrated), showing that the phosphorylation was not catalyzed by a contaminating protein kinase. The better labeling of NIPP-1b, relative to NIPP-1a (Fig. 1A), can be explained by repeated observations that NIPP-1b represents by far the most abundant species after purification (9). Maximal phosphorylation by PKA resulted in the incorporation of 1.5 ± 0.3 mol of phosphate/mol of NIPP-1 (mean ± S.E. for 5 preparations of NIPP-1). The maximal extent of phosphorylation by PKA was not affected by a preincubation of NIPP-1 with PP-1c plus PP-2Ac (not illustrated), indicating that purified NIPP-1 was already maximally dephosphorylated.

Limited action of S. aureus V8 protease on PKA-phosphorylated NIPP-1a or NIPP-1b resulted in the generation of three major phosphopeptides of smaller molecular mass (Fig. 1B). The two smallest phosphopeptides were identical for both inhibitors, as judged by their molecular mass on SDS-PAGE (3.6 and 5.4 kDa). The difference in the molecular mass of the largest phosphopeptide (7.4 versus 6.1 kDa for NIPP-1a and NIPP-1b, respectively) corresponds to a similar difference in the mass of the purified inhibitors. These data confirm the structural homology between NIPP-1a and NIPP-1b.

### Table I

**Extraction of the nuclear particulate fraction at different temperatures**

| Extraction temperature | NIPP-1 (units/g thymus) | Phosphorylase phosphatase (units/g thymus) |
|------------------------|-------------------------|-----------------------------------------|
| 0 °C                   | 6.9 ± 1.6               | 26.9 ± 4.8                              |
| 30 °C                  | 14.7 ± 0.85             | 26.3 ± 2.1                              |
| Spontaneous            | 36.0 ± 4.8              | 61.3 ± 5.0                              |
| After trypsin          | 5.7 ± 0.6               | 7.1 ± 0.7                               |

**RESULTS**

**Partial Purification of PP-1N**—The particulate fraction of 2.5 g of nuclei was extracted with 0.3 M NaCl (9), and the dialyzed against a buffer containing 50 mM Tris at pH 7.5 and 0.5 mM dithiothreitol. The dialysate was clarified by centrifugation (10 min at 10,000 x g) and applied to a column of aminohexyl-Sepharose 4B (10 x 1 cm) equilibrated in the same buffer. The column was eluted with a linear salt gradient (0–0.8 M NaCl). All the phosphorylase phosphatase activity eluted in a single peak at about 0.4 M NaCl. The peak fractions were pooled, dialyzed against buffer without salt, and applied to a Mono Q column (5 x 0.5 cm), equilibrated in the same buffer. The column was eluted with a linear salt gradient (0–0.4 M NaCl) and assayed for spontaneous and trypsin-revealed phosphorylase phosphatase activity (see Fig. 6).
NIPP-1b, as noted previously (9). Phosphoamino acid analysis showed furthermore that both inhibitors were phosphorylated on Ser as well as Thr residues (Fig. 1C). In spite of its higher acid stability (16), phosphothreonine accounted for only 30% of the recovered radioactivity. Combined with an average maximal stoichiometry of 1.5 mol of phosphate incorporated/mol of NIPP-1, this implies that at most half of NIPP-1 was Thr-phosphorylated by PKA.

Kinetics of Phosphorylation—With a $K_v$ of 0.1 $\mu M$ (Fig. 2A), NIPP-1 belongs to the best substrates of PKA. For example, using the same preparation of PKA, a $K_v$ of 5 $\mu M$ was obtained for histone IIA (Fig. 2B). The superior substrate quality of NIPP-1 is also apparent from the $V_{\text{max}}$ (15.8 nmol of phosphate incorporated/min/µg of kinase), which was 10 times higher than the $V_{\text{max}}$ obtained for phosphorylation of histone IIA (Fig. 2B).

Effects of Phosphorylation—Phosphorylation by PKA turned NIPP-1 into a less potent inhibitor of PP-1c (Fig. 3). As a mean, the concentration of NIPP-1 required for half-maximal inhibition of PP-1c ($IC_{50}$) was increased 8-fold after phosphorylation. It is noteworthy that phosphorylation increased the $IC_{50}$ 8-fold, irrespective of the concentration of the phosphatase during the assay, in the range from 0.1 to 2 nM (not illustrated). The inhibitory potency of NIPP-1a and NIPP-1b (separately pooled after Mono Q) decreased to a similar extent by phosphorylation (not illustrated). Furthermore, this phosphorylation effect was observed with both phosphorylase (Fig. 3) and with casein (not shown) as substrate for the phosphatase.

There was a close temporal relationship between the phosphorylation of NIPP-1 and the loss of its inhibitory potency (Fig. 4). An inverse linear relationship between the phosphorylation level and activity of NIPP-1 was also obtained when the concentration of PKA was varied rather than the incubation time (Pearson’s correlation coefficient $r = 0.99; p = 0.002$). Taken together, these data strongly suggest a causal relationship between phosphorylation and inactivation of NIPP-1.

We have also investigated whether the decreased inhibitory power of PKA-phosphorylated NIPP-1 (Fig. 3) results from a decreased affinity of the inhibitor for PP-1c or from a lesser inhibition of PP-1c by bound phospho-NIPP-1. Gel filtration experiments on Superdex 75 (Pharmacia LKB) did not give a clear answer, probably due to the rather small difference in apparent molecular mass between PP-1c (35 kDa) and the complex with NIPP-1 (50 kDa). In search for an alternative approach, we found that NIPP-1 is efficiently (>90%) retained by PP-1c, covalently bound to Sepharose (Table II). In contrast, after prior phosphorylation by PKA only a minor part of NIPP-1 (about 30%) was retained by PP-1c-Sepharose, whether measured by activity assays (Table II) or by autoradiography after Tricine-SDS-PAGE (not illustrated).

Reactivation of NIPP-1—The basal activity of NIPP-1 was not affected by a preincubation with protein phosphatases-1 and -2A (9). However, PKA-inactivated NIPP-1 could be completely reactivated by incubation with the catalytic subunit of PP-2A (Fig. 5). With phosphorylase as reference substrate, the type-1 catalytic subunit was far less efficient in dephosphorylating NIPP-1, even when present in a 3-fold molar excess over the inhibitor. The addition of 1 mM MnCl$_2$ did not affect the NIPP-1 phosphatase activity of PP-1c (not illustrated).

Identification of NIPP-1 as a Subunit of PP-1N$_{\alpha}$—All the phosphorylase phosphatase activity that was extracted from the nuclear particulate fraction by incubation with 0.3 M NaCl was retained on aminohexyl-Sepharose and eluted as a single peak at 0.4 M NaCl (not shown). However, subsequent chromatography on Mono Q revealed three phosphatase activity peaks (Fig. 6). While the second and third peak contained...
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FIG. 2. Kinetics of phosphorylation by PKA. Various concentrations of NIPP-1 (panel A) or histone IIA (panel B) were incubated for 20 min at 30 °C in the presence of 0.1 mM γ-32P-labeled ATP, 1 mM magnesium acetate, and either with 27 pM PKA (A) or 270 pM PKA (B). The incorporation of radioactivity into these substrates was measured as explained under "Experimental Procedures," and used to calculate reaction velocities (nmol of phosphate incorporated/min/μg of kinase). The graph shows Lineweaver-Burk plots of the means ± S.E. for 4 experiments.

FIG. 3. Inactivation of NIPP-1 by phosphorylation with PKA. NIPP-1 was incubated for 60 min at 30 °C in the presence of 0.1 mM ATP and 1 mM magnesium acetate, without (○) or with (●) 80 nM PKA. Subsequently, the incubation mixtures were heated (2 min at 90 °C), and assayed at various dilutions, corresponding to the indicated activities of NIPP-1 before incubation, for their power to inhibit 1 nM PP-1c. One unit of NIPP-1 corresponds to about 2 pmol. The concentration of NIPP-1 is shown on a log scale. The results represent the means ± S.E. for 13 experiments.

spontaneously active enzyme, the first peak contained a largely latent phosphatase, whose activity could be revealed by a preincubation with trypsin. Several lines of evidence indicated that this latent enzyme, termed PP-1Na, represents a heterodimer of the type-1 catalytic subunit and NIPP-1. First, the phosphatase activity and the heat-stable inhibitory activity co-eluted during subsequent gel filtration on Superdex 75 (not shown). Actually, the migration of PP-1Na coincided with that of an in vitro reconstituted complex (50 kDa) of purified NIPP-1 and catalytic subunit (not shown). Second, the heat-stable inhibitory activity behaved like NIPP-1a (18 kDa) and NIPP-1b (16 kDa) during SDS-PAGE, blotting, and elution from polyvinylidene difluoride membranes (9). Third, consistent with the inactivation of NIPP-1 by phosphorylation, we found that PP-1Na was activated 6.2 ± 0.6-fold (n = 4) by a preincubation with protein kinase A and MgATP.

DISCUSSION

Reversible Phosphorylation of NIPP-1 in Vitro—We have shown here that NIPP-1 from calf thymus is inactivated
TABLE II
Effect of phosphorylation on binding of NIPP-1 to PP-1c.

NIPP-1 was incubated as described in Fig. 3, in the absence (NIPP-1) or presence (phospho-NIPP-1) of 80 nM PKA. After heating (2 min at 90 °C) the incubation mixtures (15 μl) were incubated for 30 min at 25 °C under regular shaking with 10 μl of either PP-1c-Sepharose or untreated Sepharose. Subsequently, the gels were spun down (1 min at 10,000 × g). The supernatants were incubated for 30 min at 30 °C with PP-2A c (100 units/ml), heated for 2 min at 90 °C, and assayed for NIPP-1 activity. The results represent the means ± S.E. for 3 experiments.

| Inhibitor       | Matrix              | NIPP-1 not retained % of control |
|-----------------|---------------------|----------------------------------|
| NIPP-1          | Untreated Sepharose | 100                              |
|                 | PP-1c-Sepharose     | 8 ± 4                            |
| Phospho-NIPP-1  | Untreated Sepharose | 105 ± 7                          |
|                 | PP-1c-Sepharose     | 67 ± 7                           |

![Graph](https://example.com/graph.png)

Fig. 5. Preferential reactivation of NIPP-1 by PP-2A c. NIPP-1 (35 units/ml) was phosphorylated by PKA as described in the legend to Fig. 3. After heating (3 min at 90 °C), aliquots of the inactivated NIPP-1 were incubated during 10 min at 30 °C with the indicated concentrations of the catalytic subunits of either PP-1 c (0) or PP-2A c (25), causing a dilution of 10%. Following a second heat treatment, the mixtures were diluted 30-fold and assayed for their power to inhibit 1 nM PP-1c. The NIPP-1 activity prior to phosphorylation has been taken as 100%. The protein phosphatase concentrations are shown on a log scale. The results represent the mean ± S.E. for 3 experiments.

through phosphorylation by PKA (Figs. 3 and 4). Remarkably, 15 years ago Huang et al. (17) reported that rat liver contains a small, heat-stable inhibitory polypeptide of phosphorylase phosphatase that could be inactivated by the action of PKA. Although this hepatic inhibitor has never been characterized in greater detail, its properties suggest that it may well represent the hepatic homologue of NIPP-1.

NIPP-1 may be the best substrate of PKA ever described. Indeed, the $K_i$ for NIPP-1 amounted to only 0.1 μM (Fig. 24a), which is 20–80 times lower than that reported for other model substrates of PKA, like histone IIa (Fig. 2B), inhibitor-1 (18), DARPP-32 (18, 19), and Kemptide, i.e., a heptapeptide that contains the phosphorylatable Ser in hepatic pyruvate kinase (20, 21). In addition, the $V_{max}$ of PKA with NIPP-1 as substrate (Fig. 2a) was similar to that with Kemptide (20) and 3–10 times higher than those with histone IIa (Fig. 2B and Ref. 22) and histone f2b (19). Efficient phosphorylation by PKA requires the presence of a pair of basic amino acids N-terminal to a Ser residue (23). At first glance, it seems paradoxical that NIPP-1 is a better substrate than Kemptide, since the latter contains the consensus sequence for optimal phosphorylation by PKA (Arg-Arg-X-Ser). It will therefore be important to elucidate the primary structure around the phosphorylated Ser and Thr in NIPP-1. However, as has been demonstrated for inhibitor-1 and DARPP-32, it is possible that other structural factors residing in domains far from the phosphorylation site may contribute significantly to efficient phosphorylation by PKA (18, 19, 24).

Unphosphorylated NIPP-1 inhibits PP-1 with extremely high affinity ($K_i < 1$ pM); hence, at 1 nM PP-1 (as in Fig. 3), the $IC_{50}$ is 0.5 nM (9). Fig. 3 shows that phosphorylation of NIPP-1 increased the $IC_{50}$ to 4 nM, suggesting a $K_i$ of similar magnitude. However, assays at widely different concentrations of PP-1 (0.1 and 2 nM) revealed also the same 8-fold difference in $IC_{50}$ between native and maximally phosphorylated NIPP-1. This indicates that, after exhaustive phosphorylation, some 90% of NIPP-1 has completely lost its affinity for PP-1, whereas a small fraction of the inhibitor was not inactivated at all. The reason for this resistance to inactivation in vivo remains to be established.

After phosphorylation by PKA, NIPP-1 could not be measurably reactivated by substoichiometric concentrations of PP-1 c (Fig. 5). This may indicate that NIPP-1, in contrast to inhibitor-1 and inhibitor-2 (25, 26), does inhibit its own dephosphorylation by PP-1 c. However, since PP-1 c remained very inefficient in reactivating NIPP-1, even when present in a 3-fold molar excess to NIPP-1, it seems more likely that PP-1 c is intrinsically an inferior NIPP-1 phosphatase. At variance with previous findings for inhibitor-1 and inhibitor-2 (25, 26), MnCl$_2$ did not improve the dephosphorylation of NIPP-1 by PP-1 c.

Antagonistic Control of Cytoplasmic and Nuclear Species of PP-1—Previous investigations have uncovered several mechanisms whereby PKA may decrease the activity of cytoplasmic species of PP-1 (discussed in Refs. 7 and 8). Best studied is the PKA-mediated phosphorylation of inhibitor-1 and DARPP-32, which turns these polypeptides into potent and specific inhibitors of PP-1 (Fig. 7). In skeletal muscle, phosphorylation by PKA of the G-subunit of the glycogen-associated PP-1 results in the release of the (less active) catalytic subunit, which may then be further inhibited by association with phospho-inhibitor-1.

In thymus nuclei virtually all the heat-stable protein inhibitor of PP-1 is associated with the particulate fraction (9), and our current data indicate that all the NIPP-1 is present as the inhibitory subunit of a heterodimeric species of PP-1, termed PP-1Na. As present in the holoenzyme, NIPP-1 is accessible to PKA, which is able to increase the phosphorylase phosphatase activity of PP-1Na 6-fold. This activation of the nuclear PP-1Na contrasts with the inhibitory effects of PKA on cytoplasmic PP-1 (Fig. 7). The data in Table II indicate that the activation of PP-1Na results from a dissociation of phospho-NIPP-1.

The role of the putative phosphorylation of NIPP-1 by PKA in vivo remains a matter of speculation. However, two general functions can be theoretically envisaged. First, activation of PP-1Na may be instrumental in the down-regulation of signals acting through cAMP in the nucleus. Such a mechanism may, for example, be involved in the reversal of the cAMP-induced gene expression mediated by the transcription factor CREB upon phosphorylation by PKA on Ser-133. Since the latter residue is dephosphorylated by PP-1N in intact cells (27), the PKA-induced inactivation of NIPP-1 could serve to curtail cAMP-induced transcription. However,
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Fig. 6. Chromatography of PP-1N on Mono Q. Following salt extraction of the particulate nuclear fraction and chromatography on aminohexyl-Sepharose, PP-1N was applied to a Mono Q column, as detailed under "Experimental Procedures." The eluted fractions (0.5 ml) were assayed for spontaneous (○) and trypsin-revealed (●) phosphorylase phosphatase activity.

Fig. 7. Opposite regulation of cytoplasmic and nuclear species of PP-1 by PKA.

since the nucleus (even the particulate fraction, cf. Fig. 6) appears to contain several species of PP-1, it is also possible that the physiological CREB phosphatase is distinct from the NIPP-1 containing PP-1Na. A second possible purpose of the putative activation of PP-1Na may be to allow cAMP to induce the dephosphorylation of specific nuclear proteins that are not phosphorylated by PKA. This mechanism could account for previous observations that cAMP antagonizes the phorbol ester-mediated phosphorylation of a subset of polypeptides in S49 cells (28).

Comparison of NIPP-1a and NIPP-1b—Although these two inhibitory polypeptides migrated slightly differently during SDS-PAGE, they could not be differentiated by their inhibitory potency and specificity (9). In this study it is shown that both forms of NIPP-1 were inactivated by phosphorylation on Ser/Thr residues (Fig. 1). Furthermore, NIPP-1a and NIPP-1b yielded a similar phosphopeptide map after limited proteolysis (Fig. 1B). These data suggest a close structural relationship between NIPP-1a and NIPP-1b.

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REFERENCES
1. Meek, D. W., and Street, A. J. (1992) Biochem. J. 287, 1-15
2. Norbury, C., and Nurse, P. (1992) Annu. Rev. Biochem. 61, 441-470
3. Silver, F. A. (1991) Cell 64, 489-497
4. Nigg, E. A., Hils, H., Eppenberger, H. M., and Dutly, F. (1985) EMBO J. 4, 2801-2806
5. Adams, S. R., Harsent, A. T., Buechler, Y. J., Taylor, S. S., and Tsien, R. Y. (1991) Nature 349, 694-697
6. Bollen, M., Beullens, M., Van Eynde, A., and Stalmans, W. (1993) Ado. Protein Phosphatases 7, 31-47
7. Cohen, P. (1989) Annu. Rev. Biochem. 58, 453-508
8. Bollen, M., and Stalmans, W. (1992) Crit. Rev. Biochem. Mol. Biol. 27, 227-293
9. Beullens, M., Van Eynde, A., Stalmans, W., and Bollen, M. (1992) J. Biol. Chem. 267, 16538-16544
10. Fernandez, A., Bresgigian, D. L., and Lamb, N. J. C. (1992) J. Cell. Biol. 116, 1421-1430
11. Ohkura, H., and Yanagida, M. (1991) Cell 64, 149-157
12. DeGuzman, A., and Lee, E. Y. C. (1998) Methods Enzymol. 159, 356-368
13. Ramachandran, C., Gori, J., Waelkens, E., Merlevede, W., and Walsh, D. A. (1987) J. Biol. Chem. 262, 3210-3218
14. Glass, D. R., Masaracchia, R. A., Ferramisco, J. R., and Kemp, B. E. (1978) Anal. Biochem. 87, 566-575
15. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368-379
16. Cooper, J. A., Selton, B. M., and Hunter, T. (1985) Methods Enzymol. 99, 387-402
17. Huang, F. L., Tao, S., and Ginsmann, M. H. (1977) Biochem. Biophys. Res. Commun. 78, 615-622
18. Hemmings, H. C., Jr., Nairn, A. C., and Greengard, P. (1984) J. Biol. Chem. 259, 14491-14497
19. Hemmings, H. C., Jr., Nairn, A. C., Elliott, J. J., and Greengard, P. (1990) J. Biol. Chem. 265, 20969-20976
20. Kemp, B. E., Graves, D. J., Benjamini, E., and Krebs, E. G. (1977) J. Biol. Chem. 252, 4888-4894
21. Kemp, B. E., and Clark, M. G. (1978) J. Biol. Chem. 253, 5147-5154
22. Heimann, E. M., and Beham, R. A. (1980) Methods Enzymol. 80, 51-55
23. Kemp, B. E., and Pearson, R. B. (1990) Trends Biochem. Sci. 15, 342-346
24. Chessa, G., Borin, G., Marchiori, F., Meggio, F., Brunati, A. M., and Pinna, L. A. (1983) Eur. J. Biochem. 139, 829-844
25. Nimmo, G. A., and Cohen, P. (1978) Eur. J. Biochem. 73, 553-565
26. Tonks, N. R., and Cohen, P. (1984) Eur. J. Biochem. 140, 61-70
27. Haywara, M., Albert, A., Brittle, P., Minko, J., Ferramisco, J., Deng, T., Katin, M., Shenolikar, S., and Montminy, M. (1992) Cell 70, 105-113
28. Kiss, Z., and Steinberg, R. A. (1985) J. Cell. Physiol. 125, 200-206