Dephosphorylation of phosphorylase kinase by a histone H1-stimulated phosphoprotein phosphatase

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A histone H1-stimulated protein phosphatase isolated from rabbit skeletal muscle dephosphorylated [^{32}P]phosphorylase kinase. The rate of dephosphorylation was markedly increased by 5–50 μg histone H1/ml. Only the α and α' subunits were dephosphorylated in the absence or presence of histone H1. This is consistent with previous results suggesting that the H1-stimulated phosphatase is a type-2 protein phosphatase. The present studies suggest that the histone H1-stimulation is the result of a direct interaction of the histone with the phosphatase.

Dephosphorylation *Phosphorylase kinase* *Histone H1* *Protein phosphatase*

1. INTRODUCTION

Recently, we described the isolation of a heat-stable activator of phosphorylase phosphatase [1], and demonstrated that it was histone H1 [2]. In subsequent studies, it was found that a histone H1-stimulated phosphorylase phosphatase from rabbit skeletal muscle could be inhibited by 0.1 mM ATP, but not by phosphatase inhibitor-1 [3]. A bovine aorta histone H1-stimulated phosphatase was also insensitive to heat-stable phosphatase inhibitor-1 and -2 [4]. Thus, these H1-stimulated phosphatases appeared to be type-2 protein phosphatases according to the nomenclature in [5,6]. To characterize further the H1-stimulated rabbit muscle phosphatase we have investigated the influence of histone H1 on phosphatase activity utilizing as substrate phosphorylase kinase phosphorylated on the α,α' and β subunits. These studies provide further evidence that the H1-stimulated phosphatase is a type-2 protein phosphatase, and suggest that stimulation by H1 is the result of its interaction with the phosphatase and not with substrates.

2. MATERIALS AND METHODS

The histone H1-stimulated phosphatase and phosphatase-1 catalytic subunit were purified from rabbit skeletal muscle as in [3]. Rabbit skeletal muscle [^{32}P]phosphorylase *a* was prepared as in [7], and phosphatase inhibitor-1 as in [8]. Phosphorylase kinase was purified from rabbit skeletal muscle by the method in [9] through the Sepharose gel filtration step, and phosphorylated with [γ-^{32}P]ATP (400–1000 cpm/pmol) and cyclic AMP-dependent protein kinase catalytic subunit essentially as in [10]. The phosphorylated phosphorylase kinases used in this study contained 0.92–1.24 μmol phosphate per μmol ($\alpha + \alpha'$)-subunits and 0.55–0.58 μmol phosphate per μmol β subunit. The standard conditions for dephosphorylation were: 0.5–1.0 μM ^{32}P as phosphorylase *a* or phosphorylase kinase ($\alpha + \alpha'$)-subunits in 100 μl of 50 mM imidazole–HCl, 1.8 mM EDTA, 0.5 mM EGTA, 2 mM dithiothreitol, 0.2 mg bovine serum albumin/ml, 2% glycerol (pH 7.2). Dephosphorylation was carried out at 30°C in the absence or presence of various concentrations of histone H1. The histone H1 was

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purified from calf thymus as in [11]. Phosphatase activity was determined by release of 10% trichloroacetic acid-soluble ^{32}P from substrates [7]. SDS-polyacrylamide gel electrophoresis was performed in 7.5% slab gels using the buffer system in [12].

3. RESULTS

3.1. *Dephosphorylation of phosphorylase kinase by a histone H1-stimulated protein phosphatase*

The skeletal muscle histone H1-stimulated protein phosphatase which was previously shown to dephosphorylate [^{32}P]phosphorylase *a* [3] also had activity on ^{32}P-labeled phosphorylase kinase. In the absence of histone, the ratio of phosphatase activities on phosphorylase kinase *vs* phosphorylase *a* was 0.33 when both substrates were assayed at the same concentration (1.0 μM accessible ^{32}P), and in the same assay buffer (see section 2). The addition of histone H1 greatly increased the rate of dephosphorylation of the phosphorylase kinase in a concentration-dependent manner (fig.1). Neither the basal activity nor the H1-stimulated activity

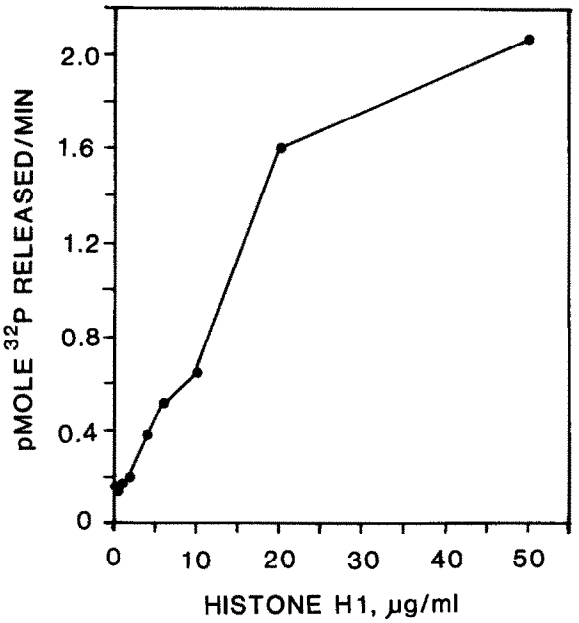

Fig.1. The effect of histone H1 on phosphorylase kinase phosphatase activity. Phosphorylase kinase phosphatase activity was determined as described in section 2 in the presence of various concentrations of histone H1. The concentration of ^{32}P-labeled ($\alpha + \alpha'$)-subunits was 0.5 μM. Each assay contained 39 munits of H1-stimulated phosphorylase phosphatase activity/ml measured in the presence of histone H1, using the unit definition and assay described in [3].

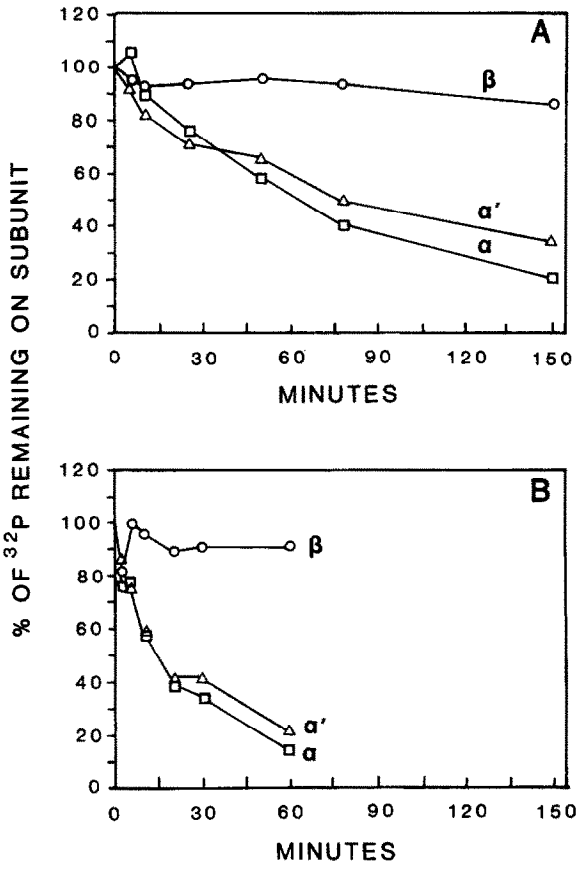

Fig.2. Specificity of the H1-stimulated phosphatase for the α and α' subunits of phosphorylase kinase. Samples of phosphorylase kinase (0.69 μM total ^{32}P) were incubated in the standard dephosphorylation buffer in the absence or presence of 8 μg histone H1/ml (panels A and B, respectively). H1-stimulated phosphatase (78 munits/ml) was added to each mixture, and at various times aliquots were removed and heated at 100°C for 2 min in the presence of 1.5% SDS and 220 mM β-mercaptoethanol. The samples were electrophoresed as described in section 2, stained with Coomassie blue, destained, and the α,α' and β subunits excised and counted in a liquid scintillation counter. The percentage of ^{32}P counts remaining in each subunit was determined by comparison to the label present in the subunits of a sample which was electrophoresed before the addition of phosphatase.

was inhibited by phosphatase inhibitor-1, but activity in the presence or absence of H1 was stimulated by 5 mM Mn^{2+} (not shown).

3.2. *Specificity toward the phosphorylated subunits of phosphorylase kinase*

Dephosphorylation of the α,α' and β subunits by the H1-stimulated phosphatase was determined by liquid scintillation counting of the Coomassie-stained subunits excised from SDS-gels (fig.2). The phosphatase was highly specific for the α and α' subunits (fig.2, panel A). The addition of histone H1 greatly accelerated dephosphorylation of these subunits, but did not allow dephosphorylation of the β-subunit (fig.2, panel B). In a similar experiment, utilizing purified catalytic subunit of type-1 phosphatase it was possible to dephosphorylate nearly totally the β-subunit without dephosphorylating the α and α' subunits (not shown).

4. DISCUSSION

The rabbit skeletal muscle histone H1-stimulated protein phosphatase was capable of dephosphorylating [^{32}P]phosphorylase kinase, and the rate of dephosphorylation was greatly increased by histone H1 (fig.1). Thus, the H1-stimulation of this phosphatase is not restricted to the dephosphorylation of phosphorylase *a*, the only other substrate previously studied [1–4]. The effect of histone H1 on both phosphorylase *a* and phosphorylase kinase dephosphorylation suggests, but does not prove, that the stimulation is not a substrate-directed effect. If phosphorylase *a* is used as substrate, the latter possibility must be considered since protamine, which activated the H1-stimulated phosphatase [3], has a direct inhibitory effect on phosphorylase *a* [13]. However, at a concentration of 15 μg/ml, histone H1, which was a better activator of the phosphatase than protamine [3], did not inhibit phosphorylase *a* activity (unpublished). This concentration of histone H1 dramatically activated the phosphatase activity with phosphorylase kinase (fig.1) or phosphorylase *a* [3] as the substrate. Thus, the activation of the phosphatase by histone H1 could be resolved from polycation-induced inhibition of phosphorylase *a*. Furthermore, maximum activation of phosphorylase phosphatase activity could be achieved in the presence of a 10-fold molar excess of phosphorylase *a* relative to histone H1 [1,3]. Since histone H1-stimulation appears to be phosphatase-directed, it is likely that histone H1 may influence the dephosphorylation of other phosphoprotein substrates in addition to phosphorylase *a* and phosphorylase kinase. For example, there may be nuclear phosphoproteins which serve as substrates for an H1-stimulated phosphatase. In recent experiments, H1-stimulated phosphorylase phosphatase activity has been detected in rat liver nuclear preparations (unpublished).

The specificity of the histone H1-stimulated phosphatase for the α and α' subunits of phosphorylase kinase (fig.2) confirms the previous suggestion that the enzyme is a type-2 phosphatase [3,4]. Among the type-2 phosphatases, only phosphatases 2B and 2C have known metabolic regulators, Ca^{2+} and Mg^{2+} respectively [6]. There is no known metabolic regulator of phosphatase 2A [6], the previously described type-2 protein phosphatase which most closely resembles the H1-activated phosphatase. It will be of interest to determine if a histone H1-like activator exists in the cytoplasm, and to establish if histone H1 is important in regulating the dephosphorylation of nuclear phosphoproteins.

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REFERENCES

[1] Wilson, S.E., Mellgren, R.L. and Schlender, K.K. (1982) FEBS Lett. 146, 331–334.
[2] Wilson, S.E., Mellgren, R.L. and Schlender, K.K. (1983) Biochem. Biophys. Res. Commun. 116, 581–586.
[3] Mellgren, R.L. and Schlender, K.K. (1983) Biochem. Biophys. Res. Commun. 117, 501–508.
[4] DiSalvo, J., Waelkens, E., Gifford, D., Goris, J. and Merlevede, W. (1983) Biochem. Biophys. Res. Commun. 117, 493–500.
[5] Ingebritsen, T.S., Foulkes, J.G. and Cohen, P. (1980) FEBS Lett. 119, 9–15.

[6] Ingebritsen, T.S. and Cohen, P. (1983) Science 221, 331–338.
[7] Killilea, S.D., Aylward, J.H., Mellgren, R.L. and Lee, E.Y.C. (1978) Arch. Biochem. Biophys. 191, 638–646.
[8] Foulkes, J.G. and Cohen, P. (1979) Eur. J. Biochem. 97, 251–256.
[9] Cohen, P. (1973) Eur. J. Biochem. 34, 1–14.
[10] Antoniw, J.F. and Cohen, P. (1976) Eur. J. Biochem. 68, 45–54.
[11] Sanders, C. (1977) Biochem. Biophys. Res. Commun. 78, 1034–1042.
[12] Laemmli, U.K. (1970) Nature 227, 680–685.
[13] Krebs, E.G. (1954) Biochim. Biophys. Acta 15, 508–515.