REGULATION OF PROTEIN KINASE AND PHOSPHOPROTEIN PHOSPHATASE BY CYCLIC AMP AND CYCLIC AMP ANTAGONIST

H. K. WASNER*

Department of Physiological Chemistry, University of Würzburg; 87 Würzburg, Koellikerstrasse 2, Federal Republic of Germany

In memoriam of Earl W. Sutherland

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1. Introduction

The activation of protein kinase [1] by cAMP is the best known mechanism by which cAMP acts. This report shows that cAMP not only activates protein kinase but also inhibits phosphoprotein phosphatase, which catalyses the reverse reaction.

Murad [2] reported on a cAMP antagonist, which inhibits the cAMP-stimulated activation of phosphorylase. In this paper is reported [3] that a cAMP antagonist, in accordance with its expected function, not only inhibits protein kinase but also activates phosphoprotein phosphatase.

Both enzymes bind cAMP under conditions where binding of the antagonist could not be observed.

The activity of the enzymes protein kinase and phosphoprotein phosphatase is regulated by cAMP and its antagonist. Thus it is possible to define a general concept of hormonal regulation by means of phosphorylation and dephosphorylation. It appears that the cAMP antagonist and cAMP are equally potent regulators, though opposite in their function.

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2. Materials and methods

ATP and cAMP were obtained from Boehringer, Mannheim; [^{3}H]cAMP and [^{32}P]phosphate from Amersham–Buchler and calf thymus histone type IIa from Sigma. [^{32}P]ATP was prepared according to Glynn and Chapell [4] and [^{32}P]phosphohistone according to Nakai and Thomas [5]. The cAMP-binding assay was performed as described by Gilman [6]; protein kinase activity was assayed by the procedure of Reiman et al. [7] and phosphoprotein phosphatase by the method of Kato and Bishop [8] following the dephosphorylation of [^{32}P]phosphohistone. Protein kinases were purified according to Kuo and Greengard [9] and the purification of protein phosphatase from beef muscle was carried out according to Kato and Bishop [8] with the following modification: The supernatant solution of the centrifuged (15 000 × *g*/10 min) homogenate was fractionated with ammoniumsulfate (60% saturation) and after extensive dialysis (24 hr/3 changes) applied to a DEAE-cellulose column equilibrated with 50 mM Tris–HCl buffer, pH 7.4 and eluted with a linear gradient of 0–500 mM NaCl. The cAMP antagonist [2,10] was purified from liver by a modified procedure to be published making use of gel filtration and anion exchange chromatography.

3. Results

3.1. *Regulation of protein kinase activity*

Protein kinase activity has been measured in the presence and absence of cAMP and antagonist. Fig.1

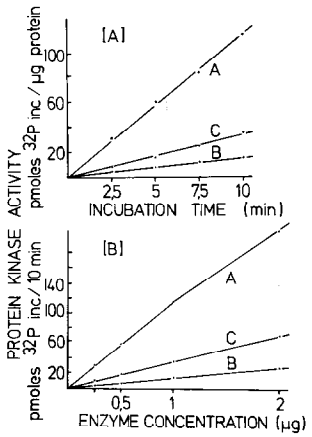

Fig.1 Phosphorylation of histone by protein kinase as a function of time (A) and enzyme concentration (B). Curves (A): 10^{-6} M cAMP present; curves B: 10 µl antagonist present; curves C: no additions.

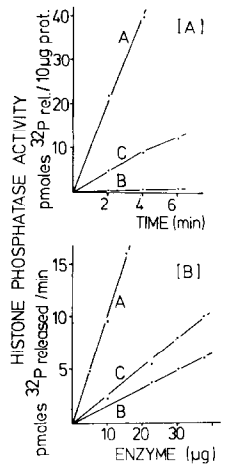

Fig.3. Dephosphorylation of phosphohistone by phosphoprotein phosphatase as a function of time (A) and enzyme concentration (B). Curves A: 10 µl antagonist present; curves B: 10^{-6} M cAMP present; curves C: no additions.

shows a linear relation of activity with respect to enzyme concentration and time. Presence of cAMP (A) results in a more active enzyme, whereas in the presence of cAMP antagonist (B) a less active enzyme is found. With no additions to the kinase assay (C) the kinase activity of the unregulated enzyme is measured. Usually protein kinase was activated by cAMP at least 2.5-fold and could be completely inhibited by the antagonist.

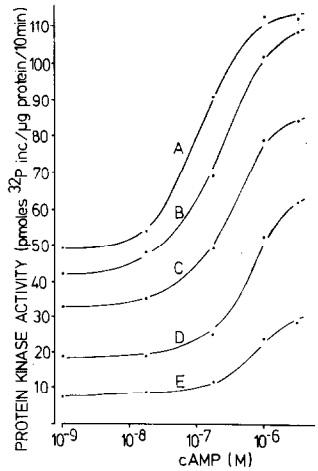

Fig.2. Effect of cAMP concentration on protein kinase activity. In the presence of 0 µl (A), 5 µl (B), 10 µl (C), 15 µl (D) and 20 µl solution of antagonist (E). (Enzyme concentration was 20 µg/ml; incubation volume was 100 µl.)

Fig.2 shows the dependence of the kinase activity from various ratios of concentrations of both effectors. Kinase activity at increasing cAMP concentrations is measured with no (curve A), 5 µl (curve B), 10 µl (curve C), 15 µl (curve D) and 20 µl antagonist (curve E). It is seen that the kinase activity can be regulated from zero to 100% activity by these two compounds. The protein kinases of beef heart, muscle, liver and brain behave the same way (unpublished).

3.2. *Regulation of phosphoprotein phosphatase activity*

The effect of cAMP and cAMP antagonist on the phosphatase has been studied. The correlation of enzyme activity with respect to time was linear for the first 4–6 minutes and started to decline thereafter (fig.3). Stimulatory effects [8] of divalent metalions were not seen. Phosphoprotein phosphatase activity is regulated by these two effectors opposite to the kinase activity. cAMP 10^{-6} M results in 100% inhibition (A in fig.4). Phosphatase could be activated up to seven-fold by the antagonist in the absence of cAMP (B in fig.4).

Brostrom et al. [11] reported that the cAMP-dependent activation of protein kinase disappears on prolonged storage. A similar desensitisation occurs with respect to the inhibition of the kinase by the antagonist. Apparently phosphatase loses its response

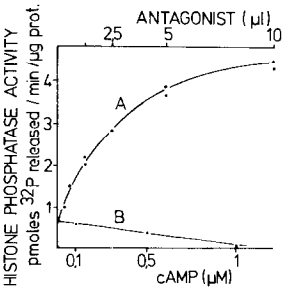

Fig.4. Effect of cAMP concentration (B) and antagonist concentration (A) on the phosphoprotein phosphatase activity. (enzyme concentration was 150 µg/ml; incubation volume was 100 µl; phosphohistone concentration was 10^{-5} M).

to the effectors more easily than the kinase. It is interesting in this context that Brandt et al. [12] have recently found a more active phosphatase after removal of an inhibitory protein.

3.3. *cAMP-binding protein*

It is well known that protein kinase is capable of binding cAMP [6]. Therefore it was of interest to see whether phosphatase also binds cAMP (fig.5). It is concluded that not only protein kinase but also phosphoprotein phosphatase binds cAMP. The occurrence of cAMP-binding sites on both enzymes is a prerequisite for their regulation by cAMP. Though inhibition of phosphatase by cAMP can easily be lost, for example by freezing and thawing, the cAMP-binding capacity appears to be more stable.

Under conditions where cAMP binding occurs, binding of radioactive labeled antagonist [3] could

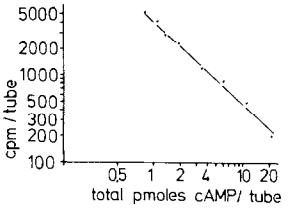

Fig.5. Standard curve for cAMP assay according to Gilman [6] performed with phosphoprotein phosphatase. All reactions were carried out at pH 4 and 0° C in a volume of 90 µl. [^{3}H]cAMP added per tube was 1 pmol. Amount of added enzyme was 5 µg. Known quantities of cAMP were added to get the total indicated amount of cAMP per tube.

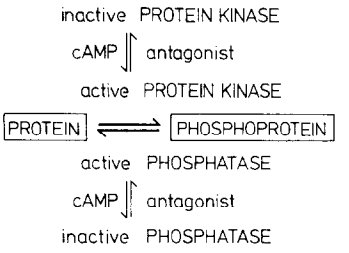

Fig.6. Regulation of the equilibrium between protein and phosphoprotein by the enzymes protein kinase and phosphoprotein phosphatase and the hormone messengers cAMP and cAMP antagonist.

not be demonstrated. No competition of antagonist and cAMP for a common binding site could be shown. Binding of radioactive labeled antagonist to kinase was, however, seen by the gel filtration method of Hummel and Dryer [13].

4. Discussion

Phosphorylation and dephosphorylation of proteins are important control mechanisms. As shown in this study both protein kinase and phosphoprotein phosphatase are regulated by cAMP and its antagonist in an opposing manner. This makes it possible to suggest a scheme of hormonal regulation where cAMP accelerates phosphorylation and decreases dephosphorylation and thus shifts the equilibrium in favor of the phosphorylated protein. Conversely the antagonist accelerates dephosphorylation and inhibits phosphorylation and thus favors dephosphorylated proteins. This regulation of the equilibrium of the phospho–dephosphoproteins by cAMP and its antagonist allows for a fine control by modulating two opposing reactions (fig.6). This could be important for the translation of hormonal signals into biological functions.

The outstanding question which needs to be clarified is the chemical structure of the antagonist. The present efforts are directed towards that goal.

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