BBA 66560

ADENOSINE 3':5'-MONOPHOSPHATE DEPENDENT PROTEIN KINASE IN THE LACRYMAL GLAND

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(Received November 8th, 1971)

SUMMARY

The properties of cyclic AMP dependent protein kinase (ATP:protein phosphotransferase, EC 2.7.1.37) of the bovine lacrymal gland were investigated. After some purification of the cell homogenate two enzyme fractions were separated by DEAE-cellulose chromatography at pH 7.5. Both fractions were activated by cyclic AMP. The cyclic AMP concentration causing 50% of the maximal activation of each enzyme was in the range of 10^{-8} M, and the maximal activation was caused by about 10^{-6} M cyclic AMP. Comparing the phosphorylation of different histone fractions, the highest cyclic AMP activation was found in the f2b histone fraction with both enzymes.

The basic tear secretion of the lacrymal gland is a constant process, but it can be increased or decreased by several hormones. It is now well known that among a number of other effects of different hormones, in some cases the regulation of secretion is also mediated by cyclic AMP[1,2]. In those few processes where we have sufficient information about the direct regulatory role of cyclic AMP, this role is generally based on the activation of a protein kinase (ATP:protein phosphotransferase, EC 2.7.1.37); moreover, the cyclic AMP dependent protein kinases have been found in several tissues[3]. Therefore, the presence of cyclic AMP dependent protein kinase in the lacrymal gland was investigated.

The cyclic AMP dependent kinase activity was measured according to the method of Kuo *et al.*[3]. The basal reaction mixture contained: 0.05 M sodium glycerophosphate (pH 6.5), 0.01 M $MgCl_2$, 0.01 M NaF, 0.002 M theophylline, 0.002 M EDTA, 0.6 nM [γ-^{32}P]ATP, about 150 000 cpm (produced by the method of Avron[4]) and 0.2 ml enzyme in a final volume of 1.6 ml. The basal reaction mixture was completed with the required amount of histone substrate and cyclic AMP. The amount of ^{32}P bound to the protein during 5 min incubation at 32 °C was measured on the basis of the Cerenkov effect in a Packard liquid scintillation spectrometer. The amount of protein was determined by the method of Lowry *et al.*[5].

Bovine lacrymal glands were removed and put in ice not later than 30 min

after slaughtering the animals. The collagen and fat were cleared away and the glands were minced and homogenized in a blendor in 2.5 vol. of 5 mM EDTA. (This and the subsequent steps were carried out at 4 °C.) The homogenate was filtered through 4 layers of gauze and centrifuged. The crude extract was purified according to the procedure of Kuo *et al.*[3] by a precipitation at pH 4.85 and another precipitation with $(NH_4)_2SO_4$. Unlike the experiments of the authors mentioned, the chromatography on DEAE-cellulose was carried out at pH 7.5 instead of pH 7.0, because at pH 7.0 only part (about 30–40%) of the cyclic AMP dependent protein kinase activity of our sample was bound by the column and this activity was eluted from the DEAE-cellulose by 0.1 M potassium phosphate buffer. At pH 7.5 (5 mM EDTA–5 mM potassium phosphate) about 90% of the cyclic AMP sensitive kinase activity was bound by the column, but only about 50% of this activity could be eluted by 0.1 M buffer. The retained activity was eluted subsequently with 0.3 M potassium phosphate. The protein content in the 0.3 M eluate was much less than that in the 0.1 M eluate, while the cyclic AMP dependent kinase activity was about the same in both fractions (Fig. 1).

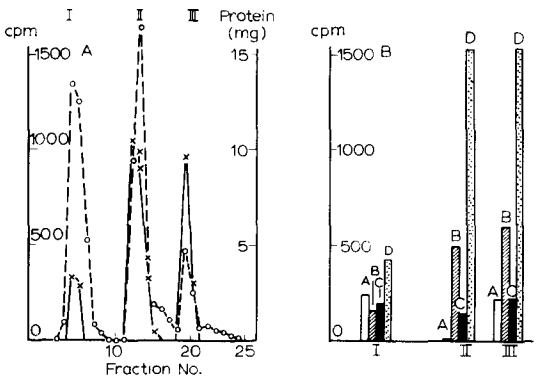

Fig. 1. (A) Chromatography on DEAE-cellulose column. The column (0.9 cm^2 × 15 cm) was equilibrated with 5 mM EDTA–5 mM potassium phosphate buffer (pH 7.5). Protein (about 100 mg) was put on the column followed by washings with I, 30 ml of the same buffer, II, 30 ml of 0.1 M buffer and III, 30 ml 0.3 M phosphate buffer. 5-ml fractions were collected. ×—× and ○---○ represent the cyclic AMP dependent enzyme activity and the protein content, respectively. (B) Histone kinase activity of the different enzyme fractions. The assay was carried out with the most active fractions of the three peaks (I, II and III). A, basal reaction mixture (for further details see the text); B, basal mixture + 160 μg f2b histone; C, basal mixture + 10^{-6} M cyclic AMP; D, basal mixture + 160 μg f2b histone and 10^{-6} M cyclic AMP. (All the values represent the means of duplicate measurements.)

So the cyclic AMP dependent protein kinase activity of the bovine lacrymal gland is separable into two major fractions by DEAE-cellulose chromatography, similar to the results of Reimann *et al.*[6] and Tao *et al.*[7]. We compared the main properties of the two fractions, Peak II and III. Since the cyclic AMP dependent activity which did not bind at pH 7.5 to the DEAE-cellulose was very low, it was neglected in further experiments.

The phosphorylation of different histone fractions was investigated and similar results were obtained with the two enzyme fractions. Using Worthington histone fractions, cyclic AMP dependent phosphorylation was found only in the f2b histone

where the increase of phosphorylation was 3–5-fold. Some activation by cyclic AMP could be observed when the substrate was mixed histone, presumably because of its f2b content. The cyclic AMP dependent phosphorylation values in the case of the other histone fractions were not considerably higher than the experimental error (Fig. 2). The apparent K_m values for Peak II and III with f2b histone fraction as substrate were $6.3 \cdot 10^{-5}$ and $1.7 \cdot 10^{-5}$ M, respectively.

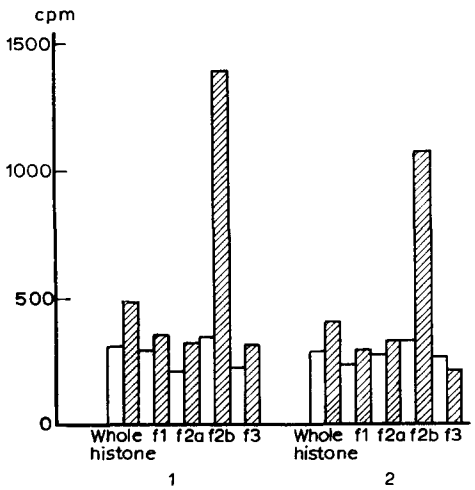

Fig. 2. Protein kinase activity using different histone fractions as substrates. The open columns show the control systems (without cyclic AMP) and the hatched ones the complete systems. Experimental conditions are the same as in the case of Fig. 1. (The histone fractions refer to the Johns code[8].) 1 and 2 represent the results obtained with the enzyme fractions eluted from the DEAE-cellulose column by 0.1 M (1) and 0.3 M (2) phosphate buffer, respectively. (All the values are the means of dupicate measurements.)

The cyclic AMP sensitivity of the two enzyme fractions proved to be very similar to each other. The cyclic AMP concentration causing 50% of the maximal activation was found to be in the range of 10^{-8} M ($6.6 \cdot 10^{-8}$ M with the enzyme fraction eluted by 0.1 M phosphate buffer and $4 \cdot 10^{-8}$ M with the enzyme fraction eluted by 0.3 M phosphate buffer), while maximal activation could be approached by 10^{-6} M cyclic AMP in both cases. These values were obtained with f2b histone fraction as substrate.

Cyclic GMP was also tested for its ability to activate the two enzyme fractions, but the activation caused by cyclic GMP was much less than that caused by cyclic AMP. In the presence of 10^{-6} M cyclic GMP the increase in the phosphorylation of f2b was only 40–50%, while cyclic AMP in the same concentration caused a 500% activation. Cyclic GMP did not increase the phosphorylation of other histone fractions. The results were similar with both enzyme fractions.

Since we have not found any significant difference between the properties of the two enzyme fractions, the question arises, is one of them only an artefact due to the purification procedure, or do the two enzyme fractions, eluted by 0.1 and 0.3 M phosphate buffers from DEAE-cellulose, represent really two different enzymes? In the latter case there is a possibility that the two enzymes are derived from different

parts of the same cells, or they may be produced by different cell types of the lacrymal gland.

Investigations for solving this problem are in progress.

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