TYPES OF cAMP-DEPENDENT PROTEIN KINASES IN SPLEEN CELLS OF IMMUNIZED ANIMALS

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Cyclic adenosine-3',5'-monophosphate (cAMP) is a highly important intracellular regulator in all mammalian tissues so far studied, including lymphoid tissue. The action of cAMP is to activate several cAMP-dependent protein kinases (cAMP-PK), which phosphorylate protein structures and thereby change their activity or the character of their interaction with other biopolymers (histones, enzymes, membrane proteins). For this reason, cAMP-activated PK are included in such important intracellular processes as mitosis and transcription [3, 6, 8, 14, 15]. Two main types of cAMP-PK are distinguished, namely I and II [3-8]. Species- and tissue-specificity has been demonstrated in the activity of cAMP-PK I and II. In the heart of rats and mice, for instance up to 90% of cAMP-PK belongs to type I, whereas in guinea pigs this proportion belongs to type II [5]. Fat cells and brain cells have cAMP-PK of type II as their main component [4]. Activity of each type of cAMP-PK fluctuates individually in the phases of the cell cycle. In a synchronizaed culture of Chinese hamster ovarian cells cAMP-PK I has high activity during mitosis and low activity in the G_1/S phase. Activity of cAMP-PK II rises sharply in the G_1/S phase and falls toward the end of the S phase of the cell cycle [6, 8]. Phosphorylation of histones and acid nuclear proteins, changing gene expression, also is observed in certain phases of the cell cycle. For instance, histone H3 is phosphorylated in the G_2 phase and in mitosis. In histone H1, ser-114 is phosphorylated in the S phase, but ser-37 is phosphorylated in the G_1 phase [6, 14]. These findings suggest differences in the functional role both of the different types of cAMP-PK and also of the nuclear proteins phosphorylated by them. This type of regulation of expression of different genes evidently also lies at the basis of the effect of the intracellular regulator cAMP on the development of immune reactions. The writers showed previously that activation of cAMP-PK in the spleen cells of animals by injecting them with cAMP and (or) glutathione increases the immune response to sheep's red blood cells (SRBC) twofold. Furthermore, if injection of cAMP or gluthathione shifts the peak of the immune response to a later day, combined injection of these substances induces an increased immune response with a peak on the 4th after immunization, characteristic of control animals [1]. The impression was obtained that there exists a series of isozyme forms of cAMP-PK, some of which may be interlinked with the intensity of proliferative processes, whereas other isozyme forms of cAMP-PK may be incorporated into the regulation of functional activity of immunocompetent cells.

To test this hypothesis, types of cAMP-PK were studied in the spleen cells of animals immunized with SRBC under normal conditions and against the background of high activity of cAMP regulatory system.

EXPERIMENTAL METHOD

(CBA × C57Bl/6)F_1 hybrid mice were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. The mice were immunized intravenously with 0.5 ml of a 5% suspension of SRBC. cAMP was injected intraperitoneally 24 h before immunization in a dose of 1×10^{-4} mole/kg, which inhibits cell proliferation [1, 3, 11]. Glutathione, an activator of cAMP-PK, was injected intraperitoneally on the day of immunization in a dose of 0.5×10^{-3} mole/kg. cAMP-PK phosphotransferase activity was determined in a supernatant

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TABLE 1. Phosphotransferase Activity of cAMP-Dependent Protein Kinases Eluted from DEAE-Cellulose in 0-0.6M NaCl Gradient [in nmoles ^{32}P transferred by 1 mg of enzymes from (γ-^{32}P)-ATP to 1 mg histone H1 in 1 min]

Peak	Fraction	NaCl concentration, M	Type of cAMP-PK	Version of experiment				
				intact animals	injection of cAMP 24 h before disease	24 h after immunization: control	24 h after immunization: injection of cAMP 24 h before immunization	24 h after immunization: injection of cAMP 24 h before immunization + glutathione on day of immunization
1	5, 6, 8, 9	—		—	1,2	8,2	1,0	2,6
2	11	0,02	I	30,2	—	—	—	—
3	21,22	0,08	I	—	14,3	—	23,5	—
4	26,25	0,11	I	—	12,8	—	7,2	43,7
5	30	0,13	I	—	—	33,7	6,6	—
6	33,34	0,16	II	—	—	15,1	3,6	38,3
7	36,38	0,17	II	—	—	13,9	6,1	32,9
8	40,41	0,20	II	—	9,2	23,4	4,2	53,0
9	43	0,22	II	—	—	—	3,9	—
10	45	0,24	II	—	—	14,2	3,1	—
11	49	0,26	II	—	—	13,2	—	—
12	50,51	0,27	II	1,6	35,5	12,4	—	—
13	61,64	0,36	II	—	—	—	9,4	—
14	69	0,39	II	—	—	—	12,6	8,9
15	76	0,44	II	—	—	—	22,5	—

of spleen cells 24 h after immunization, by the method of Nesterova et al. [12]. The supernatant was prepared and eluted from DEAE-cellulose by the method of Corbin et al. [4], with slight modifications. Spleen cells from 20 animals were freed from red blood cells with 0.84% NH_4Cl, frozen, and homogenized after freezing in 3 ml of buffer containing 5 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 3 mM $(CH_3COO)_2Mg$. All manipulations were carried out at 4°C. The homogenate was centrifuged at 23,000g and applied in a protein concentration of 12 mg to a column (10 × 100 mm) packed with DEAE-cellulose and equilibrated with the same buffer. Elution was carried out with this buffer in a 0-0.6M NaCl gradient at the rate of 15 ml/h. Fractions of 1.5 ml were collected and tested for cAMP-PK activity. The type of cAMP-PK (I and II) was determined by the method of Corbin et al. [4]. The protein concentration was measured by Lowry's method [10]. Histone H1 was obtained from the Institute of Molecular Biology, Academy of Sciences of the USSR. The (γ-^{32}P)-ATP was supplied by the Tashkent "Izotop" Inter-Republican Organization.

EXPERIMENTAL RESULTS

Types of cAMP-PK were studied in the spleen cells of intact animals and of animals 24 h after immunization with SRBC under normal conditions and in the presence of high activity of the intracellular cAMP regulatory system. The cAMP system in the spleen cells was activated by injecting the animals with cAMP alone or with cAMP together with glutathione. On injection of these substances into the animals the phosphotransferase activity of cAMP-PK in the spleen cells was increased by 3.8 times after 24 h. To study the types of cAMP-PK, the property of the regulatory subunits of cAMP-PK II to form stronger bonds with catalytic subunits than with the regulatory subunits of type I cAMP-PK was utilized. For this reason, during chromatography on DEAE-cellulose the cAMP-PK I was eluted at low ionic strength — in the presence of 0.05-0.15M NaCl, whereas cAMP-PK II was eluted with NaCl in a concentration of 0.15-0.25 M [4-8].

The results of chromatography on DEAE-cellulose are given in Table 1 and Fig. 1A, E. When describing the activities discovered, the peak No. was taken in order of elution of the peaks in all versions of the experiments. When the type of cAMP-PK in the active fraction was identified, another feature distinguishing cAMP-PK of type II was used. It was found that cAMP-PK II, unlike cAMP-PK I, dissociates slowly on incubation in the presence of 0.5M NaCl (30°C, 10-30 min). Under these conditions the cAMP-PK dissociates rapidly and the ratio of activity without cAMP to activity in the presence of 5 μM cAMP became close to 0.8-0.9 [4]. The results of incubation are given in Fig. 2 in the form of the ratio of activity without cAMP to activity in medium containing 5 μM cAMP. The following results were obtained. Two peaks of phosphotransferase activity were discovered in the spleen cells of intact animals, one of them (peak No. 2) belonging to cAMP-PK I, the other (peak No. 12)

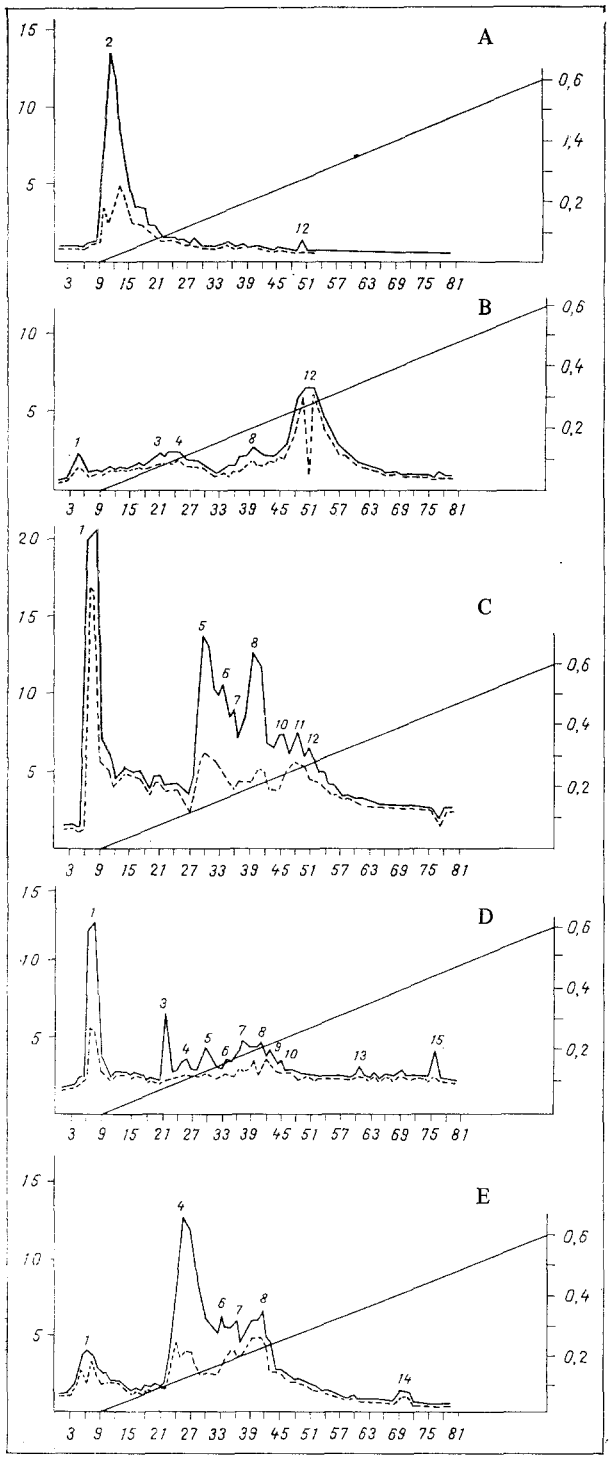

Fig. 1. Chromatography of cAMP-PK in a supernatant of mouse spleen cells from (CBA × C57Bl/6)F_1 hybrids, on DEAE-cellulose. Continuous line — activity of fraction during incubation in presence of 5 μM cAMP, broken line — enzyme activity during incubation without cAMP. 1-15) Peak Nos. Abscissa, nos. of fractions eluted from DEAE-cellulose; ordinate, on left: activity in cpm·10^{-3}, on right: NaCl concentration (in M). A) Spleen cells from intact animals, B) 24 h before sacrifice cAMP was injected in a dose of 1×10^{-4} mole/kg, C) 24 h after immunization, D) cAMP in a dose of 1×10^{-4} mole/kg was injected 24 h before immunization. Animals killed 24 h after immunization, E) injection of cAMP in a dose of 1×10^{-4} mole/kg 24 h before immunization + glutathione in a dose of 0.5×10^{-3} mole/kg on day of immunization. Animals killed 24 h after immunization.

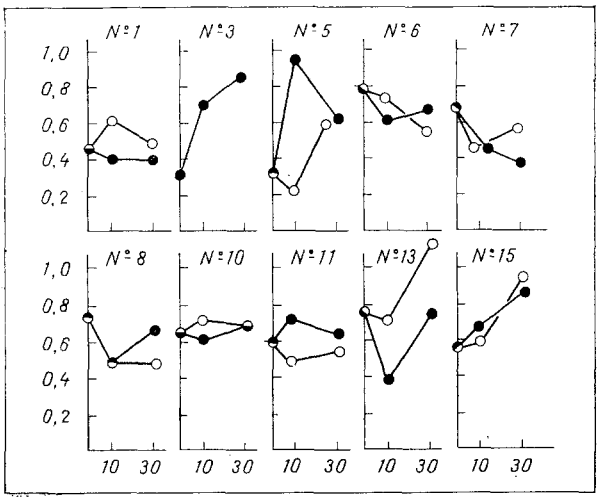

Fig. 2. Effect of 0.5 M NaCl on phosphotransferase activity of fractions eluted from DEAE-cellulose. Empty circles — incubation of active fraction without NaCl, filled circles — incubation of active fraction with 0.5 M NaCl. Abscissa, incubation time (in min); ordinate, ratio of activity of fractions after incubation without cAMP to activity in presence of 5 μM cAMP. Serial numbers correspond to peaks of activity shown in Fig. 1 and Table 1.

belonging in the character of elution and incubation with NaCl to cAMP-PK II (Fig. 1A, Fig. 2). About 96% of activity was represented by cAMP-PK I (Table 1). Replacement of activity of cAMP-PK I with one isozyme form was replaced by two others, eluted in the presence of a higher NaCl concentration (from peak No. 2 to peaks No. 3 and 4) was found 24 h after injection of cAMP into the animals in a dose of 1×10^{-4} mole/kg. Activity of peak No. 12 increased from 4 to 55% (see Fig. 1B, Fig. 2, and Table 1). An isozyme form of cAMP-PK II which eluted in peak No. 8 in the presence of NaCl in a concentration of 0.2 M, appared.

It can be tentatively suggested that cAMP-PK eluted in peaks Nos. 2-4, 8, and 12 carry regulatory functions and are incorporated into one of the mechanisms controlling the size of the pool of cell populations in the spleen. In spleen cells of the animals 24 h after immunization with SRBC eight isozyme forms of cAMP-PK were discovered, one of which (peak No. 5) belongs to cAMP-PK I. Peaks Nos. 2-4 could no longer be detected. Activities of cAMP-PK I and II, eluted in peaks Nos. 5-7 and 10-11, was found only after antigenic stimulation (see Fig. 1C, Fig. 2, and Table 1). The possibility cannot be ruled out that their synthesis takes place at this time. On immunization of the animals under conditions of an activated cAMP regulatory system (24 h after injection of cAMP), a decrease in the activities of cAMP-PK I and II, eluted in peaks Nos. 5-11, was found. At the same time, activity of different forms of cAMP-PK II was induced (peaks Nos. 13-15), also characteristic of antigen-stimulated tissue only (see Fig. 1D, Fig. 2, and Table 1). Possibly these isozyme forms of cAMP-PK II (peaks Nos. 13-15) constitute a "reserve pool" and are activated only in extremal situations during the development of an immune response. Combined injection of cAMP and glutathione activated cAMP-PK I (peak No. 4) and three isozyme forms of cAMP-PK II (peaks Nos. 6-8) in the spleen cells of immune animals. Activities of peaks Nos. 9-13 and 15 disappeared (Fig. 1E, Table 1). In this case also changes were observed in the activity of those cAMP-PK which were discovered in the spleen cells of immune animals.

The results suggest that isozyme forms of cAMP-PK, active in the spleen cells of immune animals, are induced for synthesis after antigenic stimulation, and may evidently have a regulatory influence on the functional activity of immunocompetent cells. The writers previously demonstrated a sharp increase in the immune response to SRBC on activation of the cAMP system by injection of cAMP and(or) glutathione into the animals [1]. In order to

discover any possible correlation between activity of the isozyme forms of cAMP-PK and the magnitude of the immune response, coefficients of correlation were determined between phosphotransferase activity of the peak and the content of antibody-forming cells (AFC) in the spleen.

The results demonstrated close direct correlation between phosphotransferase activity of peaks Nos. 4, 6-8 and the magnitude of the immune response. It will be noted that it was these peaks which were sharply activated if the animals were immunized under conditions of an activated cAMP system (by injection of cAMP together with glutathione). Peaks of activity of cAMP-PK Nos. 3, 9, 13, and 15 exhibited almost complete negative correlation with mitotic processes in AFC precursors. It can be postulated that shifts of the peak of the immune response to a later day on activation of the cAMP system in the spleen cells by injection of cAMP of glutathione [1] is connected with the activity of precisely these isoenzyme forms of cAMP-PK.

It must be noted that the results are preliminary in character and require experimental verification. However, there is no doubt at this stage that immunization of animals induces a whole spectrum of specific isozyme forms of cAMP-PK, strictly specialized in their function, in spleen cells. Some cAMP-PK are mutually connected with proliferative processes, whereas other cAMP-PK are evidently interconnected with functional activity of immunocompetent cells and with the magnitude of the immune response.

LITERATURE CITED

1. V. M. Novikova and V. A. Kozlov, Immunologiya, No. 5, 40 (1980).
2. I. N. Trakht, I. D. Grozdova, E. S. Severin, et al., Biokhimiya, No. 4, 636 (1980).
3. C. V. Byus, G. P. Klimpel, D.O. Lucas, et al., Nature, 268, 63 (1977).
4. J. D. Corbin, S. L. Kelly, and C. R. Park, J. Biol. Chem., 250, 218 (1975).
5. J. D. Corbin and S. L. Kelly, J. Biol. Chem., 252, 910 (1977).
6. M. Costa, E. W. Gerner, and D. H. Russle, J. Biol. Chem., 251, 3313 (1976).
7. M. Costa, J. M. Fuller, D. H. Russel, et al., Biochim. Biophys. Acta, 479, 416 (1977).
8. M. Costa, E. W. Gerner, and D. H. Russle, Biochim. Biophys. Acta, 538, 1 (1981).
9. J. M. Danse, J. M. Egly, and J. Kempf, FEBS Lett., 124, 84 (1981).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. J. J. Marchalonis and P. Smith, Aust. J. Exp. Biol. Med. Sci., 54, 1 (1976).
12. M. V. Nesterova, L. P. Sashchenko, V. Yu. Vasiliev, et al., Biochim. Biophys. Acta, 377, 271 (1975).
13. J. C. Pisano, J. Reticuloend. Soc., 20, 50 (1976).
14. E. S. Severin, L. P. Sashchenko, S. N. Kochetkov, et al., Proc. FEBS Meet., 54, 101 (1979).
15. E. S. Severin, M. V. Nesterova, N. N. Gulyaev, et al., Adv. Enzyme Regul., 14, 407 (1975).