DECYCLIZING PHOSPHODIESTERASES ASSOCIATED WITH PLASMA MEMBRANES OF THE CHICK CHORIOALANTOIS

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Received July 3rd, 1980

Two different enzymatic activities hydrolyzing 2',3'-cyclic phosphates of purine and pyrimidine ribonucleosides to give 2'- or 3'-ribonucleotides have been detected in plasma membranes of the chick chorioalantois. The enzymes differ in their temperature optima: At lower temperatures, cleavage to the 2'-isomers predominates.

Enzymes, capable of hydrolyzing ribonucleoside 2',3'-cyclic phosphates *I* without cleaving internucleotide bonds, have been detected in animal, plant and bacterial cells. Depending on the kind of the enzyme, the products are either 2'-ribonucleotides *II* (decyclizing nucleotide 3'-phosphohydrolase) or 3'-ribonucleotides *III* (decyclizing nucleotide 2'-phosphohydrolase). Enzymes of the first type – 3'-phosphohydrolases – have been found in calf spleen homogeneates[1,2], beef pancreas[3] and brain[4], in higher plants[5], in plasma membranes of rabbit brain myeline[6], in rat brain glial cells[7], in C-6 line cells from the chemically induced astrocytom of rat brain[8] and in erythrocytes[9]. On the other hand, 2'-phosphohydrolases have been studied in detail *e.g.* in higher plants[1,10] and in bacterial cells[11,12] and they were found also in the venom of *Crolatus adamanteus* and in calf intestinal mucose[1]. In our previous work[13] we proved in plasma membranes of the chick chorioalantois also the presence of enzyme activity which hydrolyzes ribonucleoside 2',3'-cyclic phosphates. The present paper concerns some of its properties.

EXPERIMENTAL

Materials and Methods

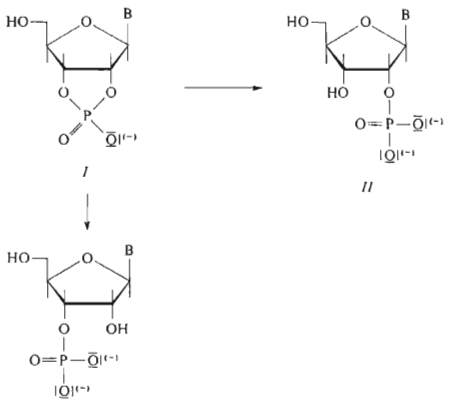
Ribonucleoside 2',3'-cyclic phosphates were prepared according to ref.[2] and transformed into lithium or sodium salts. The 2'(3')-nucleotides were Boehringer (F.R.G.) and Calbiochem Behring (U.S.A.) products. Plasma membranes were isolated from 10—11 day old chicken embrya according to ref.[13]. Proteins were determined by a described method[14] using bovine serum albumin as standard.

Enzymatic activity determination: The incubation mixture (0·2 ml) contained 150—200 μg of the substrate and 200 μg of membrane proteins in 0·05M-Tris-HCl buffer (pH 7·2). After incubation for 120 min at 30°C or at 60°C, the reaction was quenched by heating on a steam bath for

5 min, the solution was extracted with a three-fold volume of chloroform and centrifuged for 15 min (4000 rpm). The extent of cleavage was determined by descending paper chromatography in the system S1, 1-butanol–ethanol–water (312 : 198 : 90) on a paper Whatman No 1, followed by elution of the spots of the starting compound and product with 3 ml of 0·1M-HCl (48 h) and measurement of UV absorbance of the eluates at the nucleotide absorption maximum. Blanks were treated in the same way, in the absence of membrane proteins.

Identification of hydrolysis products: The spots of products after chromatography in the system S1 were eluted with water (0·5 ml) and rechromatographed on Whatman No 1 paper; isomeric adenosine and guanosine nucleotides were separated in the system S2, saturated ammonium sulfe solution–1M ammonium acetate–2-propanol (79 : 19 : 2); separation of cytidine 2′, and 3′-phosphates was carried out in the system S3, saturated ammonium sulfate solution–1M ammonium acetate–2-propanol (80 : 18 : 2) in ascending arrangement on Whatman No 3 MM paper. The products were identified by comparison with authentic compounds and their ratio was determined spectrophotometrically in the eluates of the spots.

Thermal inactivation: The membrane protein (ref.[13]) (200 μg) was pre-incubated for 2 h (at 30—60°C) or 15 min (at 70°C), cooled and the substrate (200 μg in 0·2 ml) was added. The extent of cleavage and isomer ratio were determined as described above.



RESULTS AND DISCUSSION

Activity of 2′,3′-decyclizing phosphodiesterases bound to plasma membranes of chicken embrya was studied on ribonucleoside 2′,3′-cyclic phosphates derived from uridine, cytidine, adenosine and guanosine. The rate of cleavage decreased in the

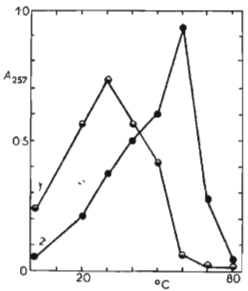


FIG. 1

Temperature Dependence of Hydrolysis of Adenosine 2′,3′-Cyclic Phosphate

1 Adenosine 2′-phosphate, *2* adenosine 3′-phosphate.

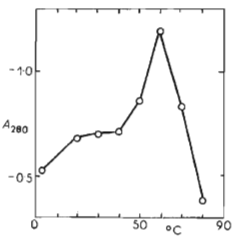


FIG. 2

Temperature Dependence of Hydrolysis of Cytidine 2′,3′-Cyclic Phosphate to Cytidine 2′- and 3′-Phosphate

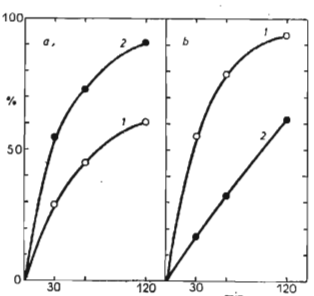


FIG. 3

Kinetics of Cleavage of Cytidine 2′,3′-Cyclic Phosphate (*a*) and Adenosine 2′,3′-Cyclic Phosphate (*b*) at 30°C (*1*) and at 60°C (*2*)

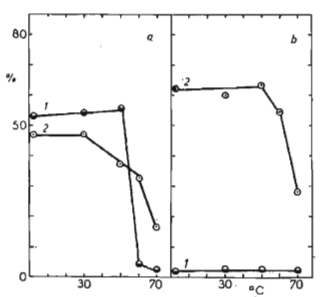


FIG. 4

Thermal Deactivation of Decyclizing Phosphodiesterase; Cleavage at 30°C (*a*) and 60°C (*b*)

1 Adenosine 2′-phosphate, *2* adenosine 3′-phosphate.

order G > A > U = C. The reaction products are 2′-nucleotides, 3′-nucleotides and ribonucleosides which arise from nucleotides by the action of non-specific nucleotidase present in the unpurified protein preparation. This nucleotidase dephosphorylates nucleotides (the rate decreases in the order A > G = U = C), with an optimum at 50°C and is completely inactivated at 65°C. It cleaves preferentially the 3′-isomers (the rate ratio 3′-AMP to 2′-AMP is 4 : 1 at 30–60°C). Accordingly, the data on cleavage by decyclizing phosphodiesterases were corrected for the presence of nucleotidase. Since the isomeric uridine 2′- and 3′-nucleotides are separated with difficulty by paper chromatography, the identification of the cleavage products was carried out for cytidine, adenosine and guanosine. The hitherto described decyclizing phosphodiesterases of the mentioned type afford invariably only one isomer; therefore the presence of two isomeric cleavage products in our case indicates the presence of both the mentioned types of decyclizing activities. This assumption is supported also by the previous observations that the level of 3′-phosphohydrolase in plasma membranes is about 1·4–2 times and that of 2′-phosphohydrolase about 20 times higher that the enzyme level in the cell homogenate. It follows therefore that the latter enzyme is preferentially bound to plasma membranes whereas the first one probably also to other cell structures[13].

Notable is the difference in the temperature optima of both types of enzymes: The cleavage of adenosine 2′,3′-cyclic phosphate to 2′-AMP proceeds already at lower temperatures and reaches its maximum at 30°C. The 3′-isomer arises also at this temperature but optimum of cleavage is at 60°C (Fig. 1). Guanosine and cytidine 2′,3′-cyclic phosphates are cleaved analogously. The formation of 3′-CMP from the latter compound has also optimum at 60°C and this isomer is practically the sole product; at 30°C both the isomeric nucleotides are present (Fig. 2) but the total

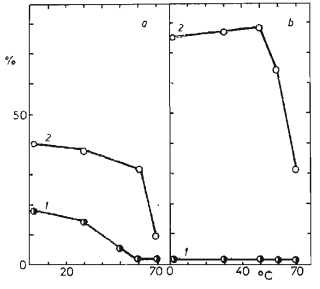


FIG. 5

Thermal Deactivation of Decyclizing Phosphodiesterase; Cleavage at 30°C (*a*) and 60°C (*b*)

1 Cytidine 2′-phosphate, *2* cytidine 3′-phosphate.

extent of the cleavage is much smaller than in the case of purine derivatives. This fact is the consequence of different affinity of enzymes toward nucleobases: The rate ratio at 30°C and 60°C is about 3·0 for adenosine 2',3'-cyclic phosphate whereas for the cytidine derivative it is about 0·5 (Fig. 3). Both the enzyme activities differ also in their thermal stability: The 2'-phosphohydrolase activity is fully retained even after incubation for 2 h at 60°C and heating to 70°C for 15 min leads only to a 50% inactivation of the enzyme. On the other hand, the 3'-phosphohydrolase is stable only up to 50°C and heating to 60°C leads to its total inactivation (Fig. 4, 5).

The effect of cations on both activities, studied at 30°C and 60°C, is parallel: Monovalent cations (Na^+, K^+ and NH_4^+ in concentrations 0·05 – 0·1M) enhance the hydrolysis (20% for cytidine derivatives, 50 – 60% for adenosine derivatives), Mg^{2+} and Mn^{2+} have no effect (at this concentration) whereas Zn^{2+} at 10^{-3}M shows a 15 – 30% inhibition. The cleavage rate is substantially affected neither by EDTA ($10^{-4} - 10^{-3}$M) nor Triton X-100 (0·1 – 1·0M).

Thus we can summarize that one of the enzymes, cleaving cyclic 2',3'-ribonucleotides, is a decyclizing ribonucleotide 3'-phosphohydrolase which gives rise to 2'--nucleotides; this enzyme has been already found in the plasma membranes of animal cells[6-9]. However, it is still not clear whether the cleavage leading to 3'-nucleotides is catalyzed by the corresponding decyclizing 2'-phosphohydrolase or ribonuclease (cyclizing ribonucleotide 2'-transferase). The presence of ribonuclease in plasma membranes of chick embrya has been already described[15]; in our case, the high temperature optimum and significant thermostability of this enzyme would speak rather in favour of a ribonuclease.

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Translated by M. Tichý.