BASE EXCHANGE REACTIONS STUDIED ON THE LEVEL OF CDP-ACTIVATED PRECURSORS OF PHOSPHOLIPIDS IN A CELL-FREE SYSTEM DERIVED FROM MOUSE FIBROBLASTS

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Received 23 October 1975

1. Introduction

The calcium-stimulated, non-energy-dependent incorporation of ethanolamine into phospholipids by various tissue preparations from different species via a base exchange reaction has been studied by several groups [1–9]. This reaction has been reported to require phospholipid acceptors [7]. Up to now, no efforts have been made to study this reaction in permanent cell lines or to find out whether a base exchange reaction may also occur on the level of CDP-choline and CDP-ethanolamine.

In this paper we describe investigations on the utilization of uniformly labeled [^{32}P]CDP-choline in exchange reactions of ethanolamine using a cell-free system derived from mouse fibroblasts. Since base exchange reactions of phospholipids have not yet been studied in a permanent cell line, experiments were also performed with [^{32}P]phosphatidylcholine and [^{3}H]ethanolamine in order to confirm this type of reaction in the given system.

2. Materials and methods

Cells from a line of SV 40-transformed mouse fibroblasts, STU 51A/232B [10], grown in suspension culture, were used in all experiments.

[^{32}P]orthophosphate, 100 Ci/mg, and 1-[^{3}H] ethanolamine hydrochloride, 320 mCi/mmole, were obtained from the Radiochemical Centre Amersham. Phosphorylethanolamine, CDP-ethanolamine, CDP-choline and HEPES (*N*-2-hydroxy-ethylpiperazine-bovine serum albumine from Behringwerke, Marburg, Germany.

2.1. *Preparation of [^{32}P]phosphatidylcholine and of uniformly labeled [^{32}P]CDP-choline*

2×10^8 STU 51A/232B cells in 60 ml of growth medium which contained HEPES buffer instead of phosphate were incubated in the presence of 10 mCi [^{32}P]orthophosphate at 37°C for 17 h. Radioactive phosphatidylcholine was isolated as described previously [11]. The sample has an original specific radioactivity of 6×10^5 cpm ^{32}P/nmole. Rechromatography of a sample containing 4×10^5 cpm ^{32}P showed no phosphatidylethanolamine detectable by autoradiography.

The upper phase of the Folch partition procedure contained the water-soluble cell components including the radioactive CDP-choline. Before chromatography of this extract on an AG 1 × 2 column [12], 50 μg CDP-choline and 500 μg CDP-ethanolamine were added to facilitate the identification of these substances in the effluent by determination of the u.v. absorption and radioactivity. The fraction containing [^{32}P]CDP-choline was further purified as described previously [11]. The uniformly labeled [^{32}P]CDP-choline sample had an original specific radioactivity of 2.9×10^4 cpm ^{32}P/nmole. The uniform label of CDP-choline was confirmed by the finding that labeled phosphatidylcholine as well as labeled CMP arouse by the action of CDP-choline: 1,2-diglyceride cholinephosphotransferase (EC 2.7.8.2).

2.2. *Preparation of the cell-free system*

2×10^9 cells, corresponding to a volume of 5 ml of packed cells, were washed once with phosphate buffered saline, resuspended in 18 ml of 0.25 M sucrose solution and disrupted in a Dounce homogenizer. Nuclei and still intact cells were removed by centrifugation at 300 g for 10 min. Only the upper half of the centrifuged homogenate was used in the experiments. Incubation mixtures were prepared by mixing 0.3 ml of the cell homogenate with 0.3 ml of the substrate solution containing the radioactive samples. The various pH steps of the substrate solution were constituted by a HEPES/glycine/NaOH buffer which covers the range of pH 5–10. The final concentrations of the individual components in each incubation mixture (total vol 0.6 ml) were 25 mM HEPES, 25 mM glycine, 2 mM $CaCl_2$, 1% bovine serum albumin, 60 units penicillin, 60 μg streptomycin, 0.2 mM ethanolamine with a specific radioactivity of 2×10^5 cpm ^{3}H/nmole, and 4.5×10^6 cpm of [^{32}P]phosphatidylcholine. Since the cell homogenate contained about 1 μmol ± 10% phosphatidylcholine per 0.3 ml, the final specific radioactivity of [^{32}P]phosphatidylcholine in the incubation mixture was 4×10^3 cpm ^{32}P/nmole. The same substrate composition with non-labeled ethanolamine was used when incubation mixtures were prepared with [^{32}P]CDP-choline. One additional pH series was performed in the presence of 5 mM ethanolamine phosphate. Each incubation mixture (0.6 ml) then contained 1×10^5 cpm of [^{32}P]CDP-choline, instead of [^{32}P]phosphatidylcholine. After 90 min of incubation with gently shaking at 37°C all samples were frozen at −80°C.

2.3. *Isolation of phospholipids and CDP-ethanolamine*

The extraction and separation of the lipids was performed as described previously [11]. The spots of the phospholipids were visualized by short exposure to iodine and by autoradiography, scraped off the plates and eluted with Bray's scintillation mixture [13]. CDP-ethanolamine was isolated from the upper phase of the Folch partition procedure using AG 1×2 column chromatography and two-dimensional thin-layer chromatography as specified above.

All radioactivities were counted in a Packard-Tri-Carb-Scintillation counter. The counting efficiency of [^{32}P] was 96%, that of [^{3}H] 17%. Determinations of the phosphorus content were performed according to the method of Bartlett [14].

3. Results and discussion

The incorporation data listed in table 1 indicate

Table 1
Incorporation of the ^{32}P-moiety of [^{32}P]phosphatidylcholine into phosphatidylethanolamine and of [^{32}P]CDP-choline into CDP-ethanolamine, phosphatidylethanolamine and phosphatidylcholine at various conditions.

Incubation mixture Radiochemicals added	Compounds added	Compounds isolated	Maximal incorporation cpm	pH
^{32}P-phosphatidyl-choline	2 mM $CaCl_2$	PE	3500 ^{32}P	8–9
+ ^{3}H ethanolamine			310 000 ^{3}H	8–9
^{32}P-CDP-choline	2 mM $CaCl_2$	PC	1980 ^{32}P	8–9
		PE	10 ^{32}P	
		CDP-E	80 ^{32}P	9
^{32}P CDP-choline	2 mM $CaCl_2$ + 5 mM EP	PC	1790 ^{32}P	8–9
		PE	10 ^{32}P	
		CDP-E	2820 ^{32}P	9

Abbreviations: PC = phosphatidylcholine, PE = Phosphatidylethanolamine, CDP-E = CDP-ethanolamine, EP = ethanolaminephosphate.

that under the given conditions [^{32}P]phosphatidylcholine is used as an acceptor in the base exchange reaction with [^{3}H]ethanolamine catalyzed by the cell-free homogenate of mouse fibroblasts. The reaction was found to have an optimum at pH 8–9. The ratio cpm ^{3}H/cpm ^{32}P found in phosphatidylethanolamine exceeded the ratio of specific radioactivities of the precursor compounds present in the incubation mixture by a factor 2. This agrees with the general findings that phospholipids other than phosphatidylcholine were also utilized as acceptors in the base exchange reaction of ethanolamine [1–9].

In order to find out whether a base exchange reaction might occur on the level of the CDP-activated bases (CDP-choline + ethanolamine ⇆ CDP-ethanolamine + choline), the utilization of the ^{32}P-moiety of uniformly labeled [^{32}P]CDP-choline (C**P**P-choline) in the biosynthesis of phosphatidylethanolamine was investigated. As a consequence of such a hypothetical base exchange, the reaction of CDP-ethanolamine: 1,2 diacylglycerol ethanolaminephosphotransferase (EC 2.7.8.1) would yield ^{32}P-labeled phosphatidylethanolamine. If this base exchange does not exist, the utilization of the ^{32}P-moiety of uniformly labeled CDP-choline may occur via back reaction of CTP: cholinephosphate cytidyltransferase (C**P**P-choline + PP ⇆ C*PPP + choline *phosphate) yields labeled CTP which can react with ethanolamine phosphate to give C**P**P-ethanolamine. From this compound, however, only a non-labeled phosphatidylethanolamine can arise.

The incorporation of the ^{32}P-moiety of uniformly labeled CDP-choline into phosphatidylcholine, phosphatidylethanolamine and CDP-ethanolamine at different pH was determined. Under conditions which favor the base exchange reactions of phospholipids, no incorporation of the ^{32}P-moiety of CDP-choline into phosphatidylethanolamine and scarcely any into CDP-ethanolamine could be detected (see table 1). The formation of labeled phosphatidylcholine and labeled CMP by the action of CDP-choline: 1,2 diglyceride cholinephosphotransferase (EC 2.7.8.2) was readily accomplished at the low Mg^{2+} concentration still deriving from cellular pools. An increase of the Mg^{2+} concentrations (2 mM) stimulated the incorporation of the ^{32}P-label into phosphatidylcholine by a factor 2 but ^{32}P-incorporation into phosphatidylethanolamine was not effected.

The small amount of ^{32}P-label found in CDP-ethanolamine might therefore not derive from a base exchange reaction of CDP-choline but rather from labeled CTP deriving from the back reaction of CTP: cholinephosphate transferase (EC 2.7.7.15) as outlined above. This reaction is drastically stimulated by adding ethanolamine phosphate (5 mM) to the incubation mixtures (see table 1). A high ^{32}P-label is found in CDP-ethanolamine but none in phosphatidylethanolamine as had to be expected.

On the basis of these results one has to conclude that a base exchange reaction of CDP-choline with ethanolamine does not occur in the given system.

Acknowledgements

The excellent technical assistance of Mrs S. Hirschle, Ms B. Chris'ophersen and Mrs B. Krafczyk is greatfully acknowledged.

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