ACTIVITY OF GUANYL CYCLASE BOUND TO SEPHAROSE

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The activity was compared of rat lung and liver guanyl cyclase in soluble form and after attachment of the enzyme to Sepharose. Both forms of the enzyme showed a similar sensitivity to the stimulating effect of Mn^{2+}-ions; neither of the two forms was stimulated by Ca^{2+}-ions; Lubrol PX in the presence of 5 mM Mn^{2+}-ions increased more the activity of the soluble enzyme than that of the enzyme bound to Sepharose.

Guanyl cyclase catalyzes the formation of cyclic guanosine 3',5'-monophosphate (cyclic GMP) from GTP. Most of the enzymatic activity of cell homogenates is present in the soluble fraction and a small part only is found in the particulate fraction. In *in vitro* studies guanyl cyclase is activated by Mn^{2+}-ions, it is not stimulated, however, by sodium fluoride or hormones[1-4]. The only exception reported so far is the stimulation of soluble liver guanyl cyclase by secretin[5].

One of the possible explanations of the absence of hormonal stimulation of guanyl cyclase in vitro can be the fact that guanyl cyclase originally bound to cell plasmatic membranes solubilizes during homogenization and activates itself by dissociating from the regulatory or repressor component of the membrane[4]. Since thus solubilized enzyme can undergo changes in configuration and interaction with the surrounding molecules and consequently differ from the original enzyme bound to membranes, the soluble enzyme bound to a solid support can serve as a model which would eliminate differences between soluble and membrane-bound enzymes. We compared therefore in this study the activity of guanyl cyclase in soluble form with the activity of the enzyme which had been bound to Sepharose.

EXPERIMENTAL

Material and Methods

Chemicals. Cyclic GMP and GTP (Sigma), cation exchanger AG 50W–X2 (200–400 mesh, H^+-form, Calbiochem), secretin (The Boots Co., 95 units), CNBr-activated Sepharose 4B (Pharmacia), and guanosine 5'-triphosphate-α[-^{32}P], trisodium salt (1348 mCi/mmol, Radiochemical Centre, Amersham) were used in our experiments.

Preparation and determination of guanyl cyclase. Rat lungs and livers were homogenized in 150 mM Tris-HCl buffer (pH 7·5). The homogenate was centrifuged at 25000 *g* and guanyl

cyclase was determined in the supernatant according to Hynie[6]. The final composition of the incubation medium (0·05 ml) was 30 mM Tris-HCl buffer (pH 7·5), 10 mM theophylline, 0·1 mM cyclic GMP, and 0·4 mM GTP. The mixture was incubated 10—15 min at 37°C; the reaction was discontinued by the addition of 50 μg of cyclic GMP in 1 ml of 0·05M-HCl and heating for 5 min. Cyclic GMP was isolated by column chromatography on cation-exchanger AG 50W (H^+-form) and by subsequent negative adsorption to zinc sulfate and barium hydroxide at pH 6·2—6·4. The recovery of cyclic GMP was determined by optical density measurement at 260 nm. The radioactive samples were added to 10 ml of aqueous solution of 1-naphthylamine (100 mg per 1 liter) and the ^{32}P-radioactivity was determined by measurement of Cerenkov radiation[6]. The protein content was determined according to Lowry and coworkers[7].

Preparation of guanyl cyclase bound to Sepharose 4B. One gram of CNBr-activated Sepharose 4B was allowed to swell and washed 15 min with 200 ml of 1 mM-HCl. Guanyl cyclase (3 ml of the preparation, 18—36 mg of the enzyme) was added to 1·5 ml of 10% NaCl, 0·5 ml of 1M Tris-HCl buffer at pH 9·0, and 1 g of the swollen gel. The mixture was stirred gently 18—20 h at 4°C. Unattached material was washed off using the coupling mixture and the remaining active groups were allowed to react with 1M ethanolamine for 1—2 h at pH 8·0. The protein not bound covalently was washed off with three alternate portions of buffer at pH 4·0 (0·1M acetate buffer containing 1M-NaCl) and at pH 8·0 (0·1M Tris-HCl buffer containing 1M-NaCl). Lastly, the enzyme bound to Sepharose was washed with 75 mM Tris-HCl buffer at pH 7·5 and allowed to stand as a suspension in the buffer (ratio 3 : 2). The quantity of the enzyme protein bound under these conditions was 70—90%.

RESULTS AND DISCUSSION

The stimulating effect of Mn^{2+}-ions on soluble rat liver guanyl cyclase and on the enzyme bound to Sepharose was compared in the first part of our study. The results given in Fig. 1 show a similar activation of both forms of the enzyme by Mn^{2+}-ions at a concentration of 0·1—10 mM.

The addition of 20 μg of secretin (0·4 U/ml) per 1 ml of the rat liver guanyl cyclase preparation did not lead to stimulation of the enzyme at any of the concentra-

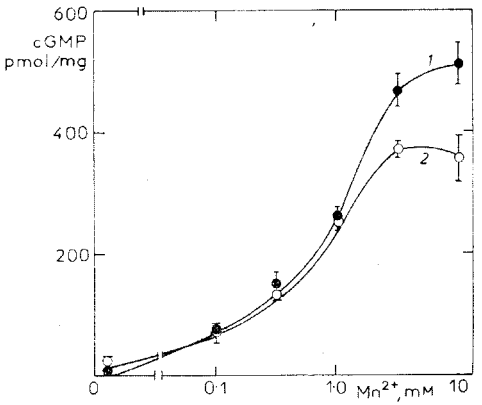

FIG. 1

Activity of Rat Liver Guanyl Cyclase in Soluble and Sepharose-bound Form in Presence of Various Concentrations of Mn^{2+}-Ions

The activity of the enzyme was determined after 10 min incubation as described in Methods. The values given are means of triplicates ±S.D.

○, Soluble enzyme; ●, Sepharose-Bound enzyme.

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TABLE I

Activity of Rat Lung and Liver Guanyl Cyclase in Soluble and Sepharose-Bound Form

The activity of the enzyme was determined as described in Methods. The values given are means of triplicates.

Additions	Lubrol PX 0·4%	Cyclic GMP, pmol/mg protein/15 min ± S.D.			
		Lung		Liver	
		soluble	bound	soluble	bound
0	—	1·1 ± 0·1	1·5 ± 0·1	0·3 ± 0·1	2·3 ± 0·2
Ca^{2+}, 5 mM	—	3·6 ± 1·8	3·4 ± 0·2	0·3 ± 0·2	5·6 ± 1·5
Mn^{2+}, 5 mM	—	322 ± 26	267 ± 28	58 ± 4	369 ± 30
Mn^{2+} + Ca^{2+}, 5 mM	—	337 ± 31	236 ± 20	66 ± 3	412 ± 25
0	+	0·4 ± 0·1	4·0 ± 0·2	0·1 ± 0·1	2·3 ± 0·2
Ca^{2+}, 5 mM	+	20·8 ± 2·6	4·8 ± 0·2	7·5 ± 2·0	5·0 ± 1·3
Mn^{2+}, 5 mM	+	1 140 ± 158	415 ± 18	128 ± 5	510 ± 25
Mn^{2+} + Ca^{2+}, 5 mM	+	1 420 ± 120	355 ± 22	185 ± 10	420 ± 31

tions of Mn^{2+}-ions used. Higher concentrations of the hormone (62 – 500 μg per ml), however, inhibited enzymatic activity in the presence of 3 mM Mn^{2+}-ions. Hence, we were not able to confirm the stimulating effect of secretin on guanyl cyclase observed *in vitro*[5].

In experiments described in Table I, the stimulating effect was examined of Mn^{2+}- and Ca^{2+}-ions and of their combination on soluble guanyl cyclase from rat lung and liver and on guanyl cyclase bound to Sepharose in the absence and presence of a detergent, Lubrol PX. The results show that both forms of the enzyme possessed a similar sensitivity to the stimulating effects of Mn^{2+}-ions which could not be replaced by calcium ions. The specific activity of the soluble enzyme and of the enzyme bound to Sepharose was very similar for the enzyme isolated from lung whereas the activity of the bound enzyme from liver was considerably higher than that of the soluble form. As demonstrated by other authors[8-10] earlier, the detergent stimulated guanyl cyclase. The addition of 0·4% of Lubrol PX during the *in vitro* determination increased the activity of the soluble preparation roughly 3-times in the presence of 5 mM Mn^{2+}-ions. The stimulating effect of Lubrol PX on the enzyme bound to Sepharose was considerably lower, both with the enzyme from lung and the enzyme from liver.

Since the reactivity of soluble guanyl cyclase is not essentially different from the activity of the enzyme bound to Sepharose, it appears that the absence of hormonal stimulation *in vitro* might be ascribed to the lack of some intermediary factors participating on the hormonal stimulation of formation of cyclic GMP in the intact tissue.

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