[Chem. Pharm. Bull. 25(6)1447—1449(1977)]

UDC 577.152.02 : 547.964.4.09

Enzyme Activities of Liver Plasma Membrane in Rat Bile after the Intrabiliary Retrograde Infusion of Triton X-100

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(Received September 3, 1976)

The activities of three bile canalicular enzymes, alkaline phosphatase, 5′-nucleotidase and ATPase, and protein concentration in bile were significantly elevated by the intrabiliary retrograde infusion of Triton X-100. The results suggest that a part of the bile canalicular site of the liver plasma membrane was solubilized by the treatment. This explains the fact that the active biliary excretion of organic anions and cations was markedly inhibited by the same treatment.

Keywords——bile canalicular enzymes; liver plasma membrane; solubilization; retrograde infusion; Triton X-100; protein concentration; ATPase; 5′-nucleotidase; alkaline phosphatase

The active biliary excretion of organic anions and organic cations was demonstrated to be strikingly inhibited by retrograde infusion of surfactants, such as sodium lauryl sulfate, Triton X–100 and sodium deoxycholate from a cannulated common bile duct in rats.[2] The amount excreted for 30 min, the concentration in bile and the bile/liver concentration ratio were reduced to 1/10 of normal values for bromphenol blue, an organic anion, and to 1/3 for acetyl procaineamide ethobromide, an organic cation, by retrograde infusion of a 4% Triton X–100 solution. In the bile collected after the treatment with the surfactants, a number of proteins which were not detected in normal bile were observed by the method of polyacrylamide gel electrophoresis.[2a] It was, therefore, assumed that these proteins were the part of the bile canalicular plasma membrane solubilized with the surfactants and were involved in the inhibitory effect on the active transport of organic compounds.

In order to verify that a part of the plasma membrane was solubilized by the treatment, we determined the enzyme activities of the rat liver plasma membrane together with the protein concentration in bile before and after the retrograde infusion of a 4% Triton X–100 solution.

Experimental

Materials——Sodium β-glycerophosphate was obtained from Merck and Co., Inc.; adenosine 5′-monophosphate, disodium salt and adenosine 5′-triphosphate, disodium salt from Kohjin Co., Ltd and the other reagents from Nakarai Chemical Co., Ltd.

Bile Collection——Male Wistar rats weighing 220—250 g were anesthetized with urethane. The abdomen was opened and the common bile duct was cannulated with a polyethylene tube. After 2 hr collection of bile, a 50 μl of 4% Triton X-100 solution in Krebs-Ringer phosphate buffer was infused retrogradely by the method previously described.[2b] After the treatment with the surfactant for 15 min, three bile samples were collected every two hours and the enzyme activities were measured immediately.

Enzyme Assay——Alkaline phosphatase was determined with β-glycerophosphate as substrate. The assay solution contained 30 mM β-glycerophosphate and 20 mM $MgCl_2$ in barbital buffer (pH 9.8). Mixture of 0.5 ml of bile sample and 0.5 ml of the assay solution was incubated for 20 min at 37°. The reaction was terminated by the addition of 1 ml of 30% trichloroacetic acid and the precipitate was removed by centri-

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fugation. To 1 ml of the supernatant, 0.1 ml of 20% sodium lauryl sulfate solution and 3 ml of water were added in order to prevent the interference of Triton X-100 with the phosphorus determination.[3] The liberated phosphorus was assayed by the Fiske and Subba Row method.[4]

5′-nucleotidase was determined with 5′-adenosine monophosphate (5′-AMP) as substrate. The assay solution contained 20 mM 5′-AMP and 20 mM $MgCl_2$ in Tris buffer (pH 7.5). Incubation of the mixture of 0.5 ml of the assay solution and 0.5 ml of bile sample, termination of the reaction and phosphorus analysis were carried out in the same manner as in alkaline phosphatase.

ATPase activity was measured under two conditions: in the presence of Mg^{2+}, Na^+ and K^+ ("total ATPase") and in the presence of Mg^{2+} alone ("Mg^{2+}-ATPase"). The assay solution contained 10 mM ATP and 15 mM $MgCl_2$ in Tris buffer (pH 7.1). For the assay of total ATPase, the mixture of the bile sample, 0.5 ml of the assay solution and 0.3 ml of Na^+, K^+ solution containing 400 mM NaCl and 67 mM KCl was incubated. Mg^{2+}-ATPase was measured in a system containing 0.5 ml of the bile sample and 0.5 ml of the assay solution. The assay procedures were the same described in alkaline phosphatase.

Protein determination was carried out by the method of Lowry, *et al.* with bovine serum albumin as standard.[5]

Results and Discussion

Alkaline phosphatase, 5′-nucleotidase and ATPase were chosen as bile canalicular enzymes because these enzymes were proved to be rich in bile canalicular plasma membrane.[6]

The protein concentrations and the activities of the enzymes in bile before and after the retrograde infusion of Triton X–100 solution are listed in Table I. Protein concentrations

Table I. Enzyme Activities,[a,b] Protein Concentrations[b] in Bile and Weight of Bile[b] before and after the Retrograde Infusion of Triton X–100

	Alkaline phosphatase	5′-Nucleotidase	Mg^{2+}-ATPase	Total ATPase	Protein (mg/ml)	Weight of bile (g)
−2—0 hr	0.4±0.4	3.5±0.4	1.1±0.7	1.6±0.7	5.3±2.3	1.83±0.30
0—2 hr	3.0±1.5	6.6±2.4	3.9±1.0	3.9±1.1	25.9±2.7	1.61±0.51
2—4 hr	3.7±1.1	5.8±1.0	2.3±0.2	2.4±0.2	25.1±3.7	1.31±0.25
4—6 hr	3.5±1.2	6.0±1.3	3.9±0.5	4.8±1.1	24.5±4.0	1.37±0.15

a) Enzyme activities are expressed in μmol per 20 min.
b) Each value is the mean±S.D. of more than four animals.

in bile were increased by a factor of five by the treatment and the level lasted at least for 6 hours without diminution. This result shows that Triton X–100 solubilized the membrane proteins to a great extent. The activities of the enzymes were also all elevated after the treatment and were not decreased for 6 hours. The elevation of the activity of alkaline phosphatase was by a factor of eight and the others by a factor of one and a half to three. The eminent elevation of the activity of alkaline phosphatase might be partly due to the solubilization of the biliary tract cell membranes with Triton X–100 because the specific activity of alkaline phosphatase in biliary tract cell is higher than in parenchymal cells.[7] The activity of Na^+,K^+–ATPase is calculated by subtracting the Mg^{2+}-ATPase from the total ATPase. In this experiment, however, no positive conclusion was to be drawn about Na^+, K^+–ATPase because the activity of total ATPase was often lower than Mg^{2+}–ATPase. Nevertheless the result is at least consistent with the previous report that Na^+, K^+–ATPase represents small percentage of total ATPase activity in canalicular membrane.[8] The activi-

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ties of Mg^{2+}–ATPase and 5′-nucleotidase in the liver plasma membrane are known to be affected by detergents.[9] Accrording to the report,[9] a 0.1% sodium lauryl sulfate solution inhibited the Mg^{2+}–ATPase and 5′-nucleotidase activities. A 0.1% Triton X–100 solution had no effect on ATPase but stimulated 5′-nucleotidase. Furthermore components in bile, *e.g.* bile salts and bilirubin, were reported to affect the activities of the enzymes.[9,10] Therefore the enzyme activity levels in this experiment are those after affected by these interfering substances. The effect of surfactants and bile components except Triton X–100 are, however, compensated because they exist in samples both before and after the treatment with Triton X–100. Only Triton X–100 might have an uncompensated effect because the initial bile sample does not contain Triton X–100 and 5′-nucleotidase was, as described above, reported to be stimulated with it. Accordingly the effect of Triton X–100 on 5′-nucleotidase was investigated by adding Triton X–100 to a new bile sample in a concentration of 0.1—1.0%. The activity of 5′-nucleotidase was not found to be changed significantly in this concentration range. Consequently the activity of 5′-nucleotidase was shown, as well as the other enzymes, to be elevated by the retrograde infusion of Triton X–100 irrespective of the interfering substances. The increase in protein concentration and the concomitant elevation of the activities of the canalicular enzymes after the treatment with Triton X–100 suggest that the part of the bile canalicular membrane was solubilized by this treatment.

Acknowledgement The authors wish to thank professor Hitoshi Sezaki for his helpful discussions.

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Chem. Pharm. Bull. 25(6)1449—1452(1977)

UDC 547.857.7.04 : 547.539.04

An Improved Procedure for the Synthesis of 1-Alkyladenines: Removal of the Ribofuranosyl Group from 1-Benzyl-, 1-(3-Methyl-2-butenyl)-, and 1-Allyladenosine Hydrobromide in Acetic Acid

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(Received September 3, 1976)

1-Benzyl- (IIIa), 1-(3-methyl-2-butenyl)- (IIIb), and 1-allyladenine (IIIc) were prepared in 77,% 44%, and 69% yield, respectively, by alkylation of adenosine (I) followed by heating the resulting 1-substituted adenosine hydrobromides (IIa,b,c) in acetic acid.

Keywords——1-alkyladenines; adenosine; alkylation; 1-alkyladenosines; acetolysis; depurinylation

1-Alkyladenines (type III) have assumed considerable importance with the finding that 1-methyladenine (type III: R=CH_3) and other 1-substituted adenines trigger the release of

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