As shown in the figure, no significant incorporation of ^{14}C-thiamine phosphates into the liver cells were observed, whereas ^{14}C-thiamine was appreciably taken up by the cells. After the addition of the medium treated ^{14}C-thiamine pyrophosphate with acid phosphatase (Takadiastase) or the addition of alkaline phosphatase to the incubation medium, the radioactivity corresponding to 5.12 and 1.36 pmoles/10^5cells of ^{14}C-thiamine, respectively, was detected in the liver cells. These results indicate that neither thiamine phosphates themselves nor their thiamine moiety are essentialy available for the liver cells. Recently, we have reported that both thiamine monophosphate and thiamine pyrophosphate could not directly translocate yeast cell membrane[15]. From these facts it is conceivable that eucaryotic cells such as yeast and hepatocytes are incapable of taking up phosphate ester of thiamine in contrast with *Escherichia coli.* Therefore, it is concluded that rat liver cells utilize only free thiamine in the plasm to synthesize thiamine coenzyme, although thiamine is present both in free and monophosphate form in rat plasm[16].

1 This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.
2 To whom reprint requests should be addressed.
3 Chen, C.P., J. Nutr. Sci. Vitaminol. *24* (1978) 351.
4 Lumeng, L., Edmonson, J.W., Schenker, S., and Li, T.K., J. biol. Chem. *254* (1979) 7265.
5 Rindi, G., and Giuseppe, L., Biochem. J. *78* (1961) 602.
6 Ishii, K., Sarai, H., Sanemori, H., and Kawasaki, K., J. Nutr. Sci. Vitaminol. *25* (1979) 517.
7 Nakayama, H., and Hayashi, R., J. Bact. *118* (1974) 32.
8 Nishimune, T., and Hayashi, R., Experientia *36* (1980) 916.
9 Schaller, K., and Holler, H., Int. J. Vitam. Nutr. Res *44* (1974) 444.
10 Iwata, H., Matsuda, T., and Baba, A., Experientia *32* (1976) 1252.
11 Seglen, P.O., Meth. Cell Biol. *13* (1976) 29.
12 Matsukawa, K., Hirano, H., and Yurugi, S., Meth. Enzym. *18A* (1970) 141.
13 Morita, M., Kanaya, T., and Mineshita, T., J. Vitaminol. *14* (1968) 77.
14 Iwashima, A., Nishimura, H., and Sempuku, K., Experientia *36* (1980) 385.
15 Nishimura, H., Sempuku, K., and Iwashima, A., J. Bact. *150* (1982) 960.
16 Rindi, G., Patrini, C., and Poloni, M., Experientia *37* (1981) 975.

0014-4754/83/050505-03$1.50+0.20/0

Detergent solubilization of cardiac 5′-nucleotidase

G. Lutoslawska, N. Jha and H.P. Baer

Department of Pharmacology, University of Alberta, Edmonton (Canada, T6G 2H7), August 19, 1982

Summary. Nonionic, anionic and zwitterionic detergents were employed for solubilization of 5′-nucleotidase from acetone powder preparations of rat heart. Zwittergents effectively solubilized the enzyme and may allow now its further identification and purification by electrophoretic techniques.

5′-Nucleotidase (EC 3.1.3.5) is a predominantly membrane-bound ecto-enzyme. Although its function in different cells and tissues is not understood, interest in this enzyme arises from the multitude of pharmacological and possible physiological effects of adenosine, the product of hydrolysis of 5′-AMP.

Several attempts to purify the enzyme from cardiac muscle have involved the use of detergents for solubilization including anionic deoxycholate and nonionic Triton X-100[1,2]. Problems encountered or expected with these detergents include modification of electrical charges of protein, and thus interference with electrophoretic techniques, and difficulty of detergent removal during and after purification[3]. Some authors have combined different types of detergents which perhaps minimizes some of these problems[4]. Recently, zwitterionic surfactants, i.e. sulphobetaine derivatives, have become available and were reported to possess effective membrane solubilization properties without accompanying charge effects thus allowing electrophoresis or isoelectric focussing of the solubilized proteins in their presence[5,6]. Zwittergent 3-14 has already been used successfully to solubilize 5′-nucleotidase from cultured fibroblasts and the subsequent identification of the enzyme by electrophoretic techniques[7].

In the following we report data concerning detergent effects on cardiac 5′-nucleotidase, comparing effects of zwitterionic with those of several ionic and nonionic detergents.

Methods and materials. Rat heart acetone powder was prepared and suspended in 50 mM TRIS-HCl (pH 7.5) containing 0.15 M KCl as described previously[8]. To 2 ml portions of suspended extract (13 mg/ml protein) detergents were added in concentrations of 1% (g/100 ml) each unless otherwise stated, and suspensions were then stirred for 4 h at 4 °C. Enzyme activity was determined in the detergent-treated suspensions and, following centrifugation at 100,000 × g for 1 h, in the supernatants and the resuspended pellets. Assays were performed with 5′-IMP as substrate and measurement of phosphate formation[8]. Assays contained, in a total of 0.25 ml, 50 mM TRIS-HCl, pH 8.5, 8 mM $MgCl_2$, 20 mM IMP and about 0.06 mg protein. Incubations were for 60 min at 37 °C. In the case of detergent-treated preparations, the detergents were diluted to 0.25% in enzyme assays and were identical in assays of suspended pellets and supernatants. Triplicate assays were done throughout and resulted in variations of less than 5%. Protein was measured by the method of Lowry et al.[9] and in the case of detergent-containing preparations no interference with protein measurements was observed at the resulting dilutions (0.02% or less) in these assays. The following detergents were used: Nonionic-Tween (type 20, 40, 60 and 85), octylglucose, Lubrol WX and Lubrol PX; anionic-deoxycholate; zwitterionic-Zwittergents 3-10, 3-12, 3-14 and 3-16. Detergents were purchased from Sigma Chemical Co., St. Louis, Mo., USA, except for Zwittergents

Effect of Zwittergents and deoxycholate on rat heart 5′-nucleotidase

Experiment	Detergent	100,000× g pellet		100,000× g supernatant		% Protein solubilization
		Specific activity*	% Activity	Specific activity	% Activity	
1	None	0.02	100	0	0	-
	Deoxycholate	0.04	100	0.05	150	60
	Lubrol PX	0.03	92	0.03	111	61
2	None	0.03	100	0	0	-
	Zwittergent 3-10	0	0	0.04	46	37
	Zwittergent 3-12	0	0	0.06	139	69
	Zwittergent 3-14	0	0	0.06	161	80
	Zwittergent 3-16	0	0	0.04	85	64
3	None	0.02	100	0	0	-
	Zwittergent 3-12 + Deoxycholate, 1%	0	0	0.10	86	21
	Zwittergent 3-14 + Deoxycholate, 1%	0	0	0.10	128	31

*Specific activity: μmole/mg/min.

which were from Calbiochem-Behring, La Jolla, Ca., USA, and Lubrol WX, which was made available by CIL Chemicals, Edmonton, Canada.

Results and discussion. The following detergents did not lead to any occurrence of 5′-nucleotidase activity in the 100,000× g supernatant and caused varying degrees of loss of enzyme activity (percent activity remaining given in parenthesis): Tween 85 (66), Tween 80 (85), Tween 60 (62), Tween 40 (43), Tween 20 (43), octylglucose (20). Lubrol WX did not cause any enzyme solubilization, but the activity remaining in the pellet increased to 130% and specific activity was doubled. The basis for either increases or decreases in enzyme activity and the reversibility of such effects seen in the presence of Lubrol WX or other detergents has not been investigated.

The effects of deoxycholate, Lubrol PX and Zwittergents on enzyme activity, specific activity and protein solubilization are summarized in the table. In the absence of detergents all of the enzyme activity present in the homogenate was recovered in the resuspended 100,000× g pellet, and in each experiment this value is taken as 100% (rows labeled 'none' in the table). Following treatment of homogenates with detergents, enzyme activity prior to centrifugation also equalled that recovered in the respective supernatant and resuspended pellet, and the activity level is expressed relative to that found in the absence of detergent (100%) in each experiment. The recoveries of solubilized protein in the 100,000× g supernatants stated in the table are also related to the amount of protein originally present in the total homogenates, as measured prior to centrifugation (100%).

Lubrol PX and deoxycholate caused partial solubilization accompanied by a doubling of total activity. Both detergents also increased the specific activity in the pellet and supernatant about 1.5- and 2-fold, respectively. In the presence of Zwittergents, enzyme activity only occurred in the 100,000× g supernatants. Zwittergent 3-10 and 3-16 reduced activity by 54 and 15%, respectively. Zwittergents 3-12 and 3-14 increased activity by 39 and 61%, respectively, accompanied by a doubling of specific activity. Combinations of deoxycholate and Zwittergents 3-12 and 3-14 also caused complete solubilization (table). The enhancement of activity caused by these combinations was less than with deoxycholate alone but specific activity was increased 5-fold in the 100,000× g supernatant.

At concentrations of 0.2%, none of the Zwittergents caused solubilization. At 5%, Zwittergent 3-16 did not result in any solubilization while Zwittergents 3-12 and 3-14 showed results comparable to those seen at 1%, and Zwittergent 3-10 caused enhancement of enzyme activity in the supernatant to 234% and thus increased the apparent sp.act. to 0.08 μmoles/mg/min. It should be noted, however, that in this case the detergent was diluted to 1.25% in enzyme assays and that enzyme activity could possibly be influenced by the detergent concentration in the assay mixture.

Our results indicate that solubilization of 5′-nucleotidase from rat heart membranes can be readily achieved with the novel zwitterionic detergents. The chain length of the hydrophobic portion of these molecules, designated by their number codes, is critical in terms of enzyme activity levels retained after exposure to these detergents, and with a given tissue or cell type the optimal detergent will have to be selected empirically. The optimal concentration for any of the Zwittergents has not been determined precisely, but is is evident that the minimum concentration must be above 0.2%. Other authors have used successfully concentrations of 0.75-2%[7,10]. It is hoped that 5′-nucleotidase solubilized by these electrically neutral detergents can now be identified and purified by electrophoretic procedures. In the meantime it was reported also that 5′-nucleotidase from rat liver can be solubilized by Zwittergent 3-14 (2%)[10] suggesting that these detergents will be generally useful for the solubilization of membrane-bound 5′-nucleotidase, opening up new possibilities for the study of isoenzymes and their roles in cell function.

1 Edwards, M.J., and Maguire, M.H., Molec. Pharmac. *6* (1970) 641.
2 Naito, Y., and Lowenstein, J.M., Biochemistry *20* (1981) 5188.
3 Helenius, A., and Simmons, K., Biochim. biophys. Acta *415* (1974) 29.
4 Riemer, B.L., and Widnell, C.C., Archs Biochem. Biophys. *171* (1975) 343.
5 Gonenne, A., and Ernst, R., Analyt. Biochem. *87* (1978) 28.
6 Hjelmeland, L.M., Nebert, D.W., and Chrambach, A., Analyt. Biochem. *95* (1979) 201.
7 Pian-Tucker, C.H., Bakay, B., and Nyhan, W.L., Biochem. Genet. *17* (1979) 995.
8 Baer, H.P., Drummond, G.I., and Duncan, E.L., Molec. Pharmac. *2* (1966) 67.
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
10 Bailyes, E.M., Luzio, J.P., and Newby, A.C., Biochem. Soc. Trans. *9* (1981) 140.

0014-4754/83/050507-02$1.50+0.20/0