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Solubilization of UDPglucose–ceramide glucosyltransferase from the Golgi apparatus

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The choice of a suitable detergent for solubilization of UDPglucose–ceramide glucosyltransferase from Golgi membranes has been investigated. Among the various classes of detergent, CHAPS, a zwitterionic detergent, was used as it produced a substantial activation of the enzyme activity. 30% of the enzyme activity and 50% of proteins were solubilized in the first attempts. Further experiments were conducted with addition of a second detergent, Zwittergent 3-14 which increased enzyme recovery to 45%. Lastly, reducing the concentrations of buffer and divalent cations Mn^{2+}, Mg^{2+} and introducing glycerol (20%, v/v) allowed 80% of proteins to be solubilized together with 68% of the ceramide glucosyltransferase activity.

Introduction

A UDPglucose–ceramide glucosyltransferase (EC 2.4.1.80) has been shown to be associated with membranes of the Golgi apparatus [1]. Topological studies led us to conclude that there is a cytoplasmic orientation of glucosylceramide synthesis [2]. Since the biosynthesis of gangliosides takes place in the lumen of the Golgi cisternae [3,4] a translocation of glucosylceramide must be considered before it can be used as precursor.

In order to understand further how translocation may occur, solubilization then purification of this enzyme is a first step towards reconstitution experiments with pure and lipid-depleted enzyme. A few reports dealing with solubilization and purification of enzymes acting on glycosphingolipids biosynthesis have been published [5,7]. Neskovic et al. [8] were successful in purifying one enzyme acting in glucocerebrosides synthesis, UDPgalactose–ceramide galactosyltransferase from rat brain, unlike Costantino-Ceccarini and Suzuki [9], but to our knowledge the UDPglucose–ceramide glucosyltransferase has not yet been purified.

In this report, results concerning the conditions for proper solubilization and stabilization of UDPglucose–ceramide glucosyltransferase from Golgi membranes are presented.

Materials and Methods

Materials

Porcine submaxillary ganglia were obtained from a local slaughterhouse. UDP [U-^{14}C]glucose (11.5 GBq/mmol) was purchased from Amersham International. Mops, Chaps were obtained from Sigma. Ceramides from bovine brain were from Serdary Research Laboratories, Canada. Zwittergent 3-12, Zwittergent 3-14, octyl β-D-glucoside and octyl β-D-thioglucoside were purchased from Calbiochem-Behring Corp. Triton X-100 with low peroxides was from Pierce.

Methods

Isolation of Golgi vesicles

Golgi vesicles were isolated from porcine submaxillary ganglia according to the procedure of Leelavathi et al. [10] as already described [1]. Golgi fraction was then suspended in 70 mM Mops buffer (pH 6.5) containing 7 mM $MnCl_2$ and 3.5 mM $MgCl_2$.

Abbreviations: BAL, British Anti-Lewisite, (2,3-dimercaptopropanol); Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; Mops, 4-morpholinepropanesulfonic acid; octyl glucoside, octyl β-D-glucopyranoside; octyl thioglucoside, octyl β-D-thioglucopyranoside; Zw 3-12, Zwittergent 3-12; Zw 3-14, Zwittergent 3-14.

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Enzyme assay

UDPglucose–ceramide glucosyltransferase assay on exogenous ceramides was adapted from Neskovic et al. [11] according to Ref. 2. Before incubation, detergent or Chaps (500 μg) and ceramides (75 μg) dissolved in chloroform/methanol (1 : 1, v/v) were introduced in the test tubes. After solvent evaporation under a gentle stream of nitrogen, 70 μl of the Golgi fraction in 70 mM Mops buffer (pH 6.5), 7 mM $MnCl_2$, 3.5 mM $MgCl_2$ then 10 μl of a mixture of 20 mM NADH and 40 mM BAL were added to the dry lipid film and vortexed vigorously twice for 30 s. The reaction was then initiated by addition of 20 μl of 260 μM UDP [U-^{14}C]glucose (70 mCi/mmol). After 10 min at 37 °C, the reaction was stopped by addition of 2 ml chloroform/methanol (2 : 1, v/v) and left for 1 h at room temperature. The lipid extract was partitioned according to the method of Folch et al [12]. The lower lipidic phase was washed twice with the upper phase, chloroform/methanol/water (3 : 48 : 47, v/v/v) and dried. Radiolabeled glucosylceramide was separated on silica-gel 60 thin-layer plates (Merck) in the solvent system chloroform/methanol/water (60 : 35 : 8, v/v/v), scraped off the plates and counted by liquid scintillation. Protein determination was according to Bensadoun and Weinstein [13].

Results

Effect of various detergents on UDPglucose–ceramide glucosyltransferase activity from Golgi membranes

Among the variety of known detergents the most effective in solubilizing lipids and proteins and the least detrimental to enzyme activity [14,15] were selected and first assayed on membrane-bound ceramide glucosyltransferase activity. They belong to the zwitterionic series: Zwittergent and Chaps which combine the polar head of zwittergents with the rigid hydrophobic part of the bile salts, or are non-ionic (Triton X-100, octyl glucoside).

Table I shows that at a concentration near their critical micellar concentration (CMC) they were inhibitors (Zw 3-12, octyl glucoside and octyl thioglucoside) or weak activators (Zw 3-14, Triton X-100) of enzyme activity. Only Chaps produced substantial activation. At concentrations higher than their CMC, they were all largely inhibitors, except Chaps. Therefore, this detergent was used for preliminary experiments of solubilization of enzyme activity.

Solubilization of UDPglucose–ceramide glucosyltransferase from Golgi membranes

Fig. 1 shows that with increasing Chaps concentrations in solubilization mixture, increasing amounts (up to 50%) of proteins were recovered after centrifugation. However, only 30% of enzyme activity could be de-

TABLE I

Effect of detergents on membrane-bound ceramide glucosyltransferase activity

In the incubation mixture, protein concentration was 1 mg/ml and detergent was introduced as indicated. Incubation was for 10 min, as described under Materials and Methods. Activity is expressed as a percent of the activity measured in the absence of detergent. Mean of at least two experiments. The average value for ceramide glucosyltransferase activity is 120 ± 20 pmol/min per mg proteins.

Detergent	CMC (mg/ml)	Quantity added (mg/ml)	Ceramide glucosyltransferase activity (%)
Zwittergent 3-12	1.2	1	0
		3	0
Zwittergent 3-14	0.12	0.08	119
		0.4	17
Octyl glucoside	7.5	1	108
		7.5	5
		10	0
Octyl thioglucoside	2.8	2.5	3.5
		7.5	0
Triton X-100	0.18	0.2	110
		5	15.4
Chaps	5	5	191
		10	145

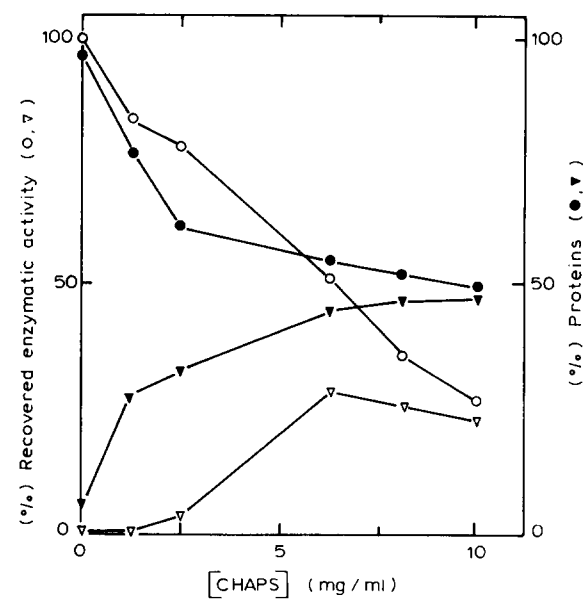

Fig. 1. Effect of Chaps concentration on the solubilization of ceramide glucosyltransferase activity. Golgi membranes (1.1 mg/ml) in 70 mM Mops buffer (pH 6.5) containing 7 mM $MnCl_2$ and 3.5 mM $MgCl_2$ were mixed with increasing Chaps concentrations (from 1 to 10 mg/ml). After 10 min at 0 °C the membrane pellet and supernatant were separated by ultracentrifugation. Ceramide glucosyltransferase activity was measured in the pellet (○) and the supernatant (▽) as described in Materials and Methods. Mean of at least three experiments. Activity is expressed as per cent of the activity in the Golgi fraction mesured in the presence of detergent. The average value for ceramide glucosyltransferase activity is 230 ± 40 pmol/min per mg proteins. Proteins in the pellet (●) and in the supernatant (▼).

tected in the supernatant with a concentration of Chaps of 6.2 mg/ml. For higher concentrations, whereas the activity detected in the pellet was decreasing, no increase of activity was obtained in the supernatant. Inhibition of the enzyme activity above CMC of Chaps was obvious.

Various treatments were then evaluated for protein solubilization and enzyme recovery. Addition of a second detergent was sometimes required to enhance solubilization of membrane proteins. Zw 3-14, octyl glucoside and Triton X-100 were therefore selected as they do not inactivate enzyme activity. At a concentration near CMC they were unable to solubilize efficiently both proteins and enzyme activity (Table II). However, when used together with Chaps while increasing only slightly protein solubilization, more enzyme activity was recovered in the supernatant: up to 45% of the ceramide glucosyltransferase activity was detected with a mixture of Chaps and Zw 3-14.

Parini and Graham [16] showed that modification of the chemical composition of the solubilization medium greatly improve the solubilizing efficiency of detergent Chaps. In our case (Table III), reduction in Mops buffer molarity from 70 mM to 20 mM and reduction of Mn^{2+} and Mg^{2+} concentrations resulted in a marked improvement of protein solubilization. Nevertheless, recovery of enzyme activity was hampered by its increasing instability as only 30% of enzyme activity was recovered in the supernatant. Addition of glycerol (20%, w/v) increased recovery of ceramide glucosyltransferase activity (up to 59% was found in the supernatant) but lowered solubilization of proteins (only 60% of proteins were recovered in the supernatant). However, glycerol introduced 10 min after detergent addition allowed 80% of proteins to be solubilized together with 68% of the ceramide glucosyltransferase.

All other treatments currently used to improve protein solubilization and enzyme recovery were ineffective: modification of pH and temperature, introduction of the substrates of enzyme or addition of essential phospholipids.

TABLE II

Effect of various detergents on the solubilization of proteins and ceramide glucosyltransferase

Golgi membranes (1.1 mg/ml protein) were prepared as described in Materials and Methods. Detergent was added at concentrations indicated: Zwittergent 3-14, 0.12 mg/ml; octyl glucoside, 7.5 mg/ml; Triton X-100, 0.18 mg/ml; Chaps, 6.2 mg/ml. The membrane pellet and supernatant were separated by ultracentrifugation. Ceramide glucosyltransferase and proteins were measured in the pellet and in the supernatant. Activity is expressed as percent of the activity in the Golgi fraction measured in presence of detergent (Materials and Methods). Mean of at least two experiments. The average value for ceramide glucosyltransferase activity is 230±40 pmol/min per mg protein.

Treatment	Solubilization (%)			
	protein		ceramide glucosyltransferase	
	pellet	supernatant	pellet	supernatant
Chaps	55	45	50	30
Zwittergent 3-14	97	5	96	0
Octyl glucoside	90	10	90	0
Triton X-100	91	9	87	0
Chaps + Zwittergent 3-14	51	51	54	45
Chaps + octyl glucoside	56	52	59	41
Chaps + Triton X-100	59	51	56	43

TABLE III

Effect of variations of solubilizing medium on the solubilization of ceramide glucosyltransferase

Golgi membranes (1.1 mg/ml protein) were prepared as described in Materials and Methods. Detergent was added at concentrations indicated: Zwittergent 3-14, 0.12 mg/ml; Chaps, 6.2 mg/ml. The membrane pellet and supernatant were separated by ultracentrifugation. Ceramide glucosyltransferase and proteins were measured in the pellet and in the supernatant. Activity is expressed as percent of the activity in the Golgi fraction measured in the presence of detergent (Materials and Methods). Mean of at least two experiments.

Treatment	Solubilization (%)			
	protein		ceramide glucosyltransferase	
	pellet	supernatant	pellet	supernatant
Chaps + Zwittergent 3-14 in 70 mM Mops (pH 6.5) + Mg^{2+} + Mn^{2+}	51	51	54	45
Chaps + Zwittergent 3-14 in 20 mM Mops (pH 6.5) minus Mn^{2+} minus Mg^{2+}	25	75	20	30
Chaps + Zwittergent 3-14 in 20 mM Mops (pH 6.5) minus Mn^{2+} minus Mg^{2+} + 20% glycerol (v/v)	40	60	39	59
Chaps + Zwittergent 3-14 in 20 mM Mops (pH 6.5) minus Mn^{2+} minus Mg^{2+} + 20% glycerol (v/v) (added 10 min after)	20	80	18	68

Discussion

Non-denaturing detergents are required for the solubilization of intrinsic membrane proteins with preservation of their native structure and biological activity. Among non-ionic and zwitterionic detergents, only Chaps produced substantial activation of ceramide glucosyltransferase activity. It is likely that this detergent allowed the hydrophobic acceptor ceramide and the hydrophilic nucleotide-sugar substrate to fit well within the active site of the enzyme. Moreover, this

detergent is efficient in solubilization of a fraction of the membrane proteins and of the ceramide glucosyltransferase activity. Addition of Zwittergent 3-14 enhanced enzyme recovery, probably by increasing stabilization of ceramide glucosyltransferase in the solubilized state.

Considering the biophysical characteristics of Chaps which make this detergent well suited for purification procedure [17] and experiments of reconstitution of membrane proteins in phospholipid vesicles [16], it was important to increase the solubilization efficiency of this detergent as well as the stability of the ceramide glucosyltransferase activity.

Modification of the solubilizing conditions by Chaps was suggested by Parini and Graham [16]. We noted a strong inhibition of ceramide glucosyltransferase activity by salts at high ionic strength (data not shown) which precluded to use their precise conditions. In our case, reducing buffer concentration and Mg^{2+} and Mn^{2+} concentrations in the solubilization mixture greatly enhanced protein solubilization. The mechanism, although unclear, may be related to membrane aggregation in medium containing divalent cations. However, enzyme lability was increased. As also reported by Parini and Graham [16], glycerol (osmolyte stabilant) appears to stabilize enzyme activity. Further tests showed that glycerol must be added a few minutes after adding detergent. If added earlier, solubilizing efficiency was decreased, perhaps by modification of the CMC of Chaps. The mechanism of rescue by glycerol (or other osmolytes) is a common finding for stabilization of membraneous proteins during solubilization process and appears to reflect a thermodynamic stabilization of the native structure in the absence of an organized lipid bilayer [18].

The following step before studies on reconstitution experiments with pure and lipid-depleted enzyme is to obtain the enzyme in a purified state. However, first attempts using common purification processes consistently led us either to inefficient purification or to low recovery of enzyme activity and a slight-fold net purification. As for other membrane-bound enzymes, it is not impossible that association of the enzyme with other components of the crude enzyme fraction is necessary for further stabilization of enzyme activity [19,20]. This is now under investigation.

References

1 Coste, H., Martel, M.B., Azzar, G. and Got, R. (1985) Biochim. Biophys. Acta 814, 1–7.
2 Coste, H., Martel, M.B. and Got, R. (1986) Biochim. Biophys. Acta 858, 6–12.
3 Yusuf, H.K.M., Pohlentz, G. and Sandhoff, K. (1983) Proc. Natl. Acad. Sci. USA 80, 7075–7079.
4 Creek, K.E. and Morre, D.J. (1981) Biochim. Biophys. Acta 643, 292–305.
5 Taniguchi, N., Yanagisawa, K., Makita, A. and Naiki, M. (1985) J. Biol. Chem. 260, 4908–4913.
6 Basu, S., Basu, M., Kyle, J.W., De, T., Das, K. and Schaeper, R.J. (1986) in Enzymes of Lipid Metabolism (Freysz, L. and Gatt, S., eds.), pp. 233–245, Plenum Press, New York.
7 Kaplan, F. and Hechtman, P. (1983) J. Biol. Chem. 258, 770–776.
8 Neskovic, N.M., Roussel, G. and Nussbaum, J.L. (1986) J. Neurochem. 47, 1412–1418.
9 Costantino-Ceccarini, E. and Suzuki, K. (1975) Arch. Biochem. Biophys. 167, 646–654.
10 Leelavathi, D.E., Estes, L.W., Feingold, D.S. and Lombardi, B. (1970) Biochim. Biophys. Acta 211, 124–138.
11 Neskovic, N.M., Sarlieve, L.L. and Mandel, P. (1974) Biochim. Biophys. Acta 334, 309–315.
12 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
13 Bensadoun, A. and Weinstein, D. (1976) Anal. Biochem. 70, 241–250.
14 Womack, M.D., Kendall, D.A. and Mac Donald, R.C. (1983) Biochim. Biophys. Acta 733, 210–215.
15 Navarrete, R. and Serrano, R. (1983) Biochim. Biophys. Acta 728, 403–408.
16 Parini, A. and Graham, R.M. (1989) Anal. Biochem. 176, 375–381.
17 Hjelmeland, L.M. (1980) Proc. Natl. Acad. Sci. USA. 77, 6368–6370.
18 Maloney, P.C. and Ambudkar, S.V. (1989) Arch. Biochem. Biophys. 269, 1–10.
19 Holmes, E.H. and Hakomori, S. (1987) J. Biochem. 101, 1095–1105.
20 Chalifour, R.J. and Spiro, R.G. (1988) J. Biol. Chem. 263, 15673–15680.