Characteristics of n-octyl β-D-thioglucopyranoside, a new non-ionic detergent useful for membrane biochemistry

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n-Octyl β-D-thioglucopyranoside, a new non-ionic detergent, was synthesized. Properties, and applicability to membrane proteins, of this detergent were investigated. The detergent was easily removed by dialysis. The solubilizing power of this detergent for Escherichia coli membrane proteins was similar to that of n-octyl β-D-glucopyranoside, which has been widely used in membrane biochemistry. No inactivation of proteins was observed after the solubilization. n-Octyl β-D-thioglucopyranoside was superior to n-octyl β-D-glucopyranoside in that it was much more stable and could be synthesized at much lower cost.

Detergents are usually required for solubilization and purification of intrinsic membrane proteins, which are highly hydrophobic. Furthermore, removal of the detergents is often necessary for the assay of the protein activity or for the reconstitution of the proteins into liposomes. For these purposes, detergents possessing the following properties are very useful: (1) high solubilizing power; (2) no denaturation of proteins; (3) high CMC; (4) non-ionic; (5) optical transparency; (6) chemical purity; (7) high solubility in water; (8) stability; (9) no effect on other assays (for example, protein assay); and (10) easily obtainable. Among the detergents currently available, few detergents satisfy these desirable properties.

Although octyl glucoside is an excellent detergent for solubilization and reconstitution of many membrane proteins (Baron & Thompson, 1975; Racker et al., 1979; Newman & Wilson, 1980; Newman et al., 1981; Tsuchiya et al., 1982a), it is not, for economic reasons, suitable for large-scale experiments. Doldecyl β-D-maltoside has also been used for purification of the lactose carrier of Escherichia coli (Wright & Overath, 1984). This detergent, however, is also very expensive. Alkanoyl N-methylglucamides are also excellent detergents (Hildreth, 1982). However, their low solubility is sometimes a problem (Hanatani et al., 1984). Since these compounds possess desirable properties as detergents, their chemical analogues are expected to possess many of the same properties. If such analogues are synthesized easily and inexpensively, they would be very useful in membrane biochemistry.

Here we report the synthesis, properties and applicability to membrane proteins of octyl thioglucoside, a compound that possesses all of the desirable properties described above.

Experimental

Synthesis of octyl thioglucoside

α-D-Glucopyranose penta-acetate was prepared by a published method (Zemplen & Pacsu, 1929). 2,3,4,6-Tetra-O-acetyl-α-D-glucofuranosyl bromide was synthesized from α-D-glucopyranose penta-acetate by a published procedure (Lemiux, 1963) and recrystallized from diethyl ether. A mixture of this compound (14 g), thiourea (3 g) and acetone (50 ml) was boiled under reflux for 30 min and cooled in an ice bath. The solid product, 2-S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-thiopseudourea hydrobromide, was collected by filtration and recrystallized from acetone. To the solution of this compound (10 g) and n-octyl bromide (4.5 g) in acetone/water (1:1, v/v) (500 ml) were added K₂CO₃ (3 g) and NaHSO₃ (2 g) in this order, and the mixture was left overnight at room temperature. The mixture was extracted with
dichloromethane (2 x 200ml). The organic extracts were combined, washed with water, dried over anhydrous Na₂SO₄, and the solvent evaporated off. The residue was dissolved in benzene and applied to a silica-gel column (Kieselgel 60, Merck), and eluted with a 0–5% gradient of acetone in benzene. The eluates were dried in vacuo. Recrystallization from n-hexane gave colourless needles of 1-S-n-octyl 2,3,4,6-tetra-O-acetyl-1-deoxy-1-thio-β-D-glucopyranoside. This compound (8g) was dissolved in 5mM-NaOH in methanol (100ml) and left overnight at room temperature. The mixture was neutralized with acetic acid and the solvent evaporated off. The residue was dissolved in dichloromethane (20ml), applied to a silica-gel column, and eluted with 5% methanol in dichloromethane. The eluates were evaporated under vacuum and freeze-dried. Thus 1-S-n-octyl 1-deoxy-1-thio-β-D-glucopyranoside (octyl thioglucoside) was synthesized in overall yield of about 80%.

Determination of glucosides

Octyl thioglucoside was determined either by g.l.c. after trimethylsilylation or by the anthrone method (Roe, 1955). Octyl glucoside and glucose were determined by the anthrone and glucose oxidase methods (Okada et al., 1981) respectively.

Solubilization of membrane proteins

Membrane vesicles of E. coli were prepared as described previously (Tsuchiya et al., 1982b). Extractability of the proteins from the membrane vesicles was determined as described by Hanatani et al. (1984).

ATPase assay

ATPase activity was measured as described by Tsuchiya et al. (1982a). One unit of ATPase activity is defined as 1µmol of ATP hydrolysed/min.

Protein assay

Protein was determined by Schaffner & Weissmann's (1973) method, with bovine serum albumin (fraction V; Sigma) as standard.

Chemicals and enzymes

Chemicals used in the present study were of reagent grade and obtained from commercial sources. β-Glucosidase (Emulsin, EC 3.2.1.21) was from Sigma.

Results and discussion

Octyl thioglucoside was synthesized with an excellent yield in several steps without using expensive materials and catalysts. The entire synthesis could be completed in several days.

Properties of octyl thioglucoside as detergent were then investigated. The CMC of octyl thioglucoside was determined to be 9mM by the method of Ross & Olivier (1959) (T. Tsuchiya & S. Saito, unpublished work). The high CMC value suggests that the detergent could be easily removed by dialysis, which is the most convenient and practical procedure for the removal of detergents. As shown in Fig. 1, 95% of the octyl thioglucoside was removed in 6 h by dialysing against 200 vol. of a buffer. Theoretical equilibrium was attained after overnight dialysis (results not shown). Octyl glucoside, the CMC of which is 25mM (Shinoda et al., 1961), was also easily removed by dialysis (Fig. 1) as reported by Baron & Thompson (1975).

One of the characteristics of octyl thioglucoside superior to that of octylglucoside is its stability. Generally the thioether bond is more stable than the ether bond. This seems to be true for octyl thioglucoside and octyl glucoside. It has been recommended to use freshly prepared octyl glucoside solution for solubilization and reconstitution of membrane proteins. It is often difficult to obtain reproducible results with solutions of octyl glucoside that have been stored for several weeks in the freezer. It is likely that degradation (hydrolysis) of octyl glucoside occurs during storage as a frozen solution. A similar situation has been reported with dodecyl β-D-maltoside (Wright & Overath,
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1984). On the other hand, octyl thioglucoside could be stored as solution for at least several months without losing its ability to give reproducible results (T. Tsuchiya, unpublished work). Thus octyl thioglucoside seems to be more stable than octyl glucoside.

In addition to its chemical stability, octyl thioglucoside was resistant to enzymic attack by β-glucosidase (whereas octyl glucoside was sensitive) (Fig. 2). The hydrolysis of salicin [2-(hydroxy-methyl)phenyl β-D-glucopyranoside, which was a standard substrate of β-glucosidase, is shown for comparison (Fig. 2). Therefore care should be taken when octyl glucoside is used for biological samples containing β-glucosidase.

The effect of octyl thioglucoside on the solubilization of E. coli membrane proteins was tested at concentrations of the detergent higher than the CMC. With increasing concentrations of octyl thioglucoside, an increasing amount of protein was solubilized (Fig. 3). The solubilizing power of octyl thioglucoside for the membrane proteins was similar to that of octyl glucoside and nonanoyl and decanoyl N-methylglucamides (Hanatani et al., 1984). We measured the H⁺-translocating ATPase activity in the solubilized fractions. The H⁺-translocating ATPase is a membrane enzyme composed of two portions, F₁ (extrinsic membrane component) and F₀ (intrinsic membrane component). The ATPase was solubilized with octyl thioglucoside (Fig. 3). The activity was sensitive to dicyclohexylcarbodi-imide (results not shown), which means that the ATPase was solubilized as F₁-F₀ complex. The specific activity of the ATPase was 2.5 units/mg of protein, which was comparable with the value shown by the F₁-F₀ complex when it is solubilized with other detergents (about 3 units/mg of protein; T. Tsuchiya, unpublished work). Solubilization and reconstitution of the melibiose carrier of E. coli membrane were also successfully performed with octyl thioglucoside (T. Tsuchiya & S. Saito, unpublished work). The specific activity of melibiose transport in the reconstituted proteoliposomes was similar to that of proteoliposomes reconstituted with octyl glucoside. Thus the solubilizing power and non-denaturing property of octyl thioglucoside on E. coli membrane proteins are similar to those of octyl glucoside.

In addition to those advantageous properties, octyl thioglucoside is endowed with other desirable properties for use in membrane biochemistry. Octyl thioglucoside is non-ionic, optically transparent, chemically pure, highly soluble in water, and does not interfere with protein determination by the method of Schaffner & Weissmann (1973,

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**Fig. 2. Action of β-glucosidase on octyl thioglucoside and octyl glucoside**

Octyl thioglucoside (●), octyl glucoside (○) or salicin (▲) (all 43 mM) was incubated with 50 mM sodium acetate buffer, pH 5.0, and β-glucosidase (0.1 mg/ml) at 37°C. Samples were taken at intervals, and concentrations of octyl thioglucoside or glucose (in the cases of octyl glucoside and salicin) were determined. Concentrations of octyl glucoside and salicin were calculated from the glucose produced.

**Fig. 3. Solubilization of membrane proteins and H⁺-translocating ATPase with octyl thioglucoside**

Membrane vesicles (0.8 mg of protein) were mixed with various concentrations of octyl thioglucoside, and after pelleting the insoluble material the protein content (●) and ATPase activity (○) of the solubilized fractions were measured.
results not shown). Thus octyl thiogluco side satisfies all of the desirable properties described above and should be very useful in membrane biochemistry.

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References