***N*-D-Gluco-*N*-methylalkanamide compounds, a new class of non-ionic detergents for membrane biochemistry**

James E. K. HILDRETH*

Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K.

(Received 29 June 1982/Accepted 17 August 1982)

N-D-Gluco-*N*-methylalkanamide detergents have been synthesized. The detergents, which were produced in high yield and at low cost, compared favourably in biochemical studies with commonly used non-ionic detergents, including a chemically related *n*-alkyl glucoside. The ease of removal by dialysis, high solubilizing power and non-denaturing properties of this new class of detergents make them valuable reagents for membrane research.

A general approach to the study of complex biological membranes involves the dissociation, characterization and re-assembly of membrane components. The most important aspect of this approach is the use of an appropriate detergent as solubilizing agent. Ideally the detergent should give maximum dissociation of membranes while preserving the enzymic, antigenic or other activities of the components. It is also desirable that the properties of the solubilizing agent include the following: (1) electrical neutrality, thus avoiding alteration of the charge properties of solubilized proteins; (2) a high CMC to permit rapid removal by dialysis; (3) optical transparency in the u.v. region to allow spectrophotometric detection of protein; (4) a well-defined chemical composition and high purity to ensure experimental reproducibility.

Very few commercially available detergents currently used in membrane biochemistry adequately combine the above properties. The most widely used detergents are the neutral non-denaturing aryl polyoxyethylene ethers, Triton X-100 and Nonidet P40. Although these compounds are excellent solubilizers that maintain native activities, they absorb intensively at 280 nm and are ineffective in breaking protein–protein interactions. The large micelle size of these detergents is incompatible with gel-filtration procedures and their low CMC (Helenius & Simons, 1975) makes removal by dialysis very difficult. Alkyl polyoxyethylene ethers

(Brij detergents) are neutral and do not absorb at 280 nm. However, these agents, which also form very large micelles and have low CMC values, are much poorer solubilizers than the aryl ethers (Helenius *et al.*, 1979). The anionic bile salts, which are optically clear at 280 nm with small micelle sizes and high CMC values (Helenius *et al.*, 1979), are also excellent solubilizers. The bile salts, however, add substantial negative charge to solubilized proteins and this profoundly affects the use of standard biochemical methods, such as isoelectric focusing and ion-exchange chromatography. The zwitterionic *N*-alkylsulphobetaines, which are electrically neutral, are extremely powerful detergents (Hjelmeland *et al.*, 1979). The use of these compounds is limited, however, because of their strongly denaturing characteristics (Hjelmeland *et al.*, 1979).

The alkyl- β -D-glucosides are homogeneous non-ionic detergents introduced into biochemical research by Baron & Thompson (1975). These compounds, especially octyl β -D-glucoside, which combines all of the desired properties listed above, have been found to be superior to other commonly used detergents for membrane research (Stubbs *et al.*, 1976; Lin *et al.*, 1979; Rosevear *et al.*, 1980). The synthesis of *n*-alkyl β -D-glucosides involves several synthetic steps and the use of a silver catalyst (Rosevear *et al.*, 1980), which makes these detergents very expensive. This consideration limits the use of these compounds to small-scale procedures.

The present paper describes the synthesis of *N*-gluco-*N*-methylalkanamide compounds and their use in membrane-reconstitution studies. The results presented show that these inexpensive reagents, chemical analogues of the *n*-alkyl β -D-glucosides, provide practical alternatives as detergents for membrane research.

Abbreviations used: CMC, critical micelle concentration; OMEGA, octanoyl-*N*-methylglucamide; MEGA-9, nonanoyl-*N*-methylglucamide; MEGA-10, decanoyl-*N*-methylglucamide.

* Present address: Department of Medicine, Johns Hopkins Medical School, Baltimore, MD 21205, U.S.A.

Experimental

Chemicals

All chemicals, purchased from the Aldrich Chemical Co., Poole, Dorset, U.K., were of the highest purity available and were used without further purification.

Synthesis

For the synthesis of *N*-D-gluco-*N*-methylalkanamide detergents, a solution of 19.5 g (0.1 mol) of *N*-methyl-D-glucamine [1-deoxy-1-(methylamino)-D-glucitol] in 150 ml of methanol was prepared in a 1-litre side-arm flask by refluxing for 30 min. Octanoic, nonanoic or decanoic acid (0.1 mol) was added to a 250 ml round-bottom flask on ice containing 10 g of pyridine in 100 ml of diethyl ether. After 10 min, ethyl chloroformate was added rapidly in slight molar excess (0.12 mol). The mixture was then left for 10 min before filtration into the 1-litre side-arm flask, equipped with a sintered-glass funnel, containing the methanolic glucamine solution at 50°C. The round-bottom flask was rinsed with an additional 50 ml of ice-cold diethyl ether, which was subsequently used to wash the filter cake. The mixture was allowed to stand for 45 min at 25°C and then overnight at 4°C. After filtration to remove recrystallized unchanged *N*-methyl-D-glucamine, the solvents were removed by rotary evaporation under reduced pressure. The oily residue (approx. 50 ml) was poured into 300 ml of diethyl ether with stirring on ice. The solid product was collected on a Buchner funnel and washed with 100 ml of ice cold diethyl ether 1–3 h later. The product, *N*-D-gluco-*N*-methylalkanamide, was obtained in 80–90% yield and was purified by two recrystallizations from methanol/diethyl ether (1:9, v/v; 10 ml/g). The following compounds were synthesized: *N*-(D-gluco-2,3,4,5,6-pentahydroxyhexyl)-*N*-methyloctanamide (octanoyl-*N*-methylglucamide; OMEGA); *N*-(D-gluco-2,3,4,5,6-pentahydroxyhexyl)-*N*-methylnonanamide (nonanoyl-*N*-methylglucamide; MEGA-9); *N*-(D-gluco-2,3,4,5,6-pentahydroxyhexyl)-*N*-methyl-decanamide (decanoyl-*N*-methylglucamide; MEGA-10).

Solubilization and reconstitution

Plasma membranes were prepared from Epstein-Barr-virus-transformed B lymphoblastoid cells exactly as described by Crumpton & Snary (1974). Purified membranes were solubilized in 50 mM-Tris/HCl, pH 8.0, containing 0.14 M-NaCl, 5 mM-iodoacetamide, 1 mM-phenylmethanesulphonyl fluoride and 0.5% (w/v) detergent at a detergent/protein ratio of 5:1 (w/w). After 30 min on ice the mixture was centrifuged at 100 000 *g* for 45 min at 4°C. The supernatant was then tested for inhibition of monoclonal antibody binding as described below or

reconstituted by overnight dialysis against 10 mM-Tris/HCl, pH 8.0, containing 0.14 M-NaCl and 5 mM-CaCl₂. Reconstituted membranes were pelleted by centrifugation at 100 000 *g* for 45 min and resuspended in phosphate-buffered saline.

Inhibition of monoclonal antibody binding

Reconstituted membranes (0.5 mg of protein/ml) and detergent extracts (5 mg/ml) were diluted serially with phosphate-buffered saline containing 0.5% bovine serum albumin or the corresponding 0.5% detergent buffer respectively. Diluted membranes or extract (50 μ l) were mixed with 50 μ l of phosphate-buffered saline/0.5% bovine serum albumin containing 50 ng of monoclonal anti-HLA-A, B, C antibody (PA 2.6; Brodsky *et al.*, 1979) that had been iodinated as described by Hunter & Greenwood (1962). After 45 min at 4°C, the mixture was centrifuged at 1500 *g* for 10 min and the residual antibody activity in 50 μ l was measured in a trace binding assay against 2×10^5 glutaraldehyde-fixed B lymphoblastoid cells (Jensenius & Williams, 1974).

Results and discussion

N-D-Gluco-*N*-methylalkanamide compounds (Fig. 1) were synthesized by using a mixed anhydride reaction. The entire synthesis could easily be completed in 1–2 days with yields ranging from 80–90% for derivatives of octanoic acid (OMEGA), nonanoic acid (MEGA-9) and decanoic (MEGA-10). The product as isolated after one recrystallization was adequate for biochemical applications. The absorption spectrum of the *N*-D-gluco-*N*-methylalkanamides consists of one major peak at 220 nm due to the secondary amide function. These detergents did not interfere with the monitoring of proteins at 280 nm.

The *N*-D-gluco-*N*-methylalkanamides were compatible with all buffers tested and their solubility was not affected by the presence of heavy-metal salts. The secondary amide function in the detergents ensures that they are non-ionic over a wide pH range. Ion-exchange chromatography of membrane glycoproteins was not affected by the presence of *N*-D-gluco-*N*-methylalkanamides (J. E. K. Hildreth, unpublished work).

Fig. 1. The chemical structure of *N*-D-gluco-*N*-methylalkanamide detergents, where $n = 5, 6$ and 7 for the octanoic, nonanoic and decanoic acid derivatives respectively

Fig. 2. Inhibition of anti-HLA-A,B,C antibody binding by detergent extracts of plasma membranes

Membranes were solubilized with 0.5% detergent solutions and after pelleting insoluble material, the supernatants were used to inhibit iodinated monoclonal antibody binding. See the Experimental section for further details. Detergents used were OMEGA (○), MEGA-9 (▲), MEGA-10 (△), octyl glucoside (●), Nonidet P40 (□) and sodium cholate (■).

The capacity of OMEGA, MEGA-9, and MEGA-10 detergents for solubilizing plasma membranes was compared with commonly used detergents. As shown in Fig. 2, MEGA-9 and MEGA-10 were as effective in releasing antigenically active membrane proteins as octyl glucoside, sodium cholate and Nonidet P40. The lower homologue OMEGA was much less effective, probably due to its shorter alkyl chain. Concentrations of the *N*-D-glucosyl-*N*-methylalkanamides as high as 20% (w/v) could be used without denaturing membrane proteins, as determined by their antigenicity. In other studies in which detergent extracts were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Laemmli, 1970) MEGA-10 proved more effective in releasing membrane proteins than any other non-ionic detergent tested, including Nonidet

Fig. 3. Inhibition of anti-HLA-A,B,C antibody binding by reconstituted membranes

Membranes were reconstituted by overnight dialysis after solubilization with MEGA-9 (□), MEGA-10 (○) or octyl glucoside (●) and compared with an equal amount of control membranes (■) for inhibition of monoclonal antibody binding. See the Experimental section for further details.

P40, Triton X-100 and Brij-96 (J. E. K. Hildreth, unpublished work).

Membranes solubilized with the *N*-D-glucosyl-*N*-methylalkanamide detergents could be reconstituted by overnight dialysis. As shown in Fig. 3, MEGA-9, MEGA-10 and octyl glucoside were sufficiently removed by this procedure to restore virtually all antigenic activity to the reconstituted membrane pellet. There was no measurable activity present in any of the supernatants. Similar results were obtained by using monoclonal antibodies against other cell-surface antigens. Although the CMC of MEGA-9 and MEGA-10 could not be measured, these results suggest that it is similar to that of octyl glucoside.

In conclusion, we have shown that *N*-D-glucosyl-*N*-methylalkanamide detergents compare favourably in membrane studies with other non-ionic detergents, including octyl glucoside. These detergents, which are easily synthesized at low cost, incorporate ideal properties previously only found in the very expensive *n*-alkyl glucoside compounds. The non-denaturing properties, ease of removal by dialysis and well-defined chemical composition of this new class of non-ionic detergents make them attractive reagents for membrane research.

I thank Professor R. R. Porter and Dr. Andrew McMichael for the use of laboratory facilities. This work was supported by a Medical Research Council Grant to Dr. McMichael. I am also grateful to the Rhodes Trust for support.

References

- Baron, C. & Thompson, T. E. (1975) *Biochim. Biophys. Acta* **382**, 276–285
- Brodsky, F. M., Parham, P., Barnstaple, C. J., Crumpton, M. J. & Bodmer, W. F. (1979) *Immunol. Rev.* **47**, 3–61
- Crumpton, M. J. & Snary, D. (1974) *Contemp. Top. Mol. Immunol.* **3**, 27–56
- Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79
- Helenius, A., McCaslin, D. R., Fries, E. & Tanford, C. (1979) *Methods Enzymol.* **56**, 734–749
- Hjemeland, L. M., Nerbert, D. W. & Chrambach, A. (1979) *Anal. Biochem.* **95**, 201–208
- Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
- Jensenius, J. C. & Williams, A. F. (1974) *Eur. J. Immunol.* **4**, 91–97
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lin, J. T., Riedel, S. & Kinne, R. (1979) *Biochim. Biophys. Acta* **557**, 179–187
- Rosevear, P., VanAken, T., Baxter, J. & Ferguson-Miller, S. (1980) *Biochemistry* **19**, 4108–4115
- Stubbs, G. W., Smith, H. G., Jr. & Litman, B. J. (1976) *Biochim. Biophys. Acta* **425**, 46–56