Synthesis of ^{14}C-labeled alkyl polyoxyethylene detergents

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Summary Procedures for the synthesis and purification of two ^{14}C-labeled alkyl polyoxyethylene detergent preparations are described. One of the preparations consisted mainly of [^{14}C]dodecyloctaoxyethylene ether, the other consisted mainly of [^{14}C] octylhexaoxyethylene ether, with critical micellar concentrations of approximately 50 μM and 10 mM, respectively. Condensation of alkyl bromides with fractionated polyethyleneglycol resulted in less polydisperse products than conventional ethylene oxide condensation. Highly purified detergent species could easily be obtained from the preparations by thin-layer chromatography. Their use as tracers greatly simplifies determination of detergent concentration during protein solubilization and fractionation.—**Fredrikson, G., L. Krabisch, and P. Belfrage.** Synthesis of ^{14}C-labeled alkyl polyoxyethylene detergents. *J. Lipid Res.* 1982. **23:** 1246–1248.

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Non-ionic detergents have become invaluable tools for solubilization and fractionation of labile amphiphilic proteins (1, 2). Triton X-100 has been commonly used, but the UV absorbance due to its aromatic group is a draw-back, e.g., when continuous monitoring of protein concentration is desired during column fractionations.

This problem is avoided with alkyl polyoxyethylene ether detergents. Several detergents of this type have been used in our laboratory in work with the lipolytic enzymes in adipose tissue (3–6). Determination of these detergents, however, is difficult, unless labeled detergents are used. In this note we describe the synthesis and purification of two ^{14}C-labeled alkyl polyoxyethylene detergents, one with a low and one with a high critical micellar concentration, and describe some of their properties. The method used for the synthesis, coupling of the alkyl chain to previously fractionated polyethylene glycol, facilitates the further purification of the detergents to homogeneous species. They can then be used also for characterization of membrane proteins in detergent solution, when homogeneous detergents are required, e.g., for studies of detergent binding.

MATERIALS AND METHODS

Labeled detergents

The procedure for the synthesis of these detergents is a modification of the method used in a report by Corkill, Goodman, and Ottewill (7). [^{14}C]$C_{12}E_8$ was synthesized as follows. [1-^{14}C]Lauric acid (The Radiochemical Center, Amersham, Great Britain) (1 mmol, 1.5 mCi) was reduced with 100 mg of $LiAlH_4$ in 20 ml of dry diethyl ether and boiled with reflux for 2 hr. The resultant [^{14}C]dodecanol (approx. 1 mmol) was dried and 0.3 ml of 48% HBr and 60 μl of H_2SO_4 were added and the mixture was boiled with reflux for 5 hr. The [^{14}C]dodecanoyl bromide was extracted with diethyl

Abbreviations: alkyl polyoxyethylene ethers with the general formula C_nH_{2n+1} $(OCH_2CH_2)_xOH$ are abbreviated C_nE_x.

ether and washed with 5% $NaHCO_3$ (w/v). Polyethyleneglycol (400, Merck, West Germany) was distilled and the fractions containing mainly octaethyleneglycol (1.2 mm Hg, approx. 300°C) were collected. The polyethyleneglycol fractions were analyzed by thin-layer chromatography as the monooleoyl esters in ethyl acetate–acetic acid–water 140:32:30 (v/v/v). (The esters were prepared as follows: 0.1 mmol polyethyleneglycol was dissolved in 1.5 ml of chloroform. Oleoylchloride (0.15 mmol) was added and the mixture was left at room temperature for 15 min, after which 0.5 ml of methanol was added for another 15 min to esterify any remaining oleoylchloride.) Two ml of distilled polyethylenglycol was dissolved in 5 ml of dioxane, 160 mg of sodium was added and the mixture was boiled with reflux overnight. The [^{14}C]dodecanoylbromide (dissolved in 5 ml of dioxane) was then added and the reaction was allowed to proceed for 4 hr under the same conditions. The molar excess of polyethylene glycol was approx. 12 times. The reaction mixture was added to 10 ml of 10% ethanol (v/v), washed four times with a total of 12 ml of light petroleum, extracted into 10 ml of chloroform, which was further washed with water, and dried with anhydrous Na_2SO_4. (A small amount of diether, less than 5% of the ^{14}C-label, was formed, but it was removed by the light petroleum washes.)

The detergent preparation obtained was subjected to preparative thin-layer chromatography on silicic acid (Kieselgel 60G, Merck, West Germany), 0.5 mm thick, in ethyl acetate–acetic acid–water 140:18:16 (v/v/v). To each plate (200 × 200 mm) 75 mg of detergent was added. The bands corresponding to C_{12E7}, C_{12E8}, and C_{12E9} (unlabeled homogeneous C_{12E5} and C_{12E8} (see below) were used as references) were scraped off and the detergent was eluted with 90% aqueous methanol (v/v). After dilution with water to 30% methanol(v/v), the detergent was extracted twice into a half volume of chloroform, which was then dried with anhydrous Na_2SO_4. The yield of labeled detergent was 140 mg with a specific activity of 2.7 μCi/mg. To obtain highly purified [^{14}C]$C_{12}E_8$, the preparative thin-layer chromatography was repeated and only $C_{12}E_8$ was collected.

[^{14}C]C_8E_6 was synthesized essentially in the same way: [1-^{14}C]octanoic acid (Radiochemical Center, Amersham, Great Britain) (4.6 mmol, 1 mCi) was reduced and the [^{14}C]octylbromide was prepared as above. Polyethylene glycol (300, Merck, West Germany) was distilled and fractions containing mainly hexaethyleneglycol (1.2 mm Hg, 210°C) were collected. After condensation and extraction as above the detergent was further purified on charcoal to remove some colored impurities, but it was not subjected to preparative thin-layer chromatography inasmuch as the initial product was less heterogeneous than the [^{14}C]$C_{12}E_8$ preparation. Two hundred fifty mg of labeled detergent was obtained with a specific radioactivity of 0.5 μCi/mg. Both labeled detergents were stored in chloroform under N_2 at −20°C.

Unlabeled detergents

Homogeneous $C_{12}E_5$ and $C_{12}E_8$ were obtained from Nikko Chemicals, Tokyo, Japan. $C_{13}E_{12}$ (Berol 058, Berol Kemi, Stenungsund, Sweden) was heterogeneous regarding the carbon chain (54% C12, 44% C14) and the polyethyleneglycol moiety. C_8E_6 was synthesized essentially as described for [^{14}C]C_8E_6, with the following modifications: octylbromide (Merck, West Germany) was used as one of the reactants and the reaction product was further purified by fractional distillation (0.25 mm Hg, 225–250°C). The final product consisted of C_8E_6 (approx. 80%) and C_8E_5 only.

Other methods

Detergent fractions were analyzed by thin-layer chromatography in ethyl acetate–acetic acid–water (140:18:16 (v/v/v). $C_{12}E_5$ and $C_{12}E_8$ were used as references (variation of the alkyl carbon chain length between C8 and C12 did not appreciably effect the R_f). The distribution of labeled detergent was determined by liquid scintillation after visualization in iodine vapor, scraping off the spots, addition of 1 ml of 50% methanol (v/v) and 10 ml Instagel (Packard Instrument Co., U.S.A.)–toluene 1:1 (v/v). Critical micellar concentration of the labeled detergents was determined by equilibrium dialysis. Sample, 1 ml, containing 0.5 mM [^{14}C]$C_{12}E_8$ (twice purified by thin-layer chromatography, cf. Fig. 1b) or 30 mM [^{14}C]C_8E_6, was dialyzed against 1 ml of water across a Spectrapor 3 membrane (Spectrum Medical Industries, U.S.A., mol wt cut off approx. 3500) at 25°C ± 1°C. Aliquots (10 μl) of the water were taken at intervals of approx. 2 hr, (10 μl was withdrawn from the sample at the same intervals) and counted by liquid scintillation in 10 ml of Instagel–toluene 1:1 (v/v). The concentration of detergent was plotted against time, resulting in a rapid and a slow phase of equilibration which approximated straight lines. The critical micellar concentration was obtained as the detergent concentration at the point of intersection between these two lines.[1] Gel chromatography was performed on Sephacryl S-200 (Pharmacia Fine chemicals, Sweden) with human immunoglobulin G (Kabi, Sweden), bovine albumin, ovalbumin, and myoglobin (Sigma, U.S.A.) as molecular weight references. The column was preequilibrated in a solution of the corresponding unlabeled detergent (0.1% $C_{12}E_8$ (w/v), 0.2% $C_{13}E_{12}$ (w/v), or 0.8% C_8E_6 (w/v), respectively).

[1] Lindström, M., and G. Fredrikson. Unpublished results.

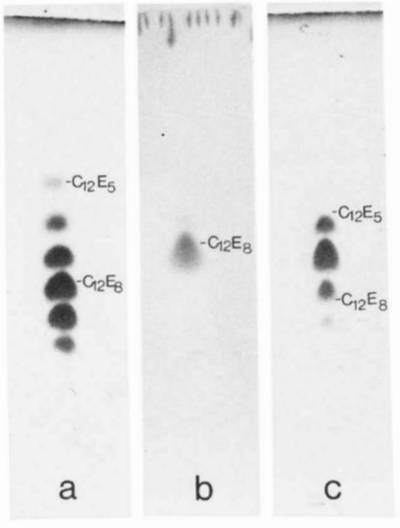

Fig. 1. Thin-layer chromatograms of the labeled detergents. The thin-layer chromatography was performed on silicic acid plates developed in ethyl acetate–acetic acid–water 140:18:16 (v/v/v) with $C_{12}E_5$ and $C_{12}E_8$ as references ($C_{12}E_5$ and $C_{12}E_8$ in the figure indicate their respective mobilities). The spots were visualized by acid charring. a, [^{14}C]$C_{12}E_8$ after first thin-layer chromatography. The distribution of the labeled detergent (see Methods) was 35% $C_{12}E_8$, 20% $C_{12}E_7$, and 25% $C_{12}E_9$; b, [^{14}C]$C_{12}E_8$ after second thin-layer chromatography, >85% $C_{12}E_8$; c, [^{14}C]C_8E_6-label distribution, 54% C_8E_6, 18% C_8E_5, 17% C_8E_7.

RESULTS AND DISCUSSION

The somewhat heterogeneous [^{14}C]$C_{12}E_8$ and [^{14}C]C_8E_6 preparations (**Fig. 1a, c**) could be used directly as radioactive tracers in the monitoring of detergent concentration during solubilization and fractionation of amphiphilic proteins. The addition of minute amounts of these labeled preparations should not significantly alter the properties of similar, unlabeled detergents such as $C_{13}E_{12}$, $C_{12}E_8$, or C_8E_6. However, in studies of the binding of detergent by protein, the use of a homogeneous detergent is required. Further purification of the labeled detergent could easily be achieved by repeated thin-layer chromatography (Fig. 1b) but at the expense of the yield. In initial studies, ^{14}C-labeled detergent was prepared by conventional ethylene oxide condensation. The labeled detergent so obtained, was, however, considerably more heterogeneous and could not be used for preparation of homogeneous detergent species at any reasonable yield.[2]

The critical micellar concentration of the labeled detergents could only be determined by equilibrium dialysis because of the limited amounts available. The values obtained, 53 μM for [^{14}C]$C_{12}E_8$ and 10 mM for [^{14}C]C_8E_6 at 25° ± 1°C were of the same magnitude as those reported for homogeneous $C_{12}E_8$, 71 μM (8) and C_8E_6, 9.9 mM (7); the differences were possibly due to the fact that surface tension measurements were used to determine critical micellar concentration in these reports.

The ^{14}C-labeled detergents were used as tracers to determine average micellar molecular weight of the unlabeled alkyl polyoxyethylene detergents $C_{12}E_8$ (homogeneous), $C_{13}E_{12}$, and C_8E_6 (see above) by gel chromatography. Values of 57,000 ($C_{12}E_8$), 98,000 ($C_{13}E_{12}$), and 17,000 (C_8E_6) were found as compared with 65,000 ($C_{12}E_8$) and 13,000 (homogeneous C_8E_6) reported by others (7, 9). The slightly higher value found for C_8E_6 may be due to the limitations of gel chromatography as a method for determination of micellar size.

The ^{14}C-labeled detergents described in this note have proved quite useful in the work with the hormone-sensitive lipase of adipose tissue in this laboratory over a number of years (3–6). They should be equally useful in similar fractionation work with other amphiphilic proteins.

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