Synthesis of 14C-labeled alkyl polyoxyethylene detergents

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Summary Procedures for the synthesis and purification of two 14C-labeled alkyl polyoxyethylene detergent preparations are described. One of the preparations consisted mainly of [14C]dodecyloctaoxyethylene ether, the other consisted mainly of [14C]octylhexaoxyethylene ether, with critical micellar concentrations of approximately 50 pM and 10 mM, respectively. Condensation of alkyl bromides with fractionated polyethylene glycol 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 14C-labeled alkyl polyoxyethylene detergents. J. Lipid Res. 1982. 23: 1246-1248.

MATERIALS AND METHODS

Labeled detergents

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 drawback, 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 14C-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.

Abbreviations: alkyl polyoxyethylene ethers with the general formula CnH2n+1 (OCH2CH2)kOH are abbreviated CnE k.
ether and washed with 5% NaHCO₃ (w/v). Polyethylene glycol (400, Merck, West Germany) was distilled and the fractions containing mainly octaethylene glycol (1.2 mm Hg, approx. 300°C) were collected. The polyethylene glycol 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 polyethylene glycol was dissolved in 1.5 ml of chloroform. Oleoyl chloride (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 oleoyl chloride.) Two ml of distilled polyethylene glycol was dissolved in 5 ml of dioxane, 160 mg of sodium was added and the mixture was boiled with reflux overnight. The [14C]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% methanol (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 Na₂SO₄. (A small amount of diether, less than 5% of the 14C-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 C₁₂E₇, C₁₂E₈, and C₁₂₉ (unlabeled homogeneous C₁₂E₅ and C₁₂E₈ 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 Na₂SO₄. The yield of labeled detergent was 140 mg with a specific activity of 2.7 μCi/mg. To obtain highly purified [14C]C₁₂E₈, the preparative thin-layer chromatography was repeated and only C₁₂E₈ was collected.

[14C]C₈E₆ was synthesized essentially in the same way: [1,14C]octanoic acid (Radiochemical Center, Amersham, Great Britain) (4.6 mmol, 1 mCi) was reduced and the [14C]octylbromide was prepared as above. Polyethylene glycol (300, Merck, West Germany) was distilled and fractions containing mainly hexaethylene glycol (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 [14C]C₁₂E₈ preparation. Two hundred fifty mg of labeled detergent was obtained with a specific radioactivity of 0.9 μCi/mg. Both labeled detergents were stored in chloroform under N₂ at −20°C.

Unlabeled detergents

Homogeneous C₁₂E₅ and C₁₂E₈ were obtained from Nikko Chemicals, Tokyo, Japan. C₁₂E₁₂ (Berol 058, Berol Kemi, Stenungsund, Sweden) was heterogeneous regarding the carbon chain (54% C₁₂, 44% C₁₄) and the polyethylene glycol moiety. C₈E₆ was synthesized essentially as described for [14C]C₈E₆, 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 C₈E₆ (approx. 80%) and C₈E₅ only.

Other methods

Detergent fractions were analyzed by thin-layer chromatography in ethyl acetate-acetic acid-water (140:18:16 (v/v/v)). C₁₂E₅ and C₁₂E₈ were used as references (variation of the alkyl carbon chain length between C₈ and C₁₂ did not appreciably effect the Rf). 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 [14C]C₁₂E₈ (twice purified by thin-layer chromatography, cf. Fig. 1b) or 30 mM [14C]C₈E₆, was dialyzed against 1 ml of water across a SpectraPore 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. 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% C₁₂E₅ (w/v), 0.2% C₁₂E₁₂ (w/v), or 0.8% C₈E₆ (w/v), respectively).

RESULTS AND DISCUSSION

The somewhat heterogeneous \(^{14}\text{C}\)C\(_{12}\)E\(_8\) and \(^{14}\text{C}\)C\(_{6}\)E\(_8\) 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\(_{12}\)E\(_8\), C\(_{13}\)E\(_8\), or C\(_{8}\)E\(_6\). 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, \(^{14}\text{C}\)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.\(^8\)

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 \(\mu\)M for \(^{14}\text{C}\)C\(_{12}\)E\(_8\) and 10 mM for \(^{14}\text{C}\)C\(_{6}\)E\(_8\) at 25\(^\circ\)C were of the same magnitude as those reported for homogeneous C\(_{12}\)E\(_8\), 71 \(\mu\)M (8) and C\(_{8}\)E\(_6\), 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 \(^{14}\text{C}\)-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\(_{8}\)E\(_6\) (see above) by gel chromatography. Values of 57,000 (C\(_{12}\)E\(_8\)), 98,000 (C\(_{13}\)E\(_12\)), and 17,000 (C\(_{8}\)E\(_6\)) were found as compared with 65,000 (C\(_{12}\)E\(_8\)) and 13,000 (homogeneous C\(_{6}\)E\(_4\)) reported by others (7, 9). The slightly higher value found for C\(_{8}\)E\(_6\) may be due to the limitations of gel chromatography as a method for determination of micellar size.

The \(^{14}\text{C}\)-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.\(^9\)

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