Chemo-Enzymatic Synthesis of Amino Acid-Based Surfactants

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Abstract: The application of lipases to the synthesis of amino acid-based surfactants was investigated. Low yields (2–9%) were obtained in the acylation of free amino acids, such as L-serine and L-lysine, as well as their ethyl esters and amides with fatty acids, owing in part to low miscibility of the reactants. When the N-carbobenzyloxy (Cbz)-L-amino acids were used in an effort to improve miscibility of the amino acid derivatives with the acyl donor, a dramatic improvement was observed for N-Cbz-L-serine (92% yield) but not for Nα-Cbz- or Nζ-Cbz-L-lysine (7 and 2% yield, respectively). As an alternative, an efficient synthesis of Nζ-acyl-L-lysines was developed, based on the regiospecific chemical acylation of copper(II) lysinate. In pursuit of a general route to amino acid–fatty acid surfactants, the utility of a polyol linker was investigated. Thus, the glycerol ester of Nα,Nζ-di-Cbz-L-lysine was prepared and evaluated as a substrate for acylation. As expected, this and other glycer-1-y1 esters of N-protected amino acids were excellent substrates for lipase-catalyzed acylation. Their reaction with myristic acid in the presence of Novozyme resulted in the regioselective acylation of the primary hydroxyl group of the glycerol moiety to afford the corresponding 1-O-(N-Cbz-L-aminoacyl)-3-O-myristoylglycerol esters of amino acids should enable manufacturers to tailor the functional properties of these surfactants to suit particular applications.

The wide range of functionalities displayed by amino acids should be a great asset where any “fine-tuning” of the surfactant’s performance is concerned. However, this characteristic is also a potential drawback from a synthetic viewpoint owing to the requirement for temporary protection of any reactive functional groups. It is somewhat surprising therefore to find that relatively few attempts to circumvent some of the synthetic difficulties, by utilizing the selectivity of enzymes, have been reported (15–17). In this communication, we report the results of our initial investigations, aimed at the development of a facile chemo-enzymatic approach to the preparation of amino acid-based surfactants. In particular, the usefulness of lipases as catalysts for acylation of glycerol esters of amino acid was investigated in some detail.

Experimental Procedures

Materials. N-Carbobenzyloxy (Cbz)-glycine, N-Cbz-L-tyrosine, N-Cbz-L-phenylalanine, N-Cbz-L-aspartic acid, N-Cbz-L-serine, and Nα,Nζ-di-Cbz-L-lysine were obtained from Sigma Chemical Company (Poole, Dorset, England). Novozyme (im-
mobilized *Candida antarctica* lipase) and Lipozyme (immobilized *Mucor miehei* lipase) were purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). L-Lysine, capryloyl chloride, caproyl chloride, lauroyl chloride, myristoyl chloride, palmitoyl chloride, stearoyl chloride, myristic acid, glycerol, formic acid, palladium on alumina catalyst (10% w/w), boron trifluoride etherate, 2,2-dimethyl-1,3-dioxolane-4-methanol (solvent), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid, d$_6$-dimethylsulfoxide (d$_6$-DMSO), EDTA disodium salt, and basic alumina (Brockmann grade 1) were acquired from Aldrich Chemical Company (Poole, Dorset, England). All solvents employed were of the highest available purity and were dried over 3A molecular sieves before use.

**Analytical methods.** Reactions were followed by reverse-phase high-performance liquid chromatography (HPLC), with a Gilson 305/306 system (Anachem Luton, United Kingdom), connected with a Gilson 231 Autosampler, an Applied Chromatography Systems (ACS; Cheshire, United Kingdom) 750/12 ultraviolet detector or an ACS 750/14 light-scattering detector, and a Hewlett-Packard Chemstation 35900 (Winnersh, United Kingdom) for data acquisition and processing. Analyses were carried out on a 0.46 × 15 cm Hichrom RP8/5-µm column (Reading, United Kingdom), maintained at 45°C and operated at a flow rate of 1 mL · min$^{-1}$. Methanol and 4:1 water/methanol, both containing 0.05% vol/vol phosphoric acid, were employed as mobile phases. Fast atom bombardment mass spectroscopy (FAB-MS) was carried out on a Kratos MS9/50T C spectrometer (Manchester, United Kingdom) with xenon at 5–7 kV and a source potential of 5.33 kV. Glycerol was used as the matrix, and spectra were recorded at 0.1 mm in positive or negative ionization mode, with polyglycerol ions as reference. Optical rotations were measured on a Thor NPL-type 243 polarimeter (Nottingham, United Kingdom) at 589.3 nm, 25°C, in a 0.5-cm cell, with methanol or 1:1 methanol/trifluoroacetic acid as the solvent. Melting points were determined with a Stuart Scientific SMP-1 apparatus (Red Hill, United Kingdom) using sealed capillaries and are uncorrected. All compounds were also fully characterized by $^1$H, $^{13}$C, DEPT, $^1$H–$^1$H correlation spectroscopy (COSY), and $^1$H–$^{13}$C COSY nuclear magnetic resonance (NMR) experiments. These spectra were recorded on a Jeol JNM EX 270 Fourier transform spectrometer (Tokyo, Japan) at 35°C, with d$_6$-DMSO as solvent. Proton spectra were recorded at 270.0 MHz and carbon-13 spectra at 67.8 MHz with broad-band proton decoupling, both by using a DMSO field lock. $^1$H–$^1$H COSY spectra were recorded in symmetrized mode.

**General procedure for acylation of free amino acids and their derivatives.** The acylation of L-serine, L-lysine, and their amide and methyl/ethyl ester derivatives was carried out as follows. The amino acid substrate (1 mmol) was dispersed in fatty acid or fatty acid methyl ester (2 or 4 mmol) at 50, 60, or 70°C, with or without the addition of solvent (5 or 10 mL where employed). Immobilized lipase (100–500 mg) was then added, and the mixture was stirred vigorously at 50, 60 or 70°C by means of a heating mantle/stirrer. Samples for HPLC analysis were withdrawn at the appropriate time intervals, quenched with methanol containing 5% vol/vol acetic acid, and analyzed by HPLC.

**Preparation of N$_3$-acyl-L-lysines.** Aqueous bis(L-lysino)coppper(II) complex was prepared by adding aqueous potassium L-lysinate (30 mL of 3.3 M solution, 100 mmol) to ice-cold aqueous copper(II) sulfate (40 mL of 1.3 M solution, 52 mmol) with vigorous stirring and cooling in an ice bath. Solid potassium bicarbonate (15 g, 150 mmol) was then added with stirring, followed by dropwise addition of the acid chloride (125 mmol in 10 mL of acetone) over a period of 1.5–2 h. The mixture was left stirring in the ice bath for a further 2–3 h, then allowed to warm to room temperature; stirring was continued for another 3–5 h, to give a viscous solution or suspension of the bis(N$_3$-acyl-L-lysinate)coppper(II) complex. The mixture was then heated to 50°C, an aqueous suspension of EDTA disodium salt (500 mL of 0.25 M, 125 mmol) was added with vigorous stirring, and the pH of the mixture was adjusted to between 4.0 and 6.0 with 2.0 M aqueous hydrochloric acid. The precipitate formed was filtered off and washed successively with 2 × 50 mL water, 2 × 50 mL of 1:1 water/ethanol, 1 × 50 mL ethanol, 1 × 50 mL of 1:1 ethanol/ether, and finally 4 × 50 mL ether, to give the required N$_3$-acyl-L-lysine as a white solid (51–64% yield). The analytical data are presented below for a representative member of this class of compounds, N$_3$-myristoyl-L-lysine (1):

N$_3$-Myristoyl-L-lysine (1). M.p. > 230°C (decomp.); [α]$_{D,25}^{20}$ +10.07° (c 1.38, 1:1 methanol/trifluoroacetic acid); FAB-MS: (M + H) calcd. for C$_{20}$H$_{41}$N$_3$O$_3$ 357.3117, (M + H) obsvd. 357.3070.

**Preparation of 1-O-(N-Cbz-L-aminoacylglycerols.** 1-O-(N-Cbz-glycyl), 1-O-(N-Cbz-L-phenylalanyl), 1-O-(N-Cbz-L-tyrosyl), 1-O-(N-Cbz-L-seryl), and 1-O-(N$_3$N$_3$-di-Cbz-L-lysyl)glycerol were synthesized from the corresponding N-Cbz-L-amino acids by esterification with glycerol and boron trifluoroetherate as catalyst (18). N-Cbz-L-amino acid (50 mmol) was dissolved in a mixture of dimethylformamide (50 mL) and glycerol (500 mL). The solution was heated to 60°C, boron trifluoroetherate (25 mL) was added over a period of 1 h to the stirred mixture, and the reaction was allowed to proceed for 20 h. Brine (300 mL) and 0.6 M sodium bicarbonate (400 mL) was then added, and the reaction mixture was extracted with 3 × 500-mL portions of ethyl acetate. The organic layers were pooled and washed successively with 4 × 50 mL of aqueous 0.1 M sodium bicarbonate containing 0.2 M sodium chloride, 4 × 50 mL of aqueous 0.1 M citric acid containing 0.2 M sodium chloride, and finally with 4 × 50 mL of 0.4 M sodium chloride. Drying over anhydrous magnesium sulfate, followed by rotary evaporation, gave the pure product in 80–90% yield.

1-O-(N-Cbz-glycyl)glycerol (2a). M.p. 68–70°C; [α]$_{D,25}^{20}$ 0° (c 1.10, methanol); FAB-MS: (M + H) calcd. for C$_{13}$H$_{17}$N$_3$O$_6$ 284.1137, (M + H) obsvd. 284.1104.

1-O-(N-Cbz-L-phenylalanyl)glycerol (3a). M.p. 43–44°C; [α]$_{D,25}^{20}$ +9.43° (c 1.96, methanol); FAB-MS: (M + H) calcd. for C$_{22}$H$_{24}$N$_3$O$_6$ 374.1606, (M + H) obsvd. 374.1588.

1-O-(N-Cbz-L-tyrosyl)glycerol (4a). M.p. 70–72°C; [α]$_{D,25}^{20}$ +5.00° (c 1.57, methanol); FAB-MS: (M + H) calcd. for C$_{20}$H$_{24}$N$_3$O$_7$ 390.1555, (M + H) obsvd. 390.1519.