ABSTRACT: Several lipoamino acids were synthesized, in which n-octadecanoic acid (stearic acid) was coupled with the α-amino group of an amino acid. The products were characterized and their identities confirmed by advanced analytical techniques like Fourier transform infrared, 1H nuclear magnetic resonance spectroscopy, and differential scanning calorimetry. Their surface properties, such as critical micelle concentration (CMC) and foaming properties, biodegradability, and antimicrobial activity were also evaluated. The N-stearoyl amino acids (NSA) had low CMC values, and some of them showed good foaming properties. They were screened for antimicrobial activity against the gram-positive bacteria Staphylococcus aureus, Micrococcus luteus, and Bacillus cereus, the gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa, and the yeast Candida albicans. All the compounds inhibited at least one of these organisms. N-Stearoyl proline was the most effective, the order of antimicrobial activity being aromatic NSA > acidic NSA > basic NSA. However, the effective inhibition by all the compounds indicates the desirability of more thorough investigation and suggests that some of these compounds may have potential utility as biostatic additives in commercial products. All NSA are highly biodegradable and can readily be removed under conditions of normal secondary sewage treatment.

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KEY WORDS: Amino acids, antimicrobial activity, biodegradability, critical micelle concentration, foaming property, lipoamino acids, surface activity.

Studies on lipoamino acids have been of great interest in the last few years because of their high degree of biocompatibility. Surfactants have a variety of applications in detergency, solubilization, emulsification, and preservation of biomaterials. Studies on surface-active properties of different types of surfactants derived from lauroyl and palmitoyl derivatives of glycine and alanine have been reported (1). Critical micelle concentration (CMC) and foaming ability can serve to characterize the physicochemical nature of any surfactant molecule. The evaluation of foaming properties of surfactants by the Ross–Miles method was reported in the literature in 1940 (2). This method involves the measurement of foam height for comparison of relative foam stability. The measured value depends on capillary penetration, time, temperature, solubility, and volume of the surfactant solutions. Thus, there is a need to develop a suitable method to measure the foaming properties of any surfactant molecules of any types, such as lipoamino acids, to keep pace with advances in the surfactant field. There is a correlation between X-ray diffraction (XRD) and differential scanning calorimetry (DSC) data for the polymorphic states of surfactants (3). Hence, an attempt has been made to measure the foaming properties of lipoamino acids by microcalorimetric techniques.

Studies of biological properties of surfactants include screening of antimicrobial efficacy and biodegradability. The quaternary ammonium compounds that are widely used as germicides (4) are strongly bacteriostatic at higher dilutions and have germicidal action in more concentrated solutions.

Earlier reports of N-palmitoylated amino acids tested for their inhibitory action against Sendai virus fusion to liposomes composed of egg phosphatidylethanolamine (5). Fatty acids of varying chain lengths are known for their antimicrobial action (6,7), primarily against gram-positive bacteria and yeast at low pH. Sheba et al. (8) studied the fungistatic action of oleic acid, which was found to be active against a wide spectrum of saprophytic molds and yeast. It has also been reported (9) in the literature that long-chain fatty acid derivatives of glycolic acid showed antimicrobial activity.

The biodegradability of surfactants has been the subject of numerous investigations in the past few years. The hydrophobic groups in all nonionic surfactants like dodecyltrimethylammonium glycol monoether can be biodegradable, except those with highly branched carbon chains. Matsumura et al. (10) studied the biodegradability of n-alkyl glucosides, mann osides, and galactosides and found that these surfactants were up to 50% biodegradable. In recent years, there has been worldwide interest in developing surface-active molecules that have both antimicrobial activity and environmental compatibility, or, in other words, that are easily biodegradable. Different kinds of surface-active molecules (11) have been synthesized, and some of them are being used in practical applications, mainly in cosmetic and food applications. Only a few studies of their properties have been reported in the literature.

N-Acyl amino acids based on a C_{18} chain have not yet been thoroughly studied with respect to their physicochemical and biological properties, in particular, their antimicrobial activity and biodegradability. In the present work, we synthesized N-octadecanoic acid-based lipoamino acids and investigated their interfacial, antimicrobial, and biodegradability properties.
EXPERIMENTAL PROCEDURES

Materials. All the amino acids used in this study (L-glutamic acid, L-aspartic acid, L-lysine, L-arginine, L-histidine, L-proline, L-phenylalanine, L-tryptophan, and L-tyrosine) were purchased from Hi Media Laboratories Pvt. Limited (Bombay, India). n-Octadecanoic acid (stearic acid) was purchased from Loba Chemie (Bombay, India), acetone from S.D. Fine Chemicals Ltd. (Bombay, India), ethyl alcohol from Bengal Chemicals (Calcutta, India), petroleum benzine from Ranbaxy Fine Chemicals Limited (New Delhi, India), and the deuterated solvent CDCl₃ from Aldrich Chemicals (Milwaukee, WI). Sodium hydroxide and sulfuric acid were obtained from Fischer Inorganic and Aromatics Ltd. (Madras, India). Double distilled water was used wherever necessary. All other chemicals used were analytical-grade reagents.

Synthesis of N-stearoyl amino acids (NSA). Synthesis of NSA involves a two-step process, i.e., preparation of the acid chlorides as reported elsewhere (12) followed by preparation of the corresponding NSA. A typical method is as follows.

To an acetone and water mixture (pH 12 with NaOH), 0.24 mol of L-amino acids and 0.2 mol of stearoyl chloride were added with stirring over 25 min at 0°C. After further stirring for 30 min, the mixture was acidified with sulfuric acid to obtain crystalline and semisolid N-stearoyl amino acid. Following washing with petroleum benzine, the crystals obtained were recrystallized from an ethanol/petroleum benzine mixture.

All the prepared NSA were characterized by Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (NMR) spectroscopy. FTIR spectra were recorded using a Shimadzu FTIR 8000 series Spectrometer (Kyoto, Japan); KBr pellets were used for solid samples, and a transmission cell with NaCl windows was used for liquid samples. NMR spectra were recorded using a Bruker 300-MHz FT-NMR spectrometer (Karlsruhe, Germany). Samples were dissolved in CDCl₃, and tetramethylsilane was used as an internal standard.

Determination of CMC. The CMC of all NSA were estimated by a conductivity method (Direct Conductivity Measurement Meter 303; cell constant = 1.0003; Systronic Electronics, Amendabad, India). NSA solutions (10 mM) were prepared in 0.1 N sodium hydroxide and added to a beaker containing 40 mL of double distilled water. The specific conductance was measured during gradual addition of the NSA solution. When differences in specific electrical conductivity were plotted against the concentration of NSA in the beakers, two straight lines were obtained and were extrapolated to obtain the concentration at their intersection, which was defined as the CMC. All the measurements were carried out in duplicate.

Determination of foaming properties by differential scanning calorimetry (DSC). All DSC measurements were performed on a DuPont 2000 Thermal Analyzer, equipped with a DSC cell (DuPont, Boston, MA). The peak areas were estimated by the DuPont Advanced DSC (V. 4.1.C) program. For analysis, 5–10 mg of the NSA was weighed into an aluminum DSC pan. The samples were analyzed under a nitrogen atmosphere at a program rate of 5°C/min up to 350°C. All samples were analyzed in duplicate. The peak integrations were performed automatically by the computer program.

Methodology for screening for antimicrobial activity. The NSA prepared in this study were screened for their antimicrobial activity against pathogenic organisms including gram-positive and gram-negative bacteria and a fungal strain. Six organisms (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus, Bacillus cereus, and Candida albicans) were used to study the ability of the test compounds (at concentrations of 10 and 20 mg/mL) to inhibit microbial growth. All the bacterial species were grown on nutrient agar (Hi Media Laboratory, Bombay, India); C. albicans was cultivated on Sabouraud dextrose media (Hi Media Laboratory). The cultures were incubated at 30°C. The following controls were employed: for E. coli, norfloxacine (5 µg); S. aureus, tetracycline (20 mg); P. aeruginosa, gentamycin (20 µg); M. luteus, erythromycin (20 µg), B. cereus, doxycycline (10 µg); and C. albicans, ketoconazole (500 µg). Twenty-four-hour slant cultures of the microorganism were used to prepare suspensions for plate inoculations. The suspensions served as the inoculum for the determination of antimicrobial activity. Agar plates were inoculated with the appropriate inoculum by placing 3 drops on the agar surface and spreading them uniformly with a sterile, bent glass rod. Filter paper discs 6.5 mm in diameter, made from Whatman No.1 filter paper, were used to evaluate the samples. The paper discs were wetted until they were completely saturated with the test compound (at concentrations of 10 and 20 mg/mL) and then placed on the surface of the agar plates inoculated with the test organisms. N,N-Dimethylformamide was used as carrier solvent. A minimum of two experiments was performed at different times, employing duplicate plates for each compound under test. All plates were incubated at the optimal growing temperature for each organism, and readings were taken after 24 h. The zones of inhibition were compared to those of the controls.

Evaluation of biodegradability. The 5-d biochemical oxygen demand (BOD) of NSA was determined by the standard oxygen consumption test (13) using activated sludge obtained from a sewage treatment plant at the Central Leather Research Institute (Adyar, India). All experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Synthesis of N-stearoyl amino acids. The NSA used in this study were prepared by conversion of fatty acids to their acid chlorides and further reaction with the L-amino acid under suitable conditions. The lipoamino acids were characterized by ¹H NMR, FTIR spectroscopy and C, H, N analysis. The characteristics of the NSA are given in Table 1.

FTIR spectroscopy. FTIR spectra of all the NSA were recorded. The band at 1460 cm⁻¹, assigned to the CH₂ scis-