Synthesis and Surface Properties of Amino Acid Surfactants from Industrial Waste Proteins

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N-Acyl amino acid surfactants (AAS) were chemically derived from industrial waste protein hydrolysates [cottonseed (CSD), silk residue (SR), and silk chrysalis (SC)] according to two methods: (1) reacting hydrolysates with alkyl acyl chloride, followed by purification and neutralization with alcoholic sodium hydroxide; (2) reacting hydrolysate with fatty alcohols in organic solvents followed by purification. The yield of purified mixed AAS (sodium salt) was ~60−75%; amino acid ester from glutamic acid was considerably higher (85−92%) than the sodium salt derivatives. Results indicate that as acyl chain length (i.e., C_{12}−C_{18}) increased, surface tension of AAS increased, critical micelle concentration (cmc) decreased, and Krafft point increased. The emulsifying power of AAS in O/W emulsion was better with *n*-decanol as an oil phase than liquid paraffin. The C_{12} derivatives of all the mixed AAS showed high foaming power. Mixed AAS from CSD exhibited the best lime soap dispersing requirement (5.7−6.5 g/100 g). The diameter of micelle increased for glutamic acid AAS (GA-AAS) and CSD-AAS as the acyl chain length increased (i.e, C_{12}−C_{18}). The hydrodynamic diameter of AAS followed the order SC > CSD > GA. Generally, AAS with C_{12} produced good surface properties.

Keywords: *Amino acid surfactants; synthesis; surface properties; industrial waste proteins*

INTRODUCTION

Pollution from industrial waste biomaterials is a major environmental problem. The problems associated with disposal of industrial waste biomaterials provide incentives to direct research effort toward identifying strategies for their recovery and use in production of value-added products. Conversion of certain industrial waste proteins into value-added products is plausible and should be explored. Such research effort would lead to effective conversion of cheap, renewable feedstock to new chemicals of high value. Because of the broad application of surfactants in many areas, our laboratory has been exploring the possibility of preparing protein-based surfactants (e.g., amino acid and peptide derivatives) from industrial waste proteins.

Amino acid surfactants (AAS) have been the subject of many studies, primarily on their applications as pharmaceuticals, biomedicals, cleaning agents, and antimicrobial agents (Xia et al., 1995). The potential use of AAS as food emulsifiers has been indicated (Nagao and Kito, 1989). At present, we are unaware of any food applications. Kabara et al. (1975) and Kabara and Haitsma (1975) reported the activity and effectiveness of acyl aminimide derivatives as antimicrobial agents. Their compounds were active against both bacteria and yeast, activity being a function of chain length (Kabara and Haitsma, 1975). The acyl derivatives tested showed maximum activity at chain lengths of C_{14} and C_{16} (Kabara et al., 1975). Recently, Molinero et al. (1988) prepared protein-based amphoteric surfactants by condensation of N^{α}-lauroylarginine with amino acids from a collagen hydrolysate. The N^{α}-lauroylarginine dipeptides had surfactant and antimicrobial properties. The Molinero research team has also prepared neutral N^{α}-lauroylarginine dipeptides from pure amino acids (Infante et al., 1989). The surfactants were reported to be very water-soluble with good surface properties and antimicrobial activity (Infante et al., 1989). Previously, we synthesized AAS of a general structure amino-(*N*-acyl)-β-alkoxypropionate (Xia et al., 1995). We found that increases in acyl chain length (i.e., from C_{10} to C_{14}) resulted in a linear reduction in surface tension as well as dramatic decreases in critical micelle concentrations (cmc). In addition, we noted that strong correlations existed between cmc values of AAS and their minimal inhibitory concentrations (MIC) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Saccharomyces cerevisiae* (Xia et al., 1995). The objective of the present work was to explore the preparation and surface properties of AAS derived from industrial waste proteins.

MATERIALS AND METHODS

Materials. *Waste Proteins.* All of the waste proteins were obtained from local factories in Jiangsu Province, China. Cottonseed proteins (CSD) were obtained from seed cakes or flakes after oil/fat and gossypols were removed by hexane−acetone solvent extraction in oil mill. The ground and dried crude CSD powders contained 50 ± 2% proteins. Silk protein (SR) was extracted from "waste silk residue" from Silk Processing Factory; extracts contained 90 ± 2% proteins. Silk chrysalis (SC), normally discarded during processing, were taken directly from the silk factory. The protein content of SC was ~50%.

Methods. *Summary of Synthesis of AAS.* Two approaches were used as follows: (a) to react protein hydrolysates with alkyl acyl chloride followed by purification and neutralization with alcoholic sodium hydroxide; (b) to react protein hydrolysates with fatty alcohols in organic solvents followed by purification. A flow chart of the procedure is presented in Figure 1.

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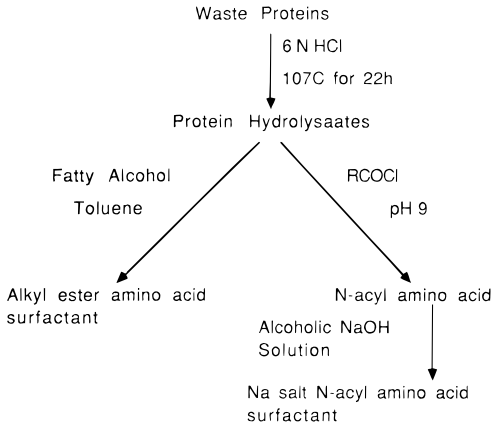

Figure 1. Flow chart of synthesis of AAS from waste proteins.

Preparation of Hydrolysates from Waste Proteins. Waste proteins (100 g) were hydrolyzed with 300 mL of 6 N HCl at 107 °C for 22 h. The hydrolysates were filtered and decolorized by activated carbon. A pale yellow filtrate was obtained. The amino acid composition of hydrolysates of waste proteins was determined by an amino acid analyzer.

Synthesis of Acyl Chloride. Fatty acids (C_{12}−C_{18}) (0.1 mol) were added into three-neck, round-bottom flasks. The fatty acids were melted by gentle heating, and then 4 mL of phosphorus trichloride (PCl_3) was added dropwise. The mixture was reacted at 70 °C for 1−2 h. The upper transparent solution contained the acyl chloride (ROCl).

Synthesis of Sodium Salt N-Alkyl Acyl Amino Acid. The waste protein hydrolysates were neutralized, concentrated, filtered, and adjusted to pH 8−10 before use. Fifty milliliters of concentrated hydrolysates was added to a round flask with funnel. Approximately 20−25 mL of acetone was added into the flask, followed by the addition of ROCl and NaOH solutions. The mixture (pH 9) was reacted at room temperature for 1 h. At this point, the mixture was viscous with a pale yellow color (colorless if pure glutamic acid was used). The pH of the reaction mixture was adjusted to ~pH 5−6 by adding 6 N HCl to allow precipitation of the product. The pale yellow solid precipitate was filtered, washed several times with petroleum ether, and then recrystallized (three times) with 90% ethyl alcohol to obtain a milk-white product, *N*-alkyl acyl amino acid.

To obtain the sodium salt of *N*-alkyl acyl amino acid, *N*-alkyl acyl amino acid (0.02 mol) was reacted with 10 mL of 2 N NaOH alcoholic solution at 60 °C for 1 h. The sodium salt derivative was distilled off and dried in the oven. A pale yellow powder was obtained.

Synthesis of Alkyl Ester of Amino Acids. Pure glutamic acid (0.1 mol) and 100 mL of toluene were added into a round flask with reflux condenser and a stirrer. After the glutamic acid was dissolved, H_2SO_4 (0.12 mol) and fatty alcohol (0.3 mol) were added and refluxed for 8 h. The toluene was distilled off and the residue purified using a solvent mixture of ether and methanol. The product (alkyl ester of glutamic acid), with a white-crystal appearance, was obtained.

If hydrolysates were used, 25 mL of the hydrolysates was reacted with 0.1 mol of fatty alcohol in toluene. In the case of hydrolysates, the final product was a pale yellow solid.

Yield and Analysis of Products. The purity and structure of representative samples were ascertained by TLC, IR, and NMR. The yields of the purified products are presented in Table 2. Structural representations of AAS, as identified and confirmed by IR and NMR, are shown in Figure 2.

Surface Tension (γ). Surface tension of AAS was determined according to the Wilhemy plate method (Hiemenz, 1977) using a Shimadzu ST-1 tensiometer in pre-equilibrated aqueous solutions (0.1%; 25 °C). In our testing, a standard glass plate with a 5 cm perimeter was utilized. The water used for preparing surfactant solution was first deionized and treated with alkaline permanganate and then distilled twice using a 50 cm condenser. The specific conductivity determined each day was about $1.1 \times 10^{-6}\ \Omega^{-1}\,cm^{-1}$. Ten minute intervals were allowed for equilibration between measurements. Critical micelle concentrations were obtained from the break-point of γ−log *C* curves (i.e., the minimum concentration of a surfactant solution at which the molecules of a surfactant are transformed from unassociated molecules to micelles). In general, maximum surface reduction occurs at the cmc. All analyses were performed in triplicate.

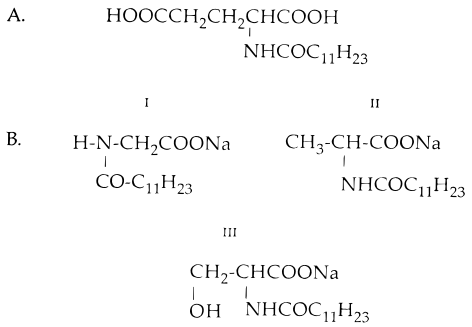

Figure 2. Structural representation of AAS based on IR and NMR spectra: (A) from C_{12} Na-GA spectra; (B) from C_{12} Na-SR spectra.

Krafft Point (°C). Krafft point was determined using a 1% solution of AAS. Krafft point is the temperature at which there is a sudden change from turbidity to transparency during gentle heating of a 1% solution of ionic surfactant. It is a useful property of ionic surfactants, and it also represents the temperature at which their solubility is equal to their cmc. All analyses were performed in triplicate.

Emulsifying Power. Performance of AAS in O/W emulsion was tested using *n*-decanol or liquid paraffin as oil phase. The emulsified layer (milliliters), established after standing for 15 min, was measured. All analyses were performed in triplicate.

Foaming Power. Foaming power was determined using a Ross Miles apparatus (1982). The foam height (millimeters), initial and after 15 min, of a 0.25% solution of each AAS surfactant in "150 ppm" hard water (Ca^{2+}:Mg^{2+} = 6:4) was measured. All analyses were performed in triplicate.

Lime Soap Dispersing Requirements (LSDR). LSDR define the tolerance of surfactants to calcium and magnesium ions as applicable to hard and soft water. LSDR of AAS were evaluated by determining the amount of AAS required to disperse calcium soap formed by 100 g of sodium oleate in "333 ppm" hard water. LSDR are expressed in percentage according to the principles of Borghetty and Bergman (1950).

Hydrodynamic Diameter of AAS Micelles. The size of AAS micelles in solution was measured by a laser scattering instrument (Malvern, CO) at a scattering angle of 90° (25 °C). All analyses were performed in triplicate.

RESULTS AND DISCUSSION

Amino Acid Composition of Waste Proteins. Table 1 shows the amino acid composition of waste proteins. As can be seen, cottonseed protein had the highest amount of polar amino acids, especially glutamic acid and arginine. Silk chrysalis was particularly high in lysine (a very reactive nucleophile), in addition to its high content of acidic amino acids.

Yield of AAS from Waste Proteins. The yield (Table 2) of purified mixed AAS from waste proteins was ~60−75% (sodium salt). This is within the yield range (Table 2) achievable using pure glutamic acid as the source of amino acid. The yield of amino acid ester from glutamic acid was considerably higher (85−92%) than from the sodium salt derivatives. The active matter contents of most of the synthesized AAS were more than 95%, an indication of their purity level.

Surface Properties of AAS. Table 3 presents the surface tension (γ), cmc, and Krafft points of AAS. Generally, as acyl chain length (i.e., from C_{12} to C_{18}) increased, surface tension of AAS increased, cmc de-

Table 1. Polar and Nonpolar Amino Acid Composition (Grams of Amino Acid /100 g of Protein) of Waste Proteins from Cotton Seed (CSD), Silk Residue (SR), and Silk Chrysalis (SC)

amino acid	waste proteins		
	CSD	SR	SC
polar			
tyrosine	0.9	5.0	2.2
lysine	3.6	2.3	8.5
arginine	10.4	2.5	7.2
threonine	2.9	3.0	5.7
serine	4.8	19.4	6.7
histidine	2.6	0.7	3.2
aspartic acid	11.8	8.6	11.8
glutamic acid	25.8	5.5	15.2
nonpolar			
alanine	4.7	20.8	7.8
glycine	4.8	26.6	6.4
isoleucine	2.2	0.6	4.0
leucine	16.3	1.2	9.4
phenylalanine	1.0	0.4	2.0
proline	2.2	0.4	nd[a]
valine	3.3	1.9	5.8
methionine	2.6	0.5	4.1
cystine	0.1	0.6	nd

[a] nd, not detected.

Table 2. Yield[a] (Percent) of Final Products[b]

type of AAS	amino acid source	acyl chain length				
		C_{12}	C_{14}	C_{16}	C_{18}	$C_{18}^{=}$
Na salt	CSD	68.9	70.0	67.3	65.1	60.1
	SR	72.7	75.3	73.4	69.1	60.5
	SC	60.5	65.4	62.8	58.8	55.6
	GA[c]	74.8	76.3	77.1	70.2	65.7
ester	GA	84.5	89.3	92.5[d]	90.7	85.4

[a] Yield represents the ratio of the weight of purified product to the calculated (theoretical) weight. [b] Purified products. [c] GA, pure glutamic acid. [d] Showed high active contents of ~95%; purity was confirmed by IR.

Table 3. Surface Tension, cmc, and Krafft Point of Sodium Acyl AAS in Aqueous Solution As Influenced by Acyl Chain Length

source of amino acid	acyl chain length	surface tension (γ)[a] (mN m^{-1})	cmc[a] (mM)	Krafft point[a] (°C)
CSD	C_{12}	29.5 ± 2.1	2.8 ± 0.02	35.0 ± 0.6
	C_{14}	33.2 ± 1.9	0.8 ± 0.03	38.0 ± 1.1
	C_{16}	35.2 ± 3.2	0.7 ± 0.1	42.5 ± 1.5
	C_{18}	39.8 ± 2.6	0.5 ± 0.01	48.4 ± 2.3
	$C_{18}^{=}$	34.8 ± 1.7	0.6 ± 0.01	
SR	C_{12}	28.0 ± 1.7	1.5 ± 0.1	31.0 ± 0.5
	C_{14}	31.2 ± 1.3	0.6 ± 0.01	41.0 ± 1.0
	C_{16}	32.3 ± 1.7	0.6 ± 0.1	50.0 ± 1.5
	C_{18}	34.0 ± 2.2	0.4 ± 0.01	57.0 ± 2.0
	$C_{18}^{=}$	33.2 ± 1.9	1.1 ± 0.01	
SC	C_{12}	30.7 ± 1.0	1.4 ± 0.02	40.0 ± 0.5
	C_{14}	32.5 ± 1.3	1.2 ± 0.05	48.0 ± 1.7
	C_{16}	35.8 ± 1.2	0.5 ± 0.03	57.0 ± 2.0
	C_{18}	36.3 ± 0.6	0.6 ± 0.01	65.0 ± 2.1
	$C_{18}^{=}$	34.8 ± 1.5	0.8 ± 0.01	
GA	C_{12}	29.2 ± 1.1	0.9 ± 0.01	37.0 ± 0.3
	C_{14}	31.1 ± 1.0	0.8 ± 0.03	45.0 ± 1.0
	C_{16}	32.0 ± 2.5	0.7 ± 0.0	54.0 ± 1.2
	C_{18}	33.9 ± 1.9	0.6 ± 0.02	62.0 ± 1.5
	$C_{18}^{=}$	30.3 ± 1.2	0.8 ± 0.03	

[a] Values represent mean ± SD of triplicate determination.

creased, and Krafft point increased. The cmc data are consistent with our previous findings (Xia et al., 1995) and with the trend reported by Rosen (1989) that as the number of carbon atoms in the hydrophobic group increases, a corresponding decrease in cmc is likely. This relationship confirms the expected behavior of homologous series of surfactants (Attwood and Florence, 1983). Notice that the use of an unsaturated acyl group ($C_{18}^{=}$) instead of C_{18} made little difference in surface tension and cmc values.

Table 4. Calculated Surface Adsorption (Γ_∞, 10^{-10} mol/cm^2) and Area/Molecule (A, Å^2) of Sodium Acyl (AAS) As Influenced by Acyl Chain Length

amino acid source	acyl chain length									
	C_{12}		C_{14}		C_{16}		C_{18}		$C_{18}^{=}$	
	Γ_∞	A	Γ_∞	A	Γ_∞	A	Γ_∞	A	Γ_∞	A
CSD	1.6[a]	103[b]	1.8	93	1.9	85	1.6	103	1.7	100
SR	1.3	129	1.4	118	1.4	118	1.3	125	1.6	103
SC	1.2	141	1.2	142	1.3	129	1.3	129	0.9	177
GA	2.6	64	2.7	61	2.9	57	2.5	65	2.8	60

[a] $\Gamma_\infty = -(1/RT)\,(d\gamma/d \ln c)$. [b] $A = 1/N\Gamma_\infty$; N = Avogadro's number; adapted from Attwood and Florence (1983).

To gain insight into the surface adsorption properties of the AAS, a derivative of the Gibbs equation was used to estimate surface adsorption (Γ) and area/molecule (A). The Gibbs equation expresses the equilibrium between the surfactant molecules at the surface or interface and those in bulk solution (Attwood and Florence, 1983; Adamson, 1976). It is a particularly useful equation since it provides a means by which the amount of surfactant adsorbed per unit area of the surface, the "surface excess", may be calculated (Attwood and Florence, 1983). Data for the calculated surface adsorption and area/molecule of AAS are presented in Table 4. The surface adsorption values of the mixed AAS derived from CSD, SR, and SC were less than those of glutamic acid surfactants. This is perhaps due to the steric effect and cross-linking between mixed AAS and other constituents in the hydrolysates. This view is supported by the larger surface area (Table 4) occupied by the mixed AAS compared to glutamic acid surfactants. The data also suggest that the molecules of glutamic acid AAS are more likely to orient at the O/W interface in a close packing arrangement than the mixed AAS.

The emulsifying power of AAS in O/W emulsion is shown in Figure 3. As can be seen, the emulsifying power of AAS in O/W emulsion was better with *n*-decanol as an oil phase than with liquid paraffin. Glutamic acid surfactants were tested only in paraffin-based emulsion due to insufficient sample.

The foaming power of AAS as affected by acyl chain length is presented in Table 5. When C_{12} was the acyl group, all of the mixed AAS from waste proteins showed high foaming abilities comparable to those of glutamic acid surfactants. Above an acyl chain length of C_{12}, considerable reduction in foaming ability of the mixed AAS was observed (Table 5).

To study the dispersibility of the AAS, their LSDR were determined. LSDR define the minimum amount of surfactants needed to disperse a definite amount of calcium soap in a visibly clear solution and depend on the structure of the surfactant, the shape of the micelle, the amount of Ca^{2+}, and the presence of other insoluble solids. Generally, a surfactant molecule containing large polar groups or more than two hydrophilic groups, such as "alcohol ethoxylate sulfate" or methyl ester sulfonate, shows good dispersing power. The smaller the LSDR, the better the lime soap dispersing power. It is seen in Table 6 that the mixed AAS from cottonseed protein exhibited the best LSDR (i.e., 5.7−6.5). These values were similar to LSDR of glutamic acid surfactants (4.8−5.6) but were less/better than literature value

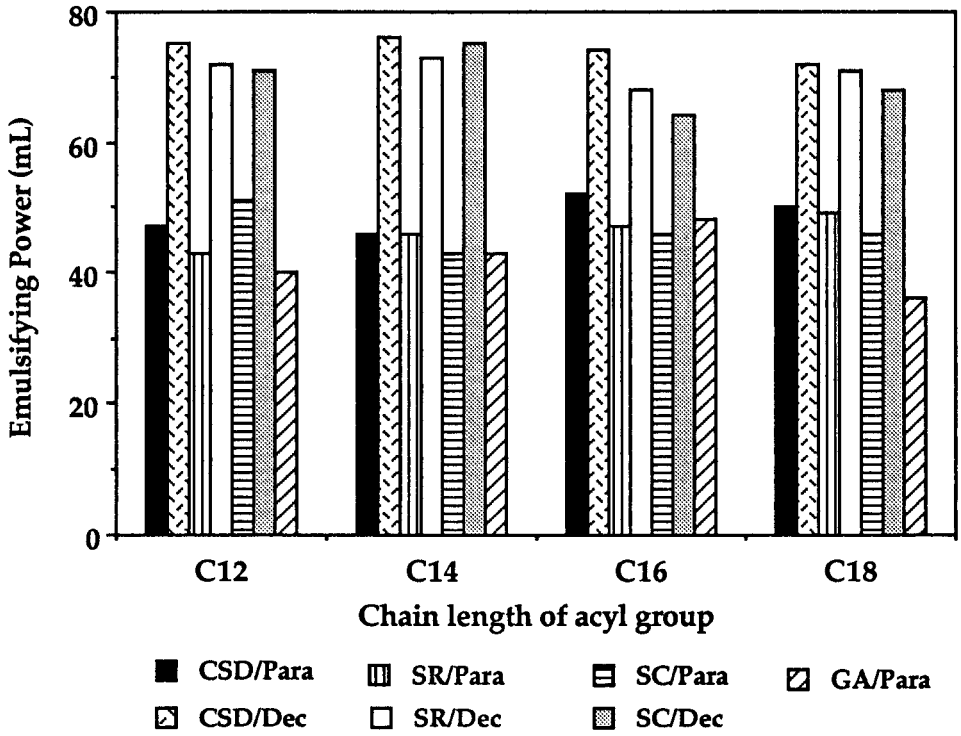

Figure 3. Emulsifying power of AAS in O/W emulsion using liquid parafin (Para) or *n*-decanol (Dec) as oil phase. Data points represent mean of triplicate measurements.

Table 5. Foaming Power (Millimeters) of Sodium Acyl AAS As Affected by Acyl Chain Length

source of amino acid	acyl chain length			
	C_{12}	C_{14}	C_{16}	C_{18}
CSD	170/165[a]	119/115	45/40	21/15
SR	155/152	32/31	28/26	21/19
SC	160/155	102/101	58/50	38/35
GA	192/189	185/182	185/180	155/155

[a] Foaming height (mm) measured at zero time/15 min after. Values represent mean of triplicate determinations.

Table 6. LSDR (Grams per 100 g) of AAS As Affected by Acyl Chain Length

source of amino acid	acyl chain length				
	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18}^{=}$
CSD	6.5[a]	6.1	5.8	5.7	19.0
SR	47.0	42.0	43.0	40.0	56.0
SC	52.0	50.0	45.0	42.0	65.0
GA	5.6	5.2	4.8	4.8	7.0
LAS[b] ($C_{12}PhSO_3Na$)	40.0[c]				
AS[d] ($C_{12}SO_4Na$)	30.0[e]				

[a] Values represent mean of triplicate determination. [b] Linear alkyl benzenesulfonate. [c] Literature value (Bistline and Noble, 1972). [d] Alkyl sulfate. [e] Literature value (Stirton et al., 1965).

for LAS (40). Even the use of unsaturated $C_{18}^{=}$ (oleic acid) as the acyl group did not change the LSDR of AAS from cottonseed protein and glutamic acid. The oustanding LSDR of mixed AAS from cottonseed protein may be attributed to its high glutamic content (Table 1). This observation suggests that the suitability of waste protein for surfactant preparation perhaps depends primarily on the amino acid composition.

The mean values for hydrodynamic diameters of micelles are presented in Table 7. No hydrodynamic diameter was determined for SR-AAS due to lack of sample. The diameter of micelle increased for GA-AAS and CSD-AAS as the acyl chain length increased (i.e, $C_{12}-C_{18}$). The hydrodynamic diameter of AAS followed the order SC > CRD > GA. In addition, the mean

Table 7. Hydrodynamic Diameter (Nanometers) of Micelles of Sodium Acyl AAS As Influenced by Acyl Chain Length

source of amino acid	acyl chain length				
	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18}^{=}$
CSD	224.3[a]	269.6	301.2	373.9	162.5
SC	1438.6	1249.2	861.5	629.2	248.9
GA	62.5	88.4	106.1	123.5	81.7
GA (disodium salt)		1124.8			

[a] Values represent mean of triplicate determinations.

diameter of the disodium salt of GA surfactant was larger than that of the monosalt. This may be explained by the difference between intramolecular and intermolecular hydrogen bonding. For an *N*-acyl amino acid, the intra-H-bond between molecules may form a large surfactant molecule so that the mean diameter of micelles becomes larger (Kazuyuki and Kaoru, 1987). For the monosalt *N*-acylglutamic acid, the possibility of forming an inter-H-bond between molecules becomes weak due to the intra-H-bond effect being a little stronger, so that the diameter of the micelle formed is relatively decreased.

By the same reason, the CSD-AAS is less than that of SC due to the high amount of glutamic acid present in the hydrolysate (Table 1). This explanation is supported by data in Table 4, which show that the adsorption surface area of SC-AAS was more than that of GA-AAS.

Cost Analysis of Waste Proteins. Cost estimates of waste proteins in China for 1994−1995 are as follows: cottonseed, $200/ton; silk residue, $1700/ton; silk chrysalis, $330/ton; glutamic acid, $1300/ton; secondary mother liquor of glutamic acid, $100/ton.

Conclusion. Amino acid surfactants made from waste proteins of cottonseed, silk residue, and silk chrysalis have important properties that can be explored further. Specifically, results from this study showed AAS yield of ~70% from cottonseed protein. Further-

more, the study suggests the suitability of cottonseed as raw material for preparation of high-quality protein-based surfactants (i.e., amino acid and peptide surfactants). Native cottonseed or its industrial waste should be readily available at a relatively low cost.

Generally, the chain length of the acyl group affected surface properties. Amino acid surfactant with C_{12} produced good surface properties.

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