⁂Preparation of Surfactants by Condensation of Fatty Acid Esters with Hydrolyzed Proteins[1]

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ABSTRACT

Products with good surface-active properties can be easily prepared by condensation of fatty-acid methyl esters with polypeptides derived from collagen proteins by hydrolysis. The condensation is done at moderate reaction temperatures (ca. 155 C) in dimethylsulfoxide solvent, and is promoted by sodium hydroxide or other alkaline reagents. The method works best with unsaturated esters, such as methyl oleate, which are more soluble in the reaction mixture, and which yield more soluble products, but also works well with the mixture of saturated and unsaturated fatty acid esters in methyl tallowate. The reaction appears to be a transamidation reaction in which incorporation of the fatty acid moiety results in partial cleavage of the polypeptide chain and in formation of methyl alcohol. Other solvents which can dissolve both fatty acid esters and collagen-derived proteins, such as ethylene glycol, can be used to advantage in this reaction.

INTRODUCTION

N-Acylpolypeptides have potential for use as surfactants, but that potential has been limited by the high cost of their commercial preparation by reaction of fatty acid chlorides with protein hydrozylates (1,2). A one-step direct reaction of fatty acids with unhydrolyzed proteins has been reported, but the reaction required rather high temperatures (225-250 C) (3). In our hands, this procedure has resulted in dark-colored, odorous products, which would limit its usefulness.

The report that transamidation between esters and amides could be effected by methoxide ion (4) led us to investigate the possibility of condensing fatty acid esters with proteins in the presence of strongly basic reagents. We have developed 2 methods by which this may be accomplished, one of which appears especially promising. We describe these methods in this communication.

MATERIALS AND METHODS

Materials

Gelatin (Baker, U.S.P. Powder) used in these experiments was dried in a vacuum oven at 110-125 C for 2 hr.

In most experiments using a more thoroughly hydrolyzed protein than gelatin, Lexein® X350 (Inolex Corp.) was used. This hydrolyzed protein has an average molecular weight on the order of 1,000-3,000 g/mol, and is supplied as a 55% aqueous solution. The Lexein solution was first adjusted to pH 9 to reduce the amount of basic catalyst neutralized by the acidic functional groups of the protein during the condensation reaction. To obtain the anhydrous protein, the solution was triturated under several portions of acetone, producing a light-colored powder, which was further dried in a vacuum oven, as described for gelatin.

In some experiments, gelatin itself was further hydrolyzed under alkaline conditions prior to use. In a typical preparation, 250 g of gelatin was dissolved with sufficient hot water to make 1 L of solution, 20 g sodium hydroxide in 50 mL water was added to it, and the resulting solution was heated at 75-80 C on a steam bath for 2 hr. During this period, the solution became less viscous. At the end of the 2 hr, the solution was cooled, the insoluble humin allowed to settle, and the supernatant liquid decanted from the precipitate. This solution was concentrated under vacuum, and then dried as described for Lexein, except that no additional sodium hydroxide was added because the solution was still quite alkaline.

The fatty acid methyl esters used in this investigation were methyl oleate (Eastman, technical grade), methyl tallowate (Fats and Proteins Research Foundation, Inc.), methyl stearate (Aldrich, 95%), methyl palmitate (Fluka, puriss), methyl myristate (Fluka, puriss), and methyl laurate (Fluka, purum), and were used as received.

Steric acid (Fisher, purified), lauric acid (Eastman, mp 43-45 C), myristic acid (Eastman), and oleic acid (Fisher, laboratory grade) were all used as received for the preparation of their ethylene glycol monoesters. The ethylene glycol monoesters of stearic, lauric and myristic acids were all prepared in the same manner. The procedure for esterification of lauric acid is given next as an example.

Lauric acid (20.4 g, 0.102 mol) was dissolved in 216 g ethylene glycol, and 2.2 mL of 18 M sulfuric acid (0.04 mol) was added. The resulting solution was heated at 140 C under house vacuum, which served to remove water as it was generated. After 4 hr, the reaction mixture was cooled and poured into 800 mL water. The water-insoluble ester was taken up by extraction with 300 mL ethyl ether, followed by extraction with 2 additional 200 mL portions of ether.

The combined ether extracts were dried first with sodium sulfate (anhyd.), and then over molecular sieves (Linde 4A). Evaporation of the ether gave the ester as a white, low-melting solid, yield 22.5 g (90% th.).

The monooleate ester of ethylene glycol was prepared by similar reaction of oleic acid (Fisher, Laboratory Grade) in an excess of ethylene glycol with catalytic sulfuric acid. However, the yield was appreciably poorer with oleic acid, and purification required vacuum distillation to separate the monoester from high boiling side-products.

All esters displayed infrared (IR) and nuclear magnetic resonance (NMR) spectra consistent with ethylene glycol monocarboxylic esters.

Reaction in Ethylene Glycol

The following procedure is typical of that used successfully with Lexein and fatty esters in glycol solvents.

To 7 mL ethylene glycol was added 0.5 g sodium hydride, and the mixture was stirred under a nitrogen atmosphere until the vigorous reaction ceased. Dried Lexein (5.0 g) was added, and the stirred mixture warmed until the Lexein dissolved at a temperature of 90 C. Ethylene glycol monooleate (2.6 g) was added and the temperature was gradually increased to 134 C over a period of 55 min. The mixture was held at this temperature for an additional 2 hr and then the ethylene glycol was distilled off under vacuum over a period of 1.5 hr while the temperature was maintained at 135-146 C. The yield of tan, brittle product was 6.78 g.

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Reaction in Dimethylsulfoxide (DMSO)

In a typical small-scale run, 14 mL DMSO and 0.9 g crushed sodium hydroxide pellets were placed in a 100-mL, 3-necked flask. The contents of the flask were heated to 155 C under a nitrogen atmosphere, and then 5.4 g dry Lexein was added with vigorous magnetic stirring. When this dissolved after brief stirring, 2.8 g methyl oleate was added, and the resulting mixture was stirred for a further 2 hr. Upon completion of this period, the DMSO was distilled off under vacuum and the last residue of DMSO was driven off by further heating at 155 C under vacuum. Essentially all of the original DMSO was recovered. The product, a tan, friable solid, weighed 8.1 g. In other runs, reaction was successfully completed in times as short as 1 hr, 20 min.

In a typical run at increased scale, 200 mL DMSO was placed in a 1-L, 3-necked reaction flask with nitrogen inlet and mechanical stirring by ground-glass sleaved stirrer. Sodium hydroxide (6.0 g) was added, and the mixture was heated to 155 C, at which point 66.6 g of dry, powdered, alkaline hydrolyzed gelatin was added with continuous stirring under a nitrogen atmosphere. Upon complete addition of the gelatin, methyl oleate (33.3 g) was added, and the reaction mixture was heated with stirring for an additional 1 hr, 20 min.

At the end of the reaction period, the flask was equipped for vacuum distillation, and the DMSO was distilled off at 0.5 mm Hg pressure, while heating was continued at 150-155 C. Much of the DMSO distilled off rapidly, but in the larger runs such as this, distillation slowed considerably when the reaction mixture thickened to the point that it was not longer possible to stir it with the equipment available. Removal of DMSO would be facilitated with equipment sufficiently powerful to continue to break up the thickening mass. After 2-3 hr distillation, the take-off of DMSO slowed to the degree that it was necessary to cool the flask and its contents, and, after breaking vacuum, open the reaction flask in order to break up its contents by hand. A final heating period at 120 C overnight in a vacuum oven effected complete removal of all residual DMSO. The yield of dry, friable product ranged from 96.0 to 101.1 g for a series of runs.

Evaluation of Products

The degree of incorporation of the fatty acid ester into the product (which reflects the extent to which the reaction is complete), as well as the degree of hydrolysis of the ester to the free fatty acid salt (soap) was determined by the following procedure prior to evaluation of the product as a surfactant. The entire product or a weighed fraction thereof was thoroughly triturated under hexane, the hexane decanted, and the product triturated again under fresh hexane. This process was repeated 3 or 4 times until evaporation of the final portion of hexane extract left little residue. The combined hexane extracts were filtered, and the hexane was evaporated under vacuum. The weight of the residue gave an upper limit to the proportion of fatty acid ester remaining unreacted, but this fraction was occasionally contaminated with lubricant from the ground-glass stirrer sleeve, particularly in early runs.

The product, after trituration with hexane, was dried to remove residual hexane, and 1 or 2 g dissolved in an appropriate volume of distilled water (usually 15-30 mL). The solution was acidified to pH 3 with dilute sulfuric acid, and extracted twice with hexane. The hexane extract was dried and filtered, and the hexane was evaporated under vacuum. The weight of the residue gave an upper limit to the amount of free fatty acid in the product, although it was occasionally contaminated with neutral material that was not completely extracted during the initial trituration.

The acidified product solution, after extraction by hexane, was briefly placed under vacuum to remove suspended hexane, and then adjusted to pH 8 with ammonium hydroxide or dilute sodium hydroxide solution. The solution was diluted to 1% (the weight of fatty acid extracted was usually negligible), filtered, and diluted to the working concentrations for evaluation (0.5% and below).

The foaming abilities of products were determined at room temperature by measuring the volume of foam remaining after 30 sec and 2 min when 5.0 mL of the dilute solution of the product was shaken vigorously in a glass-stoppered, 10-mL graduated cylinder and allowed to stand.

Surface tensions of these dilute solutions were determined at room temperature with a Fisher Du Nouy-type interfacial tensiometer.

In some experiments, after 2 g of the product was triturated, dissolved, and the resulting solution acidified and extracted as already described, the solution was made 6 M in hydrochloric acid, refluxed overnight, and extracted with hexane again. The hexane extract was dried, and the hexane evaporated under vacuum. The residue represented that portion of the fatty acid incorporated into the product and released by hydrolysis.

RESULTS AND DISCUSSION

We have developed 2 related methods for the preparation of surfactants by the condensation of fatty acid esters with protein. The first uses the protic, nucleophilic solvent ethylene glycol, the conjugate base of which is generated by addition of sodium hydride. This solvent required use of the fatty acid monoesters of ethylene glycol as the ester component, because methyl esters were insufficiently soluble. The second method uses a nonprotic solvent, dimethyl sulfoxide. Either sodium hydride or the much cheaper sodium hydroxide have been used successfully as the basic catalysts, and the methyl esters of the fatty acids may be used.

The 2 methods have much in common: a polar solvent capable of dissolving both the collagen-derived protein substrates and the fatty acid derivatives, alkaline catalysts, and fairly short reaction times at moderate temperatures. However, there are significant differences, as well, and each method has both advantages and disadvantages relative to the other.

Reaction in Ethylene Glycol

The reaction in ethylene glycol requires use of ethylene glycol monoesters of the fatty acids, because the methyl esters do not seem to be sufficiently soluble. The glycol monoesters are more difficult to prepare, and much less readily available. Upon storage, they are subject to disproportionation to the free glycol and the glycol diester, which would be less soluble in the reaction mixture. For these reasons, a process requiring glycol monoesters can be expected to be appreciably more expensive than one using methyl esters. However, given the glycol monoester, gelatin, as well as more highly hydrolyzed collagen protein products, can readily be condensed with fatty acids to give soluble surfactants. Under these alkaline conditions, it seems likely that the gelatin is broken down by reaction with the conjugate base of the ethylene glycol, yielding peptide derivatives of lower molecular weight, because the product increases in solubility as the reaction time is extended.

In runs using commercial collagen hydrozylate, short reaction times were possible. There was no evidence for substantial incorporation of ethylene glycol into the

TABLE I

Reaction of Lexein[a] with Fatty Acid Esters of Ethylene Glycol

Fatty acid[b]	(Protein:fatty acid equivalent ratio)[c]	Surface tension (0.1% solution)[d]
Lauric	4.9:1	37.3 dyne/cm
Lauric	2:1	32.7[e]
Myristic	5.4:1	37.7
Stearic	7.2:1	45.9[f]
Oleic	5:1	28.4[e]

[a]Surface tension of unreacted Lexein (0.1% solution) is 49.0 dyne/cm at 32 C.

[b]As the ethylene glycol monoester.

[c]Ratio of equivalents of fatty acid to equivalents (mol amino acid moieties) of protein, assuming an average equivalent weight of 125 g/eq for the amino acids composing the collagen protein (3,5).

[d]All surface tensions were determined at room temperature.

[e]After removal of free fatty acid soap by acidification and extraction.

[f]Determined at 30 C; product was poorly soluble.

product, and the total weight of the product was usually slightly less than the combined weight of the reactants (e.g., yield 6.8 g vs 7.6 g reactants). This is compatible with a reaction mechanism in which ethylene glycol is displaced from the fatty-acid acyl carbon by a nucleophilic group from the protein. Such a group could be either a free amino functionality, or an amide nitrogen activated by removal of a proton by the basic catalyst. Mechanisms such as the latter have been proposed for transamidation reactions between esters and amides under alkaline conditions (4). Evidence that a similar displacement of the corresponding methyl group of the methyl esters occurs during the reaction in DMSO will be reviewed in the discussion of that process.

Acidification and hexane extraction of a solution of the product produced from ethylene glycol monooleate indicated that up to one-third of the oleate was not incorporated into the product, but was converted into sodium oleate, isolated as oleic acid. Even freed of its sodium oleate component, the product was an excellent surfactant, a 0.1% solution of which had a surface tension of 28.4 dyne/cm at room temperature. Similar quantities of soaps were produced in the reaction of other fatty acid glycol monoesters done in ethylene glycol.

Table I gives the surface tension for solutions of the products of several fatty acids produced in this way. The stearate product was much less soluble than the others, resulting in considerably higher surface tensions for its "solutions," even at temperatures somewhat elevated above room temperature.

Because efforts to insure that the reagents and solvents were anhydrous did not reduce the proportion of ethylene glycol monoester converted to the free fatty acid salt, we do not believe that the latter was formed through cleavage of the ester by water. Rather, we postulate a β-elimination mechanism, promoted by strong base.

Reaction in DMSO

Methyl esters cannot undergo such an elimination and are cheaper and more readily available than the ethylene glycol monoesters. Our efforts were concentrated, therefore, on experiments using methyl esters. Ethylene glycol was not a suitable solvent for reaction of the methyl esters for 2 reasons: (a) the methyl esters are poorly soluble in ethylene glycol; and, (b) transesterification of the methyl esters in ethylene glycol would again give the ethylene glycol monoester, subject to elimination.

Gelatin could be used directly in ethylene glycol, although longer reaction times were required than for the more completely hydrolyzed Lexein. However, in DMSO, gelatin often formed an insoluble gel upon reaction with the methyl esters, apparently because of crosslinking of the peptide chains. Lexein did not lead to such difficulties because of its initially shorter peptide chains, and was the principal protein substrate used in that solvent. Gelatin which had undergone an initial hydrolysis in alkaline solution could also be used successfully.

Early experiments showed that ca. 74% of the initial methyl ester could be incorporated into alkaline Lexein by reaction in DMSO over a period of 9-10 hr using sodium hydride (expt. 1, Table II). The importance of this reagent was made evident (expt. 2) when the sodium hydride was omitted. Most of the ester (84%) was not incorporated, and the product, which was much darker than usual, had very poor surfactant properties.

Experiment 3, in which sodium hydroxide was used in place of the equivalent amount of sodium hydride, established the efficacy of sodium hydroxide as alkaline reagent. At least 90% of the tallow fatty acid present was incorporated into the product, which exhibited excellent surface-active properties. As sodium hydroxide is both cheaper and more convenient than the reactive sodium hydride, it was used in subsequent experiments. Interestingly, very

TABLE II

Reaction of Lexein with Fatty Acid Methyl Esters in DMSO

Expt. #	Rxn. temp./time (C/hr)	"Unreact. ester" (%)	Free acid (%)	Hydrol. acid (%)	Surf. tension (dyne/cm) @ .1%	Foam (mL) @ .1%
1[a]	130–140/9.5	17	9	–	29	0.2
2[b]	130–140/9.5	73	11	–	49	0
3	130–140/9.5	3	6	–	31	2.0
4	150/2.5	8	5	89	32	1.8
5	155/1.5	5	9	97	34	2.2
6[c]	155/2	2	2	–	33	3.4
7[d]	155/1.3	5	14	–	27.6	0.7
8[e]	155/2	4	3	–	28.6	2.6

[a]Catalyst used in this run was 0.54 g sodium hydride; 10.9 g alkaline hydrolyzed collagen, and 5.6 g methyl tallowate (5:1 equivalent ratio) were used with 1.8 g NaOH except where noted.

[b]Reagents same as in a, except that no catalyst was added.

[c]This run was done at one-half the scale, with 2.8 g methyl oleate and 0.9 g sodium hydroxide.

[d]This run was done with 10 g hydrolyzed collagen, 10 g methyl oleate, and 1.8 g sodium hydroxide.

[e]This run was done at one-half scale, using gelatin hydrolyzed in alkaline solution, and methyl oleate. Only 0.45 g NaOH was required.

little saponification of the ester seems to take place.

Reaction time was reduced to 2.5 hr at 150 C (expt. 4), and reduced further to 1.5 hr at 155 C (expt. 5). At least 86-97% of the tallowate was incorporated into the product in these runs. As with reaction in ethylene glycol, the final weight of the dried product was less than the total of the reactants, but was 96% of the theoretical yield calculated by assuming loss of water derived from the hydroxide and loss of methanol from the methyl ester.

The differences in surface-tension-lowering ability of these products is slight (Table II), and all compare well with commercial surfactants such as sodium lauryl sulfate and Maypon 4C (39 dyne/cm and 28 dyne/cm at .3% concentration, respectively) (3). With this in view, only the product resulting from expt. 2, in which no basic catalyst was used, stands out as significantly worse than the others.

However, it should be noted that methyl oleate and tallowate esters worked best in this regard. The results were less satisfactory with methyl laurate, reaction of which resulted in recovery of substantial quantities of lauric acid upon acidification of the product. The same was true to a lesser degree in the reaction of methyl myristate, although a product of good surfactancy was obtained from the methyl myristate (27.2 dyne/cm at 0.1%). These difficulties may result from differences of solubility in the reaction medium between the lower fatty acid esters and the higher esters. Methyl palmitate reacted well, but produced a product that had poor solubility in cold water.

The critical micelle concentration of these products falls in the range of .1-.01%, as indicated by the point of increasing surface tension with decreasing concentration (Fig. 1).

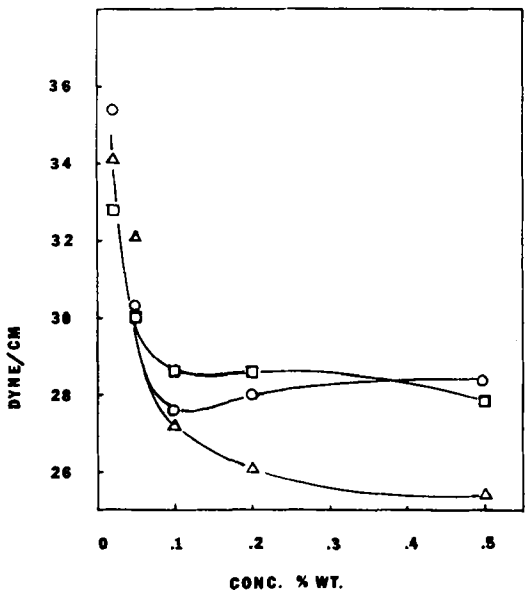


FIG. 1. Surface tensions vs concentration for aqueous solutions of the products from condensation in DMSO of: commercial hydrolyzed collagen/methyl myristate, 2:1 by wt (△); commercial hydrolyzed collagen/methyl oleate, 1:1 by wt (○); and alkaline-hydrolyzed gelatin/methyl oleate, 2:1 by wt (□).

Characterization of the Product

There is no indication that substantial DMSO is incorporated into the condensate produced in that solvent, because virtually all of it is recovered during the vacuum distillation. On the other hand, it is almost certain that the fatty acid is bound to the protein by amidation of the ester acyl carbon. This is indicated by the very small amount of unreacted ester or free fatty acid that can be isolated from the crude product by simple extraction, whereas most of the fatty acid can be recovered only after strenuous hydrolysis.

In experiments 4 and 5, a 2-g sample of the product was washed with hexane, dissolved in water, acidified, and the resulting solution extracted again as described for the routine analysis of the products. This series of extractions was followed by reflux overnight in 6 M hydrochloric acid to effect complete hydrolysis of the product. A third extraction with hexane then gave, upon evaporation of the solvent, the fatty acids incorporated into the product, which ranged from 89 to 97% of the amount of fatty acid expected from the ester initially present in the reaction mixture.

The amidation of the original fatty acid ester is confirmed by analysis of the IR spectra of the products (Fig. 2). While the esters absorb strongly at 1745 cm^{-1} and at 1170 cm^{-1} (C=O and C-O-), there are no absorptions at those frequencies in the products, even though they are apparent in a simple physical mixture of methyl stearate and hydrolyzed collagen in the same ratio as in the product. The amide absorption at 1640 cm^{-1}, however, is quite strong in the product, as it is in the original partially

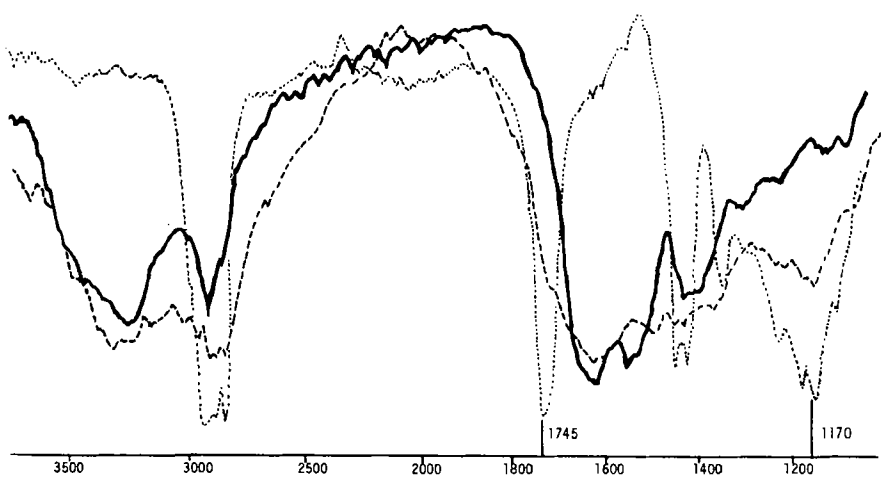
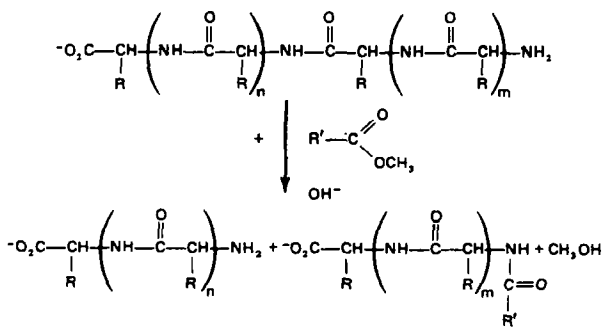


FIG. 2. Infrared spectra (scale in cm^{-1}) of: commercial hydrolyzed collagen/methyl tallowate (2:1 by wt) condensation product, reaction in DMSO (——); methyl tallowate (· · · ·); and a physical mixture of dry, hydrolyzed collagen with methyl stearate in the same proportions as in the condensation product (- - - -).

hydrolyzed collagen substrate. Such evidence shows that the ester functionality is not present (nor the methoxy group, therefore). No alternative to amide bonding of the fatty acid is apparent.

Titration of the product from pH 12 to pH 7 indicates there are as many basic functional groups (most of them undoubtedly $-NH_2$) in the product as in the hydrolyzed collagen. If it is the free amino groups that initially amidate the ester, as seems likely, then there must be regeneration of such amino groups during the course of the reaction. A tentative equation illustrating the nature of the condensation reaction can be written as (Scheme I):



SCHEME I

This is not to say that no C-terminal methyl ester is formed; rather, its formation is not the predominant path. The reaction is undoubtedly complex, and may include some crosslinking of the peptide, as well as the peptide cleavage shown in Scheme I.

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