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Spontaneous Polypeptide Formation from Amino Acyl Adenylates in Surfactant Aggregates

Sir:

Micelles and liposomes are known to mimic certain functions of the biological ensemble.[1,2] These aggregates bind appropriate molecules and catalyze their reactions.[3,4] There are numerous examples of organic and inorganic reactions which are substantially affected by micelles.[1-6] A close examination of the literature, however, reveals that the vast majority of these reactions are simple and/or degradative in nature (i.e., hydrolyses, association-dissociation, complex formation, etc.).[1-6] Although significant information has resulted from these studies, micelles have not been utilized to catalyze the condensation of biologically important molecules. We have previously shown the selective uptake of amino acids,[7] nucleotides,[8] and polynucleotides[9,10] in micelles. In addition, the formation of oligonucleotides in reversed micelles has been demonstrated.[11] The purpose of the present communication is to report the spontaneous conversion (up to 95%) of alanyl adenylate to polypeptides, containing up to 41 amino acid units, in functional micelles. To the best of our knowledge, no previous colloidal system afforded comparable spontaneous polymerization. Importantly, micelles are well characterized and are, therefore, readily amenable to controlled and reproducible experimentation.[12] An additional significant feature of the present work is the unambiguous identification of the polypeptides by ^{252}Cf plasma desorption mass spectroscopy (PDMS).[13]

Figure 1 illustrates the extent of the polycondensation of [^{14}C]alanyl adenylate[14] in 0.30 M aqueous $NaHCO_3$ adjusted to pH 8.8 (the control), in aqueous 0.10 M hexadecyltrimethylammonium bicarbonate[15] at pH 8.8 (aqueous micelles), and in 0.40 M water, solubilized by 0.225 M sodium di-2-ethylhexylsulfosuccinate (Aerosol-OT), and 0.10 M hexadecyltrimethylammonium bicarbonate in benzene (reversed comicelles).[16] The corrected yields in the control, in aqueous, and in reversed micelles are 30% (mainly dimers and trimers), 40% (oligopeptides and polypeptides in the mol wt $\simeq$ 159-2000 range), and 94.5% (polypeptides in the mol wt 350-3000 range), respectively. The yield and degrees of polymerization in aqueous buffer are in good agreement with that reported previously.[17] Micelles are seen to enhance polycondensation. Not unexpectedly, the effect is greater in reversed micelles where the concentration and polarity of the surfactant solubilized water are restricted.[2] The high molecular weight products, formed on larger scale polycondensation of alanyl adenylate in aqueous and reversed micelles, were separated on a Sephadex LH-50 column (Figure 2).[18] Rechromatography of the isolated higher molecular weight peptides from the reversed micelle reaction (peak 1 in Figure 2) removes any contaminating adenylic acid and hexadecyltrimethylammonium bicarbonate. No free or bound adenylic acid could be detected (UV absorption spectroscopy) on the polyalanine. ^{252}Cf plasma desorption mass spectroscopy of the isolated

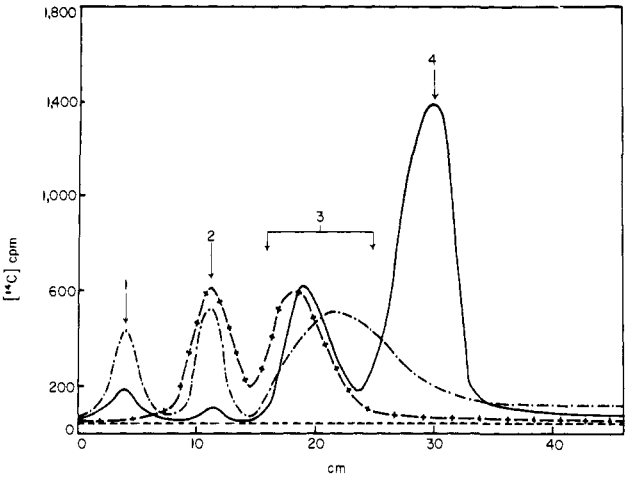

Figure 1. Paper chromatographic separation of the products formed in the room temperature condensation of [^{14}C]alanyl adenylate in aqueous 0.3 M $NaHCO_3$ buffer at pH 8.8 (-+-), in aqueous micelles at pH 8.8 (-·-), in reversed comicelles (—) and background radiation (- - -). Peaks correspond to alanine (2), low molecular weight peptides (3), high molecular weight polypeptides (4), and an unidentified compound, possibly diketopiperazine (1). See text and ref 16 for experimental details.

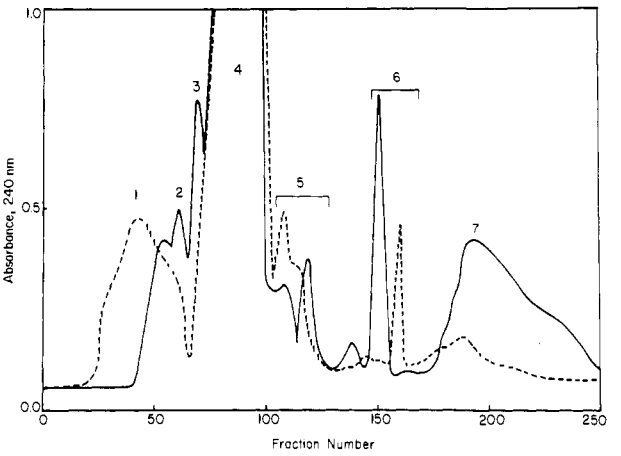

Figure 2. Separation of products on a Sephadex LH-50 column (2.5 m × 3.0 cm) formed from the condensation of alanyl adenylate in the presence of aqueous (—) and reversed comicelles (- - -). Peaks correspond to alanine (7), low molecular weight peptides (2, 3, and 4), AMP (also 4), high molecular weight polypeptides (1), and surfactants (5 and 6).

product (from peak 1, Figure 2) established the presence of polyalanines in the molecular range of ~2000-3000 daltons. The distribution maximum peak was at 2650 daltons, which corresponds to a degree of polymerization of ~36 alanyl residues.[19] Formation of these high molecular weight peptides at ambient temperature is one of the most dramatic micellar effects observed to date.

Apparently, polypeptides in micellar systems can be formed either by the reaction(s) of an amino (or peptide) acyl adenylate, A~P, or A_i~P, or A_j~P, with each other

$$A_i\sim P + A_j\sim P \rightarrow A_i\sim P + P \quad (1)$$

or by the interaction of a free amino acid or peptide, A_i, with the adenylate:

$$A_i + A_j\sim P \rightarrow A_{i+j} + P \quad (2)$$

Amino acids and/or peptides are formed by the hydrolysis of A~P or A_i~P or A_j~PP. Indeed, it was found that added alkylamines (i.e., dodecylamine) would preferentially react with amino acyl adenylates forming the amino acid surfactant. Polypeptide formation terminates when the last adenylate has either reacted or hydrolyzed. Conversely, polypeptide for-

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mation on clays was rationalized *only* in terms of eq 1 and a terminating step which involved the formation of an inactive adenylate ester bond (A-P).[20-24] Equation 1 is likely to describe the polymerization of adenylates on clays since the adenylate binds to them while amino acids do not. In reversed micelles, however, eq 2 cannot be ignored since amino acids formed from the hydrolysis of the amino acyl adenylates remain in the aqueous core of the reversed micelle in close contact with other adenylates. The chain termination step of the clay mediated polymerization (ester formation, A-P) rested upon the observed high percentages of peptides which contained terminal adenylic acid.[20] Our inability to detect terminal adenylic acid in the peptides in the present work (either by absorption or ^{252}Cf plasma desorption mass spectroscopy) may imply different mechanisms in the clay and micellar systems. Alternatively, inactive amino acid esters of AMP (A-P) could have been present as impurities in the amino acyl adenylates (A~P) used in the clay experiments.[21,24] A-P could react, of course, with A~P to give A_2-P and P. It has been shown that the dicyclyhexylcarbodiimide, DCC, mediated synthesis of amino acyl adenylates, the method used to prepare A~P in the clay experiments,[21,24] contained up to 20% A-P impurity.[17] The reaction of this ester impurity with the amino acyl adenylates (or peptide acyl adenylates) would result in peptides with a terminal AMP ester. Conversely, the method of preparation used in this work for A~P does not lead to A-P contamination.[14]

The micelle performs at least three functions which result in the formation of high molecular weight peptides: (1) they concentrate A~P, (2) they maintain the reaction at a pH >7, and (3) they minimize competing hydrolysis. The kinetics and mechanism of the condensation of amino acyl adenylates to high molecular weight peptides is the subject of our current and intensive investigation. The use of more complex functional micelles and colloidal aggregates is expected to result in even more efficient polycondensations.

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(15) In water at pH <7, amino acyl adenylates hydrolyze. However, condensation to oligopeptides, as well as hydrolysis, occurs in buffered solutions at higher pH.[17] The role of functional surface active hexadecyltrimethylammonium bicarbonate surfactant is to buffer itself. This surfactant was obtained by dialysis of 0.30 M cetyltrimethylammonium bromide in a supersaturated solution of $NaHCO_3$. One dialysis replaces ~82% of Br^- with HCO_3^-. Two dialyses replace ~96% of Br^- with HCO_3^-. The dialized solution must be lyophilized, as the surfactant decomposed easily and is very hygroscopic. The dried surfactant, above its critical micelle concentration, gives a pH of 8.8 in aqueous solution and buffers itself.
(16) In all three systems, 30 mg of [^{14}C]-L-alanyl adenylate was stirred in 5.0 mL of the appropriate solution for 30 min at room temperature. All reactions were completely homogeneous, although the adenylate dissolved more slowly in the reversed micelle reaction. Aliquots from each finished reaction were spotted on Whatman No. 1 chromatographic paper. Descending chromatography (with 12:2:1, 2-propanol–acetic acid–water, v/v, as solvent) was run. The co-chromatographed standards (consisting of the alanine monomer, dimer, trimer, tetramer, pentamer, and polyalanine with a molecular weight distribution of 1000–5000, purchased from the Sigma Chemical Co.) and the monomer alanine from the reactions could be located on the dried chromatogram by spraying with ninhydrin. Radioactive products were located and quantified both by a Hewlett-Packard Model 7201 radiochromatographic scanner or alternatively by scintillation counting the ^{14}C eluted (4 M HCl) from each 2-cm portion of the chromatogram. Completion of the reaction was demonstrated by the lack of formation of hydroxamic acid derivatives upon the addition of hydroxylamine.
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(18) The products of five reversed micellar reactions (containing a total of 250 mg of alanyl adenylate) were rotary evaporated to a viscous liquid. The remaining liquid was redissolved in 25 mL of methanol-water solution (67:33, v/v). This solution was placed on a 2.5 m × 3.0 cm Sephadex LH-50 column previously equilibrated with the MeOH–H_2O 67:33, v/v, solution; 10.0-mL fractions were collected on a Gilson automatic fraction collector. The absorbance of each fraction was detected manually (240 nm) on a Cary 118C UV–visible spectrophotometer.
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A Diborane(6) Bridged Diiron Hexacarbonyl: Preparation of $B_2H_6Fe_2(CO)_6$

Sir:

The ferraboraneS, $B_4H_8Fe(CO)_3$[1] and $B_5H_9Fe(CO)_3$,[2] have been produced in good yield from the co-pyrolysis of B_5H_9 and $Fe(CO)_5$.[3] Both the geometrical[1,2] and electronic structures[4] of these compounds demonstrate, in agreement with Wade's predictions,[5] that the equivalent boranes, B_5H_9 and B_6H_{10}, are useful models for these compounds. We are interested in exploring this analogy as the number of iron atoms in the cage increases. In addition we have demonstrated that the $Fe(CO)_3$ fragment provides a means of photochemical modification of the cage[6] and we are interested in the behavior of species with two such fragments. Herein we report the preparation and characterization of the diiron ferraborane, $B_2H_6Fe_2(CO)_6$.[7]

In a typical preparation, the addition of 2.2 mmol of $Fe(CO)_5$ and 3.0 mmol of B_5H_9 to a clear solution of 2.0 mmol of $LiAlH_4$ in 19.7 mL of diethyl ether resulted in the rapid formation of a deep red solution and a dark precipitate.[8] After standing at room temperature overnight, the nonvolatiles were removed at −196 °C. On treatment with 7.0 mmol of HCl, ~6.8 mmol of noncondensibles was evolved and the solid dissolved. Fractionation yielded unreacted starting materials, $B_4H_8Fe(CO)_3$, and a less volatile compound identified as indicated below as $B_2H_6Fe_2(CO)_6$. The reaction has been carried out several times on 2- and 8-mmol scales and the yield varies from 1 to 10% depending on the length of time and method of workup. The new compound is a yellow-brown liquid at room temperature and is very air sensitive.

The new compound has the molecular formula $Fe_2B_2C_6O_6H_6$ ($^{56}Fe_2{}^{11}B_2{}^{12}C_6{}^{16}O_6{}^{1}H_6{}^+$, calcd 307.9048 amu, obsd 307.9066 amu; $^{56}Fe_2{}^{11}B_2{}^{12}C_5{}^{16}O_5{}^{1}H_6{}^+$, calcd 279.9099 amu,

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