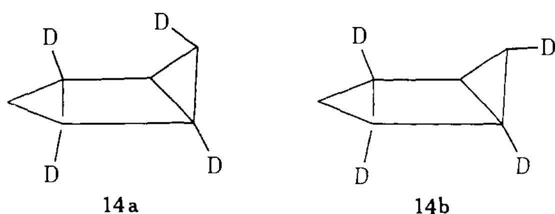
**13a** and 8% **13b**[6] which corresponds a 5:1 preference[7] for Scheme II over I for the formation of this product. In the NMR spectrum of the bicyclo[3.1.0]hexane-$d_4$ that was isolated, the intensities of the absorptions at $\delta$ 0.15, 1.2, and 1.6 were in the ratio 1:2:3 instead of 2:3:5 as in bicyclo[3.1.0]hexane. This showed that there was one deuterium atom each in the methylene and one of the methine groups on the cyclopropane ring and two deuterium atoms at C-2. In order to find out if the compound was homogenous in terms of its deuterium distribution, the NMR spectrum was studied at high resolution (220 MHz). In the spectrum of bicyclo[3.1.0]hexane, the two protons at C-6 occur at $\delta$ 0.10 and 0.23. The former which is presumably the endo hydrogen is coupled less strongly ($J$ = 4 Hz) to the two methine protons than the exo hydrogen ($J$ = 8 Hz). The geminal coupling is ~4 Hz.[8] In the spectrum of the tetradeuterio derivative, absorptions due to both protons at C-6 are present, but the geminal coupling between them is absent. The intensities of these absorptions correspond to the composition **14a**:**14b**::1:2. This distribution of the deuterium at C-6 indicates much stereoselectivity in the formation of this product




which in turn argues against the intermediacy of a diradical as in Scheme III. It is not surprising that carbene **10** does not lead to bicyclo[3.1.0]hexane, a fact that is well known.[3] If the formation of bicyclo[3.1.0]hexane is also included, the partition of the excited state of cyclohexene between carbenes **10** and **11** will be 1:14.

The intriguing question that remains unanswered is the factor (or factors) which determines the direction of the excited state between these two pathways.

**Acknowledgment.** The authors thank Drs. J. J. Wynne and J. A. Armstrong for their advice and encouragement.

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(4) Deuterium-labeling experiments with cycloheptene-*1,2-d*$_2$ were conducted in ref 2a and 2c. The formation of only one kind of methylenecyclohexane was reported. No stereochemical information on the bicyclo[4.1.0]heptane-$d_2$ that was also formed was given.
(5) Cyclohexene-*3,3,6,6-d*$_4$ was obtained from Merck & Co. Its isotopic purity as verified by NMR was >98%. Its NMR spectrum was unchanged after a part of it had been photoisomerized.
(6) In the NMR spectrum of methylenecyclopentane, the absorptions corresponding to the three kinds of protons are distinctly separate. The composition of the mixture of **13a** and **13b** can be estimated from the area of any of these peaks (if the total = 6 H). The composition that was calculated was internally consistent to ±1%.
(7) It is uncertain what, if any, isotope effect should be attributed to Schemes I or II. The preference reported here assumes that no isotope effect was operative.
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(9) IR 870, 1675 cm$^{-1}$; NMR $\delta$ 4.22 (2 H), 2.88 (2 H), 2.24 (1 H), 1.2–1.8 (5 H).

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## Two Antiparallel Tripeptide Chains Stabilized by an SS Unit: *S,S'*-Bis(Cbz-L-Ala-L-Cys-L-Ala-OMe) in Chloroform Solution

*Sir:*

Recent developments[1,2] in the study of folding pathways for SS-containing globular proteins indicate the importance of thermodynamic stability of the peptide conformations before and after disulfide bond formation. Studies on relative thermodynamic stabilities of different disulfide intermediates have been needed in this area. This report concerns the fundamental aspect of conformational stability of SS-containing oligopeptides. It has been known that many natural proteins containing cystine take antiparallel arrangements in the close proximity of the SS linkage, and that the most stable dihedral angle of the CSSC group is restricted to ~90°.[3] Restricted rotation of this group significantly reduces the number of possible conformers of cystine itself, and, more importantly, as herein reported, it stabilizes intramolecular hydrogen bonds between open oligopeptide chains leading to an antiparallel conformation. Although our study was made on specific cases, the problem of antiparallel conformation in a globular protein can be understood more comprehensively than previously by combining the present results with information on antiparallel chain-forming processes without an SS linkage (e.g., $\beta$ turn or association).[4]

We investigated the solution conformation of open-chain tetra- and hexapeptides having a cysteinyl SS linkage and found that a hexapeptide, *S,S'*-bis(Cbz-L-Ala-L-Cys-L-Ala-OMe) (I), readily takes an antiparallel ladder-like conformation possessing four intramolecular hydrogen bonds between the tripeptide chain moieties. In contrast, tetrapeptides containing cystine and alanine, i.e., *S,S'*-bis(Cbz-L-Ala-L-Cys-OMe) (II) and *S,S'*-bis(Cbz-L-Cys-L-Ala-OMe) (III), have decreasing trends in forming such specific conformations. Hence, we suggest that at least three amino acid residues in each chain are required to stabilize an antiparallel sheet structure containing hydrogen bonds in close proximity to a cystine SS linkage.

The oligopeptides were prepared stepwise from cystine by mixed anhydride methods, carefully preventing cleavage of the SS group, and resulting in powdery crystallines after recrystallization. The purity of the products was checked by high-speed liquid chromatography and elemental analysis.

In $CDCl_3$ solution, the hexapeptide I shows a $^1$H NMR spectrum (Figure 1) corresponding to an almost entirely symmetrical structure. The Cys-NH proton signal depends

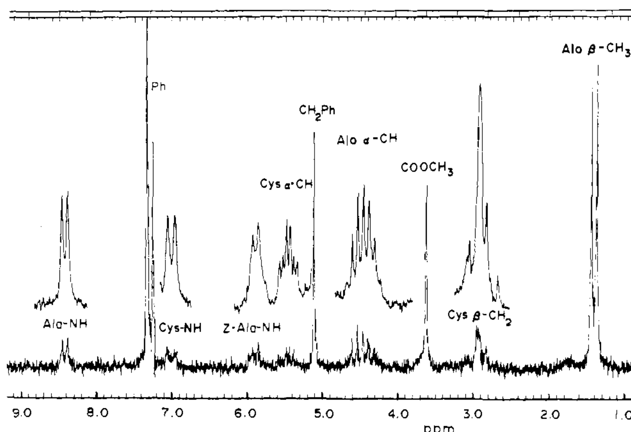


Figure 1. 100-MHz $^1$H NMR spectrum of *S,S'*-bis(Cbz-L-Ala-L-Cys-L-Ala-OMe) (I) in $CDCl_3$ solution: concentration 2.4 × 10$^{-2}$ M, 37 °C. The spectrum was recorded with a Varian XL-100 spectrometer. The inset spectra (×4 intensity) were taken by changing the radio frequency from 60 to 70 dB, the filter value from 4 to 1 Hz, and the sweep time from 500 to 1000 s, so that the noise level of the spectra was minimized.

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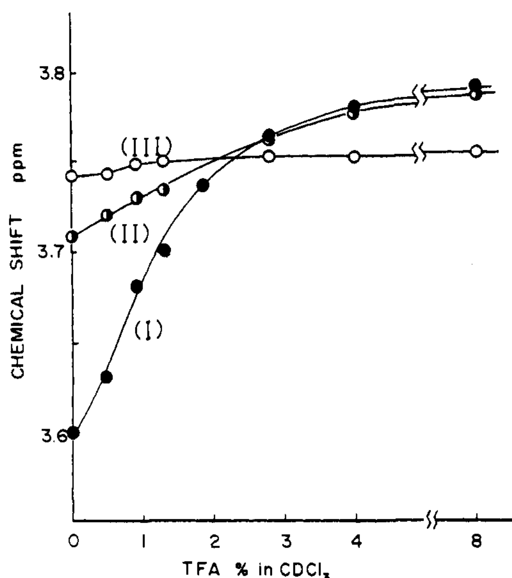


**Figure 2.** Variation of chemical shift values of the terminal methoxyl methyl proton signals as a function of TFA content in $CDCl_3$ solutions: *S,S'*-bis(Cbz-L-Ala-L-Cys-L-Ala-OMe) (I), *S,S'*-bis(Cbz-L-Ala-L-Cys-OMe) (II), and *S,S'*-bis(Cbz-L-Cys-L-Ala-OMe) (III).

**Table I.** Concentration Dependence of NMR NH-Proton Chemical Shift of I in $CDCl_3$

| Concn, M | δ, ppm MeO-Ala-NH | Cys-NH | Cbz-Ala-NH | $CHCl_3$ [a] |
|---|---|---|---|---|
| $2.4 \times 10^{-3}$ | 8.40 | 6.88 | 5.86 | 7.25 |
| $2.4 \times 10^{-2}$ | 8.43 | 7.01 | 5.89 | 7.25 |
| Δδ | 0.03 | 0.13 | 0.03 | 0.00 |

[a] Used as the internal reference.

greatly upon concentration but the two Ala-NH proton signals are much less sensitive to this change (Table I). The IR amide I band of compound I in $CHCl_3$ is observed at 1656 $cm^{-1}$, compared with 1677 and 1673 $cm^{-1}$ for II and III, indicating the presence of intramolecular hydrogen bonding in I. From these observations we can conclude that in pure $CDCl_3$ solution the Cys-NH proton is responsible for intermolecular association, and the two Ala-NH's contribute to intramolecular hydrogen bonding.

The NMR spectrum obtained in pure $CDCl_3$ shows that the methoxyl methyl proton of compound I is highly shielded compared with those of the tetrapeptides II and III (Figure 2). Addition of TFA (trifluoroacetic acid) reduces this shielding effect and the chemical shift value of the methoxyl methyl proton signal reaches a value similar to that of compound III. A less prominent shielding effect in pure $CDCl_3$ is observed for compound II, and virtually no shielding effect is present in compound III.

The temperature dependence of the methoxyl methyl proton signal is remarkable only for compound I, in which stronger shielding occurs at lower temperature (δ 3.3 ppm at −60 °C). The shielding effect would be due to the Cbz group in the neighboring peptide chain. Strong deshielding of the Cys-α-CH proton in compound I in pure $CDCl_3$ (Figure 3) indicates that two Cys-α-CH protons in both chains are located firmly in positions deshielded from the neighboring amide π system. This deshielding effect decreases drastically when >~1 mol % of TFA is added. Importantly, addition of less TFA than this amount causes no significant cleavage of the intramolecular hydrogen bonds involving C-terminal Ala-NH protons which are in closest proximity to the SS bridge as shown in Figure 4. The dihedral angle of the H-N-C-H unit

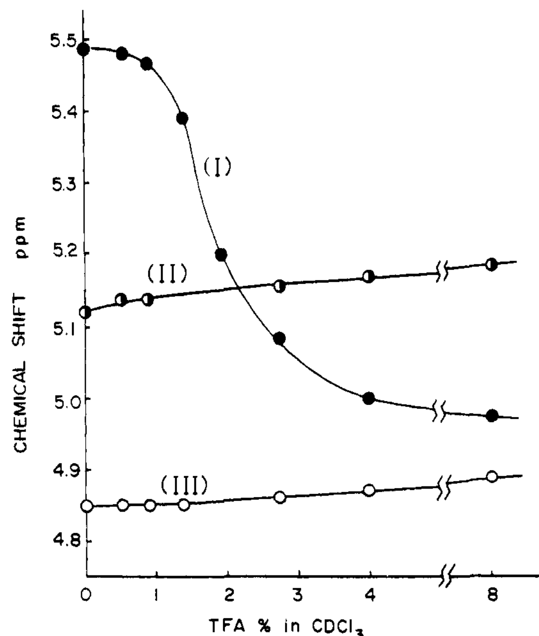


**Figure 3.** Variation of chemical shift values of Cys-α-CH proton signals as a function of TFA content in $CDCl_3$ solutions. For I, II, and III, see Figure 2.

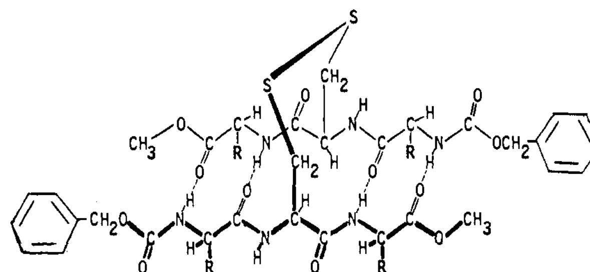

**Figure 4.** Proposed conformation of *S,S'*-bis(Cbz-L-Ala-L-Cys-L-Ala-OMe) (I) in $CDCl_3$ solution (R = $CH_3$).

in the cystine residue is estimated at ~160° when calculated from the observed coupling constant of the protons involved (9.6 Hz). This angle is reasonable for the molecular conformation illustrated.

Further support of the conformation was obtained from the CD spectra. The ellipticity of the transition at 220 nm (probably n-π*) for I is strongly solvent dependent: an intense CD band is observed in less polar solvents and is weakened by the addition of TFA. In contrast, compound II and III give only weak bands in all solvents studied.

Examination of a space-filling model based on these observations for compound I confirms the postulated structure as being highly probable. The antiparallel chain arrangement is related to the [2,7-cystine]-gramicidin S compound studied by Schwyzer et al.[5] As far as we know, compound I seems to be the first example[6] of the antiparallel stabilization of cystine-containing oligopeptide chains. We are continuing studies on the peptide conformation induced by the SS linkage.

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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

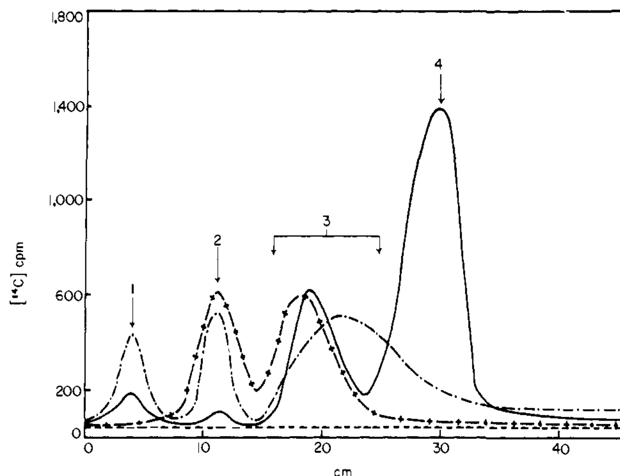



**Figure 1.** Paper chromatographic separation of the products formed in the room temperature condensation of [$^{14}$C]alanyl adenylate in aqueous 0.3 M $NaHCO_3$ buffer at pH 8.8 (-+-), in aqueous micelles at pH 8.8 (-•-), in reversed comicelles (—) and background radiation (- - -). Peaks correspond 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.

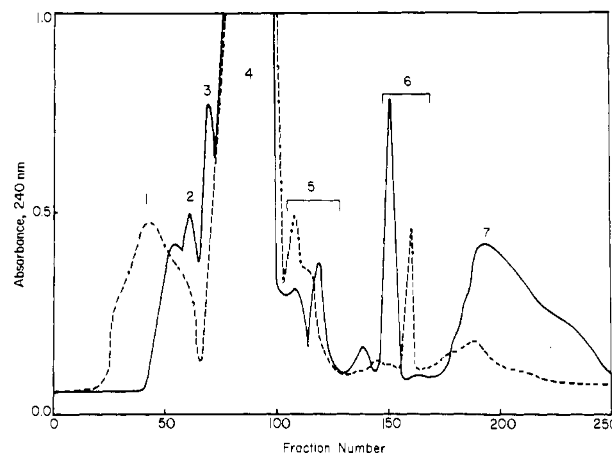


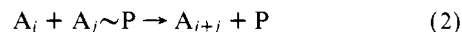

**Figure 2.** Separation of products on a Sephadex LH-50 column (2.5 m × 3.0 cm) formed from the condensation of alanyl adenylate in the presence of aqueous (—) and reversed comicelles (- - -). Peaks correspond to alanine (7), low molecular weight peptides (2, 3, and 4), AMP (also 4), high molecular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