mode is attributed completely to bond compression. 3. Again for $Fe(CN)_6^{3-/4-}$, Δa_{CN} is 0.01 ± 0.01 Å[13] vs 0.012 Å for **1**. It is worth noting that the C≡N distortion occurs at the limit of precision in the X-ray measurements,[13] but that it is rather easily detected and quantitated by postresonance Raman. We have remarked elsewhere, however, that the Raman-based Δa values could be in error by as much as +20% due to spin-orbit coupling and solvent contributions to σ^2 in eq 3.[5]

From the normal coordinate or bond distortion data, individual contributions (χ_i'; Table I) to the vibrational reorganization energy can be calculated:

$$\chi_i' = (1/2)b(\Delta a)^2 f = (1/2)\Delta^2 \nu \qquad (5)$$

From the table the following points are worth noting: 1. Both ends of the mixed-valence ion participate in vibrational trapping. 2. Nearly half of the trapping or reorganization energy comes from modes assigned to the bridging ligand. 3. Local modes remote from the metal center (i.e., C≡N stretches) can contribute measurably to the Franck-Condon energy. 4. The low-energy metal-ammine bend (which is generally overlooked) is as significant as the higher energy Ru-NH_3 stretch. It is important to realize that point 2, in particular, would be difficult to establish by any conventional X-ray structural method and that the Raman method appears, at present, to provide the only quantitative route to such information.

We intend to report shortly on a more general study of $(NC)_5M$-CN-$M'(NH_3)_5^-$ systems where M = Fe, Ru, Os and M' = Ru, Os. We also have carried out preliminary studies of symmetrical mixed-valence ions by using near infrared laser excitation. Our current effort is directed toward ion-paired metal monomers which exhibit outer-sphere charge-transfer transitions.

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(15) The comparison to $Fe(CN)_6^{3-/4-}$ may be less than completely appropriate since the extent of backbonding certainly will differ for M = Ru vs M= Fe. Unfortunately, independent structural data for $Ru(CN)_6^{3-/4-}$ are lacking.

Peptide Bond Formation at the Micellar Interface†

D. Ranganathan,* G. P. Singh, and S. Ranganathan

Department of Chemistry, Indian Institute of Technology, Kanpur 208016, India

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The highly preorganized reverse micellar system harboring water pools—formed by adding 100 mM AOT (bis(2-ethylhexyl)sodium sulfosuccinate) to isooctane containing 2% water—appeared to us as an ideal take off point to study the possibility of selective peptide bond formation.

Extensive work[1] has shown that such a micellar system can be best represented as shown in Figure 1a.

It was conjectured that a prerequisite for the peptide bond formation under nonenzymatic conditions would require the introduction of a cosurfactant harboring a carbodiimide moiety.

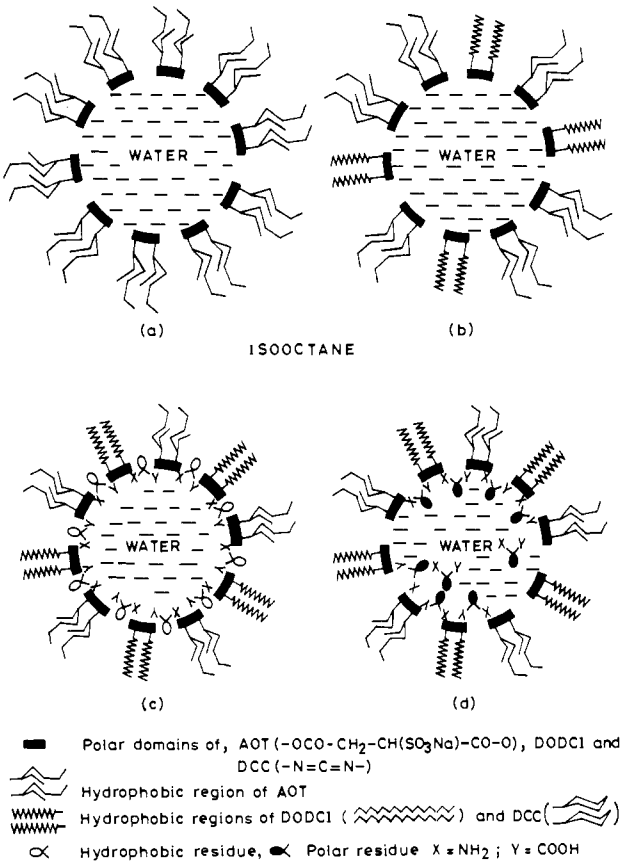

Figure 1. Schematic illustration of possible stages in the formation of peptide bond at the interface of the reverse micelle with hydrophobic and polar amino acids: (a) The highly organized reverse micellar system harboring water pools—formed by addition of 10 mM AOT to 100 mL of isooctane containing 2 mL of water; (b) The alignment of either DCC (structurally similar to AOT) or DODCI (possessing long hydrophobic tails) as cosurfactant; (c) The arrangement of hydrophobic amino acids at the micellar surface and their diffusion to the interface. Both properties promote the formation of the peptide bond; (d) The distribution of polar amino acids in the water pool. The peptide formation here is linked to the concentration in the immediate micellar environment.

Dicyclohexylcarbodiimide (DCC)[2] whose hydrophobic region bears a striking resemblance to that of AOT (Figure 1a) and the novel reagent, dioctadecylcarbodiimide (DODCI), possessing extended hydrophobic tail segments,[3] were considered appropriate for the study. It was envisaged that the introduction of either DCC or DODCI into the reverse micellar system would lead to an ordered arrangement as shown in Figure 1b. This model appeared to us to be sufficiently attractive to study the peptide bond formation from amino acids possessing hydrophobic and polar residues.

The hydrophobic amino acid residues can be expected to align at the micellar interface in proximate relationship to DODCI/DCC (Figure 1c), thus facilitating the formation of the activated ester, the key step in the formation of the peptide bond. On the other hand, amino acids carrying polar side chains would be distributed in the water pool, and therefore peptide bond formation here would be governed by the extent of their interaction with the condensing agents (DCC/DODCI) at the micellar interface (Figure 1d). Thus, based on models presented in Figure 1c and 1d, peptide bonds involving hydrophobic residues would be more favored than those with polar ones.

† This work is respectfully dedicated to Professor Sir D. H. R. Barton on the occasion of his 70th birthday.

(1) *Reverse Micelles*; Luisi, P. L., Straub, B. E., Eds.; Plenum Press: New York, 1984. Luisi, P. L. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 439. Luisi, P. L.; Magid, L. J. *CRC Critical Rev. Biochem.* **1986**, *20*, 409. Luisi, P. L., Steinmann-Hofmann, B. *Methods Enzymol.* **1987**, *136*, 188.

(2) We are most grateful to a referee for this valuable suggestion.

(3) DODCI, mp 48 °C, was prepared in 77% overall yield by the condensation of octadecylamine (20 mM) with CS_2 (10 mM) in dry EtOH to thiourea followed by HgO oxidation in CS_2 and Na_2SO_4 (anhydrous). DODCI is a useful condensing agent for peptide synthesis.

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Table I. Synthesis of Dipeptides at the Micellar Interface

$$\text{Bz-aa}_1 + \text{aa}_2\text{-OMe} \xrightarrow{\text{i}} \text{Bz-aa}_1\text{-aa}_2\text{-OMe}$$

(aa = amino acid; i. isooctane, water (2%), AOT, DODCI/DCC)

		Bz-aa$_1$-aa$_2$-OMe isolated yield %				Bz-aa$_1$-aa$_2$-OMe isolated yield %	
aa$_1$(ϕ)[a]	aa$_2$(ϕ)[a]	DODCI	DCC	aa$_1$(ϕ)[a]	aa$_2$(ϕ)[a]	DODCI	DCC
Trp (4.5)	Trp (4.5)	61	60	Leu (2.4)	Phe (3.4)	25	
Phe (3.4)	Leu (2.4)	64	67	Pro (1.8)	Phe (3.4)	51	59
Phe (3.4)	Pro (1.8)	64		Glu-γ-OMe (−2.1)	Glu-γ-OMe (−2.1)	28	
Phe (3.4)	Phe (3.4)	36					
Leu (2.4)	Leu (2.4)	40		Asp-β-OMe (−3.1)	Asp-β-OMe (−3.1)	26	

[a] ϕ: relative hydrophobicity, kcal mol^{-1} (Gly = 0); suitable corrections have been made for Glu-γ-OMe, Glu di-OMe, Asp-β-OMe, and Asp di-OMe (Von Heijne, G.; Blomberg, C. *Eur. J. Biochem.* **1979**, *97*, 175).

Table II. Selective Peptide Formation under Competitive Conditions

Set I: $\text{Bz-aa}_1 + \text{aa}_2\text{-OMe} + \text{aa}_3\text{-OMe} \xrightarrow{\text{i}} \text{Bz-aa}_1\text{-aa}_2\text{-OMe} + \text{Bz-aa}_1\text{-aa}_3\text{-OMe}$

(aa = amino acid; i. isooctane, water (2%), AOT, DODCI)

			isolated yield (%)	
aa$_1$	aa$_2$	aa$_3$	Bz-aa$_1$-aa$_2$-OMe	Bz-aa$_1$-aa$_3$-OMe
Glu-γ-OMe	Leu	Glu-γ-OMe	23	9
Asp-β-OMe	Leu	Asp-β-OMe	19	4
Leu	Leu	Glu-γ-OMe	27	13

Set II: $\text{Bz-aa}_1 + \text{Bz-aa}_2 + \text{aa}_3\text{-OMe} + \text{aa}_4\text{-OMe} \xrightarrow{\text{i}} \underset{(1,3)}{\text{Bz-aa}_1\text{-aa}_3\text{-OMe}} + \underset{(1,4)}{\text{Bz-aa}_1\text{-aa}_4\text{-OMe}} + \underset{(2,3)}{\text{Bz-aa}_2\text{-aa}_3\text{-OMe}} + \underset{(2,4)}{\text{Bz-aa}_2\text{-aa}_4\text{-OMe}}$

(aa = amino acid; i. isooctane, water (2%), AOT, DODCI/DCC)

				isolated yields (%) of							
				(1,3)		(1,4)		(2,3)		(2,4)	
aa$_1$	aa$_2$	aa$_3$	aa$_4$	DODCI	DCC	DODCI	DCC	DODCI	DCC	DODCI	DCC
Leu	Glu-γ-OMe	Leu	Glu-γ-OMe	17	19	11	16	5	6	10	12

The overall outcome of the present study,[4,5] summarized in Table I and Table II, conforms to these expectations.[6,7]

The results obtained here show for the first time the use of reverse micelles for the formation of peptide bonds under nonenzymatic conditions[8] and have potential for application in different domains.[9] The correlation between isolated yields and the rate at which the dipeptide is formed has been demonstrated. Thus, a study of the concentration variation as a function of time in the most favorable example leading to Trp-Trp peptide bond as well as the least favorable one giving rise to Glu-Glu peptide linkage has yielded, by least-squares fitting and error estimates of 95% confidence level, k_1 values,[10] respectively, 47 ± 6 and 8.8 ± 0.8 mmol^{-1} h^{-1}.

Selective peptide bond formation in a competitive environment was also explored. Two sets of competition experiments were carried out. In the simpler version, two basic residues competed for peptide bond formation via opening of activated ester formed with a common partner, and, in the more complicated case, two basic residues competed for peptide bond formation from two activated esters.[11]

The results obtained from the above study, presented in Table II, reflect, preferences based on the availability of the substrates at the micellar interface.

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(4) Blank experiments were carried out in a nonmicelle forming environment, precisely as described under ref 5, but without AOT and water. Peptide bond formation, as could be monitored by the disappearance of the α-amino esters (TLC), was poor. In two representative experiments, the resulting dipeptides were isolated by chromatography. In one of the experiments where DCC was used, another run was made without AOT but with water (2%), in a two-phase system. The results are summarized and compared below:

dipeptide	condensing agent	isolated yields(%) without (AOT + H_2O)	without AOT	with AOT + H_2O
Bz-Phe-Leu-OMe	DODCI	14		64
Bz-Trp-Trp-OMe	DCC	11		60
Bz-Trp-Trp-OMe	DCC		09	60

(5) All amino acids used were of the L configuration. In a typical experiment, 0.5 mM of N-protected amino acid was mixed with 10 mL of stock solution (prepared by adding 10 mM AOT to 100 mL of isooctane containing 2 mL of H_2O) and 0.5 mM of DODCI or DCC and stirred for 0.5 h, and the reaction mixture was added to 0.5 mM of freshly prepared amino acid methyl ester, left stirred for 2 days, mixed with 5 mL of 2 N H_2SO_4, extracted with EtOAc, organic extract washed with water, and saturated sodium bicarbonate, dried ($MgSO_4$), and evaporated; the residue on preparative TLC, using PhH:EtOAc (70:30) as eluent, afforded pure dipeptide, identical in all respects with that of an authentic sample prepared by standard procedures. Satisfactory spectral and analytical data have been obtained for all new compounds.

(6) A good correlation between the relative hydrophobicities and the yield of dipeptide obtained is evident from Table I. The yield profile of the four possible Phe/Leu dipeptides, Phe-Phe (36%), Phe-Leu (64%, 67%), Leu-Phe (25%), and Leu-Leu (40%) suggests maximum effectiveness of Phe in the activated ester formation and Leu in the generation of the peptide bond.

(7) Unlike DODCI/DCC, the water soluble 1-cyclohexyl-3(3-(dimethylamino)propyl)carbodiimide metho-*p*-toluene sulfonate cannot fit well with AOT. Use of this reagent, in place of DODCI/DCC, under conditions described in ref 5 gave neither Bz-Leu-Leu-OMe nor Bz-Glu(γ-OMe)-Glu-di OMe from appropriate precursors.

(8) Reverse micelles have been used for the enzymatic synthesis of peptides (Luthi, P.; Luisi, P. L. *J. Am. Chem. Soc.* **1984**, *106*, 7285).

(9) The present methodology would be particularly attractive for the linking of water soluble carrier proteins to hydrophobic residues (Janda, K. D.; Lerner, R. A.; Tramontano, A. *J. Am. Chem. Soc.* **1988**, *110*, 4835).

(10) Concentrations were monitored by HPLC and corroborated by isolation of products (ref 5). The k_1 values were arrived at on the basis of steady-state approximations. We thank Professor N. Sathyamurthy for suggestions.

(11) Equimolar amounts of substrates were used in all the competitive experiments and products isolated by procedure outlined in ref 5.