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Demonstration of Exclusive α-Peptidation at the Micellar Interface

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Abstract: Microreactors, dispersed in iso-octane, harboring water pools and crafted from AOT and co-surfactant condensing agent DODCI, exclusively direct α-peptidation, thus reversing the normal preference of glutamic acid and aspartic acid for side chain carboxyl group amidation.

Our continuing efforts to use crafted environments to accomplish selectivity [1] have now led to a rational, practical and the only direct strategy thus far, for the chemoselective, naturally observed, α-peptidation of coded dicarboxylic amino acids, glutamic(Glu) and aspartic(Asp) acid and arising from anticipated smooth coordination of an array of self organizing molecules that generate a defined interface. An interesting aspect of Glu and Asp is that, although directed synthesis in Nature invariably involves the peptidation of the α-COOH unit, the preference is largely reversed under *in vitro* conditions, leading to peptide bond formation involving the less sterically encumbered side chain carboxyl group[2].

The highly pre-organized reverse micellar system harboring water pools—formed by adding 100 mmol of AOT [bis(2-ethylhexyl)sodium sulfosuccinate] and 1100 mmol of water to 1L iso-octane—has been used by us for preferential peptide bond formation involving hydrophobic amino acids *via* incorporation of the novel dioctadecyl carbodiimide (DODCI) as the co-surfactant[1]. It was considered logical to use this system to realize chemoselective peptidation of Glu and Asp.

Peptide formation in the above described assembly, involving either Bz-Glu or Bz-Asp with Leu-OMe was examined[3]. Chemoselectivity was anticipated on the basis of preferential organization as illustrated in Figure 1. The presence of the benzoyl N-protecting group was anticipated to favour the alignment of the amino acid at the micellar interface. Consequently, the α-COOH unit of Glu/Asp would be more favourably disposed to form the activated ester with the proximately aligned carbodiimide grouping. Complementary placement of Leu-OMe, would promote and complete chemoselective α-peptidation leading to BzGlu/Asp (γ/β-OH)-Leu-OMe.

The model envisaged in Figure 1, functions quite well. Indeed, this reverses the normal preference for side chain carboxyl peptidation. Thus, exclusive α-peptidation, in 52% yields, resulted from Bz-Glu and Leu-OMe. Similar results were secured from Bz-Asp and Leu-OMe, leading to 60% exclusive α-peptidation. Repeats were performed on both the sets, with similar results[4].

In the absence of surfactant AOT, but otherwise all conditions unchanged, Bz-Glu/Bz-Asp was recovered, with or without water addition, as a result of insolubility. However, with DCC in place of DODCI in iso-octane admixed with water (2%) and in the absence of AOT, the reaction of Bz-Glu and Leu-OMe resulted in 14% of α-peptidation and 62% of γ-peptidation. In sharp contrast, in presence of AOT, and as envisaged in Figure 1, the corresponding yields were respectively, 64% and 10%[5,6]. We believe that crafted environments of the type described in Figure 1, have excellent practical potential[7].

The importance of the reverse micellar surface in directing chemoselectivity was revealed in an unexpected manner. With the objective of reversing the α-peptide selectivity, the apolar condensing agent DODCI was replaced with the highly water soluble, 1-cyclohexyl-3-[3-dimethyl-aminopropyl] carbodiimide metho-p-toluene sulfonate (CDMAPCI-MTS). The resulting organization would be anticipated to have a profile envisaged in figure 2. Here,the CDMAPCI - MTS, dispersed largely in water pool, would promote side chain group activation. In the case of Bz-Asp, such terminal activation can lead to either the anhydride from intramolecular cyclization or peptide bond formation with Leu-OMe. Side chain carboxyl activated Bz-Glu, would, in addition to the above two modes afford Bz-Pyro-Glu. In the event, Bz-Asp, in these environments was largely recovered. Taken in conjunction with results from use of DODCI (Figure 1), the recovery mostly arises from anhydride formation. Surprisingly, Bz-Glu, under similar conditions, in addition to the recovered product, afforded a novel dipeptide, which was subsequently identified as Bz-pyro-Glu-Leu-OMe, by comparison with authentic sample[8]. The yield of the dipeptide was a modest 5%, which, considering the path involved[9], is noteworthy.

In a broad sense the chemoselectivity of the α-peptidation observed can be attributed to directed immobilization of the substrate and the condensing agent by the micellar interface (Figure 1). From this vantage, the model resembles similar peptidation in Nature, arising from specific activation of the α-COOH unit by the preformed amino acyl transferase-ATP complex.

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FIGURE 1

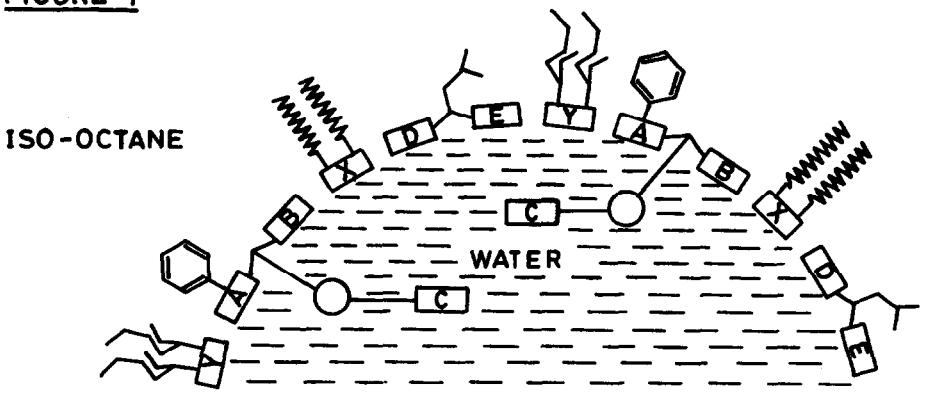
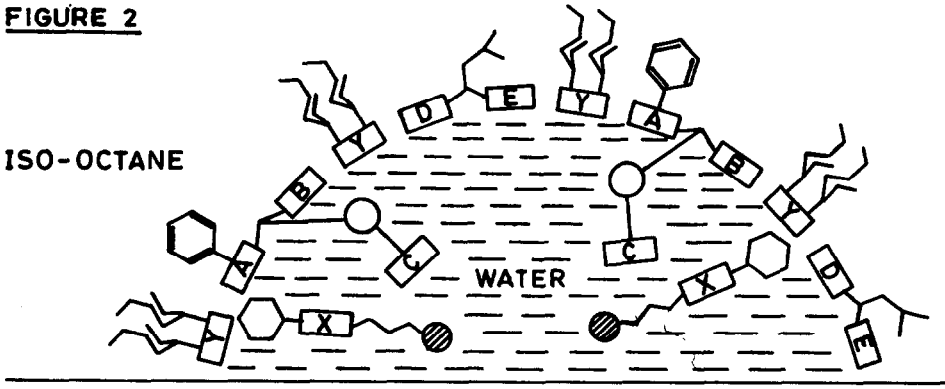


FIGURE 2



A = -CONH; B = α-COOH; C = ω-COOH = Hydrophobic region of AOT

D = $-NH_2$; E = $-COOCH_3$; X = -N=C=N-

Y = $-OCOCH_2-CH(SO_3Na)COO-$ = Hydrophobic regions of DODCI and DCC

○ = $(CH_2)_n$ n = 2 or 1; Glu: n = 2; Asp: n = 1; ● = $-\overset{+}{N}(Me)_3\bar{O}Ts$

REFERENCES AND NOTES

1.Ranganathan,D.; Singh,G.P.; Ranganathan,S. J. Am. Chem. Soc., 1989, 111, 1144.
2.Bodanszky,M.; Martinez,J. in "The Peptides: Analysis, synthesis and Biology",Eds. Gross,E.; Meinhofer,J. Academic Press, New York, Vol 5,p.111.
3.To the best of our knowledge, N-acylated Glu/Asp has not been directly used in peptide synthesis.
4. In a typical experiment, 1 mmol of N-benzoyl dicarboxylic amino acid (Glu/Asp), was mixed with 20 ml of stock solution [prepared by addition of 10 mmol of AOT to 100 ml of iso-octane, admixed with 111 mmol of water] and 1 mmol of DODCI, left stirred for 0.5h, added to 1 mmol of freshly prepared Leu-OMe and left stirred for 2d at rt. The reaction mixture was

admixed with 2N sulfuric acid (5ml), water (10ml), extracted with EtOAc (2x50ml), the organic extract washed with water, dried and evaporated in vacuo. The residue was taken up in MeOH, treated with excess ethereal diazomethane and the resulting diester purified by preparative tlc, using EtOAc:benzene as the developer.

Similar protocol was followed for blank experiments and products isolated and characterized as diesters.

Since the protected Glu/Asp-Leu dipeptides related to the present work are not reported, their identity was completly established (mp, ir, nmr, optical rotation) by comparison with independently prepared authentic samples (Please see (i) → (iv), Ref. 5).

5.Preparation of the authentic samples: Bz-Glu(γ-OMe)-Leu-OMe(i) and Bz-Asp(β-OMe)-Leu-OMe(ii) by condensation of the side chain methyl esters of Bz-Glu/Bz-Asp with Leu-OMe [DCC, HOBT; CH_2Cl_2/DMF]. BZ-Glu(α -OMe)-OH and BZ-Asp(α-OMe)-OH were prepared from Bz-Pro-OMe and Bz-Trp-OMe in respectively, 65% and 69% yields, with in situ generated Ru(VIII) (Ranganathan, S.; Ranganathan, D.; Bhattacharyya, D. J. Chem. Soc. Chem. Commun., 1987, 1085). These were condensed with Leu-OMe as above to afford, Bz-Glu(α-OMe)-Leu-OMe(iii) and Bz-Asp(α-OMe)-Leu-OMe(iv). Satisfactory elemental analysis have been obtained for compounds (i)→ (iv)

Selected data:[a. yield%; b.mp°C; c.$[\alpha]_D^{27}$; d. ^{1}H nmr (80 MHZ, $CDCl_3$) δ]: (i): a. 53; b.104-106; c.-23.74 (c 0.36,MeOH); d. 0.91(6H,d, J = 5Hz), 1.67(3H,m), 2.26(2H,m), 2.64(2H,m), 3.74(3H,s), 3.83(3H,s), 4.61(1H,m), 4.93(1H,m), 7.25-8.06(7H,m). (ii): a. 75; b. 103-104; c.-17.20 (c 0.56, MeOH); d. 0.90(6H,d,J = 5Hz), 1.64 (3H,m), 2.93(2H,m), 3.70(3H,s), 3.77 (3H,s), 4.58(1H,m), 5.13 (1H,m), 7.22-8.03(7H,m). (iii): a. 61; b.122-124; c.-10 (c 3.33,$CHCl_3$); d. 0.93(6H,d,J= 5Hz), 1.67 (3H,m), 2.35 (4H,m), 3.74(3H,s), 3.87(3H,s), 4.74(2H,m), 6.83(1H,d, J=7.5 Hz), 7.19- 8.16 6H,m).(iv): a. 55; b.149-150; c.-23.07 (c 0.09,MeOH); d.0.90(6H,d,J =5Hz), 1.62 (3H,m), 3.00(2H,m), 3.71(3H,s), 3.84(3H,s), 4.59(1H,m), 5.06 (1H,m), 6.31 (1H,d, J=7.5 Hz) 7.25-8.0(6H,m).

6.The optical rotation of compounds (i)→ (iv), prepared as above, matches well with those reported for diversely protected Glu/Asp-Leu di-peptides, in accordance with expectation for the chiral integrity in the α-amidation protocol, described in the present work: Z-Glu(γ-O^{t}Bu)-Leu-OMe,$[\alpha]_D^{22}$=-23.0 (c 2.0, EtOH)$_{23}$ (Jager,G.; Konig,W.; Wissmann,H.; Geiger,R. Chem.Ber. 1974, 107,215): $[\alpha]_D^{23}$= -27.5 (c 1.02, MeOH) (Sarasua,M.M.; Scott,M.E.; Helpern, J.A.; Ten Kortenaar,P.B.W.; Boggs III,N.T.; Pedersen,L.G.; Koehler,K.A.; Hiskey, R.G. J.Am.Chem.Soc., 1980, **102**, 3404). Boc-Glu(γ-OBzl)-Leu-OMe, $[\alpha]_D^{26}$=-29.2 (c 1.03, MeOH) (Yanaihara,N.; Kubota,M.; Sakagami,M.; Sato,H.; Mochizuki, T.; Sakura,N.; Hashimoto,T.; Yanaihara,C. J.Med.Chem.,1977, 20, 648). Z-Glu(α-OBzl)-Leu-OEt, $[\alpha]_D^{20}$= -9.46 (c 1.06, THF) (Barth,A. Ann. Chem.,1965,686,221). Z-Asp(β-OBzl)-Leu-OBzl, $[\alpha]_D^{19}$=-22.5 (c 1.38,acetone), -29.8 (c 1.41,EtOH) (Bryant,P.M.; Moore,R.H.; Pimlott,P.J.; Young,G.T. J.Chem.Soc., 1959,3868)

7.For an excellent account of organic molecules that operate collectively, see Menger, F.M. Angew. Chem. Int., 1991, **30**, 1086.

8 Bz-pyro-Glu-Leu-OMe was prepared by condensation of pyro-Glu with Leu-OMe (DCC,HOBT,DMF/CH_2Cl_2) followed by acylation (BzCl in pyridine; usual procedures in aqueous medium led to ring opening); yield 60% mp 135-136° C; $[\alpha]_D^{28}$= -4.08 (c 3.3,$CHCl_3$); ms: m/z 360(M)$^+$; ^{1}H nmr (80 MHZ, $CDCl_3$ δ: 0.87 (6H,d, J = 5Hz), 1.56 (3H,m), 2.06-3.0(4H.m), 3.68(3H,s),4.65 (2H,m), 6.25 (1H,d, J=7.5 Hz) 7.25-7.62 (6H,m).

9.Activation of both the α and γ carboxyls are required for the formation ofBz-pyro-Glu-Leu-OMe.

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