Chiral Selectivity in Elementary Peptide–Peptide Interactions

G. Michael Blackburn, Terence H. Lilley, and Peter J. Milburn

Department of Chemistry, The University, Sheffield, S3 7HF, U.K.

Measurement of the heats of dilution of aqueous solutions of uncharged amide derivatives of some amino acids and dipeptides at 298.15 K reveals that chiral recognition is energetically significant for diastereoisomeric, pairwise interactions involving monomer–monomer, monomer–dimer, and dimer–dimer species containing L- or D-alanine.

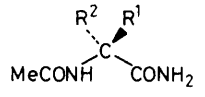
Chirality is one molecular characteristic which is a most definite index of life.[1] The general existence of molecular dis-symmetry, first recognised by Pasteur[2] in stereohomogeneous form, could in principle have resulted from the pre-biotic formation of optically-active molecules with one form in enantiomeric excess, *e.g.* by the influence of magnetism or polarised light,[3] or be a consequence of the non-conservation of parity.[1,4,5] These factors are easily over-exaggerated and, especially in view of the modest spontaneous rate of racemisation of α-amino acids *in aquo*,[6] it can be supposed that the pre-biotic soup was essentially optically inactive.[7] Consequently, this dis-symmetry must have grown out of chiral recognition between molecular species at a primitive stage of evolution, probably at the level of organised polymers, a scene dominated by the α-amino acids.

Hitherto, a degree of stereoselectivity has been identified in systems pairing α-amino acids with other species. Sheardy and Gabbay[8] have shown that lysyl-dipeptides containing a *C*-terminal aromatic amino acid exhibit stereoselective intercalative association with DNA preferential for the L,L-isomer. Chiral synthesis in the reduction of ethyl benzoylformate has been demonstrated by Endo *et al.*[9] through the use of mono- and di-peptide amides of dihydronicotinamide. Yet, notwithstanding demonstrations of chiral recognition between amino acid species in non-aqueous media,[10,11] attempts to identify stereoselective interactions between zwitterionic dipeptides *in aquo* have so far failed.[12,13] We therefore decided to extend our studies on the energetics of pairwise interactions of amino acids and peptides[14–17] in aqueous solution to a differential analysis of species containing either D- or L-alanine residues in an attempt to secure an experimental basis for chiral recognition in α-amino acid systems.

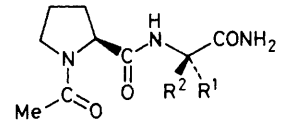
We elected to measure the enthalpies of dilution for solutions of isomeric combinations of mono- and di-peptide species. Such data lead to the excess enthalpic properties[14] of three principal types of system; (i) monomer–monomer, (ii) monomer–dimer, and (iii) dimer–dimer interactions. We inter-related these through the alternate use of D- and L-alanine moieties, a function we selected partly as a result of early manifestations of significant co-operativity in the pairwise interactions of L-alanine peptides.[15,16] We excluded coulombic effects by the use of *N*-terminal acetyl and *C*-terminal amide derivatives for monomers and dipeptides alike.† The data (Table 1), obtained by standard methods,[14,16] clearly demonstrate stereoselectivities in interactions within each of the three groups examined.

At the monomer level, the excess enthalpies of homotactic‡ interactions of Ac-L-AlaNH$_2$ (*S*-**1**) and Ac-D-AlaNH$_2$ (*R*-**1**) are sensibly identical, as is expected for enantiomeric pairings in an experiment devoid of dis-symmetric perturbation. The heterotactic interaction of the (*R*-**1**)–(*S*-**1**) pair significantly differs from the homotactic interaction, by some 60 J kg mol^{-2}, as is expected for a diastereoisomeric pairing. This result establishes that our experimental method *can* detect stereoselective interactions between D- and L-peptide monomers and contrasts with the results of Hedwig's group,[12] at least when coulombic effects are suppressed.

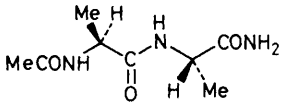
The heterotactic interactions between mono- and di-peptide species are of two types. Dipeptides which are constitutively diastereoisomeric form diastereoisomeric interaction pairs with a single chiral monomer. The interactions of (*S*,*S*-**2**) and (*S*,*R*-**2**) with (*S*-**1**) are related in this way and show a rather smaller energy difference between the two heterotactic



(*S*-**1**) R^1 = Me, R^2 = H
(*R*-**1**) R^1 = H, R^2 = Me



(*S*,*S*-**2**) R^1 = Me, R^2 = H
(*S*,*R*-**2**) R^1 = H, R^2 = Me



(*S*,*S*-**3**)

Table 1. Pairwise enthalpy of interaction parameters for peptide systems relating L-alanyl and D-alanyl residues.

Solute A	Solute B		h_{AB}[a] /J kg mol^{-2}
Ac-L-AlaNH$_2$ (*S*-**1**)	Ac-L-AlaNH$_2$ (*S*-**1**)	Homotactic	269 (5)[b]
Ac-D-AlaNH$_2$ (*R*-**1**)	Ac-D-AlaNH$_2$ (*R*-**1**)	Homotactic	277 (5)
Ac-D-AlaNH$_2$ (*R*-**1**)	Ac-L-AlaNH$_2$ (*S*-**1**)	Heterotactic	337 (10)
Ac-L-Ala-L-AlaNH$_2$ (*S*,*S*-**3**)	Ac-L-AlaNH$_2$ (*S*-**1**)	Heterotactic	488 (17)
Ac-L-Ala-L-AlaNH$_2$ (*S*,*S*-**3**)	Ac-D-AlaNH$_2$ (*R*-**1**)	Heterotactic	578 (9)
Ac-L-Pro-L-AlaNH$_2$ (*S*,*S*-**2**)	Ac-L-AlaNH$_2$ (*S*-**1**)	Heterotactic	769 (49)
Ac-L-Pro-L-AlaNH$_2$ (*S*,*S*-**2**)	Ac-D-AlaNH$_2$ (*R*-**1**)	Heterotactic	748 (16)
Ac-L-Pro-D-AlaNH$_2$ (*S*,*R*-**2**)	Ac-L-AlaNH$_2$ (*S*-**1**)	Heterotactic	803 (22)
Ac-L-Pro-L-AlaNH$_2$ (*S*,*S*-**2**)	Ac-L-Pro-L-AlaNH$_2$ (*S*,*S*-**2**)	Homotactic	1629 (24)
Ac-L-Pro-D-AlaNH$_2$ (*S*,*R*-**2**)	Ac-L-Pro-D-AlaNH$_2$ (*S*,*R*-**2**)	Homotactic	1399 (8)
Ac-L-Pro-L-AlaNH$_2$ (*S*,*S*-**2**)	Ac-L-Pro-D-AlaNH$_2$ (*S*,*R*-**2**)	Heterotactic	1515 (43)

[a] Figures in parentheses give the standard deviations of the fits to experimental data. [b] Taken from ref. 14.

† All compounds described were prepared (ref. 18) by standard synthetic methods and their structure, purity, and optical purity fully established by spectroscopic and analytical methods.

‡ Homotactic interactions involve like–like solute pairs while heterotactic interactions involve like–unlike solute pairs.

interactions, some 34 J kg mol^{-2}, than that for the Ala–Ala interactions.

The interaction of a single dipeptide with a pair of enantiomeric monomers, *e.g.* (*S,S*-**3**) with (*R*-**1**) and (*S*-**1**), constitutes a second class of heterotactic interaction between dimers and monomers. Our data show a clear difference between the energies of interaction of Ac–L-Ala–L-AlaNH$_2$ (*S,S*-**3**) with (*R*-**1**) and (*S*-**1**), some 90 J kg mol^{-2}, although there seems to be no significant difference between the interactions of these monomers with Ac–L-Pro–L-AlaNH$_2$, (*S,S*-**2**).

Finally, the dipeptide derivatives Ac–L-Pro–L-AlaNH$_2$ and Ac–L-Pro–D-AlaNH$_2$ are a pair of diastereoisomers whose energies of interaction show both a sizeable difference (230 J kg mol^{-2}) between the two homotactic interactions, and also a heterotactic interaction which, as a third diastereoisomeric encounter, is manifest in a unique value for the excess enthalpy parameter, h_{AB} (Table 1).

All of the stereochemical effects described above operate at a level which, like those of sequence dependence (which will be discussed elsewhere[19]), is below that where a group additivity analysis[14,16,17] could become applicable. For this reason, and because enthalpy–entropy compensation has been shown to render insensitive the free energy properties of isomeric systems to changes in molecular geometry,[10] we have for the present confined our studies to excess enthalpy measurements.

Three conclusions emerge. First, in the absence of coulombic interaction terms, differences in energetics of interaction between stereoisomeric peptide species in aqueous solution at ambient temperature can be surely identified. Secondly, these appear to grow larger with the number of chiral centres in the interacting system. Thirdly, there is no general trend at the level of complexity we have studied to indicate an energetic convergence favouring pairwise interactions within one family (D- or L-) of α-amino acids rather than those for mixed systems which incorporate α-amino acid residues of both chiral families.

We thank the S.E.R.C. for provision of a C.A.S.E. Research Studentship (to P. J. M.) and Dr C. H. Hassall, Roche Products Research Ltd., Welwyn Garden City, for valuable support.

Received, 30th November 1984; Com. 1687

References

1 J. D. Bernal, 'The Origins of Life,' Weidenfeld and Nicholson, London, 1967, p. 144.
2 L. Pasteur, 'Recherches sur la Dissymétric Moléculaire, Oeuvres I,' 1860, 314.
3 T. L. V. Ulbricht, *Quart. Rev.*, 1959, **13**, 48.
4 S. F. Mason and G. E. Tranter, *J. Chem. Soc., Chem. Commun.*, 1983, 117.
5 S. F. Mason, *New Scientist*, 1984, No. 1393, 10.
6 L. E. Orgel, 'The Origins of Life,' Chapman and Hall, London, 1973, p. 162.
7 J. L. Bada, 'Non-equilibrium Systems in Natural Water Chemistry,' ed. J. D. Hein, Am. Chem. Soc., Washington D.C., *Adv. Chem. Ser.*, 1971, **106**, 309.
8 R. D. Sheardy and E. J. Gabbay, *Biochemistry*, 1983, **22**, 2061.
9 T. Endo, Y. Hayashi, and M. Okuwara, *Chem. Lett.*, 1977, 391; T. Endo, H. Kawasaki, and M. Okuwara, *Tetrahedron Lett.*, 1979, **20**, 23.
10 M. T. Cung, M. Marraud, and J. Neel, *Biopolymers*, 1976, **15**, 2081.
11 A. Dobahi and S. Hara, *Tetrahedron Lett.*, 1983, **24**, 1509.
12 M. K. Kumaran, G. R. Hedwig, and I. D. Watson, *J. Chem. Thermodyn.*, 1982, **14**, 93.
13 *cf.* E. J. Cohn and J. T, Edsall, 'Proteins, Amino Acids, and Peptides,' Reinhold, New York, 1943, p. 194.
14 G. M. Blackburn, T. H. Lilley, and E. Walmsley, *J. Chem. Soc., Faraday Trans. 1*, 1980, **76**, 915.
15 G. M. Blackburn, T. H. Lilley, and E. Walmsley, *J. Chem. Soc., Chem. Commun.*, 1980, 1091.
16 G. M. Blackburn, T. H. Lilley, and E. Walmsley, *J. Chem. Soc., Faraday Trans. 1*, 1982, **78**, 1641.
17 G. M. Blackburn, T. H. Lilley, and P. J. Milburn, *J. Chem. Soc., Faraday Trans. 1*, submitted for publication.
18 P. J. Milburn, Ph.D. Thesis, Sheffield University, 1984.
19 G. M. Blackburn, H. E. Kent, T. H. Lilley, and P. J. Milburn, unpublished work.