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ORIGINS OF THERMODYNAMIC STEREOSELECTIVITY IN THE PROTONATION OF SOME DIPEPTIDES

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ABSTRACT

ΔH^0 and ΔS^0 protonation values of some couples of diastereoisomeric dipeptides have been determined by calorimetry in aqueous solution at 25°C and $I = 0.1$ mol dm^{-3} (KNO_3). The obtained data have allowed the role played by non-covalent interactions on the thermodynamic stereoselectivity in the investigated systems to be ascertained.

INTRODUCTION

Solvophobic [1] or hydrophobic [2,3] interactions are known to occur in biomolecules and to contribute to the formation of distinct structural conformations, which provide the specificity required in most biological processes [4]. These interactions occur between two aliphatic or alicyclic groups, between two aromatic groups, and between aliphatic and aromatic groups.

Recently, the stereoselectivity in the proton complex formation of LL or LD pairs of dipeptides has been interpreted as resulting from differences in the ease of folding of the two diastereoisomers [5]. Nakon and Angelici [6] introduced the concept of a hydrophobic "bond" to account for the increasing stereoselectivity with increasing size of the side chain. Kaneda and Martell [7] observed similar effects in the stereoselectivity of two diastereoisomeric dipeptides. Moreover, they gave prominence to their results on the basis of a conformational analysis carried out with the aid of molecular models.

Bearing in mind that the use of the hydrophobic bonding concept "to account for the observed trends in pK_a values must be regarded as tentative" [6] and considering the perplexity advanced by Pettit and Hefford [8] on Kaneda and Martell's approach [7], we decided to investigate the origins of the stereoselectivity in the protonation of dipeptides by means of calorimetric techniques.

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ΔH^0 and ΔS^0 values can be used to recognize the presence of interligand solvophobic interactions between two aromatic or heteroaromatic groups of biofunctional molecules coordinated to metal ions [9–11].

We report here the enthalpy and entropy changes at 25°C and $I = 0.1$ mol dm^{-3} (K NO_3) of the proton complex formation of L,L(pure) and D,L(mixed) diastereoisomers of alanylananine(Ala-Ala), leucylleucine(Leu-Leu) and leucyltyrosine(Leu-Tyr), starting from the pertinent ΔG^0 values obtained under the same experimental conditions [6]. This approach to the problem is very useful in order to demonstrate the role of the solvophobic forces in the stereoselectivity of dipeptide protonation.

EXPERIMENTAL

All the dipeptides were obtained from Serva (Heidelberg, F.R.G.) except the leucylleucine diastereoisomers which were purchased from Sigma (Munich, F.R.G.). The sample solutions were prepared from the dipeptides after dehydration over phosphorous pentoxide in a vacuum desiccator. All peptides were found to be at least 99.9% pure on the basis of potentiometric measurements using the experimental procedure described elsewhere [12]. Moreover, in order to ascertain that hydrolytic reactions did not occur, the purity of the investigated dipeptides was checked before, during and at the end of each titration, by thin-layer chromatography on pre-coated Merck (Darmstadt, F.R.G.) cellulose or silica-gel plates. The following solvent systems were used: (1) BAW, *n*-butanol–acetic acid–water (12 : 3 : 5); (2) PhW, phenol–water (3 : 1). Chromogenic reagents were: (a) 0.1% ninhydrin in acetone; (b) 0.2% isatin in acetone.

The calorimetric measurements were carried out at 25 ± 0.001°C with a LKB 8700 precision calorimeter or a Tronac 550 apparatus. In the latter case the enthalpies of protonation were obtained using the continuous titration

TABLE 1

Experimental details of calorimetric measurements at 25°C and $I = 0.1$ mol dm^{-3} (KNO_3)

L	Titration vessel [a] C_L	Titrant C_{HNO_3}(mol dm^{-3})	No. of titrations	pH [b]
L-Ala-L-Ala	2.0–5.0	0.3623, 0.2980	9	8.5–2.3
L-Ala-D-Ala	2.2–5.0	0.3623, 0.2980	8	8.6–2.3
L-Leu-L-Leu	2.1–5.0	0.3623, 0.2980	8	8.1–2.4
L-Leu-D-Leu	2.0–5.0	0.3623, 0.2980	7	8.3–2.5
L-Leu-L-Tyr	0.8–1.6	0.3623, 0.2980	11	8.4–2.4
L-Leu-D-Tyr	0.9–1.6	0.3623, 0.2980	12	8.5–2.3

[a] Concentrations in mmol dm^{-3}

[b] Adjusted by adding KOH.

calorimetric method [13]. The results obtained by means of incremental (LKB) or continuous (Tronac) titrations were identical within experimental error. Experimental details are reported in Table 1. The dipeptide concentrations ranged from 2 mmol dm^{-3} to 5 mmol dm^{-3} except for Leu-Tyr, where, owing to its low solubility, the concentration ranged from 0.8 to 1.6 mmol dm^{-3}. The accuracy of the calorimetric equipment was checked by the ΔH_w^0 determination; the found value of 13.5 kcal mol^{-1} (1 cal = 4.184 J) is in agreement with the accepted value [14–17].

Computations pertinent to the purity of the dipeptides, E^0 determination, and calculations of the concentrations of the standard solutions were made by means of the ACBA least-squares computer program [18], which refines all the parameters of an acid–base titration. The enthalpies of protonation were determined by means of the DOEC least-squares program [19].

Throughout the paper the uncertainties of the thermodynamic parameters are expressed as $\pm 3\sigma$. Other details are as reported previously [20].

RESULTS AND DISCUSSION

ΔG^0, ΔH^0 and ΔS^0 values for the protonation of dipeptides are reported in Table 2. As can be seen, the protonation of the amine group is favoured on enthalpy grounds, while that of the carboxylate only reveals a favourable entropy contribution. The overall negative ΔH^0 value of amine protonation is due to several factors; in particular, there is an exothermic contribution due to the nitrogen–hydrogen bond formation that is larger than the endothermic desolvation contributions of both the amine group and the H_3O^+ ion. The positive contribution of ΔS^0 in the case of the carboxylate oxygen takes place from the desolvation processes of the anion as well as of H_3O^+ and from the charge neutralization of the resulting protonated species. In the case of the protonation of the amine group the solvation process

TABLE 2

Thermodynamic parameters of proton complex formation of some diastereoisomeric dipeptides at 25°C and I = 0.10 mol dm^{-3} (standard deviation (3σ) in parentheses)

System	$-\Delta G^0$		$-\Delta H^0$		ΔS^0	
	(NH_2)	(CO_2^-)	(NH_2)	(CO_2^-)	(NH_2)	(CO_2^-)
L-Ala-L-Ala	11.14	4.50	10.64(6)	−0.26(8)	1.7(2)	15.9(3)
L-Ala-D-Ala	11.34	4.34	10.26(7)	−0.63(9)	3.6(2)	16.7(3)
L-Leu-L-Leu	10.78	4.71	10.21(7)	−0.36(9)	1.9(2)	17.0(3)
L-Leu-D-Leu	11.18	4.16	10.92(5)	−0.77(6)	0.88(16)	16.5(2)
L-Leu-L-Tyr	10.68	4.41	10.43(8)	−0.0(1)	1.0(3)	14.6(4)
L-Leu-D-Tyr	11.32	4.03	11.15(7)	−0.1(1)	0.6(2)	13.9(3)

ΔG^0 and ΔH^0 in kcal mol^{-1}; ΔS^0 in cal mol^{-1} deg^{-1} (1 cal = 4.184 J).

occurs more easily than for that of the carboxylate one, and this accounts for the less favourable entropy contribution. Analogously, the several processes of desolvation and consequent cleavage of bonds with solvent explain the resulting endothermic contribution of the carboxylate protonation.

Although the above considerations allowed correct interpretation of the general trend of thermodynamic data concerning the proton complex formation of the dipeptides reported here, there are still some points which need clarification. In particular, we shall discuss the difference in the thermodynamic parameters between the diastereoisomers of each couple, as well as the different behaviour of the Ala-Ala system with respect to the others. As regards this latter point it must be pointed out that the higher stability of the L,D derivative is due mainly to a more positive entropy contribution, while for the other two dipeptides the order is reversed, i.e., the "mixed" derivative is favoured by a more negative enthalpy change.

The discussion of the obtained thermodynamic parameters which follows is based on the assumption that the investigated dipeptides are in a β-type conformation in their acidic, neutral and basic species. In such a β-conformation, both the α-H bonds lie in the same plane of the amide bond. This assumption is justified on the basis, among other evidence [21,22], of NMR results [23], for the alanylphenylalanine and phenylalanylalanine diastereoisomer couples and for glycyl-L-phenylalanine. As a consequence of the β-conformation, the L,D-dipeptide has a shorter end-to-end distance than the L,L-dipeptide.

In the case of L-Ala-D-Ala the protonation of the amine group is favoured by the electrostatic interaction between the NH_3^+ and COO^- group (see Fig. 1). Consequently the degree of neutralization of the overall charge is greater than that occurring in the case of the L,L-derivative. The greater desolvation of the protonated amine group gives rise not only to a more positive entropy contribution, but also to a lower enthalpy change, due to cleavage of solvent bonds, which is not balanced by the NH_3^+ $-COO^-$ electrostatic interaction. Thus in this case the stereoselectivity is due to a gain in entropy because of the conformation in these peptides.

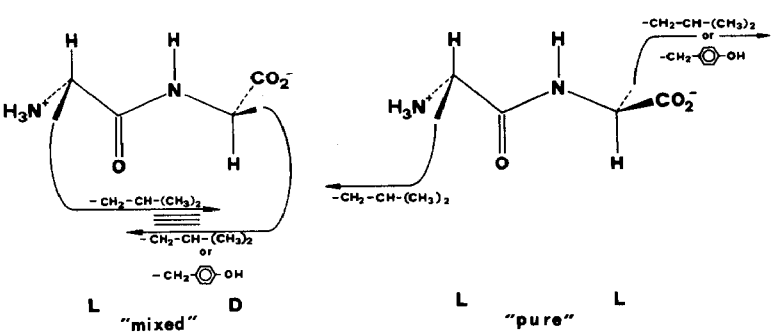

Fig. 1. Hypothesized structures of L,L- and L,D-dipeptides.

As regards the other two dipeptides, unlike the Ala-Ala system, we can observe an enthalpy stabilization of the L,D- with respect to the L,L-derivative (see Table 1). We consider that the greater enthalpy stabilization is due to the presence of a solvophobic interaction in the L,D-derivative. In fact, in the "mixed" isomer the large side-chains of this dipeptide lie on the same side of the molecule and therefore may interact with each other and with the amide group, unlike in the "pure" isomer where they point in opposite directions.

Calorimetric studies [9–11,24] have shown that solvophobic interactions are favoured on enthalpy grounds. Hence for the Leu-Tyr and Leu-Leu couples the stereoselectivity is probably due not only to the favourable electrostatic interaction, such as is found in the Ala-Ala system, but also to the presence of this "secondary bond".

The occurrence of such solvophobic interaction in the L,D-dipeptides, possible in all protonation states, becomes more effective and stronger in the ampholytic state, owing to the electrostatic interaction that should make the molecule more rigid and the side-chain groups closer.

Consequently, in the "mixed" isomer the neutral species will be stabilized favouring the protonation of the amine group and disfavouring that of the carboxylate group compared with the "pure" isomer.

The non-relevant exothermic difference between the pairs of diastereoisomers results from the negative enthalpy contribution of the solvophobic interaction, which is partially counterbalanced by the positive contribution of the electrostatic interaction and which must be considered larger than that present in the case of L,D-alanylalanine.

Hence it is possible to state that:

(1) only taking into account the β-conformation as resulting from experimental evidence, it is possible to obtain a non-misleading rationalization of the ΔH^0 values of protonation;
(2) the solvophobic interaction plays a determining role in the thermodynamic stereoselectivity. This is in agreement with what was recently suggested on the basis of ΔG^0 values only [6]; and
(3) our experimental results show that the solvophobic forces are stabilizing on enthalpy grounds but not on entropy grounds, in agreement with the view of Sinanoglu [1], but in disagreement with the theoretical results of other authors [25].

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