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INFLUENCE OF NON-COVALENT INTERACTIONS ON THE THERMODYNAMIC STEREOSELECTIVITY OF THE PROTONATION OF SOME DIPEPTIDES

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ABSTRACT

ΔG^0, ΔH^0 and ΔS^0 protonation values of some pairs of diastereoisomeric dipeptides have been determined by potentiometry and calorimetry in aqueous solution at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3). On the basis of the results obtained it has been possible to assess the role played by two different non-covalent interactions, namely the electrostatic interaction and the solvophobic interaction, on the thermodynamic stereoselectivity in the proton complex formation, shown by the systems investigated.

INTRODUCTION

Non-covalent interactions, namely hydrogen bonds, electrostatic interactions, hydrophobic or stacking forces, have been invoked mainly as a "key to biological flexibility and specificity" [1].

It has been shown that these intramolecular interactions have a determining role in: (1) typical bimolecular chemical reactions of small molecules in aqueous solution [2]; (2) resolution of D- and L-amino acids by HPCL [3,4]; (3) molecular complexes of drugs [5]; (4) fading reaction of dyes [6]; (5) stereospecific reactions of the hydrolysis of phenyl esters [7].

Recently, it has been shown that ΔH^0 and ΔS^0 values can be used to recognize the presence of intraligand solvophobic [8] (or, according to more classical denominations, hydrophobic or stacking [9,10]) interactions between two aromatic or heteroaromatic groups of biofunctional molecules coordinated to metal ions [11,12].

The thermodynamic approach has also been used in order to evaluate the role played by the electrostatic and the solvophobic interactions, respectively, in the stereoselectivity of the proton complex formation of the L,L-dipeptide with respect to its L,D-diastereoisomer [13].

Here, the ΔG^0, ΔH^0 and ΔS^0 values of the protonation reaction of "pure" and "mixed" diastereoisomers of alanylphenylalanine (Ala-Phe), alanylleu-

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cine (Ala-Leu) and leucylphenylalanine (Leu-Phe) at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3) are reported.

The aim is to obtain further insight into the influence of side chains of different size on the thermodynamic stereoselectivity.

EXPERIMENTAL

L-Alanyl-L-phenylalanine, L-alanyl-L-leucine and D-alanyl-L-leucine were Sigma (Munich) products, while L-leucyl-L-phenylalanine was a Serva (Heidelberg) product. L-Alanyl-D-phenylalanine and L-leucyl-D-phenylalanine were synthesized by the method reported elsewhere [14].

The sample solutions were prepared from the dipeptides after dehydration over phosphorous pentoxide in a vacuum desiccator. All the peptides were found to be at least 99.9% pure on the basis of potentiometric measurements using an experimental procedure described elsewhere [15]. Potentiometric measurements were carried out using an Orion 801 A meter equipped with an EIL glass and an Ingold saturated calomel electrode, the potentiometer being connected with an Amel timer-printer (model 882) controlling the addition of titrant delivered from an Amel digital dispenser (model 232), with the titrations thus being performed automatically. The electrode couple was standardized on the $pH \equiv -\log c_{H^+}$ scale by titrating HNO_3 (0.01–0.005 mol dm^{-3}) with KOH at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3). Other details are as previously reported [16].

The calorimetric measurements were carried out with a Tronac 550 apparatus, using the continuous titration calorimetric method [17]. The accuracy of the calorimetric equipment was checked by the ΔH_w^0 determination; the value found was 13.48 kcal mol^{-1}, which is in agreement with the accepted value [18–21].

Calculations pertinent to the electrode checks, the purity of the peptides and the protonation constants were performed by means of the least-squares computer program ACBA [22]. The enthalpies of protonation were determined by means of the DOEC least-squares program [23].

Throughout this paper the uncertainties of the thermodynamic parameters are expressed as $\pm 3\sigma$. Other details are as previously reported [13].

RESULTS AND DISCUSSION

ΔG^0, ΔH^0 and ΔS^0 values for the protonation of the dipeptides studied here are reported in Table 1 together with the data concerning other previously studied dipeptides [13], given for comparison.

From the above results it can be seen that the ΔG^0 values concerning the protonation of an amine group are always more negative for the L,D-peptides

TABLE 1

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

System	$-\Delta G^0$ (kcal mol^{-1})		$-\Delta H^0$ (kcal mol^{-1})		ΔS^0 (cal mol^{-1} K^{-1})	
	(NH_2)	(CO_2^-)	(NH_2)	(CO_2^-)	(NH_2)	(CO_2^-)
L-Ala-L-Phe	10.820(4)	4.275(4)	10.51(4)	−0.05(6)	1.0(1)	14.5(2)
L-Ala-D-Phe	11.165(4)	4.029(4)	10.64(5)	−0.39(6)	1.8(1)	14.6(2)
L-Ala-L-Leu	10.939(3)	4.557(3)	10.68(6)	−0.24(8)	0.9(2)	16.1(3)
D-Ala-L-Leu	11.246(4)	4.261(4)	10.58(7)	−0.77(7)	2.2(2)	16.9(2)
L-Leu-L-Phe	10.495(4)	4.342(4)	10.42(5)	−0.08(6)	0.3(2)	14.3(2)
L-Leu-D-Phe	11.114(4)	3.940(4)	11.0(1)	−0.1(2)	0.3(4)	13.4(6)
L-Ala-L-Ala [13]	11.14	4.50	10.64	−0.26	1.7	15.9
L-Ala-D-Ala [13]	11.34	4.34	10.26	−0.63	3.6	16.7
L-Leu-L-Tyr [13]	10.68	4.41	10.43	−0.0	1.0	14.6
L-Leu-D-Tyr [13]	11.32	4.03	11.15	−0.1	0.6	13.9

compared to those for the corresponding L,L-isomers. The opposite trend is shown in the protonation of a carboxylic group. This behaviour has been observed in many pairs of dipeptide diastereoisomers [13,24], and this stereoselectivity increases as the size of the side chain increases [25].

Our systems show this behaviour: in fact the difference in the ΔG^0 values is a maximum in the case of Leu-Phe dipeptides.

However, this trend is not followed by the separate ΔH^0 and ΔS^0 contributions. In particular, as regards the protonation of the amine group, the ΔH^0 is more negative for the L,D- than the L,L-Leu-Phe diastereoisomer, opposite to that observed for the two diastereoisomers of Ala-Ala (alanyl-alanine; Table 1).

On the basis of the β-conformation [26], that constrains the COO^- and NH_3^+ groups on the same side of the molecule of the L,D-diastereoisomers, it was possible to explain the thermodynamic quantities of Ala-Ala dipeptides as only due to the stronger electrostatic interaction present in the L,D- with respect to the L,L-diastereoisomers [13] since it was known [27–29] that peptides containing some D-amino acids show a shorter end-to-end distance than do all L-peptides.

To explain the opposite behaviour exhibited by the two Leu-Phe dipeptides, it is necessary to invoke a second interaction, namely a solvophobic interaction between the isobutyl and the phenyl side chains, that in the L,D-diastereoisomer lie on the same side of the molecule. Thus, the behaviour of the two Leu-Phe dipeptides is similar to that exhibited by the Leu-Tyr (leucyltyrosine) diastereoisomeric pair [13]. In these cases, the differences obtained in the thermodynamic parameters, in particular in ΔH^0 values between each pair of diastereoisomers, should be interpreted as resulting from the algebraic sum of the contribution due to the solvophobic interaction and the contribution, of the opposite sign, due to the electrostatic interaction.

While in the Leu-Phe system, the solvophobic interaction certainly prevails and the stereoselectivity appears to be enthalpically driven, as regards the other two couples of dipeptides the behaviour is less straightforward.

In the Ala-Phe systems, a higher enthalpy contribution in the protonation of the amine group of the L,D-diastereoisomer is observed, like in the cases of Leu-Phe and Leu-Tyr. However, in this case the difference is far smaller and it might appear hazardous to hypothesize a solvophobic interaction between the methyl group and the phenyl group only by this difference. However, on the basis of NMR data, it has been reported [30] that the methyl group should project towards the plane of the phenyl group at a distance of about 5 Å and perpendicular to the plane of the ring. The electrostatic interaction between the NH_3^+ and the COO^- groups should serve as a stabilizing influence for this conformation. As regards Ala-Leu peptides, the stereoselectivity appears to be entropy driven and, therefore, on the basis of the above considerations, it may be supposed that in this case the solvophobic interaction between the two alkylic groups involves an exothermic enthalpy variation lower than the endothermic effect due to the electrostatic interaction, resulting from the lower degree of solvation that this interaction causes.

CONCLUSIONS

Some concluding remarks may be made, concerning the systems reported here and the systems previously investigated [13]:

(1) the thermodynamic stereoselectivity is due to two different kinds of non-covalent interactions and to the algebraic sum of their effects;

(2) ΔH^0 and ΔS^0 values have proved diagnostic to evaluate the relative weight of these interactions;

(3) the enthalpy changes may also reflect the occurrence of solvophobic interactions when the responsible groups are significantly far from each other, thus showing a "sensitivity" comparable to spectroscopic approaches (like NMR).

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