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PEPTIDE–LANTHANIDE CATION EQUILIBRIA IN AQUEOUS SOLUTION. IV. ISOMORPHOUS SUBSTITUTION, CONFORMATIONS AND THERMODYNAMICS OF COMPLEX FORMATION *

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ABSTRACT

Induced shifts (IS) by Ln(III) (Dy, Ho, Yb) on the proton resonances of glycyl-L-phenylalanine (GF) and L-anserine (L-ans) are studied as a function of the temperature. From induced bound shift (IBS) data, a T^{-2} dependence of dipolar shifts is confirmed and absolute values of geometric factors are established. Results indicate that minor displacements of the Ln(III) cations occur with respect to the carboxylate moeity. For each system, isomorphous replacement and axial symmetry are not removed, even though the side chain and the main chain interact with the COO^{-}–Ln^{3+} moeity.

From ^{1}H NMR data, thermodynamic parameters for the series of Ln^{3+}–L-carnosine, Ln^{3+}–GF, Ln^{3+}–L-ans complexes have been established. Positive $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ values confirm the strongly ionic character of lanthanide bonding.

INTRODUCTION

Metal ions interacting in specific ways with biological materials play an important role in life processes. Although rare earth elements occur in trace amounts in organisms, their role is not well established at this time.

The ability of Ln cations to replace Ca^{2+} isomorphously in a complex results from similarities in their chemistry: spectral properties of the system are modified with no change in the physiological activity of the complex. Thus, Ln^{3+} analogs of calcium or magnesium have been used to probe the metal binding environment of peptides or proteins. However, less attention has been given to the use of lanthanide induced shifts (LIS) for thermochemical studies.

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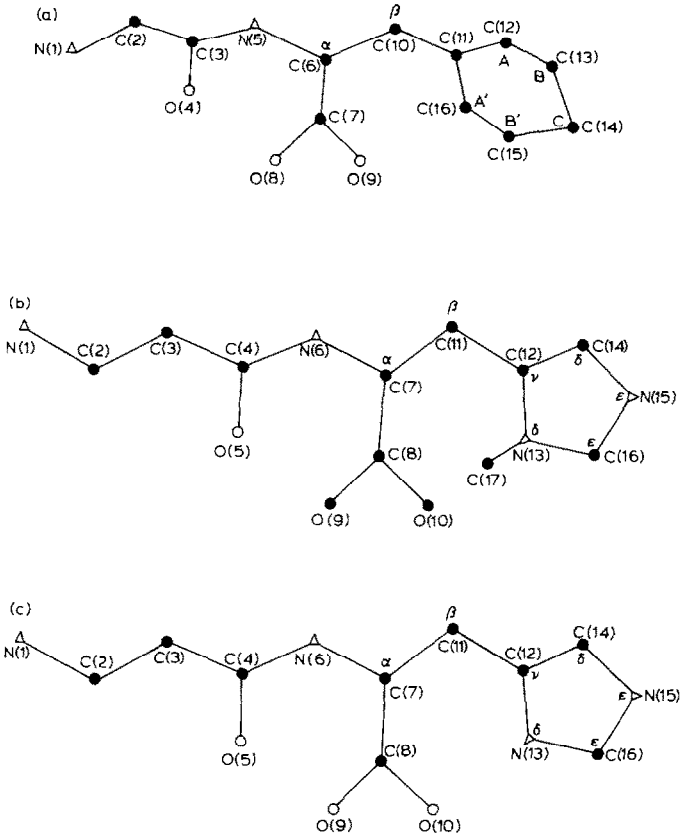

Fig. 1. IUPAC–IUB numbering of (a) glycyl-L-phenylalanine, (b) L-anserine, (c) L-carnosine.

Studies of the interactions of Dy and Yb cations with the peptides glycyl-L-phenylalanine (GF) and L-anserine (L-ans) by measurements of the induced NMR shifts are reported. In addition, the deprotonation constants are determined by potentiometric titrations. With known binding constants and lanthanide induced bound shifts values (LIBS), conformational changes and thermodynamic parameters for a series of complexes with L-carnosine (L-car), GF and L-ans (Fig. 1) are established.

EXPERIMENTAL SECTION

Lanthanide chloride salts were prepared from lanthanide oxides (Fluka puriss.). L-carnosine, glycyl-L-phenylalanine and L-anserine (Interchim, puriss. grade) were used without further purification. The pH (pD = pH + 0.4) was measured using a Metrohm EA 120-type microelectrode. For all samples the pH was adjusted within ± 0.02 pH unit with KOD and DCl. All measurements on standardized solutions of peptides at 10^{-2} M, maintained

under N55-grade argon, were done at constant ionic strength (2 M KCl) and at constant temperature (298 K).

Proton NMR spectra for all systems were recorded on a Bruker Fourier transform spectrometer operating at 200 MHz. Measurements were performed on samples containing peptide in D_2O in the presence of various amounts of $LnCl_3$. Eight samples were used at each temperature value for Yb (C_M/C_L was varied from 0.0 to 5.0). Only six were used for the Dy and Ho systems (C_M/C_L was varied from 0.0 to 2.5 with L-car and L-ans and from 0.0 to 5.0 for GF). Lanthanide induced shifts were measured relative to internal TSP-d_4 (2-(trimethylsilyl) propionic acid, sodium salt).

RESULTS

Potentiometry

The values of pK for L-car given in Table 1 were established as previously reported [1].

The following equations describe the deprotonation equilibria for both GF and L-ans

$$H_2P \rightleftarrows HP + H \qquad (K_2)$$

$$HP \rightleftarrows P + H \qquad (K_1)$$

The pK values obtained (Table 1) agree with known values [2–4] for carboxylate and NH_3^+ of the GF.

Nuclear magnetic resonance

The temperature dependence of the LIS

The approach of the data analysis has been previously described and discussed in detail [5] for L-car systems. The ytterbium titrations produce an

TABLE 1

pK values of glycyl-L-phenylalanine (GF), L-anserine (L-ans) and L-carnosine (L-car) at 298 K

References		pK_2	pK_1
This work	GF	2.99	8.16
	L-ans	2.97	9.42
GF	[2]	3.12	8.16
	[3]	3.09	8.10
	[4]	2.99	8.16
		pK_3	pK_2
[1]	L-car	2.96	7.08

TABLE 2

^{1}H Induced downfield (HA, HA′, HB, HB′, HC) and highfield shifts of GF at $T = 298$ K, pD = 3.53. Values in Hz ± 0.3 Hz (200 MHz)

C_M/C_L	CH_2N (1)	CH_2N (2)	Hα	Hβ	Hβ	HA=HA′	HB=HB′	HC
0.5				12.4	11.6			
1.0				25.6	24.3			
1.5	10.1	11.5	66.1	38.4	37.0	2.6	4.0	2.5
2.0	13.2	14.9	87.1	50.3	47.4	3.2	5.2	3.2
2.5	16.2	18.5	108.6	62.3	59.4	4.0	6.5	3.9
3.0	19.4	22.1	128.3	74.1	70.7	4.8	7.8	4.7
4.0	25.8	29.4	172.4	99.5	94.7	6.4	10.4	6.3
5.0	32.1	36.7	213.0	121.8	115.6	7.9	13.2	7.7

TABLE 3

Geometric factors (ppm) of GF–Ln^{3+} systems (MHP) at $T = 298$ K

Ln^{3+}	CH_2N (1)	CH_2N (2)	Hα	HA=HA′	HB=HB′	HC	Hβ	Hβ
Dy^{3+}	0.35	0.37_6	3.08	0.58	0.05	0.04		
Ho^{3+}	0.37	0.44_6	3.18	0.24	−0.11	−0.12		
Yb^{3+}	0.45	0.53	3.07	0.02 [a]	-0.24_5 [a]	−0.19 [a]	1.68	1.76

[a] Computed from induced shift ratios.

upfield shifting of most of the resonance lines of GF and L-ans. Only phenyl (Table 2) and *N*-methyl imidazole ^{1}H resonance lines are shifted downfield at 298 K. It is clear from these ^{1}H NMR titrations that the Yb cation folds the peptide side chains; besides, this is confirmed by the negative geometric factor (G_i) values of ^{1}H rings (Table 3). The phenyl ring is in a nearly symmetrical position with respect to the magnetic axis, as $G_B = G_{B'}$ and $G_A = G_{A'}$, whereas the *N*-methyl imidazole ring takes a dissymmetrical position (Table 4).

With Dy, all ^{1}H G_i values are positive as shown in Tables 3 and 4. The *N*-methyl imidazole and the phenyl ring reside both in the inner part of the cone defined by the magic angle θ.

TABLE 4

Geometric factors (ppm) of L-ans–Ln^{3+} systems (MH_2P) at $T = 298$ K

Ln^{3+}	CH_2N	CH_2C (1)	CH_2C (2)	Hα	Hβ	Hβ	CδH	CεH	CH3
Dy^{3+}	0.03	0.12*		1.0	0.51	0.5	0.12_8	0.02_6 [a]	0.01_5
Yb^{3+}	0.04	0.12_4	0.13_8	1.0	0.63	0.59	−0.04	[b]	-0.12_8

[a] Computed from induced shift ratios. [b] Not measurable; negligible induced shifts values.

TABLE 5

Thermodynamic data for the L-car–Ln^{3+}, GF–Ln^{3+} and L-ans–Ln^{3+} complexes

	Ln^{3+}	$\Delta G^{\ominus}$ (cal mol^{-1}) (298 K)	$\Delta H^{\ominus}$ (cal mol^{-1})	$\Delta S^{\ominus}$ (u.e.) (298 K)
L-car	Dy^{3+}	−1087.4	3809.5	16.4
	Ho^{3+}	−1151.2	5132.3	21.1
	Yb^{3+}	−806.5	4403.4	17.5
GF	Dy^{3+}	−738.0	7054.0	26.1
	Ho^{3+}	−704.0	6977.0	25.7
	Yb^{3+}	−242.0	7615.0	26.4
L-ans	Dy^{3+}	−1026.8	3615.9	15.6
	Yb^{3+}	−691.3	4822.1	18.5

Thermodynamic parameters of the complexation of Ln^{3+} by peptides

The formation in aqueous solutions of lanthanide complexes with aliphatic carboxylic acids has been studied extensively [6], but less attention has been given to the complexation of Ln^{3+} with aromatic acids [7]. The determination of the stability constants and thermodynamic parameters for the formation of 1 : 1 complexes were made by potentiometry [6–11], calorimetry [12] or by both techniques [13–15]. No determination has been made to date by means of NMR. ^{1}H NMR studies reported pK values [16–18] or have provided information on the complexes present in solution [12].

Determination of thermodynamic parameters by NMR requires β values in a well chosen temperature range. For complexes of Dy, Ho and Yb in the MH_2P form (L-car) and in the MHP form (GF, L-ans), the following relationships have been used: $\Delta G^{\ominus} = -RT \ln \beta$; $d(\ln \beta)/d(1/T) = -\Delta H^{\ominus}/R$; and $\Delta G^{\ominus} = \Delta H^{\ominus} - T\Delta S^{\ominus}$. All data are given in Table 5. Positive values for $\Delta S^{\ominus}$ and $\Delta H^{\ominus}$ are consistent with other results obtained on amino acids–lanthanide or amino acids–actinide systems [19].

DISCUSSION

Consistent results established for each system indicate that Dy, Ho and Yb cations have nearly identical positions relative to the carboxylate moiety, with only minute differences among them (Table 6). With Yb and Ho, the structures are folded and aromatic rings are close to the lanthanide cation (folded structure). However with Dy, aromatic rings are contained in the spatial domain corresponding to $\theta < 54.7°$ (extended structure).

^{1}H NMR spectra of the free GF shows that the CH_2N protons in acidic pH, are magnetically non equivalent and constitute an AB system. Dy and Yb cations preserve this system, even in the presence of various amounts of

TABLE 6

Bound shift ratios $(\Delta_j)_j/(\Delta_j)_{j'}(j' = \text{Dy})$ of L-ans–Ln^{3+} and GF–Ln^{3+} complexes for the same nucleus i in the case of an interaction restricted to pseudo-contact only (deduced from Tables 3 and 4), compared to the Bleaney factors.

		Dy	Ho	Yb
Bleaney C_j^D		−100	−39	22
GF	Hα	−100	−40.2	22.0
	Hα	−100		21.3
L-ans	CH_2C [a]	−100		24.3
	CH_2N	−100		23.3

[a] The peaks shifted by Yb (see Table 2) have been averaged for a suitable comparison with the Bleaney C_j^D factor.

$LnCl_3$; whereas Ho cation transform this AB system to the simplified A_2 system (singlet). $\rho_{ii'}$ ratios ($i = H\alpha$, $i' = CH_2N$) established at different temperatures are nearly constant for each system but differ from one system to another (Table 3). The $CH_2NH_3^+$ part of the glycine residue is involved in an "end to end" type interaction with the COO^-–Ln^{3+} moiety.

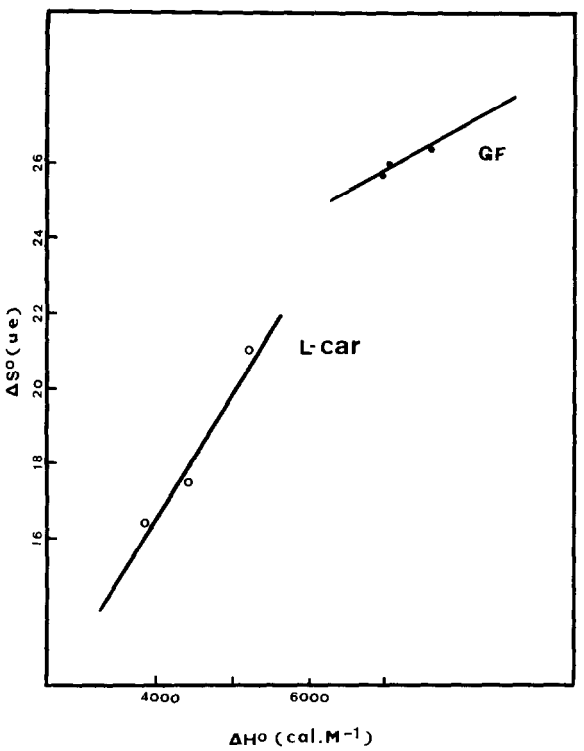

Fig. 2. Correlation of $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ of formation of MH_2P and MHP complexes.

According to the previous results presented above, isostructural geometry of the COO^{-}–Ln^{3+} site and axial symmetry are retained even though the side chain and the main chain of GF could interact differently throughout the Ln^{3+} cations.

In solution only the most electronegative donor atoms displace the water molecules of the solvation sphere; this is most probably the result of the strong ionic character of the lanthanide compounds. Their average number decreases under complexation, even with minute stability constants. This is related to a positive entropy term resulting from the increase of randomness of the system. As H_2O–H_2O and Ln^{3+}–H_2O bonds are broken in the hydration sphere (inner) perturbed by the linkage of the entering ligand, an endothermic enthalpy contribution is expected.

The observed changes reflect the net result of opposing contributions. This may or may not be accompanied by the side chain effects (folding). We could then infer that $\Delta S^{\ominus}$ and $\Delta H^{\ominus}$ will be correlated in the series of Ln cations if the ligand and other conditions are kept identical; this is based on the hypothesis that the contribution of the dehydration is largely predominant in the overall energetic balance of the complexation process. Such a linear correlation is seen in Fig. 2, although limited to the three elements studied in the present series of experiments. The folding of the side chain towards the cation does not appear to have a significant influence in the case of Yb and Ho. The rationale for this should be sought in a net change of the number of hydration molecules, or may be due to differences in the state of hydration of the free aquo ions.

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