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Lanthanide complexation with amino acids: Eu(III) with alanine in aqueous methanol

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Abstract

In water and aqueous methanol up to 90% methanol–10% water and an ionic strength of 1.0 M (perchlorate), europium(III) forms a 1:1 complex with alanine. As methanol is added to the solvent, the complexation constants increase by less than 50% by changing the solvent from water to 90% methanol. This is different from the Eu(III)–glycine complex where more than an order of magnitude increase in the complexation constant was observed. The results are consistent with inner sphere Eu(III)–glycine and outer sphere Eu(III)–alanine complexes. © 1998 Elsevier Science S.A.

Keywords: Europium complexes; Alanine; Inner sphere complexes; Outer sphere complexes

1. Introduction

The use of lanthanide probes to detect calcium binding in biological molecules is now a standard technique using a variety of methods, such as NMR, luminescence, X-ray and electrochemistry [1]. The lanthanides also are important MRI contrast agents [2]. Our long-term interests include using ultrasonic relaxation to detect coordination number changes upon complexation [3] and using UV-Vis measurements to distinguish between inner and outer sphere complexes [4]. We have recently initiated studies on the binding of lanthanides to amino acids in water and aqueous methanol [4–6]. Both equilibrium and ultrasonic studies of Eu(III) complexation with glycine in water were consistent with the formation of a 1:1 inner sphere complex [5], as predicted earlier [7–9]. Our study [4,5] was carried out between pH 2 and 5, below the isoelectric point for glycine. The addition of methanol to the solvent increased the complexation constants and, in 90% aqueous methanol, a bis-glycinato complex formed [5]. In water, Eu(III) also forms a 1:1 complex with alanine and the complexation constant is very close to the glycine value, but the complexation thermodynamics are consistent with outer sphere complexation [6]. Eu(III) complexation with alanine has been previously studied and the equilibrium constant increased at higher pH values, consistent with the formation of a chelate at the higher pH values [10–14]. Since the previous studies were carried out using pH titrations or potentiometry, we decided to use spectrophotometric methods for this study [4–6]. As expected, no complexation difference was observed for D-alanine and L-alanine with Eu(III) [6]. This study in water/methanol solvent systems was carried out to determine if the complexation between Eu(III) and the two amino acids differs as the environment changes from a hydrophilic medium (water) to a more hydrophobic medium (methanol).

2. Experimental

Eu_2O_3 (Molycorp) was dried and dissolved in a slight excess of $HClO_4$ to make stock solutions. The stock solutions were standardized by EDTA titrations [4–6]. L-Alanine (99%, Aldrich) was used without further purification. Since preliminary data indicate that no proton is released upon complexation within our pH range, the alanine acts as a neutral ligand, and complexation should not affect the ionic strength. Thus, the ionic strength in all solutions was kept at 1.0 M in perchlorate media by using 0.167 M $Eu(ClO_4)_3$ with small quantities of $HClO_4$ or NaOH to adjust the pH. The Cary 3 spectrometer was used for the spectrophotometric measurements and the techniques used to convert deviations from Beer's Law into extinction coefficients and equilibrium constants were described previously [4]. Measurements were carried out between 300 and 800 nm, with absorption at 464.8 nm used to determine the complexation constants. The addi-

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tion of excess $NaClO_4$ to Eu(III) at this wavelength does not change the absorbance, whereas the addition of alanine results in an absorbance increase, attributed to complex formation.

Stability constants were measured using the Benesi–Hildebrand method [15], originally developed to study the reactions of iodine with aromatic hydrocarbons. In these experiments, difference spectra are obtained, with the reference solution containing the metal ion, ionic media, and the same solvent as the test solution, which differs only in having the ligand, alanine, present. The experiment uses matched 1.00 cm cells (Helma). If M_o and L_o represent the total concentration of Eu(III) and alanine, respectively, and if the complex is symbolized by Eu–ala, the complexation constant is given by:

$$K = [\text{Eu–ala}]/[\text{Eu(III)}][\text{ala}]$$

$$= [\text{Eu–ala}]/(M_o - [\text{Eu–ala}])(L_o - [\text{Eu–ala}]) \qquad (1)$$

Here, Eu(III) and ala represent free europium and free alanine in the system. Under the conditions of excess ligand, $L_o \gg$ complex, and the expression simplifies to:

$$K = [\text{Eu–ala}]/(M_o - [\text{Eu–ala}])(L_o) \qquad (2)$$

Substituting the absorbance difference, $\Delta A = \Delta\epsilon b[\text{Eu–ala}]$, we can replace [Eu–ala] by $\Delta A/\Delta\epsilon$ in matched cells where $b = 1.00$ cm, obtaining:

$$K = (\Delta A/\Delta\epsilon)/(M_o - (\Delta A/\Delta\epsilon))(L_o) \qquad (3)$$

Algebraic manipulation yields:

$$1/\Delta A = (1/M_o K\Delta\epsilon L_o) + (1/M_o\Delta\epsilon) \qquad (4)$$

Thus, a plot of $1/\Delta A$ as a function of $1/L_o$ gives a straight line with an intercept equal to $(1/M_o\Delta\epsilon)$ and a slope equal to $(1/M_o K\Delta\epsilon)$, thereby allowing the calculation of the complexation constant by dividing the intercept by the slope. If more than one complex is present, then systematic deviations from linearity occur at low values of $1/L_o$. Fig. 1, the Benesi–Hildebrand plot in 90% methanol–10% water, is consistent with only one complex present.

3. Results and discussion

Our measurements were carried out between a pH of 2.5 and 5 and, under these conditions, alanine is predominantly present as the zwitterion, and the alanine absorption spectra did not change with pH, consistent with the observations on glycine [4]. As shown in Fig. 1, the Benesi–Hildebrand plots were linear at each temperature indicating that only the 1:1 complex forms, consistent with the earlier studies [6,9–14]. Thus, within experimental error, europium(III) tends to form a single complex with alanine at all solvent mixtures up to 90% methanol–10% water. Solubility limitations restricted the range of alanine concentrations accessible for this solvent composition, resulting in a large uncertainty in the intercept, which is reflected in the uncertainties in the equilibrium constants in 90% methanol–10% water.

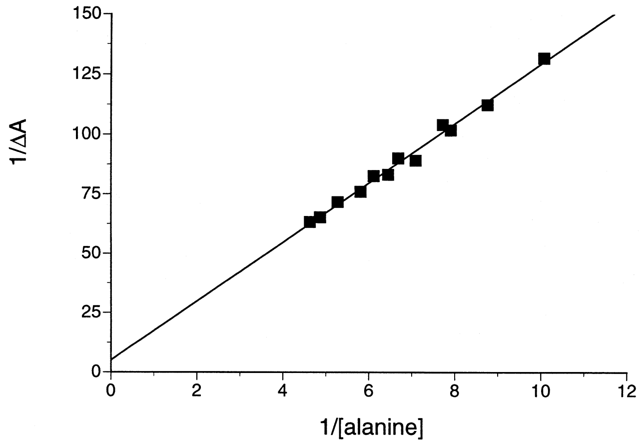

Fig. 1. The Benesi–Hildebrand plot for 0.167 M Eu(III) with L-alanine at 25°C in 90% methanol–10% water.

Table 1 summarizes the equilibrium constants for the L-alanine system with Eu(III) as a function of temperature and solvent. The variation of the stability constants with increasing methanol content was smaller for lanthanide complexation with alanine than was measured by similar techniques with an inorganic ligand like nitrate or an amino acid like glycine [3,5]. Since this study and the complexation with glycine [5] were carried out under the same experimental conditions, a comparison of the two results is shown in Fig. 2. In water the magnitudes of the complexation constants are similar, but the addition of methanol causes the stability constant to increase for glycine, in a manner similar to that observed with nitrates [3,5]. Clearly the variation is much smaller when alanine is the ligand (Table 1 and Fig. 2). An examination of the ligand acidity constants [16] shows no significant difference between the two ligands. Structurally in comparing glycine with alanine, the major difference is the replacement of a hydrogen on the α-carbon by a methyl and since this group does not appear to present steric hindrance to an incoming metal ion on the carboxyl group, this should not be the major difference between the two ligands. An alternate suggestion is that the solvation around the Eu(III) in aqueous methanol could cause a significant difference in

Table 1
The complexation constants for Eu(III) with alanine as a function of temperature and solvent

% water	Stability constants			
	15°C	20°C	25°C	37°C
100	0.54±0.07	0.38±0.07	0.33±0.06	0.23±0.07
80	0.53±0.05	0.47±0.16	0.46±0.07	0.44±0.06
60	0.44±0.06	0.41±0.06	0.38±0.05	0.33±0.05
30	0.70±0.15	0.51±0.11	0.42±0.07	0.35±0.18
10	0.71±0.43	0.55±0.32	0.40±0.25	0.29±0.26

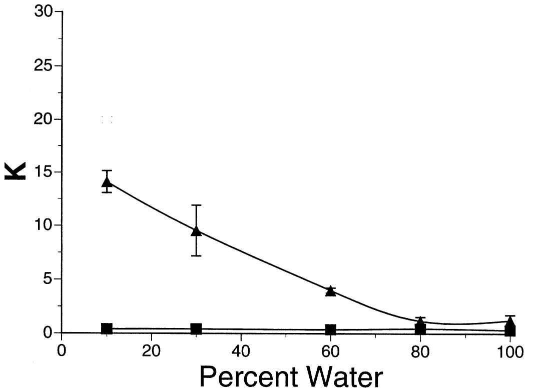

Fig. 2. The variation of the complexation constant for Eu(III) with glycine and alanine at 25°C.

steric strain between a hydrogen and a methyl group on the α-carbon[1]. However, in aqueous methanol above a water mole fraction of 0.1, a condition met by each mixture except the 90% methanol solution, the Eu(III) ion is preferentially solvated by water over methanol [17]. Alternately, the amino acids may have a different composition of inner sphere solvent as methanol is added depending upon the substituents on the α-carbon, but this cannot explain why glycine forms inner sphere, whereas alanine forms outer sphere complexes in water. If the complexation mechanisms were different with the two similar ligands, then this should show up in the complexation enthalpy and entropy, shown in Figs. 3 and 4 for the alanine system. Although numerical differences are found, the enthalpy and entropy reach a maximum value near 60% water for glycine and 80% water for alanine, suggesting that the complexation mechanism is similar. However, since the signs of the complexation enthalpy and entropy are consistent with inner sphere complexation in the glycine system and with outer sphere complexation with alanine, this may be the major factor in the observed differences.

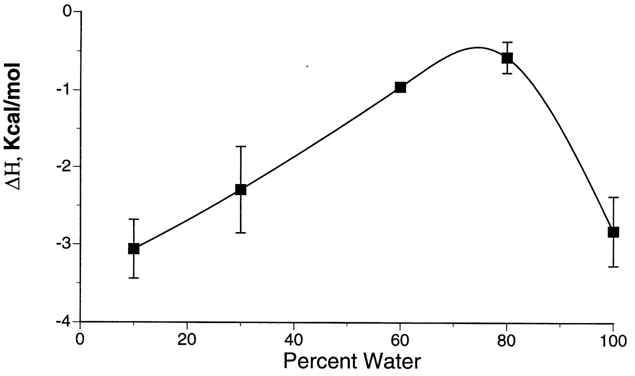

Fig. 3. The complexation enthalpy for Eu(III) with alanine as a function of solvent.

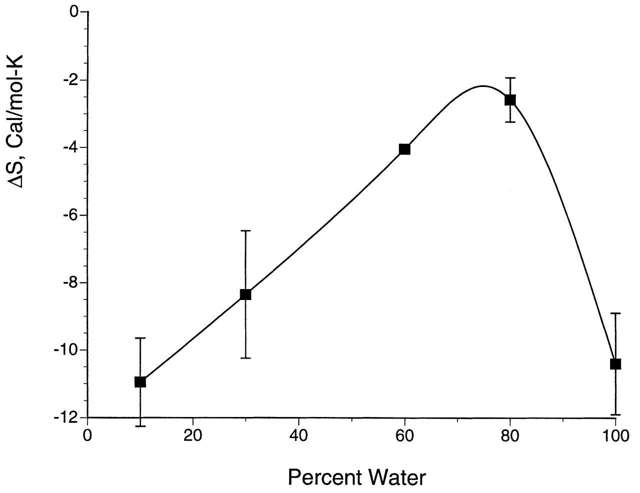

Fig. 4. The complexation entropy for Eu(III) with alanine as a function of solvent.

The lanthanide complexation with acetate ion has been studied many times in water [16], and the logarithm of the stability constants in water are approximately equal to 2, which differs from the constants for these two amino acids by two to three orders of magnitude. The reason for this difference is easily understood, in that near pH 5, acetic acid is present as the negative acetate ion which attracts the plus-three lanthanide ion. Both glycine and alanine are present in the pH range of our study as the zwitterion, with a positive amino group close to the carboxyl group. This would certainly decrease the ionic nature of the complex formation. In order to ascertain what structural parameters within the amino acid series contribute to these differences, we are undertaking investigations of europium complexation with other simple amino acids.

Acknowledgements

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