Inorganica Chimica Acta, 139 (1987) 119–120

Thermodynamics of Ln(III) Complexation with AMP and ATP*

S. M. SHANBHAG and G. R. CHOPPIN**

Department of Chemistry, Florida State University, Tallahassee, Fla. 32306-3006, U.S.A.

The adenosine phosphates are present in all forms of life in 2–10 millimolar concentrations. The enzymatic hydrolysis reactions of adenosine 5-triphosphate (ATP) require either Mg(II) or Ca(II) as cofactors. There is substantial evidence that the substrate is a metal nucleotide complex and not the free nucleotide, but the actual role of the metal ion and the structure of the complex are still not clear. The spectroscopic inertness of these two cations has hindered development of a satisfactory understanding of the complexes formed by these alkaline cations and the adenosine phosphates.

Lanthanides can serve to probe Ca and Mg binding sites in biomolecules and, indirectly or directly, allow spectroscopic measurements. To study the validity of using Ln(III) as probes of Ca(II) and Mg(II) binding to adenosine phosphates, the thermodynamics of complexation have been measured for the ML and MHL species.

Controversy persists on whether M(II) ions interact with the heterocyclic rings at all and which and how many of the phosphate groups are binding. A study led to the conclusion that while Ni(II) is bound to the adenine ring in ATP, Co(II) and Mn(II) show no evidence for such an interaction [1]. Granot and Fiat [2] based on NMR shifts, proposed that Mg(II), Ca(II), Ni(II) and Co(II) bind ATP predominantly through the phosphate chain, but in the protonated complexes some metal–ring interaction occurs.

Experimental

All chemicals were reagent grade. Each solution of metal and of ligand was adjusted to 0.1 M ionic strength with $NaClO_4$. The adenosine monophosphate (AMP) solutions showed no change in titration behavior, but after about 4 days the ATP solutions had slight changes, probably due to hydrolysis. Therefore, fresh solutions were prepared for each set of experiments and kept in the dark.

The potentiometric titrations to obtain the stability constants were performed by pH measurement under an atmosphere of N_2 in a jacketed titration vessel in which the temperature was maintained by circulating constant temperature (±0.1 °C) water.

The Ln(III)–AMP complexes were not sufficiently soluble and their stability constants were measured by solvent extraction distribution at tracer concentrations and different temperatures. The ΔH (and ΔS) values were obtained for the Ln–AMP and Ln–ATP complexation from the variation of $\log \beta_{101}$ and $\log \beta_{111}$ with $1/T$ (Tables I and II).

Ca and Mg complexation thermodynamics were determined by pH titration and calorimetry [3]. The results are presented for comparison with the Ln(III) data (Table III).

Discussion

For Mg and Ca, the $\log \beta$ values of ML compare well with the values for the analogous polyphosphate anions. This plus the variation in the ΔS values indicate that the cations bind only to the phosphates in AMP, ADP and ATP with stronger interaction with the β and γ groups in ATP. However, in the MHL complexes, the ΔH and ΔS values are consistent with folding of the ring system with metal–ring interaction [3].

The relatively large and positive ΔH and ΔS values for LnAMP formation are consistent with binding of the metal to the phosphate group alone. For example,

TABLE I. Thermodynamic Parameters for Formation of Lanthanide–AMP Complexes

Complex	$\log \beta$	$-\Delta G$ (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})
(a) Ln + L = LnL				
YAMP	4.35	24.81	23.36 ± 1.50	161.5 ± 5.2
LaAMP	3.78	21.56	17.90 ± 0.51	132.3 ± 1.9
NdAMP	4.09	23.23	20.87 ± 1.22	147.9 ± 4.3
EuAMP	4.50	25.71	18.81 ± 0.73	149.3 ± 2.6
DyAMP	4.57	26.08	20.43 ± 0.41	156.0 ± 1.6
TmAMP	4.46	25.51	20.60 ± 1.81	154.3 ± 6.2
(b) Ln + H + L = LnHL				
YHAMP	9.05	51.68	−12.89 ± 3.81	130 ± 13
LaHAMP	9.05	51.68	−12.89 ± 3.01	130 ± 10
NdHAMP	9.03	51.56	−3.43 ± 0.99	162 ± 4
EuHAMP	9.01	51.45	−10.82 ± 2.78	136 ± 10
DyHAMP	9.00	51.39	−9.73 ± 3.18	140 ± 11
TmHAMP	8.99	51.33	−8.33 ± 6.22	144 ± 21

---

*Paper presented at the Second International Conference on the Basic and Applied Chemistry of f-Transition (Lanthanide and Actinide) and Related Elements (2nd ICLA), Lisbon, Portugal, April 6–10, 1987.

**Author to whom correspondence should be addressed.

0020-1693/87/$3.50

TABLE II. Thermodynamic Parameters for Formation of Lanthanide–ATP Complexes

Complex	log β	$-\Delta G$ (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})
(a) Ln + L = LnL				
YATP	6.29	35.90	−14.72 ± 1.00	71.0 ± 3.5
LaATP	5.97	34.07	−9.69 ± 0.40	81.8 ± 1.5
NdATP	6.23	35.61	−16.71 ± 0.50	63.4 ± 1.9
EuATP	6.31	35.98	−16.60 ± 0.75	65.0 ± 2.7
DyATP	6.37	36.36	−11.76 ± 0.33	82.5 ± 1.3
TmATP	6.45	36.82	−12.33 ± 0.57	82.1 ± 2.1
(b) Ln + H + L = LnHL				
YHATP	10.15	57.91	−13.40 ± 1.40	149.3 ± 5.1
LaHATP	10.03	57.24	−10.13 ± 0.88	158.0 ± 3.3
NdHATP	10.14	57.84	−12.26 ± 1.17	152.9 ± 4.3
EuHATP	10.16	58.03	−14.72 ± 0.63	145.2 ± 2.4
DyHATP	10.17	58.07	−9.25 ± 0.75	163.7 ± 2.9
TmHATP	10.18	58.12	−12.64 ± 0.84	152.6 ± 3.2

TABLE III. Thermodynamic Parameters for Formation of Mg^{2+} and Ca^{2+} Complexes

Complex	log β	$-\Delta G$ (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})
(a) M + L = ML				
MgAMP	2.10	11.99 ± 0.03	5.71 ± 0.10	59
MgADP	3.28	18.72 ± 0.03	17.53 ± 0.10	121
MgATP	4.03	23.01 ± 0.04	18.08 ± 0.10	138
CaAMP	2.03	11.61 ± 0.03	4.23 ± 0.10	53
CaADP	2.90	16.58 ± 0.02	13.80 ± 0.10	101
CaATP	3.70	21.13 ± 0.04	13.45 ± 0.10	116
(b) M + H + L = MHL				
MgHADP	8.31	47.45 ± 0.06	−10.01 ± 0.20	126
MgHATP	8.63	49.28 ± 0.06	−14.65 ± 0.20	116
CaHADP	7.00	39.97 ± 0.06	−8.62 ± 0.20	105
CaHATP	8.55	48.82 ± 0.06	−13.04 ± 0.20	120

for $EuSO_4^+$ formation, $\Delta S = 140$ J K^{-1} mol^{-1}. Since the ΔH becomes negative upon protonation with a small negative change in entropy, backfolding and lanthanide-ring system binding presumably occurs in LnHAMP.

The stability constants for $LnATP^-$ are larger than for $LnHP_3O_{10}^-$, indicating Ln–ring interaction, probably through a water molecule. The low ΔS values and negative ΔH support this. This outer sphere Ln–N interaction is not inconsistent with the NMR results [4]. In the LnATP complex, the lanthanide is bound primarily to the phosphate chain but interaction with the heterocyclic base increases the net binding. Having a water molecule between the α-phosphate and the lanthanide also reduces the steric constraints on metal–water–ring interaction.

The LaHATP complexes have larger, positive ΔS values, with little change in ΔH. These data support a model of Ln interaction with both the phosphate and the adenosine ring system. The larger ΔS values may reflect direct Ln–ring interaction (*i.e.*, no water molecule).

These results indicate that CaATP and LnATP most likely have different structures with no metal–adenosine interaction for Ca but an Ln–H_2O–adenosine structure for Ln. Such differences must be taken into account if Ln(III) are to be used as Ca(II) replacement probes in biological molecules.

Acknowledgements

This research was supported by a contract with the Division of Chemical Sciences, OBES-USDOE.

References

1 T. J. Swift, T. A. Glassman, C. Cooper and L. W. Harrison, *Biochemistry, 10,* 843 (1971).
2 J. Granot and D. Fiat, *J. Am. Chem. Soc., 99,* 70 (1977).
3 S. M. Shanbhag and G. R. Choppin, *Inorg. Chem.*, submitted for publication.
4 P. Tanswell, J. M. Thornton, A. V. Korda and R. J. P. Williams, *Eur. J. Biochem., 57,* 135 (1975).