Asian Journal of Chemistry Vol. 14, No. 1 (2002), 236–242

Absorption Difference Spectroscopy of Lysozyme with Nd(III) in Presence of Zn(II), Cd(II), Ca(II) and Mg(II) Involving 4f-4f Transition

S.B. MEHTA* and M.K. SHAH
Department of Chemistry, Bhavanagar University, Bhavanagar-364 002, India

The absorption difference and comparative absorption spectrophotometry of neodymium(III) with lysozyme at different pH (2, 4, 6) in different solvents such as methanol, dimethylformamide, acetonitrile and t-butanol in presence and absence of chemically different metal ions like Ca(II) and Mg(II) on one hand as well as Zn(II) and Cd(II) on the other hand have shown that the binding capacity of lysozyme is better at pH 2 than that observed at pH 4 and 6, because lysozyme has been known to exhibit biological activities in the mildly acidic medium while the higher range of pH does not contribute remarkably towards binding. Among different solvent compositions maximum intensification takes place when solvent comprises of DMF and MeOH. Acetonitrile causes very minor and t-BuOH leads to a very small variation. The effect of hard metal ions like Ca(II) and Mg(II) on hetero metal compositions of Nd(III)-lysozyme results in lowering of the intensities of 4f-4f bands of Nd(III) ions. As Ca(II) and Mg(II) both are hard metal ions like Nd(III), hence they enjoy identical binding atom preference and therefore compete effectively for lysozyme identical coordinating sites. Contrary to these, Cd(II) and Zn(II) both being soft metal acceptors, they prefer soft donor site of lysozyme and stimulate the binding of Nd(III) to lysozyme.

Key words: Absorption, spectroscopy, lysozyme, Nd(III), 4f-4f transition.

INTRODUCTION

The 4f-4f transitions absorb electric dipole, magnetic dipole or even higher electric multiple radiations. Judd[1] and Ofelt[2] independently have developed the model for deriving the oscillator strengths of the intra 4f-4f transitions of lanthanides considering the mixing of the excited state of parity difference. The oscillator strength (P) of an electric dipole transition from ground state to an excited state can be expressed by the equation given below.

$$P_{E.D.} = \chi \frac{8\pi^2 m}{3h} \sum_{\lambda = 2, 4, 6} T_\lambda \langle f^n \alpha [SL] J \| U^{(\lambda)} \| f^n \alpha [S'L'] J' \rangle$$

where χ = Lorenz field correction for the refractivity of the medium, m = electronic mass, $U^{(\lambda)}$ is the reducible matrix element of unit tensor operator of the rank λ and T_λ are the parameters containing all details of lanthanide ligand field interactions known as Judd-Ofelt parameters, which are found to be highly sensitive to the coordination environment around lanthanide ion and symmetry of complex species.

The present study mainly confines to the quantitative spectral intensity and

energy interaction analysis involving relative sensitivity of different 4f-4f transitions and their correlation with Judd-Ofelt intensity parameters in Nd(III) complexation with lysozyme in aqueous and aquated organic solvent at different pH, in presence and absence of other biologically important metal ions like Ca(II), Mg(II), Cd(II) and Zn(II). The present work reports the ligand mediated pseodohypersensitivity of $^4I_{9/2} \rightarrow {}^4G_{7/2}$, $^4I_{9/2} \rightarrow {}^4F_{7/2}$ and $^4I_{9/2} \rightarrow {}^4F_{5/2}$ transitions of Nd(III) and utilized the magnitude and variation of the oscillator strength (P) and Judd-Ofelt electric dipole intensity (T_λ) parameters in complexes of Nd(III) with lysozyme. The variation of coulombic (F_k) spin orbit (ξ_{4f}) and nephelauxetic (β, δ, $b^{1/2}$) parameters have also been calculated to support the intensity data.

EXPERIMENTAL

Neodymium(III) chloride of 99.9% purity from Indian Rare Earth Ltd., India was used for synthesis and spectral studies and the concentration of Nd(III) ion = 10 mM was used. Lysozyme was kept throughout in deep freezer in nitrogen atmosphere and always a fresh solution of 10^{-3} M concentration was prepared before use. All spectroscopic measurements were carried out on Perkin-Elmer Lambda-2 spectrophotometer upgraded with attachment to improve the resolution and expansion of the scale. The region 980–480 cm^{-1} was explored. The intensity of the absorption band was measured by the experimental oscillator strength (P_{exp}) which is directly proportional to the area under the absorption curve (Gaussian curve) (Fig. 1) and can be expressed in terms of molar extinction coefficient (ε_n) and half width band* $\Delta\nu_{1/2}$ by the relationship:

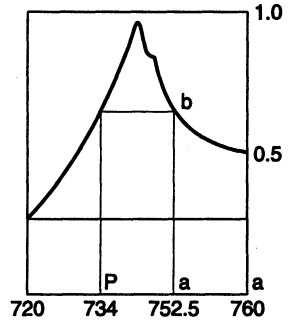

Fig. 1. Schematic representation of the Gaussian curve analysis of an expanded single peak

$$P_{exp} = 4.31 \times 10^{-9} \times \varepsilon_n \times \Delta\nu_{1/2}$$

where $\Delta\nu_{1/2} = \left[\frac{1}{P} - \frac{1}{Q}\right] \times 10^7$ (half width band*)

$$\varepsilon_n = \frac{\text{Absorbance}}{\text{concentration} \times L}$$ (where L = path length of the cell in cm).

RESULTS AND DISCUSSION

Our approach has been quite parallel to the studies made by Devlin *et al.*[3–5] We have used not only absorption difference spectroscopy but also included in

*Fig. 1 shows the schematic representation of the Gaussian curve on an expanded single peak of $^4I_{9/2} \rightarrow {}^4F_{7/2}$ transition of Nd(III) spectrum. A basic line 'a' has been drawn at the foot of the curve showing maximum absorbance and another horizontal line 'b' indicates the exact centre of the band. The wavelength region showing from 734.0 to 752.5 (P to Q is the peak range half width band.

our studies comparative absorption spectrophotometry involving the comparison of oscillator strength of electric dipole 4f-4f multiplet to multiplet transition manifolds and consequently the magnitude and variation Judd-Ofelt (T_λ) parameters in complexes of Nd(III) with structurally related ligands (more specifically the biomolecules). Since Ln(III) ions resemble very closely to one of the most abundant, and equally most important, metal ion Ca(II) in our body system in terms of ionic size and coordinated characteristics, we studied Ln(III) ions to understand the reaction of Ca(II) taking place in our body. Ca(II) ion being diamagnetic and hence not giving spectroscopic signals, using paramagnetic Ln(III) ions to substitute isomorphously Ca(II) from the biomolecules mostly does not induce any noticeable change in the structure and conformation of biomolecules, so much that the biological properties of the biomolecules are also retained. This makes Ln(III) comparative absorption and absorption difference spectroseopy involving 4f-4f transitions an effective tool in following the biochemical reactions involving Ca(II) and Mg(II). We have therefore used 4f-4f transition absorption intensity analysis and absorption difference and comparative absorption spectroscopy in understanding the binding behaviour of biomolecule lysozyme in solution, both in aqueous and in aquated organic solvent at varying pH.

The lanthanide(III) ions are hard metal ions in Pearson's scheme prefer hard donor ligands like oxygen, halogen and sometimes nitrogen under certain experimental/physiological conditions. The multidentet large biological molecules comprise of several potential donor sites with oxygen, nitrogen, sulphur, halogen and phosphorus donor atoms in the form of functional groups. Since biological systems as such are multi-metal multi-ligand systems where different endogenous metal ions do compete for physiological ligands, at the same time the biologically active functional groups of the large biological molecules go for coordination with different metal ions and hence the complexation becomes highly complexed. Which particular metal ion will coordinate to which particular binding site depends upon a number of factors and this binding is responsible for the biological activation and for the probable of the ion in biological processes. While analyzing quantitatively, the absorption spectra of Nd(III) complexation with lysozyme, we have observed some noteworthy structural information regarding lysozyme molecule, the flexibility of the enzyme present and several coordinating positions available for multi-complexation. The binding of the Ln(III) ion to lysozyme brings about changes in the energies of 4f-4f transitions. As usual we have observed noticeable red shift of the band. The magnitude of the red shift signifies the lowering in inter-electronic repulsion (Fk's) parameters and consequently leads to the nephelauxetic effect which is represented by parameter β, called nephelauxetic ratio.

$$\beta = \frac{F_k^c}{F_k^f} \qquad k = 2, 4, 6$$

Smaller the value of β greater the nephelauxetic effect $(1 - \beta)$. Nephelauxetic effect causes the shortening of the ligand distance and hence probably enhances the probability of orbital overlap. This is also a measure of covalence δ.

$$\delta = \frac{1-\beta}{\beta} \times 100 \qquad \text{where } \delta = \text{percentage covalence parameters.}$$

$b^{1/2}$ binding parameter which is often used to measure the extent of involvement of metal-4f orbital in binding is known as mixing coefficient or binding parameter is also related to β.

$$b^{1/2} = \frac{[1-\beta]^{1/2}}{2}$$

All these three parameters β, δ, $b^{1/2}$ are used in defining the degree of covalence in predominantly ionic Ln(III) band. The values of these parameters obtained from quantitative absorption spectral analysis for the complexation of lysozyme with Nd(III) in presence of DMF, CH_3CN, MeOH, t-BuOH at three different pH 2, 4 and 6 were calculated. The computed data is given in the Tables 1–4. In general, we find the binding capacity of lysozyme better in acidic medium (at pH 2) than that observed at pH 4 and 6. This is quite different from that found in complexation in other ligand to Ln(III) where increase in pH leads to better complexation of ligand. Lysozyme has been known to exhibit higher biological activities in mildly acidic medium (pH 1 and above) while range 4–6 does not contribute noticeably towards binding, so that binding of lysozyme to Ln(III) remains unchanged when H^+ ion concentration varies significantly. A look at the table listing the computed and observed values of spectral parameters, both energy interaction (spin orbit interaction, coulombic interaction, nephelauxetic parameters) and intensity parameters (oscillator strength and Judd-Ofelt intensity (T_λ) parameters) demonstrate the sensitivity of 4f-4f transitions towards even minor coordination changes around Ln(III) immediate coordination. The variation and magnitudes of these spectral parameters also speak about some confirmation changes in the structure of lysozyme when the biomolecules bind to Ln(III) at pH as low as 1 to as high as 6. The different intensities of 4f-4f bands observed in the spectra (of Ln(III) lysozyme system) recorded in different solvent composition speak about the different coordinating powers of different solvents. In general we find minimum intensification when the solvent comprises of DMF or MeOH. Acetonitriles cause only minor variation while t-butanol leads to very small variation. It is interesting to note that tertiary butanol though is provided with oxygen donor site yet it appears to be a weak donor ligand for Ln(III) in presence of water in equal proportion. We have selected two hard metal ions like Mg(II) and Ca(II) and two soft metal ions like Cd(II) and Zn(II) to investigate the heterometal complexation of lysozyme with Nd(III) as primary metal ions while Mg(II)/Ca(II)/Cd(II)/Zn(II) are chosen as secondary metal ions. The effect of hard metal ions like Ca(II) and Mg(II) on heterometal complexation of Nd(III)-lysozyme results In lowering of the intensities of 4f-4f band while the soft metal ions like Zn(II) and Cd(II) result in the enhanced intensities of 4f-4f bands of Nd(III) ion, such behaviour is understandable as Ca(II) and Mg(II) both are hard metal ions like Nd(III) and hence enjoy identical coordination sites. Contrary to this, Zn(II) and Cd(II) both being soft metal acceptors, prefer soft donor sites of lysozyme and therefore stimulate the binding of Nd(III) to lysozyme. Even the energy interaction, nephelauxetic, covalency and bonding

parameters show different patterns when heterometal complexation of lysozyme involves Ca(II)/Mg(II) and Cd(II)/Zn(II) ion and Nd(III) ion.

TABLE-1
COMPUTED VALUES FOR INTER-ELECTRONIC REPULSION (f_k, k = 2, 4, 6) NEPHELAUXETIC RATIO (β), BINDING ($b^{1/2}$) AND COVALENCY (δ) PARAMETERS OF Nd(III) LYSOZYME: Zn(II)/Cd(II)/Ca(II)/Mg(II) SYSTEM AT DIFFERENT pH VALUES

In DMF:

System	pH values	F_2	F_4	F_6	β	(1 – β)	$b^{1/2}$	δ
Nd(III) +lysozyme	2	363.38	45.12	5.17	0.9959	0.0041	0.0451	0.409
	4	363.02	45.36	5.17	0.9968	0.0032	0.0451	0.327
	6	360.67	45.55	5.17	0.9978	0.0022	0.0451	0.322
+ Zn(II)	2	363.30	45.33	5.17	0.9967	0.0033	0.0451	0.330
	4	362.63	45.33	5.17	0.9968	0.0032	0.0451	0.327
	6	362.78	45.02	5.17	0.9980	0.0020	0.0451	0.196
+ Cd(II)	2	362.97	45.43	5.17	0.9965	0.0035	0.0451	0.344
	4	362.87	45.38	5.17	0.9970	0.0030	0.0451	0.323
	6	361.07	43.71	5.17	0.9977	0.0023	0.0408	0.335
+Ca(II)	2	362.87	45.35	5.17	0.9969	0.0031	0.0408	0.313
	4	362.87	45.38	5.17	0.9970	0.0030	0.0408	0.323
	6	363.44	44.91	5.17	0.9981	0.0019	0.0408	0.193
+Mg(II)	2	362.15	45.56	5.17	0.9965	0.0035	0.0408	0.346
	4	362.29	45.17	5.17	0.9978	0.0022	0.0408	0.222
	6	363.44	44.91	5.17	0.9981	0.0019	0.0408	0.193

TABLE-2

In MeOH:

System	pH values	F_2	F_4	F_6	β	(1 – β)	$b^{1/2}$	δ
Nd(III) + lysozyme	2	359.90	43.98	5.17	0.9970	0.0030	0.0389	0.304
	4	361.22	45.94	5.17	0.9957	0.0043	0.0417	0.424
	6	358.18	44.10	5.17	0.9965	0.0035	0.0417	0.349
+ Zn(II)	2	358.85	44.12	5.17	0.9969	0.0031	0.0393	0.310
	4	359.26	43.99	5.17	0.9967	0.0033	0.0407	0.332
	6	358.89	44.04	5.17	0.9967	0.0033	0.0407	0.332
+ Cd(II)	2	356.69	44.43	5.17	0.9969	0.0031	0.0393	0.311
	4	359.20	44.06	5.17	0.9969	0.0031	0.0394	0.312
	6	358.76	43.94	5.17	0.9963	0.0037	0.0432	0.374
+Ca(II)	2	358.85	44.12	5.17	0.9969	0.0031	0.0393	0.310
	4	359.20	44.06	5.17	0.9969	0.0031	0.0394	0.311
	6	358.22	44.12	5.17	0.9966	0.0034	0.0411	0.339
+Mg(II)	2	358.18	44.22	5.17	0.9969	0.0031	0.0391	0.306
	4	359.20	44.06	5.17	0.9969	0.0031	0.0394	0.611
	6	358.89	44.04	5.17	0.9967	0.0033	0.0407	0.332

TABLE-3
COMPUTED VALUES FOR INTER-ELECTRONIC REPULSION (f_k, k = 2, 4, 6) NEPHELAUXETIC RATIO (β), BINDING ($b^{1/2}$) AND COVALENCY (δ) PARAMETERS OF Nd(III) LYSOZYME: Zn(II)/Cd(II)/Ca(II)/Mg(II) SYSTEM AT DIFFERENT pH VALUES

In CH_3CN:

System	pH values	F_2	F_4	F_6	β	(1 – β)	$b^{1/2}$	δ
Nd(III) + lysozyme	2	360.82	46.01	5.17	0.9956	0.0044	0.0407	0.434
	4	358.11	44.17	5.17	0.9967	0.0033	0.0404	0.326
	6	358.99	44.05	5.17	0.9968	0.0032	0.0403	0.326
+ Zn(II)	2	356.99	44.44	5.17	0.9970	0.0030	0.0386	0.230
	4	357.39	44.35	5.17	0.9970	0.0030	0.0389	0.303
	6	357.38	44.25	5.17	0.9967	0.0033	0.0406	0.331
+ Cd(II)	2	355.48	44.26	5.17	0.9971	0.0029	0.0381	0.297
	4	356.73	44.46	5.17	0.9970	0.0030	0.0387	0.300
	6	356.88	44.15	5.17	0.9967	0.0033	0.0408	0.334
+Ca(II)	2	357.01	44.44	5.17	0.9970	0.0030	0.0386	0.300
	4	357.48	44.30	5.17	0.9969	0.0031	0.0391	0.320
	6	356.88	44.26	5.17	0.9967	0.0033	0.0406	0.331
+Mg(II)	2	356.88	44.43	5.17	0.9970	0.0030	0.0386	0.299
	4	357.24	44.66	5.17	0.9969	0.0031	0.0391	0.307
	6	356.19	44.10	5.17	0.9967	0.0033	0.0398	0.331

TABLE-4

In t-BuiOH:

System	pH values	F_2	F_4	F_6	β	(1 – β)	$b^{1/2}$	δ
Nd(III) + lysozyme	2	358.63	44.17	5.17	0.9970	0.0030	0.0388	0.301
	4	357.54	44.33	5.17	0.9970	0.0030	0.0388	0.303
	6	359.96	43.99	5.17	0.9967	0.0033	0.0404	0.328
+ Zn(II)	2	356.27	44.57	5.17	0.9972	0.0028	0.0377	0.285
	4	401.01	44.32	5.17	0.9970	0.0030	0.0398	0.301
	6	357.52	44.21	5.17	0.9965	0.0035	0.0451	0.346
+ Cd(II)	2	355.84	44.62	5.17	0.9971	0.0029	0.0380	0.289
	4	358.30	44.18	5.17	0.9968	0.0032	0.0397	0.316
	6	358.14	44.15	5.17	0.9967	0.0033	0.0408	0.334
+Ca(II)	2	355.42	44.67	5.17	0.9971	0.0029	0.0382	0.293
	4	356.73	44.46	5.17	0.9970	0.0030	0.0387	0.300
	6	357.09	44.26	5.17	0.9965	0.0035	0.0418	0.350
+Mg(II)	2	355.85	44.62	5.17	0.9971	0.0029	0.0380	0.290
	4	358.30	44.18	5.17	0.9968	0.0032	0.0397	0.316
	6	357.76	44.15	5.17	0.9965	0.0035	0.0420	0.353

ACKNOWLEDGEMENT

The authors are thankful to Prof. S.N. Misra, Professor and Head, Department of Chemistry, Bhavnagar University, Bhavnagar for research facilities and inspiration for completing this work in the Department of Chemistry, Bhavnagar University, Bhavnagar.

REFERENCES

1. B.R. Judd, *Phy. Rev.*, **127**, 750 (1961).
2. G.S. Ofelt, *J. Chem. Phy.*, **37**, 571 (1962).
3. N.T. Devlin, E.M. Stephens F.S. Richardson, *Inorg. Chem.*, **27**, 1517 (1988).
4. ———, *Inorg. Chem.*, **26**, 1208 (1987).
5. ———, *Inorg. Chem.*, **26**, 1204 (1987).
6. E.Y. Wong, *J. Chem. Phy.*, **35**, 544 (1961); **38**, 976, (1963).
7. W.T. Carnall, P.R. Fields and B.G. Wybourne, *J. Chem. Phy.*, **42**, 3797 (1965).
8. S.N. Misra, G. Joseph and K. Anjaiah, *Indian J. Chem.*, **29A**, 245 (1990).

(*Received*: 7 August 2001; *Accepted*: 9 October 2001) AJC-2483