A NMR Study of Lanthanide-NOTA Chelates as Aqueous Paramagnetic Shift and Relaxation Probes*

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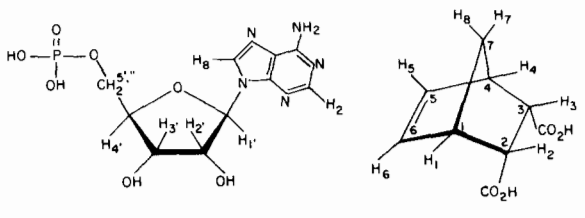
One of the major limitations in the use of paramagnetic lanthanide(III) chelates as NMR aqueous shift reagents to probe dynamic molecular structures [1, 2] has been their lack of structural or effective axial symmetry [3, 4]. In an effort to optimize the characteristics of water soluble NMR shift and relaxation reagents, we have recently [5] studied the paramagnetic shifts and solution structure of the Ln(NOTA) chelate series in aqueous solution near pH 7 (Ln = lanthanide; NOTA is the axially symmetric macrocyclic triaza ligand 1,4,7-triazacyclononane-*N,N',N''*-triacetic acid) and its complexes with the monocarboxylate ligand cyclopropane carboxylic acid [6]. In this work we report a similar study of the binding of the Ln(NOTA) chelates to the flexible mononucleotide adenosine 5'-monophosphate (5'-AMP) which contains one phosphate binding group, and to the rigid *endo-cis*-bicyclo-[2.2.1]hept-5-ene-2,3-dicarboxylic acid (DCA), which has two carboxylate groups oriented in a favorable way to bind lanthanide chelates (Fig. 1). The symmetry of the dipolar shifts and the geometry of the adducts formed are discussed.



5'-AMP DCA

Fig. 1. Chemical structure and numbering scheme of adenosine 5'-monophosphate (5'-AMP) and *endo-cis*-bicyclo-[2.2.1]hept-5-ene-2,3-dicarboxylic acid (DCA).

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Experimental

The preparation of NOTA and the 1:1 Ln(NOTA) complexes have been described previously [5]. 5'-AMP and DCA were obtained from Sigma and Aldrich, respectively. The NMR spectra were obtained on either a Varian XL-200 or JEOL FX-200 spectrometer. T_1 measurements were made using the standard inversion–recovery sequence.

Results and Discussion

LIS studies on 5'-AMP, previously undertaken using aqueous Ln^{3+} cations and Ln(EDTA) chelates [7] (EDTA is ethylenediaminetetraacetic acid), were extended to the entire Ln(NOTA) chelate series. Exchange broadening of the proton 5'-AMP resonances was observed at 25 °C for all Ln(NOTA) chelates; the early Ln(NOTA)–AMP complexes appear to be in rapid exchange at 85 °C but the heavy Ln(NOTA)–AMP complexes are in slow exchange at all temperatures. The Ln(EDTA) chelates cause extensive exchange broadening at 25 °C only for the latter half of the series and not at all at 85 °C [8]. The ^{31}P resonances show similar exchange broadening. The exchange rate differences between the two mixed complexes, Ln(NOTA)–AMP *versus* Ln(EDTA)–AMP, must reflect the larger association constants in the former complexes and hence longer bound lifetimes, τ_M. Thus, the Ln(EDTA)–AMP complexes come closer to meeting fast exchange conditions ($w_M\tau_M \ll 1$) at a given temperature than do the Ln(NOTA)–AMP complexes.

Table I compares the proton shift ratios determined for 5'-AMP protons for the light Ln(NOTA) and Ln(EDTA) chelates at 85 °C. Since conditions of fast exchange apply at this temperature, the shift ratios were obtained in the usual way [7] from NMR titration curves of 5'-AMP with the chelates. The two sets of data agree with each other quite well and both agree with calculated ratios based on axially symmetric dipolar shifts determined from an average 5'-AMP conformation previously described [7] involving chelation at the phosphate groups. Table I also shows the measured proton spin–lattice relaxation rate ratios induced by binding of Gd(NOTA) and Gd(EDTA), which depend on the inverse sixth power of the distances of the protons to the paramagnetic center. The overall data generally support the 5'-AMP adduct structure described previously [7].

The mixed complexes formed by the dicarboxylate ligand DCA with aqueous Ln^{3+} cations and various chelates, including Ln(EDTA) and Ln(HEDTA) (HEDTA is *N*-hydroxyethylenediamine-

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TABLE I. Lanthanide-induced Proton Shift and Relaxation Ratios[a] for 0.05 M 5'-AMP at pH 7.5 and 85 °C, using Ln(NOTA) and Ln(EDTA Chelates as Probes

Perturbation	Lanthanide chelate	H_8	H_2	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$
Shift	Pr(NOTA)	36	−4	15	34	44	36
	Nd(NOTA)	30	−5	5	30	32	27
	Eu(NOTA)	42	2	13	22	b	b
	Pr(EDTA)	32	−1	7	24	35	31
	Nd(EDTA)	30	−2	10	25	35	28
	Eu(EDTA)	30	−1	8	28	40	32
	Calculated[c] (axial)	32	1	13	28	38	33
Relaxation	Gd(NOTA)	97	5	7	b	b	b
	Gd(EDTA)	47	5	7	8	15	19
	Calculated[c]	41	2	3	10	9	13

[a]Relative to $H_{5',5''} = 100$. [b]Not measured. [c]From ref. 7.

tetraacetic acid) have been studied previously using LIS and relaxation techniques [9]. We extended these studies to obtain ^1H and ^{13}C LIS and relaxation data using the Ln(NOTA) chelates.

The ^1H spectra of the Ln(NOTA)–DCA complexes at 25 °C indicate that the Ce → Eu complexes are in fast exchange whereas the Tb → Yb complexes show exchange broadening even at 85 °C. For ^{13}C nuclei, fast exchange conditions applied at 25 °C throughout the entire lanthanide series. Titration curves were consistent with formation of 1:1 Ln(NOTA)–DCA adducts which show increased stability over the corresponding Ln(EDTA)–DCA adducts [9]. Shift ratios and relaxation enhancements for ^1H and ^{13}C nuclei are given in Tables II and III, respectively.

The ratios of induced relaxation rates obtained with Gd(NOTA) and a variety of Ln(HEDTA) chelates (including Gd) are quite similar and agree well with relaxation ratios estimated from a structure for the DCA–lanthanide chelate adduct as described in the literature [9]. ^{13}C relaxation rate ratios induced by Gd(NOTA) also agree quite well with calculated values obtained, in a similar way, from molecular models (Table III). This indicates that DCA forms adducts of very similar structure with all lanthanide complexes studied, including the present NOTA chelates. Therefore, the large variations in shift ratios obtained for ^{13}C LIS values (Table III) do not result from variations in molecular geometry [9]. They could instead result from (a) contact contribution to the measured LIS; (b) non-axial symmetry of the dipolar shifts; or (c) variations of the direction of the axial symmetry axis along the lanthanide cation series. Previous workers [9] have shown that (c) does not apply and proposed (b) as the explanation for the observed changes in proton shift ratios along the lanthanide series. Unfortunately, we were not able to measure proton shift ratios for all Ln(NOTA)–

TABLE II. Lanthanide-induced Proton Shift and Relaxation Ratios[a] for 0.05 M DCA at pH 7.6 and 25 °C, using Ln(NOTA) Chelates as Probes

Perturbation	Lanthanide chelate	H_2, H_3	H_1, H_4	H_5, H_6	H_7	H_8
Shift	Ce(NOTA)	100	29	28	35[b]	
	Pr(NOTA)	100	46	36	36[b]	
	Nd(NOTA)	100	42	19	31	27
	Sm(NOTA)	100	16	22	21[b]	
	Pr(EDTA)[c]	100	42	22	39	28
	Calculated[c] (axial)	100	42	25	43	32
Relaxation	Gd(NOTA)	100	41	70	18[b]	
	Gd(HEDTA)[c]	100	37	63	18	14
	Calculated[c]	100	30	65	16	12

[a]Relative to $H_{2,3} = 100$. [b]Average value for H_7 and H_8. [c]From ref. 9.

TABLE III. Lanthanide-induced ^{13}C Shift and Relaxation Ratios[a] for 0.05 M DCA at pH 7.6 and 25 °C, using Ln(NOTA) Chelates as Probes

Perturbation	Lanthanide chelate	C_1, C_4	C_7	C_2, C_3	C_5, C_6	CO_2
Shift	Ce(NOTA)	40	16	100	14	90
	Pr(NOTA)	26	24	100	23	77
	Nd(NOTA)	7	10	100	6	−30
	Eu(NOTA)	13	13	100	7	−191
	Tb(NOTA)	155	41	100	123	498
	Dy(NOTA)	108	70	100	91	420
	Ho(NOTA)	220	122	100	175	835
	Er(NOTA)	−100	53	100	−93	−66
	Tm(NOTA)	−70	37	100	−51	220
	Yb(NOTA)	0	85	100	10	195
Corrected dipolar shift	(Ce → Eu)	40	24	100	20	312
	(Tb → Yb)	42	38	100	33	220
	Calculated (axial)	50	36	100	32	220
Relaxation	Gd(NOTA)	39	33	100	46	833
	Calculated	30	14	100	40	745

[a]Relative to $C_{2,3} = 100$

DCA complexes because of exchange broadening. Nevertheless, the sign and magnitude of the ^{13}C LIS values, especially for those nuclei near the lanthanide binding site on DCA, indicate that the contact contribution to the LIS values cannot be zero. Using standard procedures [10], we separated the contact and dipolar contributions to the measured LIS values. The proton LIS values for Ce → Sm were found to be overwhelmingly dipolar, and the LIS ratios agreed quite well with the ratios calculated from the published structure using the axial symmetry model (Table II). The analysis of the ^{13}C data, after purification of the contact contribution to each shift, gave similarly good agreement with the axial symmetry model (Table III).

In conclusion, dipolar shifts induced by Ln(NOTA) chelates compare quite well with values calculated assuming axial symmetry and the previously defined solution structures for the 5'-AMP and DCA adducts of lanthanide chelates [7, 9]. This contrasts with non-axial contributions to the LIS values observed in some cases when non-axially symmetric chelates like Ln(EDTA) or Ln(HEDTA) have been used [9]. Therefore, the fact that the axial symmetry of the Ln(NOTA) chelates [5] is preserved when these form adducts with mono- [8] or even polyfunctional ligands like DCA indicates that the symmetry of NOTA determines the overall symmetry of the dipolar induced shifts on the second ligand. This observation makes Ln(NOTA) an attractive series of chelates to be used as aqueous shift probes of molecular structure. Their main limitations seem to result from extensive problems of exchange broadening for the second half of the lanthanide series, caused by stronger interactions with phosphate or carboxylate groups than what is found, e.g., with Ln(EDTA) chelates.

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