Ethylenediaminetetra-acetato-lanthanate(III), -praesodimate(III), -europate(III), and -gadolinate(III) Complexes as Nuclear Magnetic Resonance Probes of the Molecular Conformations of Adenosine 5′-Monophosphate and Cytidine 5′-Monophosphate in Solution

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The use of lanthanoid complexes of ethylenediaminetetra-acetic acid, $[M(edta)]^-$ (M = La, Pr, Eu, and Gd), as n.m.r. shift and relaxation probes has enabled the determination of the conformations of adenosine 5′-monophosphate (amp) and cytidine 5′-monophosphate (cmp) at pH 7·5. The conformations are the same as those found for the molecules at pH 2·0 using lanthanoid aquo-ions as probes.

THE use of lanthanoid ions as probes for conformational studies in solution has been restricted because the uncomplexed ions are insoluble above a pH of *ca.* 6. In addition, complexes of lanthanoid cations with the molecules in which we are interested are often soluble over even more limited pH ranges. Complexes with nucleotides such as cytidine 5′-monophosphate are soluble only below pH 3. The complexes with flavin mononucleotide are totally insoluble. To overcome these restrictions we have made use of water-soluble lanthanoid complexes in much the same way as complexed lanthanoids are used in organic solvents.[1] In this paper we describe studies with ethylenediaminetetra-acetate complexes $[M(edta)]^-$ (M = La, Pr, Eu, and Gd) which have the advantages of known 1 : 1 stoicheiometry and ready solubility over a wide pH range. The method used for the determination of the conformations in solution has already been described.[2-4]

EXPERIMENTAL

Lanthanoid(III) oxides were obtained from Koch–Light, and corresponding chlorides were prepared as described previously.[2,3] Adenosine 5′-monophosphate (amp) and cytidine 5′-monophosphate (cmp) were obtained from Sigma Chemical Company, and the disodium salt of ethylenediaminetetra-acetic acid (EDTA) from B.D.H. Ltd. The nucleotides and EDTA were lyophilised from D_2O (99·8%) and the solids dissolved in D_2O. The complexes $[M(edta)]^-$ (M = La, Pr, Eu, and Gd) were prepared by mixing stoicheiometric quantities of lanthanoid(III) chloride and EDTA solutions. The concentration of amp or cmp was held at 30 mM and $[M(edta)]^-$ concentration varied up to 50 mM. Nominal pH values were adjusted to 7·5 ± 0·1 using NaOD and DCl.

N.m.r. spectra were run on a Brücker HX 90 (90 MHz) spectrometer employing a Nicolet 1085 computer for Fourier-transform procedures. 3-(Trimethylsilyl)propanesulphonic acid, sodium salt (tss), was used as internal standard.

RESULTS

In a separate publication we shall describe work on the $[M(edta)]^-$ (M = La, Pr, and Eu) complexes which have been studied thoroughly by n.m.r. methods. The positions of the resonances from these complexes are therefore well known and, as they do not interfere with observation of the resonances in which we are interested, we shall not be concerned with them in this paper.

Shifts induced in n.m.r. resonances of both amp and cmp by the lanthanoid complexes were effectively linear as the $[M(edta)]^-$ concentration was increased. Some broadening of the nucleotide resonances occurred with both the ions $[Eu(edta)]^-$ and $[Pr(edta)]^-$, but this was not great at temperatures higher than 25 °C. These facts show that the complexes bind to the nucleotides and that fast exchange between bound and unbound forms of the nucleotides is occurring. Shifts induced by $[Eu(edta)]^-$ are upfield for nuclei inside the shift cone as with the Eu^{III} aquo-ion,[2,3] but in the opposite direction to that found with organic shift reagents.[5,6] The magnitudes of shifts induced by the $[M(edta)]^-$ complexes at pH 7·5 at a given concentration were greater than those induced by the uncomplexed ions at pH 2, for the same protons of the nucleotides. Shift magnitudes decreased slightly on increasing the temperature.

TABLE 1

amp Shift ratios relative to H_5' with $[M(edta)]^-$ ions

M	t/°C	H_8	H_2	H_1'	H_2'	H_3'	H_4'
Eu	18	0·33	0·02	0·11	0·30	0·37	0·34
Eu	25	0·29	−0·02	0·08	0·29	0·37	0·31
Pr	18	0·29	0·00	0·12	0·30	0·39	0·31
Pr	25	0·31	−0·01	0·10	0·27	0·39	0·30
Pr	42	0·29	−0·01	0·10	0·27	0·39	0·32

TABLE 2

cmp Shift ratios relative to H_5' with $[M(edta)]^-$ ions

M	t/°C	H_6	H_5	H_1'	H_2'	H_3'	H_4'
Eu	25	0·46	−0·07	0·05	0·19	0·35	0·30
Eu	85	0·41	−0·11	0·08		0·38	0·32
Pr	25	0·46	0·07	0·10	0·23	0·40	0·32
Pr	85	0·43	0·04	0·11	0·21	0·37	0·33

The ratios of shifts induced in resonance positions of all the protons of the nucleotides, relative to the shift of the H_5' protons (which have very nearly the same shift), are

[1] 'Nuclear Magnetic Resonance Shift Reagents,' ed. R. E. Sievers, Academic Press, 1973.
[2] C. D. Barry, J. A. Glasel, A. C. T. North, R. J. P. Williams, and A. V. Xavier, *Nature*, 1971, **232**, 236.
[3] C. D. Barry, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, following paper.
[4] C. D. Barry, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *J. Mol. Biol.*, 1974, **84**, 471.
[5] C. C. Hinckley, *J. Amer. Chem. Soc.*, 1969, **91**, 5160.
[6] C. D. Barry, C. M. Dobson, L. O. Ford, D. A. Sweigart, and R. J. P. Williams, ref. 1, p. 173.

given in Tables 1 (amp) and 2 (cmp) for several temperatures. These shift ratios were independent of the concentration of the lanthanoid complex. The ion [La(edta)]$^-$ caused negligible shifts in spectra of both amp and cmp and so the paramagnetic shifts did not need correction for diamagnetic effects. The shift ratios were the same for both the ions [Eu(edta)]$^-$ and [Pr(edta)]$^-$, except for a

TABLE 3

Mean shift ratios (relative to H_5' protons)

	H_8	H_2	H_1'	H_2'	H_3'	H_4'
(*a*) amp at 25 °C						
pH 2·0	0·31	−0·02	0·08	0·24	0·38	0·32
pH 7·5	0·30	−0·01	0·09	0·28	0·38	0·31
	H_6	H_5	H_1'	H_2'	H_3'	H_4'
(*b*) cmp at 25 °C						
pH 2·0	0·49	0·06	0·09	0·20	0·41	0·28
pH 7·5	0·46	0·00	0·08	0·21	0·38	0·31
(*c*) cmp at 85 °C						
pH 2·0	0·42	0·04	0·10	0·20	0·39	0·32
pH 7·5	0·42	−0·03	0·10	0·21	0·38	0·33

very small change in the ratio for the cmp H_5 proton. In addition broadening studies with the ion [Gd(edta)]$^-$ were carried out at pH 7·5. Relative broadenings of different protons for amp were $H_5' \sim H_8 \gg H_1' > H_2$ and $H_5' > H_6 > H_5 \gg H_1'$ for cmp.

DISCUSSION

The [M(edta)]$^-$ (M = La, Pr, Eu, and Gd) complexes are clearly very effective shift and relaxation probes at pH values higher than those at which the uncomplexed ions can be used. Observed shift ratios for both amp and cmp at pH 7·5 are virtually independent of the lanthanoid used, and are identical to ratios obtained at pH 2·0 using the uncomplexed ions as shown in Table 3. The relaxation data are also very similar at the two pH values.[2,3] Thus the [M(edta)]$^-$ complexes must bind to the phosphate group of each nucleotide.

The effective symmetry of the metal ion in lanthanoid–nucleotide complexes was shown to be axial at pH 2·0.[2-4] Thus must also be true at pH 7·5 for the [M(edta)]$^-$–nucleotide complexes as the shift ratios are the same in the two systems. We have observed that ratios of shifts induced in resonance positions of EDTA protons by Pr^{III} and Eu^{III} are very different, and very dependent on temperature. Thus the [M(edta)]$^-$ system itself does not appear to have axial symmetry, and from this we conclude that the symmetry of the metal ion or complex used need not control the effective symmetry of the complexes formed with the nucleotides. This will be discussed fully in a subsequent paper (but see also refs. 6 and 7).

Computer-based analysis of the data obtained at pH 7·5 (which are identical to those obtained at pH 2·0) must give the same families of conformations as found at pH 2·0. This raises some general questions as to the nature of the observed conformations. The n.m.r. shift and relaxation probes used in these studies can define a 'conformation' of a molecule by using a procedure which deliberately seeks for a fit between observed perturbations and those predicted for computer-generated conformations. For both amp and cmp, searching over a vast number of possible conformations has been shown to yield a very limited family of acceptable solutions in each case. However the n.m.r. data are a reflection of the average conformation in solution observed for a given molecule. We must ask therefore whether or not the generated family of conformations genuinely relates to this average, for the experimental data could arise from gross averaging on the n.m.r. time scale over many widely different conformations. We have stated previously, from the studies at pH 2·0, the reasons why we believe gross averaging cannot be occurring.[2-4] The results given in this paper support this conclusion and lead to new conclusions about the nature of the averaging process.

The first conclusion is that the conformation of the base ring is *anti* with respect to the ribose ring in both amp and cmp. This is true at both pH values studied, despite a change of charge on the base and the use of different probe complexes. It would be quite inconsistent with both shift and relaxation data to postulate any significant contribution from *syn*-forms of either amp (a pyrimidine) or cmp (a purine) in the complexes studied. This is most clearly illustrated by the very small degree of relaxation induced in the H_2 proton of amp,[4] indicating that this proton spends only a very small proportion of time close to the metal (as would be the case in the *syn*-conformation). There must be considerable restraints about the C_1'–N_1 bond in cmp and the C_1'–N_9 bond in amp. There is little effect of temperature on these observations.

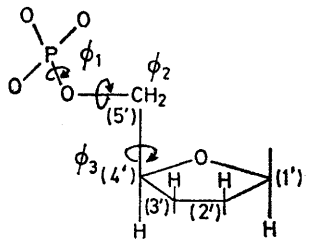
Secondly the shift data, and therefore the conformations, of the ribose rings are virtually the same in cmp and amp at both pH values and over the temperature range 25—85 °C (Table 3). We have provided a single family of conformations to fit the n.m.r. data. We do, however, recognise that this interpretation of the data is in conflict with that of n.m.r. coupling-constant data for ribose rings,[8] but at the present stage of refinement of our methods we have no reason to suppose that more than one family of conformations can contribute *significantly* to the overall picture. This family we will call the conformation below.

Thirdly, the position in space of the metal relative to the phosphorus atom is known with considerable confidence as there are gross limitations on the positions in space of the two H_5' protons. The fact that exactly the same n.m.r. data have been obtained with the two different probes (the uncomplexed lanthanoid ions and their EDTA complexes) strongly indicates that the position of these two H_5' protons is relatively fixed with respect to the metal atom. We stress that the

[7] J. H. Briggs, G. P. Moss, E. W. Randall, and K. D. Sales, *J.C.S. Chem. Comm.*, 1972, 1180.

[8] C. Altona and M. Sundralingam, *J. Amer. Chem. Soc.*, 1973, **95**, 2333.

conformation is one in which the initial section of the molecule is virtually fully extended.[2-4] There are several bonds in this part of the molecule about which free rotation is conceivable (Figure). Even a rather



Rotatable bonds in the ribose phosphate section of the nucleotides

restricted rotation about ϕ_1 from the positions shown in the Figure would move the two H_5' protons relatively closer to the metal ion than the other protons, and this would fit neither the shift nor relaxation data. The relative distance of the phosphorus and the H_5' protons is very well established.[3,9] Rotation about ϕ_2 is not so critical with respect to the H_5' protons but grossly affects the ribose protons. The other possible free rotation (ϕ_3) is that about the C_5'–C_4' bond. Such rotation again moves the metal atom with respect to the ribose ring and the base. To stress this point, only one family of conformations fits the n.m.r. data for both amp and cmp.

A further piece of evidence that gross averaging is not occurring is provided by the previous studies on dinucleoside phosphates.[10] A molecule such as adenylyl-(3′ ⟶ 5′)cytidine (ac), can be considered as a 5′ nucleotide (cmp) in which one of the phosphate oxygen atoms is bound to another nucleoside (adenosine). Ratios of shifts induced by lanthanoid ions for protons of the cytidine 5′-phosphate section of the molecule are the same as those found for the free nucleotide (cmp).[10] Thus the family of conformations and the lanthanoid binding mode must be the same in both systems. It is difficult to see how averaging of conformations over ac could lead to the same result as averaging over cmp.

It is also of interest to consider the binding modes and strengths of complexes formed by [M(edta)]$^-$ with various molecules. The crystal structure[11] of the lanthanoid complex shows that one side of the cation is hydrated by several water molecules. The latter must be displaced by the phosphate group of phosphate monoesters, and the co-ordination between the phosphate oxygen atoms and the lanthanoid cation of [M(edta)]$^-$ must have exactly the same geometry as that of the lanthanoid aquo-ion with phosphate monoesters. At pH 7·5, the phosphate ester binds as a dianion, whilst at pH 2·0 it binds as a monoanion. We have shown elsewhere that the lanthanoid aquo-ions can bind phosphate diesters [*e.g.* dinucleoside phosphates[10] or cyclic adenosine 3′ : 5′-monophosphate (camp)[12]], but we have observed that there is no significant binding of [M(edta)]$^-$ to these ligands. In addition we have observed no significant binding to singly charged carboxyl groups but there is binding to the diphosphate group of flavin adenine dinucleotide. This selectivity in binding can be of great use, for example in adenylyl-(3′ ⟶ 5′)adenosine 3′-phosphate (aap) [M(edta)]$^-$ ions bind only to the terminal 3′-phosphate. By careful selection of complexed lanthanoids, the selectivity of binding, such as is seen here, could well become of great value. For example specific-site reagents could be devised for large systems such as t-RNA or proteins.

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[10] C. D. Barry, J. A. Glasel, A. C. T. North, R. J. P. Williams, and A. V. Xavier, *Biochim. Biophys. Acta*, 1972, **262**, 101.
[11] J. L. Hoard, B. Lee, and M. D. Lind, *J. Amer. Chem. Soc.*, 1965, **87**, 1611.
[12] C. D. Barry, D. R. Martin, R. J. P. Williams, and A. V. Xavier, *J. Mol. Biol.*, 1974, **84**, 491.