Mössbauer-Effect Studies on Metal Binding in Purine Compounds[1a]

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Abstract: The nature of the binding site of transition metals in nucleotides and nucleic acids is still largely unsettled. Mössbauer effect spectra have been obtained on Fe(II) and Fe(III) chelates of nucleotides, nucleic acids, and EDTA-type chelating agents. Spectra have also been obtained for Sn(IV) chelates of nucleotides. The spectra of the Fe(III)–EDTA-type chelates are analyzed with respect to a previously offered suggestion of an experimental Mössbauer effect test for covalency. The nucleotide and nucleic acid spectra are similarly discussed in terms of degree of covalency of binding and the geometry of the binding site. The Mössbauer effect data support the view that Fe(II) and Fe(III) can bind to the purine nitrogens at neutral and basic pH, but not at acidic pH. The Mössbauer effect results complement quite satisfactorily epr spectra obtained from the same compounds. Correlations with infrared and nmr results are also discussed.

Emphasis in the study of the structure and reactivity of metal complexes of ATP[2] has recently shifted to studies of reaction kinetics and detailed analyses of the electronic charge configuration of the complexes. Fukui, *et al.*,[3] have surveyed some of the concepts involved, and Fukui, *et al.*,[4] have summarized the possible models for metal chelation to ATP. Metal binding to naturally occurring polymers of nucleotides, such as RNA, is known to affect the conformation and physical properties of the RNA molecule,[5] although it is not yet clear whether the naturally occurring heavy metals in RNA have any effect upon the biological function of the molecule.[6–8]

Cohn and Hughes[9] have studied the proton and phosphorus nuclear magnetic resonance (nmr) spectra in metal complexes of ADP and ATP. They found that some metals bind only to the β and γ phosphates of ATP and some to the α, β, and γ phosphates, and that zinc may bind to the nitrogen(s) of the purine ring as well as to the β and γ phosphates. Alteration of pH was shown to affect the position of the resonance peaks. Fe(II) also binds to the purine rings as well as to the phosphate chain.[10] Eisinger, *et al.*,[11] determined nuclear relaxation times of protons in metal–DNA complexes and concluded that whereas Mn(II), Cu(II), and Cr(II) are bound to exterior phosphate groups, Fe(III) is bound to interior purine coordination sites. Singer[12] studied the firmness of binding of various metals to tobacco mosaic virus RNA by repetitive precipitations in alcohol and concluded that Fe(III), among others, binds to the bases, whereas Ca(II), among others, binds only to the phosphates. Maling, *et al.*,[13] found that Mn(II) and Fe(III) gave similar electron paramagnetic resonance (epr) signals in complexes with ATP and RNA suggested that Mn(II) may be bound to the base or ribose moiety as well as the phosphates in these compounds. Patten and Gordy,[14] however, found that the epr signals from Mn(II)–RNA complexes were similar to those obtained with Mn(II) salts in solution.

The Mössbauer parameters of greatest interest to the chemist, the isomer shift (IS) and quadrupole splitting (QS), are sensitive to changes in nearest-neighbor interactions with the Mössbauer atom. Since at least one of the binding sites for Fe(III) in nucleotides is thought to be the polyphosphate chain, the Mössbauer spectra of $Fe_2(PO_4)_3$, $Fe_4(P_2O_7)_3$, and Fe(III)–adenine nucleotides were studied in detail. In addition, since the possible models for metal chelation by nucleotides include structures with four or five oxygen ligands together with two or one bonding interactions with nitrogen atoms, respectively,[4] a series of model iron chelates having these structures was studied *via* their epr, infrared, and Mössbauer spectra. The model compounds chosen were of the "EDTA family"; *i.e.*, nitrilotriacetate (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and cyclohexanediaminetetraacetic acid (CDTA). In the order given, the Fe(III) *vs.* Fe(II) stability constant increases and the oxidation–reduction potential decreases in the pH range 5–9, which indicates that the

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(2) Abbreviations used in this paper are AMP, ADP, ATP for adenosine mono-, di-, and triphosphate, respectively. RNA = ribonucleic acid. Other abbreviations used are given in the Experimental Section in parentheses, after the full term appears.

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(4) K. Fukui, K. Morokuma, and C. Nagata, *ibid.*, 36, 1450 (1963).

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(9) M. Cohn and T. R. Hughes, Jr., *J. Biol. Chem.*, 237, 176 (1962).

(10) Personal communication from Professor M. Cohn; methods used are given in ref 9.

(11) (a) J. Eisinger, R. G. Shulman, and W. E. Blumberg, *Nature*, 192, 963 (1961); (b) J. Eisinger, R. G. Shulman, and B. M. Szymanski, *J. Chem. Phys.*, 36, 1721 (1962). (c) After submittal of this paper further nmr studies of metal binding to nucleotides and nucleic acids were reported in a series of papers by R. G. Shulman, *et al.*, *J. Chem. Phys.*, 43, 3116, 3750 (1965).

(12) B. Singer, *Biochim. Biophys. Acta*, 80, 137 (1964).

(13) J. E. Maling, L. T. Taskovich, and M. S. Blois, Jr., *Biophys. J.*, 3, 79 (1963).

(14) R. A. Patten and W. Gordy, *Nature*, 201, 361 (1964).

Fe(III) chelate in these compounds is stabilized relative to the Fe(II) chelate.[15] This provides an independent measure of "covalency" against which another proposed experimental test for "covalency" based on Mössbauer analysis[16] was investigated, as discussed below.

Results and Discussion

As shown in Table I, there is no quadrupole splitting in the Fe(III) nucleotides at pH 1 to 3 except for sample 5 which will be discussed below, or in $Fe_4(P_2O_7)_3$; in contrast, the QS in ferric phosphate is easily resolved from the resonance spectra. The crystal symmetry of $Fe_2(PO_4)_3$ is D_3^4 or D_3^6,[17] for which the screw axis does not allow perfect octahedral symmetry around the Fe(III) atom. This noncubic nearest-neighbor environment gives rise to a finite electric field gradient which is experimentally observed as a splitting of the first excited nuclear energy level in Fe^{57}. The isomer shift and quadrupole splitting values for $Fe_2(PO_4)_3$, as shown in Figure 1a and Table I, are in good agreement with those reported by Fluck, *et al.*[18] The crystal symmetry of $Fe_4(P_2O_7)_3$ has not been reported in the literature, but early crystallographic work on tetravalent metal salts (including Sn(IV)) of pyrophosphoric acid[19] and a more recent report on $Na_4P_2O_7$[20] lead to the expectation that the iron atom in $Fe_4(P_2O_7)_3$ has six nearest-neighbor oxygen ligands in octahedral symmetry. This symmetry should result in a zero electric field gradient tensor and hence to the absence of quadrupole splitting. The Mössbauer spectrum of $Fe_4(P_2O_7)_3$, shown in Figure 1b, has a large resonance effect, and there is no resolved quadrupole splitting although the line width is about 2.5 times greater than the expected upper limit for an Fe^{57} absorption line.[21] For the nucleotides at low pH, then (except perhaps for sample 5), the Mössbauer spectral data support the view that the Fe(III) atom is surrounded by an octahedral arrangement of oxygens, although it is not immediately possible to say which of these oxygens are bound to phosphorus atoms and which to hydrogen atoms in water molecules.

The isomer shift values for all of the nucleotides at all pH values, and for all of the chelate model compounds, are in the range expected for ionic Fe^{2+} or Fe^{3+} compounds. For discussions of the Mossbauer systematics of iron compounds, see, for example, Wertheim,[22] or Gol'danskii.[23] Among the chelate compounds studied, however, there is available chemical evidence of covalent bonding interactions, and the Mössbauer spectral parameters of these compounds were examined to see if any systematic qualitative differences could be observed which could lead to quantitative descriptions of the bonding involved. Until this latter is achieved use is made in this paper of the more noncommittal Fe(II) or Fe(III) notation.

(15) J. Bond and T. I. Jones, *Trans. Faraday Soc.*, **55**, 1311 (1959).
(16) L. M. Epstein, *J. Chem. Phys.*, **40**, 435 (1964).
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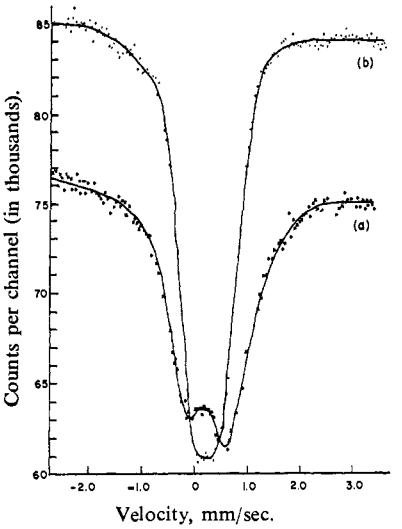

Figure 1. Mössbauer spectrum of (a) $Fe_2(PO_4)_3$, 300°K; (b) $Fe_4(P_2O_7)_3$, 77°K.

For the EDTA series of Fe(III) chelates, which have similar binding site symmetry and nearest-neighbor ligand atoms, systematic IS values were found. It has been suggested[16] that as the 3s character ("covalency") of an iron atom increases within a series of closely related compounds, a shift toward more negative values for the IS parameter should be observed. As shown in Table I, samples 23 to 28, this seems generally to be the case for the series Fe(III)–NTA, –EDTA, –DTPA, –CDTA, where independent evidence[15] indicates that the covalent character of the iron atom increases in the order given.

Some of the dangers of a too facile interpretation of Mössbauer data, as well as some of the interesting potentialities for this technique, may be seen in a closer analysis of the EDTA family of chelates. NTA, for example, is known to have a slight chelating tendency toward sodium ions[24] and this may be reflected in the different isomer shifts of Fe(III)–NTA when potassium or sodium are the counterions. The different isomer shifts could be due to the competitive effect of sodium bonding forcing the iron to adopt more than one binding site so that the Mössbauer spectrum would consist of two superposed resonances effectively changing the observed IS and QS values. It seems less likely that the IS value, arising from the electronic charge density at the iron nucleus, would be differentially affected by an electrostatic field of sodium as opposed to potassium ions. The former effect should be concentration dependent and it was found that within a wide range of sodium concentration there was no appreciable difference in the Mössbauer parameters of Fe(III)–ATP at low pH (samples 6 and 7, Table I). Sodium was therefore used as a convenient counterion in all of the other complexes investigated.

When the ATP:Fe(III) molar concentration ratio was increased from 1:1 to 10:1 at low pH, a surprising bumpy spectrum was obtained, as seen in Figure 2 (sample 8). A similar spectrum showing unresolved fine structure, with more than one major resonance, was observed by Epstein for Fe(III)–EDTA, and it was

(24) G. Schwarzenbach and W. Biedermann, *Helv. Chim. Acta*, **31**, 331 (1948).

Table I

	Sample	Concn, *M*	pH	Temp, °K	Line width, mm/sec	IS,[a] mm/sec	QS, mm/sec
1	$Fe_4(P_2O_7)_3$	Fe = 1.8×10^{-2} $Na_4P_2O_7$ = 1.8×10^{-2}, ppt obtained	3.0	77	1.16	0.70	
2	$Fe_2(PO_4)_3$	Ppt with 1 *M* NaH_2PO_4 and $FeCl_3 \cdot 6H_2O$	2.0	300		0.69	0.66
3	Fe(III)–AMP	Fe = 3.0×10^{-3} AMP = 3.6×10^{-3}	1.9	77	0.92	0.53	
4	Fe(III)–ADP, Chelex-treated	Fe = 2.4×10^{-3} ADP = 3.7×10^{-3}	2.0	77	1.14	0.81	
5	Fe(III)–ADP	Fe = 8.3×10^{-3} ADP = 1.2×10^{-2}	1.8	77		0.70	0.48
6	Fe(III)–ATP	Fe = 2.3×10^{-3} ATP = 2.8×10^{-3} ionic strength (NaCl) = 1.0	3.2	77	1.36	0.71	
7	Fe(III)–ATP	Fe = 6.3×10^{-3} ATP = 7.2×10^{-3} ionic strength, (NaCl) = 3.5×10^{-4}	1.6	77	1.12	0.71	
8	Fe(III)–ATP	Fe = 1.8×10^{-3} ATP = 1.8×10^{-2}	1.6	77	1.13	0.64	
9	Fe(III)–ATP	Fe = 1.3×10^{-3} ATP = 8.3×10^{-3}	7.1	77		0.70	0.61
10	Fe(III)–ITP	Fe = 1.3×10^{-3} ITP = 8.3×10^{-3}	7.1	77		0.75	0.51
11	Fe(III)–ATP in 70% glucose	Fe = 9.4×10^{-3} ATP = 1.1×10^{-2}	6.0	77		0.71	0.69
12	Fe(III)–ATP	Fe = 1.8×10^{-3} ATP = 1.8×10^{-2}	9.8	77		0.68	0.48
13	Fe(III)–ATP in 70% glucose	Fe = 1.2×10^{-3} ATP = 1.2×10^{-2}	9.7	77		0.74	0.69
14	Fe(III)–ADP	Fe = 1.3×10^{-3} ADP = 1.1×10^{-2}	7.1	77		0.88	0.63
15	Fe(III)–AMP	Fe = 6.3×10^{-4} AMP = 8.0×10^{-3}	7.1	77		0.79	0.74

[a] As stated in the Experimental Section, the isomer shifts for the iron samples are with respect to a standard absorber of $Na_2Fe(CN)_5NO \cdot 2H_2O$. For Co^{57} (Pd) sources (used in this work), this amounts to adding 0.442 mm/sec to each IS value. [b] No independent determinations of molecular weight were made for these molecules. An estimated molecular weight for poly A (Calbiochem) would be 200,000 and for

suggested[25] that there were two or more discrete binding environments for the Fe(III) in EDTA. Other examples of such complex spectra, exhibiting hyperfine (nuclear Zeeman) interactions between the magnetic

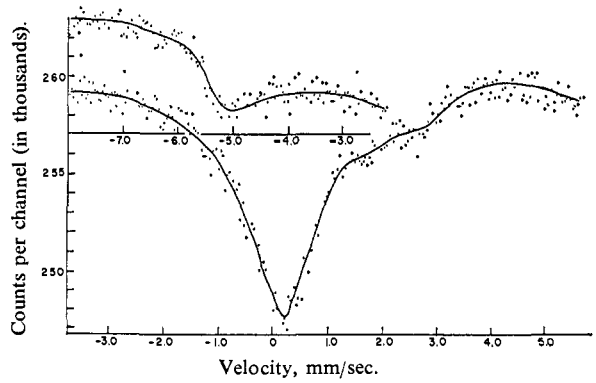

Figure 2. Mössbauer spectrum of Fe(III)–ATP, pH 1.6, 77°K; the molar ratio of nucleotide to Fe is 10:1.

moment of the iron nucleus and the surrounding magnetic field, have been reported, notably in ferrichrome and ferrichrome A by Wickman, *et al.* (quoted by Gonser and Grant[26]). A theoretical treatment of the effect on Mössbauer resonances of fluctuating electric and magnetic fields produced by the relaxation of paramagnetic ions or by the fluctuation of the environment surrounding the nucleus has been reported by Blume.[27] The spectrum of ferrichrome A at 77°K[26] closely resembles the spectrum shown in Figure 2, and in the case of ferrichrome A the temperature dependence of the hyperfine splitting indicated that the fluctuations in the internal magnetic field were due to a long electronic spin–lattice relaxation time affecting the Mössbauer resonance.[26] In the case of sample 8 of Fe(III)–ATP, the concentration dependence of the hyperfine structure indicates that the fluctuations are due to electronic spin–spin relaxation, although the temperature dependence has not been checked. The ATP:Fe(III) concentration ratio of the complex at pH 7.1 (sample 9) is not exactly the same as that at pH 1.6 (sample 8), but there is no trace of hyperfine splitting. This supports the argument given below that the binding sites are different at these two pH values. A further complication in the analysis of the Fe(III)–EDTA Mössbauer spectrum, and, by implication, the other chelates of this family, is X-ray diffraction evidence in

(25) L. M. Epstein, *J. Chem. Phys.*, **36**, 2731 (1962).

(26) U. Gonser and R. W. Grant, "Mössbauer Effect Methodology," Vol. I, Plenum Press, New York, N. Y., 1965, pp 36–37.

(27) M. Blume, *Phys. Rev. Letters*, **14**, 96 (1965).

	Sample	Concn, *M*	pH	Temp, °K	Line width, mm/sec	IS,[a] mm/sec	QS, mm/sec
16	Fe(III)–poly A	1.0 mg of Fe 102.0 mg of poly A[b]	7.0	77		0.72	0.74
17	Fe(III)–Me-poly A	0.7 mg of Fe 75.0 mg of Me-poly A[b]	7.0	77		0.77	0.79
18	Fe(III)–S-RNA	1.0 mg of Fe 101.0 mg of S-RNA[b]	7.2	77		0.63	0.68
19	Fe(II)–ATP	$Fe = 1.7 \times 10^{-3}$ $ATP = 1.7 \times 10^{-2}$	1.9	77		1.58	3.10
20	Fe(II)–ATP	$Fe = 1.4 \times 10^{-3}$ $ATP = 1.4 \times 10^{-2}$	7.2	77		(i) 0.56[c] (ii) 1.77	0.72 2.42
21	Fe(II)–ADP	$Fe = 2.0 \times 10^{-3}$ $ADP = 2.0 \times 10^{-2}$	1.8	77		1.61	2.90
22	Fe(II)–AMP	$Fe = 1.8 \times 10^{-3}$ $AMP = 1.9 \times 10^{-2}$	3.0	77		1.53	3.02
23	Fe(III)–NTA potassium salt of NTA	$Fe = 6.1 \times 10^{-2}$ $NTA = 6.7 \times 10^{-2}$	6.0	77		0.77	1.39
24	Fe(III)–NTA sodium salt of NTA	$Fe = 1.0 \times 10^{-2}$ $NTA = 1.2 \times 10^{-2}$	5.3	77		0.55	1.68
25	Fe(III)–NTA	$Fe = 1.0 \times 10^{-2}$ $NTA = 1.2 \times 10^{-2}$	9.1	77		0.66	0.70
26	Fe(III)–EDTA	...[d]		300		0.62	
27	Fe(III)–CDTA	$Fe = 7.8 \times 10^{-3}$ $CDTA = 4.4 \times 10^{-2}$	7.3	77	1.82	0.62	
28	Fe(III)–DTPA	$Fe = 7.1 \times 10^{-3}$ $DTPA = 4.0 \times 10^{-2}$	7.0	77		0.39	0.56
29	Fe^{III} (A–H) I_2	...[e]		77		0.79	1.62
30	$Fe^{III}B(OH)(ClO_4)_2$	...[e]		77		0.80	0.85
31	Sn(IV)–AMP Mg_2Sn source	$SN = 1.5 \times 10^{-2}$ $AMP = 1.7 \times 10^{-2}$	1.5	300	1.00	−1.79	
32	Sn(IV)–ADP Mg_2Sn source	$Sn = 1.4 \times 10^{-2}$ $ADP = 1.6 \times 10^{-2}$	1.5	300	1.10	−1.88	
33	Sn(IV)–ATP Mg_2Sn source	$Sn = 1.1 \times 10^{-2}$ $ATP = 1.2 \times 10^{-2}$	1.6	300	1.16	−1.89	
34	Sn(IV)–ATP SnO_2 source[f]	$Sn = 1.1 \times 10^{-2}$ $ATP = 1.2 \times 10^{-2}$	1.6	77	1.44	−0.04	

S–RNA, 25,000. [c] Interpretation of the two resonance effects given in text. [d] *Cf.* ref 29. [e] *Cf.* ref 28. [f] A SnO_2 source was substituted for the Mg_2Sn source used for samples 31 to 33 after the Mg_2Sn source disintegrated in midrun for sample 34. The velocity conversion factor for Mg_2Sn source to a SnO_2 source is +1.76 mm/sec.

favor of seven rather than six coordination of the iron atom.[28]

Because the geometry of the binding site for Fe(III) in nucleotides is not definitely known, and because electric field gradients in ionic Fe^{3+} compounds are very sensitive to small changes in bond lengths and angles as well as to postulated ligand charges,[22] crystal-field models of the binding sites, yielding information about the strength of the ligand field and values for crystal-field splittings, are not advanced here. Once the molecular parameters are accurately known, quantitative statements about the extent of the deviation from a purely ionic Fe^{3+} nucleotide structure may be made from the Mössbauer data. It is possible, however, to detect changes in the electric field gradient in a given compound when one ligand is substituted for another. The difference, for example, in the QS between Fe(III)–NTA at pH 5.3 and pH 9.1 (samples 24 and 25, Figure 3), may be due to one aquo ligand being replaced by a hydroxo ligand.[24,29] The structural differences between the pH 5.3 and pH 9.1 Fe(III)–NTA chelates can also be observed in the infrared spectra of the KBr pellets of the powdered solids,[30] although a quantitative treatment of the infrared spectra was not attempted in view of the difficulties involved, as discussed by Nakamoto.[31] Epr spectra of the two chelates showed only marginal differences, which is perhaps to be expected with powder samples.

The absence of resolved quadrupole splitting in Fe(III)–CDTA (sample 27) is a further caution not to equate "chemical" with electric field symmetry.[32] A more complex example of this is that of the Mössbauer spectra of two Fe(III) chelates, prepared by Curry and Busch[33] (samples 29 and 30, Figure 4). As with the EDTA family of chelates, these two chelates are presumed to be six-coordinate, but instead of having one or more electronegative oxygen atom ligands replaced by electropositive nitrogen atoms, the situation is reversed. Both chelates are low-spin Fe(III) chelates and the first sexadentate chelate (sample 29), all the ligands of which are nitrogen atoms, is a highly strained structure with low symmetry[33] which would therefore be expected to show quadrupole splitting, as observed. The second chelate (sample 30)

(28) J. L. Hoard, M. Lind, and J. V. Silverton, *J. Am. Chem. Soc.*, **83**, 2770 (1961).
(29) R. I. Gustafson and A. E. Martell, *J. Phys. Chem.*, **67**, 576 (1963).
(30) I. N. Rabinowitz, *Dissertation Abstr.*, **24**, 171 (1964).
(31) K. Nakamoto, "Infra-Red Spectra of Inorganic and Coordination Compounds," John Wiley and Sons, Inc., New York, N. Y., 1963.
(32) R. H. Herber and H. A. Stöckler, *Trans. N. Y. Acad. Sci.*, **26**, 929 (1964).
(33) J. D. Curry and D. H. Busch, *J. Am. Chem. Soc.*, **86**, 592 (1964).

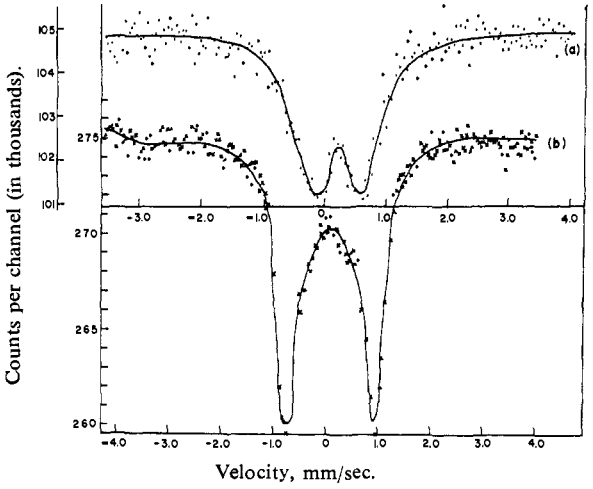

Figure 3. Mössbauer spectrum of (a) Fe(III)–NTA, pH 9.1, upper velocity scale and counting range 101,000 to 105,000; (b) Fe(III)–NTA, pH 5.3 (both at 77°K). Note: There are some Mössbauer curves with more than one abscissa and more than one set of numbers giving "counts per channel." This is because the total counting rate was not always the same for all samples and because the velocity scale (the abscissa) therefore would necessarily be different for compounds which were measured when the apparatus had differing velocity constants. It is nearly impossible to put all of the curves on one standard abscissa.

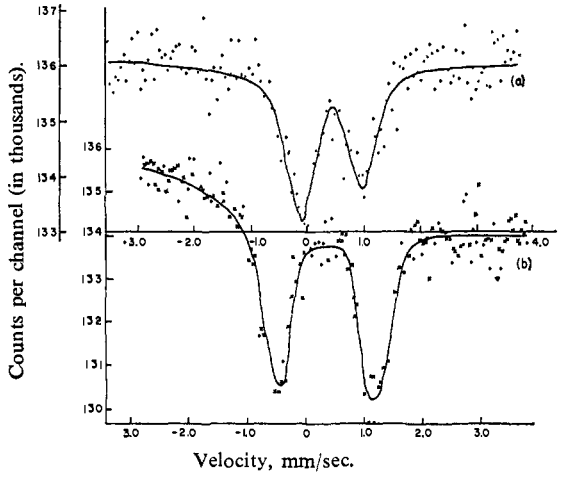

Figure 4. Mössbauer spectrum of (a) $Fe^{III}B(OH)(ClO_4)_2$, upper velocity scale and counting range 133,000 to 137,000; (b) Fe^{III}-(A-H) I_2 (both at 77°K).

differs from the first in the substitution of a hydroxo ligand for an imino nitrogen.[33] The two chelates differ markedly in the magnitude and asymmetry of their quadrupole splittings.

Line asymmetry can be an important parameter in deducing binding site symmetry and structure from the Mössbauer spectra. The task lies in clearly distinguishing from among the possible causes of line asymmetry. There is first the preferential orientation of the polycrystalline sample in the absorber holder;[34] then there is the Gol'danskii effect,[35] in which bond strengths stronger in one direction than another give rise to an

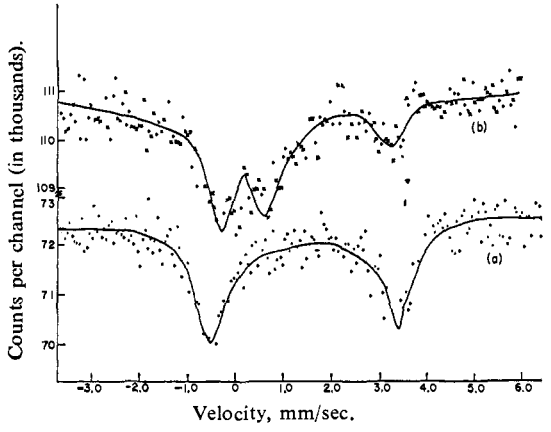

Figure 5. Mössbauer spectrum of (a) Fe(II)–ATP, pH 1.9; (b) Fe(II)–ATP, pH 7.2 (both at 77°K).

anisotropic Debye–Waller factor, or recoil-free fraction. This effect is probably responsible for the asymmetry of the QS in samples 29 and 30. The last cause of line asymmetry would be unresolved nuclear Zeeman interactions as mentioned above. Definite identification of the correct cause of line asymmetry will depend on concentration and temperature studies of a polycrystalline sample and on obtaining Mössbauer spectra from single crystals.

With the results of the model chelates in mind, we may try to rationalize the increase in the values of the isomer shift for the series AMP, ATP, ADP, at low pH as follows. The iron atom electron orbitals in an octahedral ligand arrangement are hybridized as d^2sp^3 and the two d orbitals used are the e_g orbitals in the nomenclature of Orgel,[36] the remaining t_{2g}[36] d orbitals accommodating the electrons originally present on the metal. If Fe(III) is bound to a diphosphate moiety in ADP and ATP, the electronegative phosphoryl oxygen atoms can donate electrons to the t_{2g} orbitals of the iron. This addition of d electrons has a shielding effect on the nucleus which reduces the attractive Coulomb potential between the nucleus and the s electrons. This gives rise to 3s wave function expansion with a concomitant decreased s electron density at the nucleus. This decrease in $|\Psi_s(0)|^2$ results in a positive change in the isomer shift[37] in ADP and ATP relative to that of AMP, which cannot bind iron to a diphosphate, except in an intermolecular complex.

The increase in isomer shift in the series Fe(III)–AMP, –ATP, –ADP (samples 3, 4, and 6) at low pH, is repeated for the series Fe(II)–AMP, –ATP, –ADP (samples 22, 19, and 21), although the data are not quite as clear-cut. The isomer shifts for the Fe(II) chelates are characteristically larger than those for the Fe(III) chelates, since the extra d electron in Fe(II) effectively decreases the s electron density in a manner similar to that discussed above.[37] The quadrupole splitting for the Fe(II) chelates is also characteristically larger than for the Fe(III) chelates, as can be seen by comparing Figure 5a with Figure 6b (samples 19 and 9). This observation, which is consistently noted for analogous Fe(II) and Fe(III) inorganic compounds, arises from the fact that the quadrupole splittings for

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(36) L. E. Orgel, "An Introduction to Transition-Metal Chemistry: Ligand Field Theory," Methuen and Co., Ltd., London, 1963.
(37) L. R. Walker, G. K. Wertheim, and V. Jaccarino, *Phys. Rev. Letters*, **6**, 98 (1961).

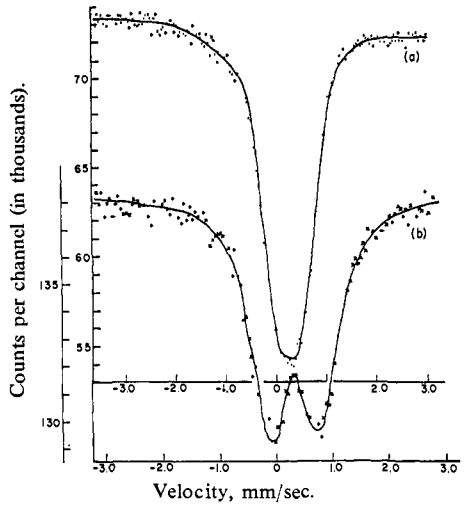

Figure 6. Mössbauer spectrum of (a) Fe(III)–ATP, pH 1.6 (sample 7 of Table I), upper velocity scale and counting range 53,000 to 74,000; (b) Fe(III)–ATP, pH 7.1 (both at 77°K).

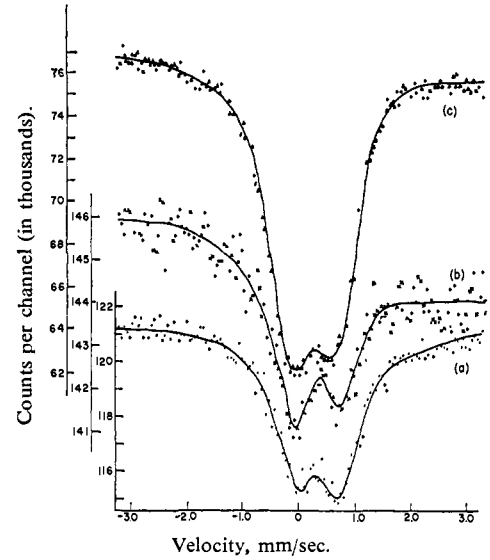

Figure 7. Mössbauer spectrum of (a) Fe(III)–ITP, pH 7.1, counting range 115,000 to 122,000; (b) Fe(III)–ATP, pH 9.7, in 70% glucose, counting range 141,000 to 146,000; (c) Fe(III)–ATP, pH 9.8, in water, counting range 62,000 to 77,000 (all samples at 77°K).

Fe(III) complexes are due to asymmetric field gradients around the spherically symmetric charge distribution on the Fe(III) ion, whereas the extra d electron present in complexes of Fe(II) introduces an asymmetric charge field on the iron atom itself. The energetic state of this extra orbital is determined by the crystal field.

When the pH of the Fe(III) chelates of AMP, ADP, and ATP is brought up to pH 7 (samples 15, 14, and 9), quadrupole splittings are observed. These data strongly suggest that one or more nitrogens of the ring have replaced oxygen ligands; *i.e.*, at low pH the Mössbauer data support a chelate model involving only the phosphate moieties of adenine nucleotides, but at neutrality and higher pH the ring nitrogens have become effective ligands. Nmr evidence seems to point to either the 6-amino and/or 7-imino position on the adenine ring as possible binding sites.[9,10] An Fe(III) chelate of inosine triphosphate was prepared in exactly the same manner as sample 9 of Fe(III)–ATP (sample 10, Figure 7a). The isomer shifts of the two samples were about the same within experimental error, and the QS values were marginally different. The asymmetry of the QS for the two cases appears to be different although the counting statistics and resolution of the Fe(III)–ITP resonance is not particularly good. In view of the results with the model chelates, a difference in line asymmetry would be expected between the two compounds if the 6-amino position is in fact an effective ligand in the Fe(III)–ATP chelate. If the pH of the Fe(III)–ATP chelate is raised to very basic values, the nitrogen of the 6-amino position should become an even more effective electron donor, and as seen in Figure 7b,c (samples 12 and 13), with good resolution, the asymmetries of the quadrupole splittings are opposite in sense to that of the Fe(III)–ITP (Figure 7a) and more pronounced than that of Fe(III)–ATP at pH 7.1 (Figure 6b).

Glucose (70%), used as a solvent for samples 11 and 13, eliminated the possibility of ferric hydroxide formation and allowed the molar ratio of iron to nucleotide to be increased so that spectra at high pH could be studied over a wider range of concentrations. At approximately neutral pH, the Mössbauer parameters for Fe(III)–ATP in both water and 70% glucose were essentially the same, including line asymmetry, which gave some reason for believing that the nearest-neighbor configuration about the iron atom was the same in both cases, at least at neutral pH.

When the pH of an Fe(II)–ATP chelate is raised to about neutrality (sample 20) the spectrum shown in Figure 5b is obtained. The resonance exhibiting quadrupole splitting and centered at 0.56 mm/sec is interpreted as being due to Fe(III), arising probably from air oxidation of Fe(II) as the pH is raised. The resonance line at about 3.1 mm/sec is then part of the quadrupole splitting doublet of residual Fe(II), the other part of the doublet being at about 0.56 mm/sec. It would be of interest to repeat this experiment under an inert atmosphere throughout to determine to what extent, if at all, Fe(III) is the stable valence state in iron nucleotides, as it is in chelates with the EDTA family.[15]

The Mössbauer spectra for Fe(III)–polyadenylic acid (sample 16, Figure 8b) and Fe(III)–methylated poly A (sample 17) resemble almost exactly the spectrum for Fe(III)–AMP (Figure 8a), which supports the thesis that the binding sites in these molecules are similar to that in AMP, *i.e.*, a site involving both the phosphate and the adenine ring. Methylated poly A is methylated at the N_1 position on the adenine ring,[38] so that these results provide more evidence that the N_1 position is not involved in metal complex formation. The spectrum for Fe(III)–S-RNA shown in Figure 8c (sample 18) exhibits a slightly different IS, QS, and line asymmetry than either Fe(III)–poly A or Fe(III)–Me-poly A, and this is to be expected as the different bases present in S-RNA allow different populations of binding sites and the resultant of the separate Mössbauer resonances should yield an effect having a different IS, QS, and line asymmetry.

Sample 5 of Fe(III)–ADP at low pH, unlike Fe(III)–AMP or Fe(III)–ATP at low pH, exhibits a clearly resolved quadrupole splitting (Figure 9a). When the ADP was first passed through Chelex resin to remove

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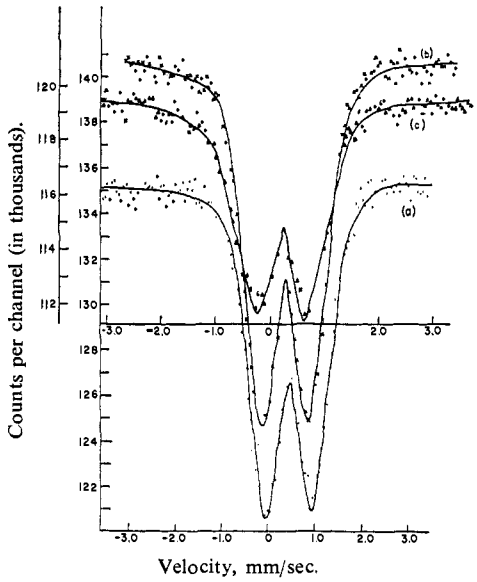

Figure 8. Mössbauer spectrum of (a) Fe(III)-AMP, pH 7.1, counting range 120,000 to 141,000; (b) Fe(III)–poly A, pH 7.0, counting range 120,000 to 141,000; (c) Fe(III)–S-RNA, pH 7.2, upper velocity scale and counting range 112,000 to 120,000 (all samples at 77°K).

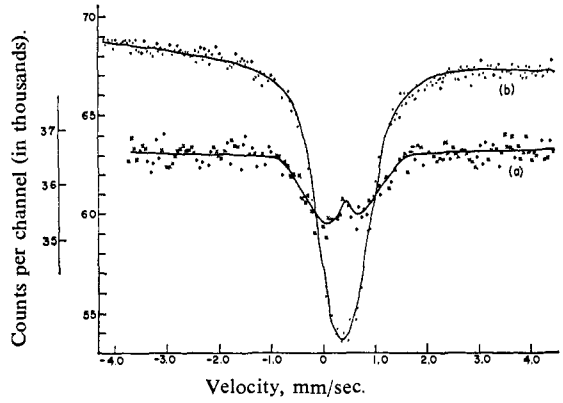

Figure 9. Mössbauer spectrum of (a) Fe(III)–ADP, pH 1.8, not Chelex treated, counting range 35,000 to 37,000; (b) Fe(III)–ADP, pH 2.0, Chelex treated, counting range 53,000 to 70,000 (both at 77°K).

approximately two-thirds of the iron "impurity"[39] (from 455 to 131 mμg of Fe/mg of nucleotide) present in the commercial sample, and then complexed with added iron, there was no quadrupole splitting (Figure 9b, sample 4) although the isomer shift became more positive. It is possible that the totality of trace element impurities in ADP is responsible for competitive binding with iron, but this seems unlikely in view of the extraordinarily high binding constant found for Fe(III) to ADP.[40] It will be recalled that at low pH, Fe(III)–ATP exhibited concentration-dependent hyperfine interactions, but not quadrupole splittings, and it has also recently been suggested[41] that Fe(III) will form complexes of the form Fe_2ATP and $Fe(ATP)_2$ at pH 2 but that Fe(III) will form only the 1:1 complex with

(39) For a quantitative survey of the amounts of trace iron found in commercially available nucleotides and a demonstration of a biochemical role for such "impurities," *cf.*, P. Hochstein, K. Nordenbrand, and L. Ernster, *Biochem. Biophys. Res. Commun.*, **14**, 323 (1964).

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(41) C. R. Goucher and J. F. Taylor, *J. Biol. Chem.*, **239**, 2251 (1964).

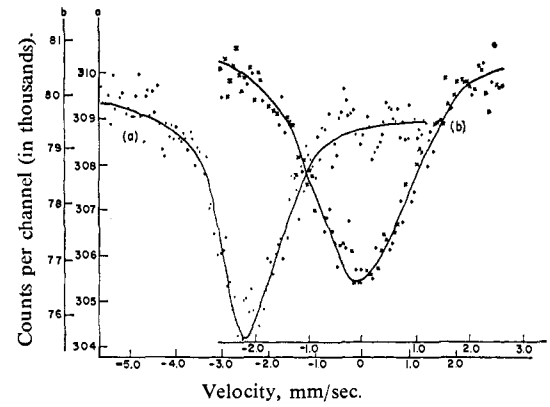

Figure 10. Mössbauer spectrum of (a) Sn(IV)–ATP, pH 1.6, Mg_2Sn source, 300°K, counting range 304,000 to 310,000; (b) same sample as (a) but with an SnO_2 source and at 77°K. Upper velocity scale and counting range 76,000 to 81,000.

ADP. The Mössbauer data would seem to advance one reason for the high binding constant of Fe(III) to ADP by suggesting that even at low pH the ring nitrogens are involved in chelation.

The spectra of the tin nucleotides (samples 31 to 34), a representative sample of which is shown in Figure 10 (samples 33 and 34), support the interpretation given for the Fe(III) nucleotides at low pH. It may also be recalled here that Sn(IV) is known from X-ray diffraction evidence to be in an octahedral binding site in stannic pyrophosphate.[19] The existence of a sizable resonance at room temperature for six-coordinate tin is taken as evidence that the tin is bonded to a polymer chain of high molecular weight[42] and the present results clearly indicate extensive polymer formation in the tin nucleotides which have been examined.

Epr spectra were obtained on many of the same complexes used for Mössbauer analysis. The difficulties of working with powdered samples have been discussed by Maling, *et al.*,[13] who found that pH and temperature differences changed the spectra of manganese–tripolyphosphate, manganese–ATP, and iron–tripolyphosphate. Similar spectral changes were observed in this study with iron nucleotides. As shown in Figure 11, for Fe(III)–ATP, an increase in pH or a lowering of temperature appears to increase the amplitude of the $g = 4$ peak relative to that of the $g = 2$ peak. Some of the nucleotides as well as some of the model compounds, *e.g.*, Fe(III)–CDTA, also showed a clear $g = 6$ component (Figure 12b), whereas $Fe_4(P_2O_7)_3$ showed only a $g = 2$ component with possibly a trace of $g = 4$ (Figure 12a). Castner, *et al.*,[43] and Griffith[44] have discussed the meaning of these g values for Fe(III) complexes, and the conclusions pertinent to the present discussion are that $g = 2$ will appear when the crystal field is weak, or in an octahedral ligand field when the ligand field is electrically symmetrical. Moreover, $g = 4$ and $g = 6$ will appear when the ligand field is made unsymmetrical by the substitution of unequal charges, although the shape of this distorted

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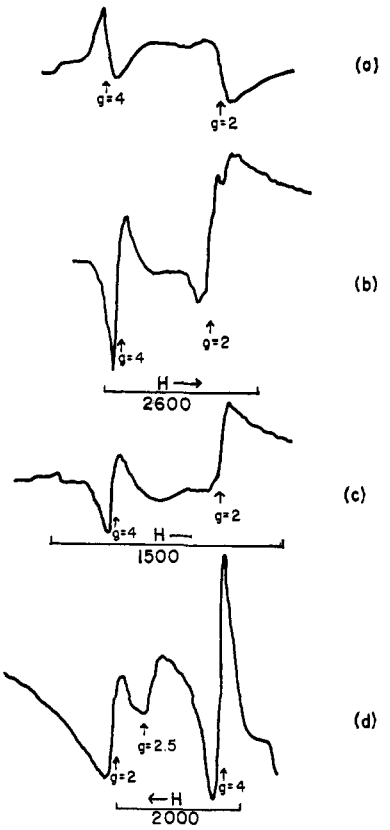

Figure 11. Epr spectrum of (a) Fe(III)–ATP, pH 2.7, 300°K; (b) same sample as in (a), but at 77°K; (c) Fe(III)–ATP, pH 7.1, 300°K; (d) Fe(III)–ATP, pH 1.6; molar ratio of nucleotide to Fe is 10:1, 300°K.

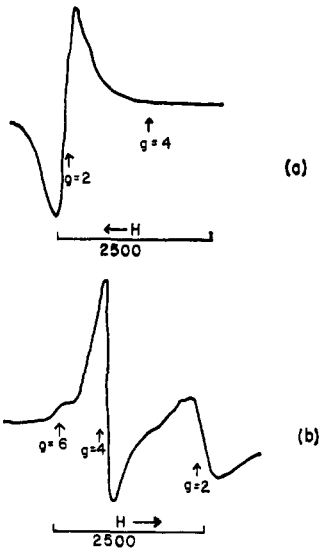

Figure 12. Epr spectrum of (a) $Fe_4(P_2O_7)_3$; (b) Fe(III)–CDTA, pH 7.3 (both at 300°K).

octahedron is not uniquely defined by the appearance of $g = 4$ and $g = 6$ resonances.[44] It is interesting that a pronounced $g = 2.5$ peak appears in the Fe(III)–ATP chelate at low pH when nucleotide: Fe(III) concentration is 10:1 (Figure 11d). This is the sample that exhibited nuclear hyperfine interactions in its Mössbauer spectrum (Figure 2). Although $g = 2.5$ peaks have been noted in nucleic acids,[45] no explanation has yet been offered which accounts for this observation.

Experimental Section

The purest available grades of adenine nucleotides were obtained from Schwarz Bio-Research, Inc., or from the Sigma Chemical Co. Inosine 5′-triphosphate (ITP) was obtained from Mann Research Laboratories, Inc., as the "chromatographically pure" sodium salt. Polyadenylic acid (poly A) was obtained from Calbiochem as the "B" grade potassium salt and used without further purification. Methylated polyadenylic acid (Me-poly A) was prepared from poly A according to the method of Ludlum, *et al.*,[38] for 13.5% methylation. Soluble ribonucleic acid (S-RNA) was prepared according to the method of Penniston and Doty[46] from fresh pressed yeast (*Saccharomyces cerevisiae*). Nitrilotriacetate was obtained from Distillation Products Industries. Cyclohexanediaminetetraacetic acid and diethylenetriaminepentaacetic acid were gifts of the Dow Chemical Co. The two compounds, $Fe^{III}(A–H)I_2$ and $Fe^{III}B(OH)(ClO_4)_2$, whose structures are described in the Results and Discussion section, were gifts from Dr. D. H. Busch of the Ohio State University. Chelex 100, 100–200 mesh, was obtained from Bio-Rad Laboratories. Chelex treatment for nucleotides was carried out at 10° using a column or batch process. Co_2-free KOH was prepared according to the method of Schwarzenbach and Biedermann[24] and stored with ordinary precautions, as was NaOH made up CO_2 free by the usual procedure from saturated NaOH and boiled distilled water.

Enriched Fe^{57} in the form of Fe_2O_3 (85% Fe^{57}) was obtained from Oak Ridge National Laboratory and converted to $FeCl_3$ with concentrated HCl. Enriched $Fe^{57}Cl_2$ was prepared from a slightly acid solution of $Fe^{57}Cl_3$ by bubbling hydrogen gas through the solution for 17 hr over small pieces of platinum wire.[47] Analyses for inorganic phosphate were carried out according to the methods of Fiske and Subbarow and Lowry and Lopez.[48] The amounts of inorganic phosphate found in the nucleotide chelates were negligible. Analyses for iron content were carried out using the "bathophenanthroline" method,[49] slightly modified for use with nucleotides.[39]

All complexes studied were prepared as soluble aqueous systems unless otherwise noted. The concentrations of the reagents, in solution, are given in Table I, and the pH given is likewise for the solution. The physical state of the compounds for all of the spectral analyses reported here was a lyophilized powder.

Infrared spectra were obtained with a Perkin-Elmer Model 21 recording spectrophotometer with sodium chloride optics.

The epr spectra were obtained using a Varian V-4500 X-band spectrometer with 100-Kc modulation and using a Varian 6-in. magnet.

The parabolic motion Mössbauer spectrometer used in this study has been described in detail elsewhere.[50,51] The samples were generally uniform powders less than 1 mm thick pressed between aluminum foil supports. The velocity constant was determined periodically (before or after a sequence of sample determinations) by determining the multi-channel analyzer addresses of the peaks in the magnetic hyperfine spectrum of metallic iron. These data can be used to obtain the Döppler shift constant (mm/sec/channel) for the spectrometer by using the precision data of Preston, *et al.*[52] The zero of motion was determined independently using a $Sn^{119}O_2$ source *vs.* SnO_2 absorber, both at room temperature. For work at 77°K, the absorber holder was attached to a cooling prong of a dewar flask. The parameters for the iron complexes

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(51) F. C. Ruegg, J. J. Spijkerman, and J. R. DeVoe, *ibid.*, **36**, 356 (1965).

(52) R. S. Preston, S. S. Hanna, and T. Herberle, *Phys. Rev.*, **128**, 2207 (1962).

were obtained using a Co^{57} source in palladium, and the isomer shift values have been converted to shifts from a standard absorber of $Na_2Fe(CN)_5NO \cdot 2H_2O$ in order to facilitate comparisons with isomer shift values in the literature.[53]

To obtain the values for the isomer shifts, the centers of the resonance lines were found using the method of chords.[54] Quadrupole splittings were measured from center to center of the resolved doublets and the isomer shift of a compound exhibiting a QS was determined by averaging the two centers. For well-resolved lines the standard deviation for the IS and QS is ±0.08 mm/sec. Curve fitting was not attempted for asymmetric quadrupole splittings with poor resolution.

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Random-Coil Configurations of *cis*-1,4-Polybutadiene and *cis*-1,4-Polyisoprene. Theoretical Interpretation

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Abstract: The rotational isomeric state model with neighbor dependence is used to calculate random-coil dimensions of the *cis* forms of 1,4-polybutadiene, $+CH_2-CH=CH-CH_2+_x$, and 1,4-polyisoprene, $+CH_2-C(CH_3)=CH-CH_2+_x$, in the limit of large x. Comparison of calculated and experimental values of the *characteristic ratio* $\langle r^2\rangle_0/nl^2$ and its temperature coefficient is used to determine intramolecular energies of various conformational sequences of the chain backbone. The form of lowest intramolecular energy closely corresponds to the conformation adopted by these two polymers in the crystalline state.

In a series of investigations over the last few years, the rotational isomeric state model has proved remarkably successful in the interpretation of configurational characteristics of chain molecules. An area of particular importance is the analysis of the chain extension, and frequently also its temperature coefficient, for polymer chains in the limit of high molecular weight. Such studies have been carried out for polyisobutylene,[1] polyethylene,[2,3] poly(dimethylsiloxane),[4] polyoxymethylene,[5] polyoxyethylene,[6] polypeptides,[7] and vinyl polymers of both tactic and atactic structure.[8] The fact that this model has also been shown to give a very satisfactory account of the dipole moments of α,ω-dibromo-*n*-alkanes[9] and oxyethylene molecules[10] in the region of short chain length strengthens confidence in its application.

The availability of experimental values of the chain extension and its temperature coefficient for *cis*-1,4-polybutadiene and *cis*-1,4-polyisoprene now permits similar analyses of these two polymers, which are of considerable importance because of their relatively simple chemical structure as well as their extensive commercial utilization. The correlation of experimental results with results calculated using a rotational isomeric state representation of these polymers should permit estimation of the energies associated with conformations accessible to the skeletal bonds.

Additionally, the conformation of these polymers in the crystalline state is known from detailed X-ray diffraction studies. It is therefore also of interest to compare this conformation with the conformation of lowest intramolecular energy, thereby gauging the effects, if any, of intermolecular interactions in the selection of a suitably regular chain conformation for adoption into a crystalline lattice.

Theory

Structure of the Repeat Units. On the basis of X-ray diffraction studies of crystalline *cis*-1,4-polybutadiene[11,12] and *cis*-1,4-polyisoprene[11,13,14] and extensive data on low molecular weight analogs,[15] the following structural parameters were adopted: $l_{C-C} = 1.53$ A, $l_{C=C} = 1.34$ A, $l_{C-H} = 1.10$ A, $\angle CH_2-CH=CH = \angle CH_2-C(CH_3)=CH = 125°$ (*e.g.*, $180° - \theta_1$, in Figure 1 and $180° - \theta_2$ in Figure 2), $\angle CH-CH_2-CH_2 = 112°$ (*e.g.*, $180° - \theta_3$ and $180° - \theta_4$ in Figure 1), $\angle CH_2-C-H = \angle CH_2-C-CH_3 = 117.5°$ (*e.g.*, θ_5 in Figure 1), and $\angle CH_2-CH-H = 110°$ (*e.g.*, θ_6 in Figure 2). The X-ray crystallographic studies of *cis*-1,4-polyisoprene by Bunn[13] suggest a somewhat smaller value of ~118° for $\angle CH_2-C(CH_3)=CH$. This variation probably represents, at least in

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