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Interactions of L-Ascorbic Acid with Transition Metal Ions in Water Solution. A ^{13}C Nmr Spin–Lattice Relaxation Study

LARS NORDENSKIÖLD, JOZEF KOWALEWSKI and NIKOLAS BENETIS

Department of Physical Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

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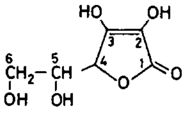
The interactions of L-ascorbic acid (*1*) with transition metal ions are known to be of considerable importance in many biological systems [1]. L-ascorbic acid has several potential sites for complex-formation with metal ions. However, the details of the interaction of ascorbic acid with transition metal ions in water solution in this respect has not yet been thoroughly investigated. In an earlier report from this laboratory [2], we described a combined potentiometric, optical spectroscopy and ^{13}C NMR study of the complex between Ni^{2+} and ascorbic acid in water solution at pH 8.5. In this communication, we report a ^{13}C NMR study of the paramagnetic effect on the spin–lattice relaxation times of the carbon-13 nuclei in ascorbic acid caused by the complex formation with Co^{2+}, Fe^{2+} and Mn^{2+}. The data obtained from this study allow determination of the binding site of the complexes formed between the metal ions and ascorbic acid at pH 8.5 and low metal concentration.



(1)

Experimental

All samples consisted of 0.8 *M* ascorbic acid at pH 8.5. The paramagnetic solutions were prepared by weighing analytical grade $MeSO_4 \cdot XH_2O$ from Merck, Darmstadt. Special precautions were taken in order to obtain the samples in a reduced and anaerobic state. The procedure for the preparation of the NMR samples has been described in reference 2. Spin–lattice relaxation time measurements were made on a Varian XL-100 as described in reference 2. The reported T_1 are mean values of at least two measurements, with 1σ standard deviations of 2–8%. T_{1P}^{-1}, the paramagnetic contribution to the observed relaxation rate, was obtained from: $T_{1P}^{-1} = T_{1,obs}^{-1} - (T_1^o)^{-1}$, where $T_{1,obs}^{-1}$ is the observed relaxation rate in the solution containing metal and $(T_1^o)^{-1}$ is the observed relaxation rate in a sample containing no metal ions, but which is otherwise identical to the paramagnetic solution. All measurements were made at 31 ± 1 °C.

Results and Discussion

If a point–dipolar interaction between the unpaired electron spin of the metal ion and the nucleus i of the ligand is assumed [3] and if the conditions of fast exchange hold [4] (which is certainly true under the present conditions for the metal ions considered here), then the paramagnetic contribution to T_{1P}^{-1} can be written as:

$$T_{1P}^{-1}(i) = \frac{p \cdot qKS(S+1)}{r_i^6} \quad 3\tau c_1 + \frac{7\tau c_2}{1 + \omega_S^2 \tau_{c2}^2} = $$

$$= \frac{pqKS(S+1)}{r_i^6} f(\tau c_j) \quad (1)$$

where $\tau_{cj}^{-1} = \tau_R^{-1} + T_{1e}^{-1} + \tau_M^{-1}$, K is a proportionality constant common for all nuclei of the same kind in a ligand and the other symbols have their usual meaning [6]. All parameters except r_i, which is the distance between the metal ion and nucleus i, are the same for all carbon nuclei in the ligand.

Table I gives the paramagnetic contribution to the observed relaxation rates for all six carbons for

TABLE I. Observed Paramagnetic Relaxation Rates for the Carbon-13 Nuclei of 0.8 *M* Ascorbic Acid at pH 8.5 containing Co^{2+}, Fe^{2+} and Mn^{2+} at 304 K.

	$[Me^{2+}]$ (mmol dm^{-3})	S	$T_{1P}(i)^{-1}$ (s^{-1})						$T_{1P}(C(2))^{-1}/S(S+1)[Me^{2+}]$ (s^{-1})
			C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	
Co^{2+}	4.8	3/2	0.22	0.43	0.44	0.10	0.02	–	0.024×10^3
Fe^{2+}	0.82	4/2	0.52	1.6	1.6	0.42	0.21	0.09	0.31×10^3
Mn^{2+}	0.10	5/2	0.56	1.8	2.0	0.31	0.21	0.11	2.0×10^3

0.8 *M* ascorbic acid with a pH value of 8.5 containing different amounts of Me^{2+}. The diamagnetic relaxation rates for the carbons C(1), C(2) and C(3) are 0.028, 0.049 and 0.052 and have been drastically enhanced in the paramagnetic solutions of all metal ions, which provides evidence of the formation of a complex between the metal ion and the ascorbic acid. It can also be noted at once that for all the metal complexes the quotient $T_{1P}(C(2))^{-1}/T_{1P}(C(3))^{-1}$ is, within the limits of the experimental error, equal to unity. Furthermore, the paramagnetic relaxation rate is largest for these two carbons, becomes shorter for carbons C(1) and C(4) and falls off considerably for C(5) and C(6). Interpreted in terms of equation (1), according to which $T_{1P}^{-1}(i)$ is a rapidly decreasing function of the distance between the metal ion and the nucleus i under consideration, these data strongly suggest that at this pH and metal concentration the ascorbic acid–metal complexes are formed by a symmetric binding of the metal ion to the oxygen O(2) and O(3). The relatively faster relaxation rate of carbon C(1) as compared to C(4) for the Co^{2+} and Mn^{2+} complexes is probably due to the fact that the unpaired electron spin of the metal ions is delocalized onto the ligand nuclei through the π-electron system of ascorbic acid, causing a considerable amount of spin density to be present at carbon C(1). Consequently, deviations from the point dipole approximation inherent in eqn. (1) can arise [7–8].

In the last column of Table I, T_{1P}^{-1} for carbon C(2) normalized with S(S + 1) and the metal concentration for the different complexes is given. It can be assumed that for low metal concentrations all metal ions present in the solution are bound in a neutral complex $MeAsc^0$ [9–11] (*i.e.*, pq in eqn. (1) equals $[Me^{2+}]/0.8$ l) and that the metal–carbon C(2) distances are approximately equal in all complexes. Under these assumptions the normalized T_{1P}^{-1} values will give a relative estimate of the function $f(\tau_{cj})$ within the brackets in eqn. (1). It can be noted that $f(\tau_{cj})$ is two orders of magnitude larger for the Mn^{2+} complex than for the Co^{2+} complex while Fe^{2+} is an intermediate case between the two. This reflects the fact that the electron spin relaxation characterized by relaxation times T_{1e} and T_{2e} in Co^{2+} is much faster than the random molecular tumbling of the complexes characterized by τ_R, which in turn is faster than the electron spin relaxation in the Mn^{2+} complex. For Fe^{2+}, the electron spin relaxation is probably about the same order of magnitude as the correlation time for the molecular tumbling [12, 13]. Consequently, for the Co^{2+} complex, the nuclear spin relaxation will be modulated by the electron spin relaxation of the metal ion ($<10^{-12}$ s) [13, 14], while for the Mn^{2+} complex the modulation will be governed by the molecular rotational reorientation correlation time ($\sim 10^{-10}$ s) [15].

Acknowledgements

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