Ternary Complexes of Gadolinium(III) and Bovine Serum Albumin – an *in vitro* Study

GRAHAM E. JACKSON

Department of Inorganic Chemistry, University of Cape Town, Rondebosch 7700, South Africa

Received December 18, 1984

Abstract

Proton relaxation enhancement has been used to study the ternary complexes formed between gadolinium(III), bovine serum albumin and a number of low molecular weight plasma ligands. Only the ternary citrate complex was formed under physiological conditions, a formation constant of 11.0 log units being obtained. Computer simulation of the complexing conditions occurring in plasma was used to construct a simplified model of human blood plasma. The [Gd·serum albumin·citrate] and [Gd·serum albumin] complexes are predicted to predominate. This model was also used to assess the effectiveness of EDTA against lanthanide(III) ion poisoning.

Introduction

A feature of mammalian plasma albumins is their ability to bind reversibly to a wide variety of organic compounds. Indeed, one of their primary functions is the transport of free fatty acids [1]. The versatility of the albumins in this regard is attributed to their flexibility, which enables any interaction with a particular molecule to be optimized.

At the same time the importance of serum albumins as reversible metal-ion chelates has been well established [2], and it is this function in which we are interested here. In a previous study [3] binary metal-ion/serum albumin complexes received particular attention. Besides the proteins, however, there are a large number of low molecular weight ligands present in blood plasma. We have shown that ternary complexes tend to be more important biologically than do the simple binary complexes [4], and hence a study of possible ternary metal-ion/serum albumin/low molecular weight ligand complexes was deemed necessary.

The experimental technique of choice in this study was proton relaxation enhancement (PRE). The technique is well documented [5] and has been used extensively in the study of metal-ion binding to macromolecular systems. Unfortunately, the most important metal ions biologically are generally not sensitive to this technique and so are traditionally replaced by more easily studied, chemically similar, elements. In magnetic relaxation studies the paramagnetic transition metal-ion, Mn(II), has been found to be most suitable as a probe for Mg(II), Cu(II) and Zn(II). However, while this is a hard metal-ion it does not show the preference for oxygen over nitrogen coordination that Mg(II) and Ca(II) do. In this respect gadolinium(III), which is also an s-state ion with a relatively long electronic relaxation time and a labile hydration sphere, is more suitable. Indeed, inhibition by trivalent lanthanide-ions of enzymatic reactions and of calcium transport across membranes [7], has been reported. In this paper we present results of an investigation aimed at establishing the most predominant gadolinium(III) complex present in blood plasma.

Experimental

Crystalline bovine serum albumin (BSA) (Fraction V, code 81-0.1-2, Lot 59M, MW 69024 g mol^{-1}) was obtained from Miles Laboratories and used without further purification. The relaxation time (T_2) of the solutions used in these experiments was not altered by the addition of serum albumin to a final concentration of 44×10^{-5} M, indicating the presence of minimal paramagnetic impurities. Solutions of $Gd(NO_3)_3$ were prepared from a 0.01 M stock solution made by the action of concentrated nitric acid on Gd_2O_3 (BDH Laboratory Chemicals). All the solutions were made 0.15 M in NaCl and the pH maintained at 7.5 using HCl or NaOH. A total volume of 10.0 cm^{-3} was used. No buffer was added, thus eliminating any Gd(III)-buffer interaction. The concentration of BSA was measured by its absorption at 280 nm [8].

NMR spectra were recorded on a Bruker WH 90 spectrometer using D_2O as an external lock and at a probe temperature of 27 °C. T_2 was measured from the spectral line width at half-height, using $1/T_2 = \pi\Delta\nu_{1/2}$.

0020-1693/85/$3.30

The data were analysed on a Univac 1106 computer. The methodology used was to guess at initial values for the stability constants and to optimize these against the experimental data. At each stage a simple Newton-Raphson iterative technique was employed to calculate the species distribution. A copy of the program is available upon request.

Results and Discussion

In this study we were interested in the possible complexes of Gd(III) existing in blood plasma. A previous study [3] established a high affinity between BSA and Gd(III), hence this ligand is likely to be a major chelator of Gd(III) in blood plasma. But besides serum albumin there are present *in vivo* a number of potential low molecular weight ligands. Gd(III) being a hard metal-ion will show a preference for oxygen donor ligands and indeed was found not to coordinate to the amino acids under physiological conditions. Instead the ligands carbonate, phosphate, citrate, malonate and lactate, which are either present in relatively high concentration in blood plasma or are potentially good chelators of Gd(III), were considered more important. Before the possible ternary systems could be studied, however, it was necessary to characterize the nuclear relaxation behaviour of the binary systems. This was achieved by measuring the change in transverse relaxation of the solvent water molecules as a function of total metal and total ligand.

If the relaxation enhancement is defined by:

$$\epsilon^* = \frac{(1/T^*_2) - (1/T^*_{2(0)})}{(1/T_2) - (1/T_{2(0)})}$$

where T_2 is the transverse relaxation time, * indicates the presence of macromolecule and the subscript the absence of paramagnetic metal-ion, under conditions of fast exchange the enhancement of the bulk solvent is given by:

$$\epsilon^* = \Sigma \epsilon_i [\text{species}]_i$$

where ϵ_i is the characteristic enhancement of a particular species and the summation is over all such species. A typical PRE curve is shown in Fig. 1 for Gd(III)/BSA. From this a formation constant of 2.2×10^4 was obtained. The binary Gd(III)/citrate system is shown in Fig. 2. Since the aquo sphere of the metal-ion decreases without the concomitant change in correlation time modulating the relaxation, in this case the enhancement, ϵ^*, decreases as complexation takes place. A computer analysis of this system yielded formation constants (Table I) for the species, [Gd·citrate], $[Gd\cdot citrate\cdot H]^+$ and $[Gd\cdot citrate\cdot OH]^-$. The solid line in Fig. 2 is the theoretical curve calculated assuming these constants to be correct. The [Gd·citrate] constant is in agreement with potentiometric studies of other lanthanide(III) ions [9]. The $[Gd\cdot citrate\cdot H]^+$ or $[Gd\cdot citrate\cdot OH]^-$ species have not been detected previously.

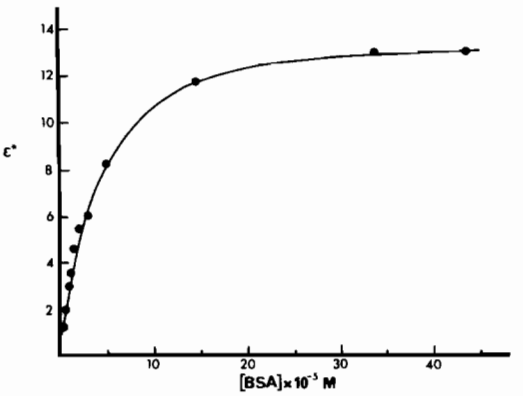

Fig. 1. Variation of observed enhancement, ϵ^*, with total BSA concentration at 60 MHz. Total gadolinium(III) concentration = 5×10^{-5} mol dm^{-3}, pH = 7.5 and T = 27 °C.

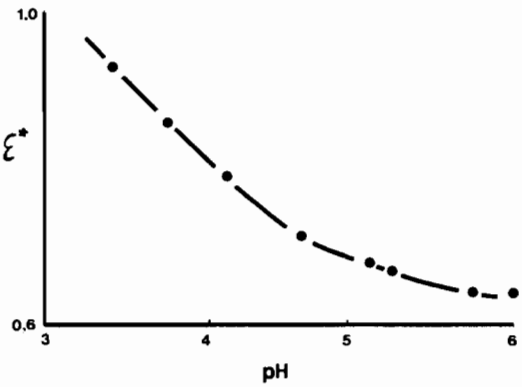

Fig. 2. pH Dependence of observed enhancement, ϵ^*, for the binary gadolinium(III)/citrate system. Total gadolinium = 1.4×10^{-3}, total citrate = 3.1×10^{-3} mol dm^{-3}, I = 0.15 mol dm^{-3} NaCl and T = 27 °C.

TABLE I. Formation Constants and Characteristic Enhancements Determined in this Study at 27 °C, I = 150 mmol dm^{-3} NaCl. For Clarity Charges have been Omitted. M = Gd(III), BSA = Bovine Serum Albumin and CIT = Citrate.

	Log (K)	ϵ_B
M·BSA	4.35 ± 0.05	14.0 ± 0.5
M·CIT	4.0 ± 0.4	0.6 ± 0.1
M·CIT·H	8.7 ± 0.2	0.6 ± 0.1
M·CIT·OH	−1.0 ± 0.2	0.6 ± 0.1
M·CIT·BSA	11.0 ± 0.3	5.0 ± 0.1
BSA·CIT	5.5 ± 0.6	0.0
$M\cdot BSA\cdot CO_3$	>8.0	–
$M\cdot BSA\cdot CO_3\cdot H$	>16.0	–

It is interesting to speculate on the structure of the $[Gd{\cdot}citrate{\cdot}OH]^-$ complex, which may arise from loss of a proton from either the hydroxyl group of the citrate or from a coordinated water molecule. Since the same characteristic enhancement is obtained for all three species the indication is that there is no change in the aquo-sphere of the metal-ion, and so the proton must be lost from the hydroxyl group. For this to occur the hydroxyl group would have to be coordinated. A similar conclusion was reached for the structure of $[Al(III){\cdot}citrate{\cdot}OH]^-$ [10]. In addition, if we assume a co-ordination number of 8 (or 9) for Gd(III), a denticity of three can be calculated for the citrate ion [3]. Such a high denticity is not consistent with the relatively low stability of the complex.

Formation of the ternary Gd(III)/BSA/citrate complex was studied by the addition of citric acid to a solution 1.0×10^{-4} M in Gd(III) and 3.8×10^{-4} M in BSA. Once again the enhancement decreased due to the displacement of coordinated water molecules (Fig. 3). A computer analysis of the

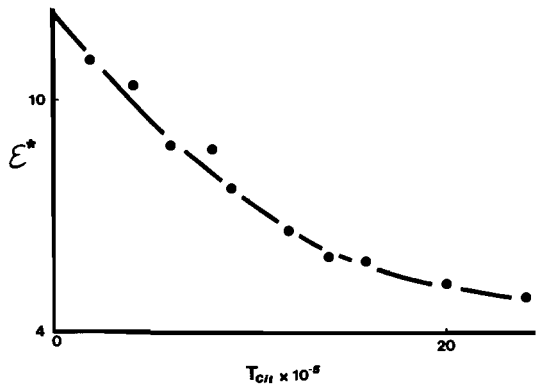

Fig. 3. Variation of observed enhancement, ϵ^*, with total citrate concentration for the ternary Gd/BSA/citrate system. Total gadolinium(III) concentration = 1.0×10^{-4} mol dm^{-3}, total BSA = 3.8×10^{-4} mol dm^{-3}, pH = 7.5, I = 0.15 mol dm^{-3} NaCl and T = 27 °C.

results yielded the constants given in Table I. Because of the nature of the protein (BSA is a transporter of free fatty acids [1]) account had to be taken of the binding of the citrate to the protein, at a site remote from the metal-ion. From the characteristic enhancement of the ternary complex a hydration number of 2 for the metal-ion was calculated. Since the binary [Gd·BSA] complex was found to have four [3] water molecules coordinated to the metal-ion, if we assume no change in the chelation of the BSA, the citrate must be bidentate. But the ternary complex is far more stable than its constituent binary complexes. This indicates that some protein-citrate binding is present in the complex.

A species distribution diagram for the system is shown in Fig. 4. Clearly low molecular weight com-

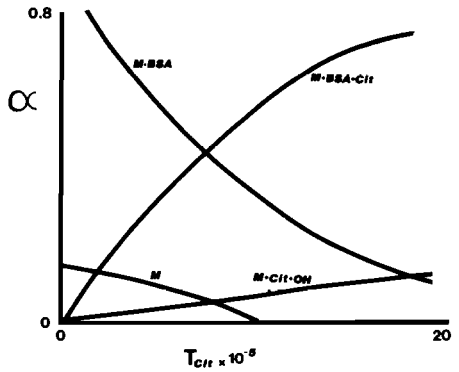

Fig. 4. Species distribution diagram for the ternary Gd(III)/BSA/citrate system calculated using the constants given in Table I. Total gadolinium(III) concentration = 5.0×10^{-5} mol dm^{-3}, total BSA = 6.5×10^{-4} mol dm^{-3} and pH = 7.5.

plexes of Gd(III) are relatively unimportant but at higher citrate concentrations the ternary [Gd·BSA·citrate] complex predominates.

Similarly, ternary histidinate, carbonate, phosphate malonate and lactate systems were studied. In each case no significant ternary complexation was detected. For the carbonate system, from the results it was possible to set upper limits for the stability of the ternary complexes. Using these constants, together with constants available from the literature [9] and tables of analytical concentrations [11], it was possible to set up a simplified model of blood plasma. This model included the most predominant low molecular weight ligands found in human blood plasma but only one metal-ion, Gd(III). The results (Fig. 5) show that under physiological conditions the main Gd(III) complex present in blood plasma should be the ternary [Gd·BSA·citrate] complex, with some [Gd·BSA]. A significant amount of $[Gd{\cdot}BSA{\cdot}HCO_3]$ is predicted to be present but this may be a result of upper limit formation constants being used in the calculation.

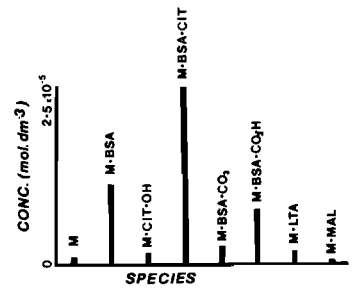

Fig. 5. Calculated concentrations of most predominant gadolinium(III) complexes present in blood plasma under physiological conditions, calculated using a computer model of plasma. Analytical concentrations were taken from ref. 11 and literature formation constants from ref. 9. Total gadolinium(III) concentration was 5×10^{-5} mol dm^{-3}. M = Gd(III), CIT = citrate, LTA = lactate and MAL = malonate.

Finally, of interest in chelation therapy is the action of therapeuticals in promoting the excretion of polluting metal-ions. The plasma mobilizing index (PMI) [12] is a measure of a drug's ability to mobilize metal-ions, from the protein bound fraction of plasma to a low molecular weight excretable form. The calculated PMI as a function of EDTA concentration is shown in Fig. 6. Curves for Cu(II) and Pb(II) are included for comparison [12]. EDTA is ineffective in promoting the excretion of Cu(II) [13] but is one of the ligands of choice in the treatment of plumbism [14]. Hence we might expect EDTA to be reasonably efficacious against Gd(III) poisoning in particular and lanthanide(III) poisoning in general.

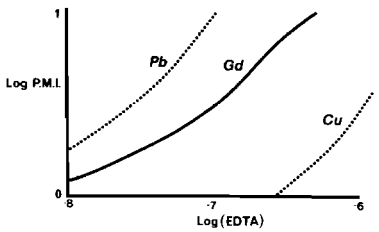

Fig. 6. PMI curves for the metal ions Cu(II), Pb(II) and Gd(II) with the chelating agent EDTA.

Acknowledgements

The author acknowledges the University of Cape Town and the C.S.I.R. for financial assistance.

References

1 T. Peters and Sjöholm (eds.), 'Albumin', F.E.B.S. 11th Meeting, Copenhagen, 1977, Vol. 50, Colloquium B9.
2 F. Friedberg, *FEBS Lett., 59*, 140 (1975).
3 G. E. Jackson and M. Singh, *S. Afr. J. Chem., 36*, 101 (1983).
4 G. E. Jackson, P. M. May and D. R. Williams, *J. Inorg. Nucl. Chem., 43*, 825 (1981).
5 R. A. Dwek, R. J. P. Williams and A. V. Xavier, in H. Sigel (ed.), 'Metal Ions in Biological Systems, Vol. 4', Marcel Dekker, New York, 1974, chap. 3.
6 V. Z. Holten, G. C. Kyker and M. Pulliam, *Proc. Soc. Exp. Med., 123*, 913 (1966).
7 L. Mela, *Biochemistry, 8*, 2481 (1969).
8 E. J. Cohn, W. L. Huges and J. H. Weare, *J. Am. Chem. Soc., 69*, 1753 (1947).
9 A. E. Martell and R. E. Smith, 'Critical Stability Constants', Plenum, New York, 1974.
10 G. E. Jackson, *S. Afr. J. Chem., 35*, 89 (1982).
11 P. M. May, P. W. Linder and D. R. Williams, *J. Chem. Soc., Dalton Trans.*, 588 (1977).
12 P. M. May and D. R. Williams, *FEBS Lett., 78*, 134 (1977).
13 J. Tripod, in F. Gross (ed.), 'Iron Metabolism', Springer-Verlag, Berlin, 1964.
14 J. J. Chisholm, *J. Pediat., 73*, 1 (1968).