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Computer simulation of Gd(III) speciation in human interstitial fluid

Jinping Wang, Haiyuan Zhang, Kuiyue Yang & Chunji Niu*
*Key Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, P.R. China. *Author for correspondence (E-mail: cjniu@ns.ciac.jl.cn)*

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Abstract

The speciation and distribution of Gd(III) in human interstitial fluid was studied by computer simulation. The results show that at the background concentration, all the Gd(III) species are soluble and no precipitates appear. However as the total concentration of Gd(III) rises above 2.610×10^{-9} mol/l ,the insoluble species become predominant. $GdPO_4$ is formed first as a precipitate and then $Gd_2(CO_3)_3$. Among soluble species, free Gd(III), [Gd(HSA)] , [Gd(Ox)] and the ternary complexes of Gd(III) with citrate as the primary ligand are main species when the total concentration of Gd(III) is below 2.074×10^{-2} mol/l. With the total concentration of Gd(III) further rising, $[Gd_3(OH)_4]$ begins to appear and gradually becomes a predominant species.

Introduction

In recent years, application of the rare earths has become very widely accepted. This has caused more and more rare earths to enter into the environment and human body *via* the food chain, etc (Evans 1990, Ni 1995). As a result, people have become concerned the influence of rare earths on human health and the environment. Therefore it is of great importance to study the biological effects of rare earths. Since biological effects of metals are controlled by its *in vivo* speciation (Jackson & Toit, 1992), research on rare earth speciation is a key to understand the distribution, metabolism and biological effects of rare earths in life systems. It is also very important to elucidating the effects of rare earths on human health and the environment. It is difficult to determine metal speciation in the human body using analytical techniques. Therefore computer simulation have been developed as a expedient method to study the speciation without disturbing the delicate equilibria (May *et al.* 1976, May *et al.* 1977, Williams 1979) in the human body.

The study on Gd(III) speciation in human blood plasma was pioneered by Jackson and his co-workers, but they used a single-phase model in which the insoluble species of Gd were not considered, and some important macromolecular complexes and ternary complexes were not included (Jackson et al. 1990). Webb *et al.* studied Gd(III) and Cm(III) in the gastrointestinal tract, but precipitates were again not considered (Webb *et al.* 1998). Yue Wang *et al.*. studied Gd(III) speciation in human blood plasma with precipitate species and some important new complexes being considered(Wang *et al.* 2001). However, Gd(III) speciation in human interstitial fluid has not been reported. Human interstitial fluid, which constitutes the environment of the cells and is regulated by body homeostasis, is very important in treating patients with abnormal plasma volume or homeostasis (Fogh-Andersen *et al.* 1995). In this work, the distribution of Gd(III) speciation in human interstitial fluid was studied by means of computer simulation.

Method

Model of human interstitial fluid

A muti-phase model of human interstitial fluid, including 3 metals, 30 ligands, such as Ca(II), Zn(II), Gd(III), PO_4^{3-}, CO_3^{2-}, HSA (Human Serum Albumin), IgG (immunoglobulin), and a lot of important low-molecular-weight ligands, and more than 350 species,

Table 1. Concentration of main metal ions and ligands in human interstitial fluid (mol/l)

Component	Concentration	Component	Concentration	Component	Concentration
Phosphate	6.050×10^{-4}	Glycinate(Gly)	2.400×10^{-4}	Salicylate(Sal)	5.000×10^{-6}
Carbonate	3.096×10^{-2}	Glutaminate(Gln)	5.200×10^{-4}	Serinate(Ser)	1.200×10^{-4}
HSA*	1.880×10^{-4}	Glutamate(Glu)	4.800×10^{-5}	Succinate(Suc)	4.200×10^{-5}
IgG*	5.400×10^{-5}	Histidinate(His)	8.500×10^{-5}	Thiocyanate(Thi)	1.000×10^{-6}
Aspartate(Asp)	5.000×10^{-5}	Isoleucinate(Ile)	6.500×10^{-5}	Threoninate(Thr)	1.500×10^{-4}
Alanate(Ala)	3.700×10^{-4}	Lactate(Lac)	1.790×10^{-3}	Tryptophanate(Trp)	1.000×10^{-5}
Arginate(Arg)	9.500×10^{-5}	Leucinate(Leu)	1.200×10^{-4}	Tyrosinate(Tyr)	5.800×10^{-5}
Asparaginate(Asn)	5.600×10^{-5}	Lysinate(Lys)	1.800×10^{-4}	Valinate(Val)	2.300×10^{-4}
Citrate(Cit)	8.470×10^{-5}	Oxalate(Ox)	1.200×10^{-5}	Ca(II)	1.551×10^{-3}
Citrullinate(Citrl)	2.700×10^{-5}	Ornithinate(Orn)	5.800×10^{-5}	Zn(III)	1.000×10^{-5}
Cysteinate(Cys)	3.330×10^{-5}	Prolinate(Pro)	2.100×10^{-4}	Gd(III)	variable

HSA = Human Serum Albumin; IgG = immunoglobulin.

was used in this work. The concentrations of metal ions and ligands in human interstitial fluid are listed in Table 1.

Almost all the stability constants of the binary and ternary complexes of Gd(III), Ca(II) and Zn(II) with the low-molecular-weight biological ligands contained in this model were determined accurately under physiological conditions (T = 37 °C, I = 0.15 mol dm^{-3}) by potentiometry in our group (Han 1999, Wang 2000 & Lu 2001). The binding constants of Gd(III), Ca(II) and Zn(II) with inorganic ligands were mostly cited from references (Sillén & Martell 1964, Sillén & Martell 1971, Rizkalla&Choppin 1994, Ni 1995). In addition, the binding constants of Gd(III), Ca(II) and Zn(II) with proteins were mainly cited from references (Duffield *et al.* 1994) and (Xu 1995). The binding constant of Gd-HSA was estimated by linear regression based on linear relationship between stability constants and lanthanide ionic radii. Since some of the binding constants were not determined at physiological conditions, Davies corrections were performed to generate new constants applicable to physiological conditions by the MINTEQA2 program. The values of binding constants of the main Gd(III) species in human interstitial fluid are listed in Table 2.

Computational method

In this work, the MINTENQA2 program which was developed by U.S. Environment Protection Agency in 1991 was used to simulate the distribution of Gd(III) speciation in human interstitial fluid.

MINTEQA2 is a geochemical model capable of calculating equilibrium aqueous speciation, adsorption, gas phase partitioning, solid phase saturation states, and precipitation-dissolution of metals. MINTEQA2 can solve a broad range of chemical equilibrium problems. The model contains an extensive thermodynamic database which involves metal-ligand-proton equilibria, hydroxide formation, solubility, adsorption, finite solid, possible solid, excluded species, and ionic strength and temperature details (Minteqa2 1991).

Results and discussion

Gd(III) speciation

The distribution of Gd(III) species is shown in Table 3. The background value of Gd(III) in human interstitial fluid for a normal man was estimated to be about 1.203×10^{-9} mol/l (Meng *et al.* 1995). At this concentration, all the Gd(III) species are soluble and no precipitate appears. With the total concentration of Gd(III) increasing , precipitate appears. $GdPO_4$ was formed first at the concentration of 1.4×10^{-9}mol/l (7.3%) and gradually becomes the predominant species. For example, at a total concentration of 2.610×10^{-9}mol/l, the percentage of $GdPO_4$ reaches 50.1% and at 1.000×10^{-6} mol/l the percentage of $GdPO_4$ attains to 99.9%. At the same time, the percentage of soluble Gd(III) varies greatly from 100% to 0.1%. When the total concentration of Gd(III) gets to 6.00×10^{-4} mol/l, another precipitate, $Gd_2(CO_3)_3$,begins to appear and gradually becomes the predominant species. At the LD_{50} concentration of $GdCl_3$ (1 mmol/l), which is estimated from reference (May *et al.* 1977), the percentage of

Table 2. Binding constants of main Gd(III) complexes in human interstitial fluid

Species	Logβ	Species	Logβ	Species	Logβ
$[Gd_2(OH)_2]$	12.200	$[Gd(Lys)(Citl)H_3]$	25.3324	[Gd(Tyr)(Suc)	7.5023
$[Gd_3(OH)_4]$	15.829	[Gd(Tyr)(Lys)H]	16.5432	$[Gd(Tyr)(Suc)H_3]$	25.4262
[Gd(His)(Trp)H]	16.937	[Gd(Cit)(Gln)H]	18.132	[Gd(Orn)(Suc)]	8.3513
$[Gd(His)(Trp)H_2]$	24.312	$[Gd(Cit)(Gln)H_2]$	26.213	[Gd(Orn)(Suc)H]	15.4976
$[Gd(His)(Trp)H_3]$	33.950	[Gd(Cit)(Lac)]	10.237	$[Gd(Orn)(Suc)H_2]$	20.2123
$[Gd(His)(Thr)H_3]$	33.380	[Gd(Cit)(Lac)H]	13.965	[Gd(Orn)(Lys)]	7.6177
[Gd(Pro)(Thr)]	9.677	$[Gd(Cit)(Lac)H_2]$	17.307	$[Gd(Orn)(Lys)H_2]$	22.0226
[Gd(Pro)(Trp)]	10.647	[Gd(Cit)(Glu)]	12.662	$[Gd(Orn)(Lys)H_3]$	24.3954
[Gd(Pro)(Trp)H]	17.977	[Gd(Cit)(Glu)H]	18.687	[Gd(Ser)]	5.227
$[Gd(Pro)(Trp)H_2]$	25.602	$[Gd(Cit)(Glu)H_2]$	23.117	[Gd(Ile)]	5.077
[Gd(Trp)(Thr)]	9.877	$[Gd(Cit)(His)H_2]$	27.727	$[Gd(Asp)_2]$ [Gd(Asp)]	10.037
$[Gd(Trp)(Thr)H_2]$	24.582	[Gd(Cit)(Leu)]	12.667	[Gd(Sal)]	5.767
$[Gd(Gly)(Ala)H_2]$	23.052	[Gd(Cit)(Leu)H]	18.167	[Gd(Cit)]	2.827
$[Gd(Val)(Glu)H_2]$	23.617	[Gd(Cit)(Asp)]	15.132	[Gd(Lac)]	5.995
[Gd(Cit)(Arg)H]	22.035	[Gd(Cit)(Asp)H]	20.827	$[Gd(Asn)_2]$	3.347
$[Gd(Cit)(Arg)H_2]$	30.017	$[Gd(Cit)(Asp)H_2]$	24.087	$[Gd(Citl)_2]$	7.267
$[Gd(Cit)(Ser)H_2]$	22.217	[Gd(Sal)]	2.827	[Gd(Suc)]	7.667
$[Gd(Cit)(Ile)H_2]$	22.557	[Gd(Gly)]	4.127	[Gd(Thi)]	3.107
$[Gd(Asp)(Ile)H_2]$	27.577	[Gd(Ala)]	4.067	[Gd(Lys)]	2.897
$[Gd(Asp)(Thr)H_2]$	25.927	[Gd(Pro)]	4.507	[Gd(Tyr)]	3.365
$[Gd(Asp)(Ser)H_2]$	26.287	[Gd(Val)]	3.457	[Gd(Orn)]	4.247
$[Gd(Cit)(Thr)H_2]$	22.347	[Gd(Thr)]	4.477	[Gd(Ox)]	3.269
$[Gd(Glu)(Ala)H_2]$	23.127	[Gd(His)]	3.087	$[Gd(Ox)_2]$	7.137
[Gd(Cit)(Ala)H]	18.423	[Gd(Cys)]	4.827	[Gd(HSA)]	10.127
$[Gd(Cit)(Ala)H_2]$	26.720	[Gd(Arg)H]	15.852	[Gd(IgG)]	6.465
$[Gd(Cit)(Ala)H_3]$	30.730	[Gd(Glu)]	4.727	$[Gd(HPO_4)]$	5.205
[Gd(Cit)(Gly)H]	18.529	[Gd(Glu)H]	11.857	$[Gd(HPO_4)_2]$	5.037
$[Gd(Cit)(Gly)H_2]$	26.911	[Gd(Gln)]	3.917	$[Gd(OH)_2]$	8.847
$[Gd(Cit)(Gly)H_3]$	30.935	[Gd(Trp)]	5.117	$GdPO_4$	6.593
[Gd(Cit)(Val)H]	18.446	[Gd(Leu)]	4.627	$Gd(OH)_3$	20.010
$[Gd(Cit)(Val)H_2]$	26.704	[Gd(Lys)(Suc)]	7.3159	$Gd_2(CO3)_3$	21.700
$[Gd(Cit)(Val)H_3]$	30.660	$[Gd(Lys)(Suc)H_3]$	23.2568		32.200
[Gd(Lys)(Citl)]	7.9325	$[Gd(Citl)(Suc)H_2]$	20.3521		
[Gd(Lys)(Citl)H]	15.4237	[Gd(Tyr)(Orn)]	8.5134		

Charges of all the species were omitted

insoluble Gd(III) species is 100% (Gd_2CO_3, 25.08%; $GdPO_4$, 74.92%), and the amount of soluble Gd(III) in the system is neglected. From mentioned above, the precipitates of $GdPO_4$ and Gd_2CO_3 remain as the predominant species in a wide concentration range. This is in good accord with the conclusion of Luckey *et al.* that lanthanides tend to form insoluble complexes with phosphate, etc (Luckey *et al.* 1975). Since the precipitate species are the predominant species of Gd(III), most of Gd(III) are not readily transported and absorbed in human body.

Soluble Gd(III) speciation

Distribution of soluble Gd(III) is shown in Table 4. When the total concentration of Gd(III) is below 5.988×10^{-4} mol/l, free Gd(III) (5.4%), [Gd(HSA)] (29.6%), [Gd(OX)] (18.2) and the ternary complexes of Gd(III) with citrate as the primary ligand are main species. The total percentage of the ternary complexes of Gd(III) with citrate is about 37.7%. Among them, the percentage of [Gd(Cit)(Lac)] is about 10.0% because of the high concentration of lactate. In the concentration range from the background concentra-

Table 3. Distribution of Gd(III) species(%)

Species	Total concentration of Gd(III) (mol/L)						
	1.203×10^{-9}	1.4×10^{-9}	2.610×10^{-9}	1.000×10^{-6}	6.00×10^{-4}	1.00×10^{-3}	2.05×10^{-2}
$GdPO_4$	0.0	7.3	50.1	99.9	99.9	74.92	5.73
$Gd_2(CO_3)_3$	0.0	0.0	0.0	0.0	0.1	25.08	94.16
Soluble	100.0	92.7	49.9	0.1	0.00	0.00	0.1
Gd(III)	(1.203×10^{-9})	(1.233×10^{-9})	(1.233×10^{-9})	(1.235×10^{-10})	(1.155×10^{-7})	(1.188×10^{-7})	(1.454×10^{-5})

The values in parentheses are the concentrations of soluble Gd(III) species

Table 4. Distribution of the soluble Gd(III) speciation (%) (pH = 7.4 I = 0.15 mol dm^{-3} T = 37 °C)

Soluble Gd(III)	Total concentration of Gd(III) (mol/L)				
	1.203×10^{-9}	1.000×10^{-7}	5.988×10^{-4}	2.074×10^{-2}	2.200×10^{-2}
Free Gd(III)	5.4	5.4	5.5	6.5	2.6
$[Gd_3(OH)_4]$	<1	<1	<1	1.0	78.9
$[Gd(Cit)(Ala)H_2]$	1.6	1.6	1.6	1.6	<1
$[Gd(Cit)(Gly)H_2]$	2.2	2.2	2.2	2.1	<1
$[Gd(Cit)(Val)H_2]$	1.4	1.4	1.4	1.3	<1
$[Gd(Cit)(Gln)H_2]$	3.1	3.1	3.1	3.0	<1
[Gd(Cit)(Lac)]	10.0	10.0	9.9	9.5	1.7
[Gd(Cit)(Glu)]	1.1	1.1	1.1	1.0	<1
$[Gd(Cit)(His)H_2]$	1.6	1.6	1.6	1.5	<1
[Gd(Cit)(Leu)]	7.9	7.9	7.8	7.4	1.2
[Gd(Cit)(Asp)]	7.7	7.7	7.6	5.3	<1
[Gd(Lac)]	1.1	1.1	1.1	1.3	<1
[Gd(OX)]	18.2	18.2	18.3	14.9	1.2
[Gd(HSA)]	29.6	29.6	29.8	33.6	8.5
[Gd(IgG)]	3.1	3.1	3.1	3.5	1.1
[Gd(OH)]	2.5	2.5	2.5	3.0	1.2

tion (1.203×10^{-9} mol/l) to 5.988×10^{-4} mol/l, the percentage of the main species is nearly unchanged. As the total concentration of Gd(III) reaches to 2.074×10^{-2} mol/l, $[Gd_3(OH)_4]$ begins to appear(1%). At the same time, the other species vary obviously, such that the percentage of [Gd(HSA)] increases to 33.6% and free Gd(III) to 6.5%, whereas [Gd(Ox)] decreases to 14.9%. Then, with the concentration of Gd(III) further increasing, Gd(III) ions were mainly bound to hydroxyl group, and $[Gd_3(OH)_4]$ gradually becomes the predominant species. At the concentration of 2.20×10^{-2}mol/l, the percentage of $[Gd_3(OH)_4]$ already reaches 78.9%, and those of other species decrease sharply. For example, the percentage of [Gd(HSA)] decreases from 33.6% to 8.5% and [Gd(Ox)] decreases to 1.2%. This could means that soluble $[Gd_3(OH)_4]$ in human interstitial fluid would play an important part in metabolism of rare earths .

Conclusion

In human interstitial fluid, when Gd(III) compounds enter into human body, precipitate species are the main species. Among them $GdPO_4$ was formed first due to its high stability, while $Gd_2(CO_3)_3$ appears later. Since the precipitates are main species, they will affect the transportation of rare earths, and affect the accumulation of rare earths in organs and tissues.

Among the soluble species, Gd(III) ions are mainly distributed as [Gd(HSA)], [Gd(Ox)], the ternary complexes with citrate as the primary ligand and free Gd(III). When the total concentration is over 2.074×10^{-2} mol/l, $[Gd_3(OH)_4]$ begins to appear and gradually becomes the predominant species. The presence of soluble species of rare earths would help the human body absorb and transport rare earths.

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References

Duffield JR, Taylor DM, Williams DR. 1994 *In*: Gschneidner KA, Eyring LR, Chopping GR, Lander G H. Eds. *Handbook on the Physics and Chemistry of Rare Earths*, vol. 18. Amsterdam: North-Holland: 591.

Evans CH. 1990 Biochemistry of Lanthanides, New York: Plenum Press.

Fogh-Andersen N, Altura BM, Altura BT. 1995 Composition of interstitial fluid. *Clin Chem* **41**, 1522–1525.

Han JF. 1999 Thesis Changchun Institute of Applied Chemistry, Academia Sinica.

Jackson GE, Wynchank S, Woudenberg M. 1990 Gadolinium(III) complex equilibria: The implications for Gd(III) MRI contrast agents. *Magn Reson Med* **16**, 57–66.

Jackson GE, Toit JG. 1992 Gadolinium (III) *in vivo* speciation. Part 2. A potentiometric study of gadolinium(III) amino acid complexes. *South Afr Tydskr Chem* **45**, 82–85.

Lu X. 2001 Thesis, Changchun Institute of Applied Chemistry, Academia Sinica.

Luckey TD, Venugopal B, Huntcheson D. 1975 Heavy metal toxicity safety and hormology, New York: Academic Press.

May PM, Linder PW, Williams DR. 1976 Ambivalent effect of protein binding on computed distributions of metal ions complexed by ligands in blood plasma. *Experientia* **32**, 1492–1494.

May P M, Linder P W, Williams DR. 1977 Computer simulation of metal-ion equilibria in biofluid: Models for the low-molecular-weight complex distribution of calcium(II), Magnesium(II), Iron(III), Copper(II), Zinc(II), and lead(II) ions in human blood plasma. *J Chem Soc Dalton Trans*, 588–595.

Meng L, Ding L, Chen HT, Zhao D Q, Ni JZ. 1999 Biological effect of rare earth(I)-Contents and distribution of rare earth in normal human plasma. *Chem J Chin Univ* **20**, 5–8.

MINTEQA2/PRODEFA2. 1991 A Geochemical Assessment Model for Environmental Systems: Version 3.0 User's Manual.

Ni JZ. 1995 Bioinorganic Chemistry of Rare Earth Elements, Beijing: Science Press.

Rizkalla EN, Choppin GR. 1994 *In*: Gschneidner KA, Eyring L eds, *Handbook on the Physics and Chemistry of Rare Earths*, vol. 15. Amsterdam: North-Holland.

Sillén G, Martell AE. 1964 Stability Constants of Metal-ion Complexes, Special Publication No. 17. The Chemical Society, London: Burlington House.

Sillén G, Martell AE. 1971 Stability Constants of Metal-ion Complexes, Special Publication No. 25. The Chemical Society, London: Burlington House.

Wang Y, Lu X, Nui CJ, *et al.* 2001 Study on effect of Gd(III) speciation on Ca(II) speciation in human blood plasma by computer simulation. *Chin Chem Lett* **12**, 161–162

Wang Y. 2000 Thesis, Changchun Institute of Applied Chemistry, Academia Sinica.

Webb LM, Taylor DM, Williams DR. 1998 Computer modeling of the chemical speciation of lanthanide and actinide elements in the human gastrointerstinal tract. *J. Alloy and compounds* **112**, 271–273

Williams DR. 1979 Complex concentrations that are too dilute to analyse-The dilemma and a solution. *Proc Analyt Div Chem Soc*, 81–82.

Xu GX. 1995 Rare earths, Beijing: Metallurgy Industry Press, 471.