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Inorganica Chimica Acta 242 (1996) 201–204

Inorganica Chimica Acta

Iron-59 complexes of lipophilic hexadentate phenolate-derivatized cyclohexanetriamine ligands

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Abstract

The compounds $(RsalH_2)_3tachH_3$ (R = H, NO_2, OMe) have been synthesized by Schiff base condensation between *cis*-1,3,5-triaminocyclohexane and a substituted salicylaldehyde, followed by reduction with KBH_4. Reaction of these compounds with iron(III) salts gives neutral hexacoordinate N_3O_3 complexes of type $Fe(RsalH_2)_3tach$. These complexes have been characterized by a combination of infrared, UV-vis and mass spectroscopy. The distribution coefficients between 1-octanol and water indicate that the complexes are lipophilic. The electronic absorption spectra of the high spin Fe(III) complexes show LMCT bands in the 450–500 nm range. These lipophilic complexes in vivo do not cross the blood-brain barrier to any significant extent.

Keywords: Iron; Multidentate N,O-ligand; Chelate complexes; Lipophilic complexes

1. Introduction

Because of the clinical importance of trivalent metal ions there is a need to synthesize chelating agents that are specifically designed for medical applications [1]. If chelators are planned for clinical use there are, however, a number of restrictions that must be met. Among these is the requirement that generally both the free ligand and its complexes should be water soluble. Also, if the ligand is to be used to scavenge a metal ion in vivo, it must be able to penetrate cell membranes, As a result the ligand cannot possess charged functional groups in solutions having physiological pH, and the resulting complexes must also be neutral. In addition to the aforementioned requirements, it is also important that the metal be tightly bound within the chelate ligand. With kinetically labile complexes the metal may be donated to endogenous high-affinity binding sites such as those located on the plasma protein apotransferrin. In order to obtain kinetically inert complexes it is usually advantageous to use multidentate chelate ligands. In general, oligodentate chelates are more kinetically inert than are bidentate chelates.

Two important applications of trivalent metal ions is in imaging with gallium(III) and indium(III), and in iron scavenging. Multidentate 'hard' ligand, such as those incorporating a phenolate anion moiety, generally form strongly coordinated complexes with these metals [2]. These ligands generally have N_3O_3 coordination, and resemble similar ones based on a macrocylic or pyramidal backbone [3,4]. If compounds are to be used as metal extraction agents, it is necessary that both they and their iron(III) complexes have an overall neutral charge and be lipophilic [5].

The gradual accumulation of iron is associated with a number of diseases such as β-thalassaemia [6]. It is important therefore to develop chelators that can be used to reduce iron overload and the deleterious effects on health associated with it. In addition chelators may also be useful for the treatment of inflammatory diseases such as rheumatoid arthritis through their action as scavengers for iron [1]. In microorganisms the iron complexing compounds that are secreted are the siderophores. These chelating agents typically have hydroxamate or catecholate groups that coordinate to the Fe^{3+} center. Therefore these functionalities are preferred ones for forming stable complexes of this ion [7–9]. We have synthesized and characterized a new set of uncharged ligands $(RsalH_2)_3$-

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0020-1693/96/$15.00
SSDI 0020-1693(95)04867-7

$tachH_3$ (R = H, NO_2, OMe) and prepared their neutral lipophilic hexacoordinate complexes with trivalent iron [10]. These ligands have been designed to avoid the presence of C = N functional groups because it is believed that such groups undergo degradation under in vivo conditions [11]. The biodistribution of these ^{59}Fe complexes in vivo shows that these lipophilic complexes are directed away from the hemopoietic organs and toward the blood-clearing tissues. This effect is particularly pronounced for $Fe(NO_2salH_2)_3tach$. The rapid clearance of these complexes from the blood indicates their potential use as iron scavenging agents [12].

The present work seeks to determine the viability of the three ligands $(RsalH_2)_3tachH_3$ (R = H, NO_2 and OMe) as chelators for use as brain imaging agents. The reason for making these studies is that these compounds have been shown to affect the neurotoxic aspects of aluminum [13]. In particular, we have found that $(NO_2salH_2)_3tachH_3$ is capable of binding aluminum from the circulation and that the resulting complex may enhance delivery of biologically active aluminum to its site of action. Because it is not known whether the effect of aluminum occurs in the blood or brain side of the blood–brain barrier, speculation on the mechanism is difficult [13]. It would be useful, therefore, to know whether complexes of these hexadentate chelates do indeed cross the blood–brain barrier. The current experiments use radioactive ^{59}Fe complexes to determine the permeability of the blood–brain barrier.

2. Experimental

2.1. Synthesis

The ligands $(salH_2)_3tachH_3$, $(NO_2salH_2)_3tachH_3$ and $(MeOsalH_2)_3tachH_3$ and their Fe complexes were synthesized and purified by previously reported methods [10]. The ligands were prepared and used in this study in their azeotropic form except for $(NO_2salH_2)_3tachH_3$, which was prepared as its tris-hydrochloride salt. Partition coefficients were measured using the procedure in the literature [10].

2.2. Radioactive iron complexes

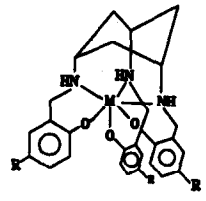
Solutions of $(salH_2)_3tach$, $(NO_2salH_2)_3tach$ and $(MeOsalH_2)_3tach$ complexed to radioactive ^{59}Fe (Amersham, Arlington Heights, IL) were prepared similarly to solutions of ligands complexed with non-radioactive iron except on a much smaller scale [10]. Radioactive solutions were prepared behind lead foil or bricks inside a fume hood. Preparations involved addition of the iron to a solution of excess ligand to which was then added excess base. Details of the syntheses of $^{59}Fe(salH_2)tach$, $^{59}Fe(MeOsalH_2)_3tach$, $^{59}Fe(NO_2salH_2)_3tach$ and the ^{59}Fe control solution have been published elsewhere, along with details of the uptake measurements [12].

3. Results

3.1. Synthesis

The phenolate-derivatized cyclohexanetriamine ligands have been synthesized by the procedure shown in Scheme 1. The compound has the *cis* stereochemistry about the cyclohexane ring, and the phenolic groups are appended in an arrangement whereby the deprotonated phenolate derivative can readily give neutral hexadentate complexes with trivalent metal ions. In their deprotonated ligating forms these compounds are abbreviated as $(salH_2)_3tach$ (R = H), $(NO_2salH_2)_3tach$ (R = NO_2) and $(MeOsalH_2)_3tach$ (R = OMe). In addition to designing a ligand with the preferred coordination and lipophilicity characteristics, the ligand has been chosen to have fully saturated bonds between carbon and nitrogen because unsaturated C=N bonds are considered to be a cleavage point for biodegradation under in vivo conditions.

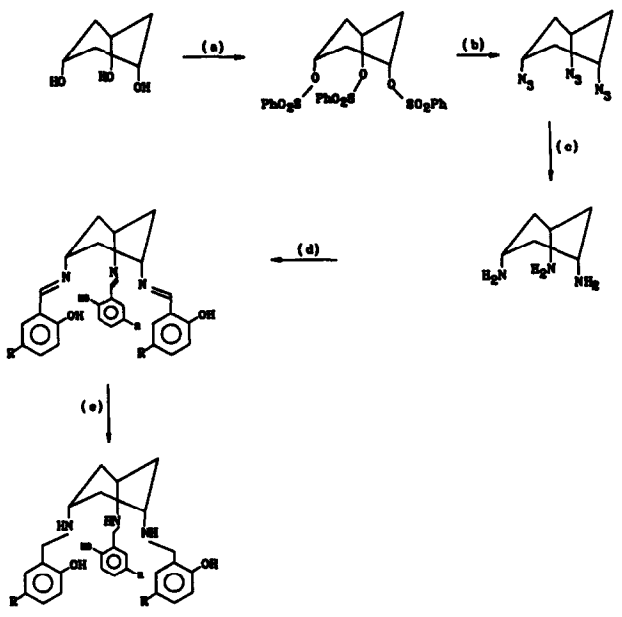
Metal complexes of trivalent iron with these ligands have been synthesized by treating salts of these ions with a 1:1 stoichiometric ratio of the ligand in methanol solvent (Eq. (1)). In all cases the yield of the complex is greater than 75%.

$$M^{3+} + (RsalH_2)_3tachH_3 \rightarrow \text{  } + 3H^+ \quad (1)$$

(M = Ga, In, Fe; R = H, NO_2, OMe)

These iron complexes are red in color. The electronic absorption spectra of the iron complexes show three absorption bands. If we assign the two short wavelength bands to π-π^* transitions that are primarily centered on the aromatic groups of the ligands, we can assign this third longer wavelength band to a transition that involves the Fe(III) center. This third absorption band is observed at 460, 500 and 484 nm respectively for $Fe(salH_2)_3tach$, $Fe(NO_2salH_2)_3tach$ and $Fe(MeOsalH_2)_3tach$. Since the extinction coefficients of this band in all three complexes are approximately 10^3 M^{-1} cm^{-1}, the absorption is too intense to be due to a d-d transition, and is likely due to a LMCT transition [14–17].

The single crystal X-ray structures of $Fe(RsalH_2)_3tach$ (R = H, NO_2, OMe) confirm that in each case the coordination geometry about the central metal ion is octahedral with N_3O_3 ligating atoms. The magnetic moments of the complexes all exhibit the high values characteristic of a high spin complex in a weak ligand field. All three complexes show Curie–Weiss behavior with a plot of $1/\chi$ against T extrapolating toward zero with very small negative Weiss constant. The complexes therefore behave as



Scheme 1. (a) PhO_2SCl, pyridine, 10°C; (b) NaN_3, diethylene glycol, 100°C; (c) $LiAlH_4$, THF, reflux; (d) **1a** R = H, salicylaldehyde, EtOH, reflux; **1b** R = NO_2, 5-nitro-salicylaldehyde, EtOH, reflux; **1c** R = OMe, 5-methoxy-salicylaldehyde, EtOH, reflux; (e) KBH_4, EtOH, reflux.

isolated d^5 centers having weak antiferromagnetic couplings. The partition coefficients for the distribution of the complexes $Fe(RsalH_2)_3tach$ between 1-octanol and water are large, with the respective values of 53, 13 and 4.8 being found for R = H, NO_2 and OMe, respectively.

3.2. *In vivo biodistribution studies*

The solutions of iron-59 complexes of the three ligands $(salH_2)_3tachH_3$, $(NO_2salH_2)_3tachH_3$ and $(MeOsalH_2)_3tachH_3$ for in vivo biodistribution studies were prepared as saturated solutions in 0.9% NaCl, with 1% BSA and 5% DMSO added to enhance solubility. The complexes have limited aqueous solubility, especially for $Fe(NO_2salH_2)_3tach$. The solubility of this complex is however significantly enhanced in 1% BSA solution.

The tissue samples that were removed after i.v. injection of the three radioactive complexes and free radioactive iron were examined to determine the amount of radioactivity per gram of tissue. These totals were then divided by the activity in serum and presented as the apparent volume of distribution (V_{dt}) at time t as expressed by

$$V_{dt} = Q/C_{pt}$$

where Q is the total amount of radiotracer/g in the tissue and C_{pt} is the amount of radiotracer per ml of serum at time t [18]. The experimental data for delivery of ^{59}Fe to the brain by the three ligands and uncomplexed iron are collected in Table 1.

Table 1
Distribution (volume of distribution in $\mu l\ g^{-1}$) for ^{59}Fe delivered to the brain

	Time (min)						
	5	10	15	30	60	120	240
$(salH_2)_3tach$	16.4	20.0	17.0	20.9	13.5	23.5	29.6
$(NO_2salH_2)_3tach$	21.6	11.6	12.3	18.0	10.5	20.5	21.1
$(MeOsalH_2)_3tach$	16.9	15.2	11.4	17.0	19.6	34.4	33.8
Uncomplexed	4.05	11.6	11.1	12.8	12.1	18.6	33.1

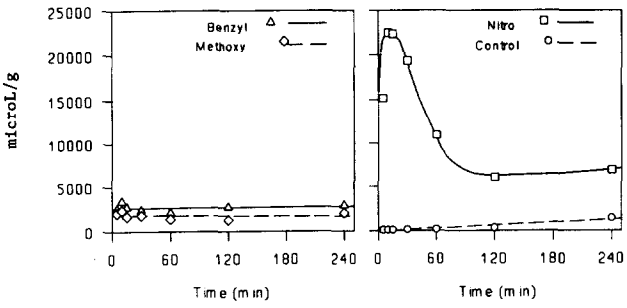


Fig. 1. Short term distribution of radiotracer in liver.

For imaging, resolution from surrounding tissues is important. Since blood is the most ubiquitous tissue, a high tissue to blood ratio, as expressed here by V_d, is ideal for an imaging agent. For therapeutics however, the total dose reaching a tissue, as expressed here as % ID g^{-1}, is more relevant. The crucial factor that differentiates V_d from % ID g^{-1} is the amount of compound in the blood. Important differences occur among the three complexes and free iron regarding concentrations and clearances from blood. For this reason, the results are expected to yield profiles and interpretations when expressed as V_d or as % ID g^{-1}. The biodistribution data in the brain for $^{59}Fe(RsalH_2)_3tach$ (R = H, NO_2 and OMe) in % ID $g^{-1} \pm$ MSE (n) over a 24 h period show the following values: $(salH_2)_3tach$, $0.107 \pm 0.007(14)$; $(NO_2salH_2)_3tach$, $0.023 \pm 0.006(10)$; $(MeOsalH_2)_3tach$, 0.129 ± 0.011; control, $0.453 \pm 0.041(12)$.

4. Discussion

In investigating a broad range of tissues we have found that there is a selectivity in the uptakes [12]. It is likely that this selectivity among the ligands and tissue types is observed because of a rapid and reversible association with the tissue which occurs immediately after injection when the complex is the predominant form in serum. As serum concentrations decrease the reversibly bound complexes reenter the bloodstream and are decomposed, releasing free radioactive iron. The free radiotracer is then redistributed throughout the body. However, because selectivity among tissue types is observed among the complexed and control groups, there likely exists a second equilibrium involving the complexed radiotracer between

reversibly and irreversibly bound states within certain tissues. Thus overall long-term tissue distributions reflect a combination of complexed iron having diffused irreversibly into tissue from reversible tissue compartments, and non-ligand bound tracer accumulating as a result of the natural iron-transport system.

The short term (up to 240 min) V_d data collected in Table 1 and the long term (24 h) % ID g^{-1} data show that these ligands do not effectively carry complexed ^{59}Fe into the brain. One small difference is in the initial 5 min V_d values. For the uncomplexed ^{59}Fe this value is low. After this time, however, the incorporation slowly increases. For the three complexes the initial 5 min incorporation does not show the reduced levels shown for the uncomplexed ^{59}Fe. After 240 min the complexed and uncomplexed ^{59}Fe show similar V_d levels in the brain, albeit relatively low. The 24 h % ID g^{-1} data show small differences between complexed and uncomplexed ^{59}Fe. The control value is up to 10 times those of the values found for the complexes. Among the complexes themselves the lowest 24 h % ID g^{-1} value is found for $^{59}Fe(NO_2salH_2)_3$-tach, which correlates with its different biodistribution behavior with other tissues and organs [12].

In conclusion it is apparent the barrier-protected organs such as the brain and testis [12] do not show significant incorporation of these ^{59}Fe complexes. This result agrees with the radiogallium biodistribution data for these complexes [12], and indicates that the effect of $(NO_2salH_2)_3tachH_3$ on the biological activity of aluminum is not likely due to the migration of $Al(NO_2salH_2)_3tach$ across the blood–brain barrier. Although these complexes have many characteristics favorable for passage through this barrier (uncharged, lipophilic, etc.), it is likely that the reason why they do not pass is that the molecular weight is too high.

Acknowledgements

We thank the Center for Bioenvironmental Research and the VA for financial support.

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