Competition of Tripositive Metal Ions for Fe(III) Binding Sites in Chelation Therapy of Iron Load

F. S. REHMANI
Department of Chemistry
Universityof Karachi, Karachi-75270, Pakistan

(Received 16th September, 2002, revised 25th June, 2003)

Summary: For the treatment of iron overload, a drug desferrioxamine mesylate has been used. It is linear trihydroxamic acid, a natural siderophore produced by streptomyces which removes the extra iron from body. Salicylhydroxamate is a simple model of hydroxamate type siderophore. In present research salicylhydroxamate was used for the complexation with tripositive metal ions which are available in biological environments such as Al(III), Cr(III) and Fe(III). The aim of our work was to study the competition reactions between Fe(III) and other tripositive ions, to calculate the thermodynamic data of chelation of these metal ions complexes with hydroxamate by computer program and comparison with iron hydroxamte complexes.

Introduction

Thalasemia and sickle cell anemia are genetic disorder in which patients need blood transfusion over a long period of time, results an excess amount of iron deposited in the organ of the body which damages the tissues. This condition is known as iron overload [1]. There are many natural mechanism for soloubilization or removal of iron, for example, the micro organism utilizes a well define iron acquisition strategy which includes the production of low molecular weight chelating agents called siderophores or microbial iron chelators to solublize and transport ferric ions in aqueous medium. These siderophores have high affinity for Fe(III) and they are better chelators for Fe(III) than Fe(II) [2]. Desferrioxamine mesylate a liner trihydroxmic acid natural siderophores produced by streptomyces have been used for the treatment of iron overload. It chelate out the excess burden of iron. There are three main tripositive ions such as Fe (III), Al (III) and Cr (III) which found in the biological system. Their ionic radii are very close to each other so these ions are involved in competition reactions [3]. For example the iron requirement of biological system may be influenced by the presence of Al (III). It binds more strongly to the iron binding sites [4]. Al (III) and Cr(III) both are involved in competition reaction of Fe(III) because of their +3 oxidation states. A chelating agent to be effective in removing a toxic metal from the body, it must satisfy second law of thermodynamic that is the free energy change for the transfer of metal ions from the binding sites to the chelating drug must be negative. To achieve this requirement, stability constant between the toxic metal and chelating drug must be greater than that of the competing ligands with the metal concerned [5]. For any significant metal sequestration to occur the affinity of the metal for the chelator must be greater than its affinity for endogenous ligands and the chelator must be faster than the rate of elimination of the chelator. If a chelator eliminated more rapidly than the dissociation of the metal endogenous Ligand complex, it may not be present in sufficient concentration for effective competition with the metal endogenous binding sites [6]. The aim of our work was to study the competition reactions between Fe (III) and other tripositive ions, to calculate the thermodynamic data of chelation of these metal ions complexes with hydroxamate by computer program and comparison with iron hydroxamate complexes.

Results and Discussion

The potentiometric data at different tempratures for hydroxamate drug and its tripositive metal ions complexes such as Al(III), Cr(III), Fe(III), were analyzed by computer program "BEST". The β values of these complexes at different temperatures were used to calculate the entropy and enthalpy values of complexes [Martell, *et.al.* 1988].the table 1. shows the competition of Al(III) ions with Iron salicylhydroxamate complexes. Table 2. shows the competition of Cr(III) ions with Iron Sâlicylhydroxamate complexes while table 3 shows competition of Fe(III) ions with Cr(III) salicyl-hydroxamate complexes. The results of table 1. shows the decrease in absorbance by the increasing concentration of Fe(III) ions which indicates that Fe(III) ions do not replace Al(III) ions from aluminum hydroxamate complexes. In the second table the concentration of Fe(III) hydroxamate keep constant and the concentration of Cr(III) ions were increased but the absorbance almost remain constant in each case. It told that Cr(III) ions are not replaceing Fe(III) ions. The result of table 3 also justify the results of table 2.

Table-1:Competition of Al(III)ions with iron salicylhydroxamte complexes Absorbance of iron salicylichydroxamic acid complex (5 x 10^{-4}M) in the presence of Al(III) ions

	Al SHA Complex Solu.	Fe^{3+} Ions solu.	Buffer pH5	$A\lambda_{500}$
1.	5ml	-	5ml	
2.	5ml	1ml	4ml	0.33
3.	5ml	2ml	3ml	0.25
4.	5ml	3ml	2ml	0.22
5.	5ml	4ml	1ml	0.19
6.		5 ml		-

Table-2:Competition of Cr(III)ions with iron salicylhydroxamte complexes. Absorbance of iron salicylichydroxamic acid complex (5 x 10^{-4}M) in the presence of Cr(III) ions

	FeSHA Complex Solu	Cr^{3+} Ion solu.	Buffer pH5	$A\lambda_{500}$
1.	5ml	-	5ml	0.35
2.	5ml	1ml	4ml	0.34
3.	5ml	2ml	3ml	0.32
4.	5ml	3ml	2ml	0.31
5.	5ml	4ml	1ml	0.30
6.		5 ml	5 ml	15

Table-3:Absorbance of Chromiuim salicyhydroxamic acid complex (5 x 10^{-4}M) in the presence of Fe^{2+} ions

	CrSHA	Fe^{2+}	Buffer pH5	$A\lambda_{500}$
1.	5ml	-	5ml	-
2.	5ml	1ml	4ml	0.21
3.	5ml	2ml	3ml	0.28
4.	5ml	3ml	2ml	0.31
5.	5ml	4ml	1ml	0.34
6.	-	5ml	5ml	-

In order to examine the results of competition reactions of metal ions, it is important to consider the stability constant and thermodynamic stability of metal ions. Such reactions are of major nutritional and toxicological importance. These reactions can be predicted on the basis of their stability constants. Similarity in these values is believed to underlie many of similar biological reactions and competition for binding sites on metalloenzyme transport and storage proteins[9]. When a foreign metal competes with or replaces a functional metal, toxicity results. This is shown by a comparison of the stability constants[10]. Our results showed, among tripositive ions Al(III) has high stability constant and thermodynamic stability values[11] therefore it will prefentially occupy binding sides of the ligands used in the present study, the other cations will only be able to win the competition, if the concentration Al(III)is reduced Fe(III) will be the second in the competition.

Experimental

All reagents used were of AR or equivalent grade and were used without further purification. Distilled water was re-distilled and subsequently passed through a column of cation exchanger (Amberlite resin IRA-401 from BDH chemicals) in order to make it free of cations. This doubly distilled and deionzed water was used in prepartion of all solutions of reagents. The salts of metals (E. Merck) taken were auminium chloride, cadmium chloride, calcium chloride, cobalt chloride, copper chloride chromium chloride, magnesium chloride, manganese chloride, nickle chloride and zinc chloride. All these metal solutions were standardized by standard method. The hydroxamate was obtained from E.Merck and was used with any further purification. NaOH salution was standardized by standard HCl solution. For all pH measurments in potentiometric titrations, Orion pH meter model SA 720, was used. A 0.05M solution of potassium hydrogen pthalate, which has pH value 4.01 at room temperature, 25°C was used to calibrate the pH meter. The titration were carried out in double walled glass cell, fitted with an air tight cork, having three holes. One for the nitrogen purging other for base added and third one for the electrode to be dipped in the solution. The temperature of the cell was kept constant through out the experiment by thermostat. The capacity of the cell was about 75 ml. The solution used for the titration were prepared in double deionized and decarbonized water, pH was measured with a combination glass electrode 0.001 pH unit. All the titrations were performed at 30°C, 35°C,40°C, 45°C, 50°C, 20ml of 0.01 M of hydroxamate were mixed with 20 ml of 0.01 ml of metal ions solution and were titrated with 0.1 M NaOH solution. The change in pH was noted with the small increment (0.05 ml) 0f base. Equilibrium conditions, determined by a constant meter reading falling with an interval of less than 0.002 pH unit was obtained for each experimental point before proceeding with the next step. The solution was stirrered with magnetic stirrer constantly. For each metal hydroxamate solution these titrations were performed twice to minimize the probable errors [7].

Determination of Log β Values and Thermodynamic Values Through Potentiometric Method by Computer Program BEST

The data obtained from pH titrations were utilized for the calculations of log β values. For this purpose computer program BEST was used. Data files FOR004.DAT was prepared for each titration. Calculated β values was refined several times, till the sigmafit values reduced upto 0.04. The date file of this program was required the following informations:-

1. Total volume of the solution.
2. Molarity of the base used for pH titration.
3. Change in pH after each step.
4. No of millimole of metal ions present in the solution
5. No. of millimole of ligand present in the solution.

The whole calculations in this program were based upon the expected β values for each species present in the solution by refining these values to get sigmafit values, the goodness of sigmafit was reflected on accuracy of K values. The K values of the complexes at different temperature was used to calculate the thermodynamics values of complexes.

$$\Delta G = -Rt \text{ in } \beta$$

$$\Delta G = \Delta H - T\ \Delta S$$

$$\text{In } B = \Delta H/T + \Delta S/R$$

A plot in β vs 1/T, slope = -ΔH/R, intercept = ΔS/R [8].

Experiments for the Determination of Competetion of Metal Ions with Iron Siderophore Complexes

Study of the competetion reactions two sets of experiments were performed with those metals whose stability constants are close to iron siderophore complexes. 7 volumetric flasks of 10 mls were selected. These flasks were filled in the following manner.

	Iron gallic acid	Metal ions solu.	buffer pH 5
1	5 ml	x	5 ml
2	"	1 ml	4 ml
3	"	2 ml	3 ml
4	"	3 ml	2 ml
5	"	4 ml	1 ml
6	"	5 ml	_
7	X	5 ml	5 ml

Another set was made in following manner

	Metal siderophore complex	Fe(III) solu.	Buffer pH 5
1	5 ml	x	5 ml
2	"	1 ml	4 ml
3	"	2 ml	3 ml
4	"	3 ml	2 ml
5	"	4 ml	1 ml
6	"	5 ml	_
7	X	5 ml	5 ml

The absorption of these solutions were noted at 50° after half an hour and after 24 hours.

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