IRON in BIOCHEMISTRY and MEDICINE, II

Edited by
A. JACOBS and
M. WORWOOD

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Heath Park, Cardiff, Wales

1980

ACADEMIC PRESS LONDON AND NEW YORK

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

British Library Cataloguing in Publication Data

Iron in biochemistry and medicine.
2
1. Iron metabolism
I. Jacobs, Allan II. Worwood, Mark
591.1'9'214 QP535.F4 73–19015
ISBN 0–12–378980–X

Filmset by Northumberland Press Ltd.
Gateshead, Tyne and Wear
Printed in Great Britain by
Fletcher and Son Ltd, Norwich

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and MEDICINE, II

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Preface

Iron in Biochemistry and Medicine was published in 1974 and had two major aims: firstly to bridge the gaps between the many disciplines which contribute to the study of iron metabolism and secondly to provide up-to-date reviews of some areas of particular activity. In preparing *Iron in Biochemistry and Medicine II* we have kept the same aims. Some topics which were not included in the first volume, or which received only brief discussion, are reviewed in this volume. The chapters on Haem Proteins and Detoxication, Sideroblastic Anaemia, Microbial Iron Metabolism and Comparative Iron Metabolism are examples. Other chapters reflect the tremendous amount of new information that has become available since 1974 and perhaps it is not surprising that the biochemistry of ferritin and the clinical problems caused by iron toxicity take up six chapters. Many of the contributions to the first volume still provide excellent sources of information and these subjects have not been included in the present volume.

We are most grateful for the hard work put into the preparation of each chapter by the contributors.

ALLAN JACOBS
MARK WORWOOD

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1. The Inorganic Chemistry of Iron Metabolism

PETER M. MAY AND DAVID R. WILLIAMS

Department of Chemistry, University of Wales Institute of Science and Technology, Cardiff, Wales

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I. Introduction

During the period between the formation of the earth and the appearance of primitive life about $3{\cdot}7 \times 10^9$ years ago, a stage of chemical evolution took place in which the prerequisite molecules and complexes were assembled (Orgel, 1973; Österberg, 1976). The elements used in these compounds were those most readily available at the site of synthesis, i.e. in seawater or on tidal beaches. Since at this time the earth had a reducing atmosphere, the iron incorporated into early biomolecules was essentially in the Fe(II) state. The use of Fe(III) became increasingly favoured as the early environment was replaced by one with a surplus of oxygen.

However, the shift in favour of higher oxidation states had an important

effect on the development of the biological chemistry of iron. Iron in the form of Fe(II) had been readily soluble but the polymeric oxides and hydroxides of Fe(III), which are precipitated at neutral pH unless the metal has been suitably chelated, tended to remove the element from water. So, although iron is very abundant in the earth's crust (in fact it is the second most common metal), its bioavailability is generally limited and higher species often exhibit deficiency states. Paradoxically, iron overload conditions also occur (Chapters 12–14).

The effects of such imbalance can be severe. In quantitative terms iron is the most important essential trace element; adult men and women contain approximately 55 and 45 mg per kg of body weight respectively. Iron's biochemical activity reflects its dual ability to co-ordinate electron donors and to participate in redox processes. These properties are widely exploited in enzyme systems to perform a variety of physiological tasks under the very mild reaction conditions which pertain *in vivo* (Chapter 2). Haemoglobin normally accounts for 60–70% of the total iron in the body while myoglobin, the cytochromes and other iron-containing enzymes comprise a further 10%. The remaining 20–30% is equally distributed between the two storage proteins ferritin and haemosiderin (the extracellular transport protein transferrin accounting for only 0·1–0·2% of the total body iron). In iron deficiency, all these metalloproteins are affected and a reduction in the amount of circulating haemoglobin can occur. On the other hand, in iron overload accumulating deposits of the metal can seriously damage organs such as the heart, liver and spleen.

In the following account we attempt to outline the solution chemistry of iron and its chelates and their relevance to human iron metabolism.

II. Iron Complexes *in vitro*

Just two oxidation states of iron exist as aquated ions in aqueous solution— $Fe(H_2O)_6^{2+}$ and $Fe(H_2O)_6^{3+}$. These octahedral complexes readily hydrolyse and polymerize at physiological pH values: at pH = 7 the maximum solubility of $Fe(H_2O)_6^{3+}$ is 10^{-17} mol/ℓ whereas the solubility of $Fe(H_2O)_6^{2+}$ permits solutions of 10^{-1} mol/ℓ (Spiro and Saltman, 1974). However, when the water molecules are replaced by other chelating ligands stable complexes are formed. The strongest complexes of Fe(III) tend to be with oxygen donor ligands, e.g. citrates, phosphates, phenols or carbohydrates whereas Fe(II) prefers nitrogen or nitrogen with oxygen donors.

The potential necessary to reduce Fe(III) to Fe(II) in a biological fluid such as plasma varies from 770 mV to 360 mV depending upon the ligands complexed to the metal ions. Further, the lipophilicity of the metals' environment and the stereochemistry of the co-ordinated groups can be

manipulated in macromolecules to produce variations in redox potential of more than 1 V. The electronic structures of these two oxidation states and the ligand characteristics which determine crystal field stabilization energies and the influence of high spin or low spin complexes have been reviewed by Spiro and Saltman (1974).

Both $Fe(H_2O)_6^{2+}$ and $Fe(H_2O)_6^{3+}$ are only stable under acidic conditions which prevent hydrolysis. On neutralization and further addition of alkali, such solutions produce green and brown gelatinous precipitates respectively. Loss of protons from the co-ordinated water molecules gives rise to bridging by oxygen atoms bound simultaneously to two metal ions. Studies involving iron at physiological pHs are often hampered by this hydrolysis which tends to be kinetically slow to reach equilibrium and is invariably thermodynamically irreversible. Even when complexed to other ligands, Fe(III) commonly exhibits hydrolytic dimerization to produce species such as $[LFe(OH)]_2^{2-}$ and $[LE_2Fe(OH)]_2^{2-}$.

Greater orders of polymerization occur amongst simple Fe(III) hydroxy species which form polynuclear structures closely related to ferritin. Difficulties in keeping low-molecular-weight iron complexes in aqueous solution have been avoided to some extent through the function of ferritin as a cellular reservoir of low-molecular-weight species. When ligands occupy all six co-ordination positions on each iron ion, hydroxypolymerization is suppressed and mononuclear complexes can exist at physiological pH values. Such species are formed by polydentate chelating agents manufactured by micro-organisms, e.g. ferrichrome A and desferrioxamine B (Chapter 15).

Biologists often need to know which metals are most likely to be influenced by the administration of an exogenous agent to human subjects. The extent to which a metal complex will be formed *in vivo* depends on many factors such as total concentrations, stereochemistries, stability constants, kinetics and competitive interactions. How these determine the actual situation from the immense range of metal ligand complexes that can occur *in vivo* is briefly outlined below (Perrin, 1964).

Firstly, the total amount of metal or ligand present imposes a limit on the amount of complex that may be formed. A corollary is that if a component concentration is changed, the availability of its co-ordinating partners will also alter (with corresponding side-effect implications).

There are certain preferred stereochemistries of bonds about metal ions which must be satisfied if a biological ligand or administered drug for complexing a metal is to be successful. With octahedral complexes having bonds which subtend an angle of 90° at the metal ion 5- or 6-membered chelate rings are preferred. Additionally, the occurrence of the ring draws benefits from the "chelate effect" wherein two donor groups on the same

ligand have greater stability than that of the groups separately. Iron, the subject of this review, prefers octahedral co-ordination for both its common oxidation states (although tetrahedral arrangements are known *in vitro* especially in non aqueous environments).

The compatibility of metal acceptor ions and ligand donor atoms ultimately depends upon the strength of the chemical bond which can be formed between them. Such bonds can be most fully described using molecular orbital theory but in practice simpler and much more rapid approaches which can be applied as rules of thumb are often used. One of these approaches is the concept of "hard" and "soft" acids and bases (HSAB). A base is defined as a species which can donate a pair of electrons to form a co-ordinate bond and an acid is a species which can accept this pair of electrons (Perrin, 1970). A ligand may be described as a "soft" base if its donor atom is of high polarizability, having empty, low energy orbitals. Such donor atoms are usually of low electronegativity and are easily oxidized because the valence electron orbitals are readily distorted. Conversely, a "hard" base has a donor atom of low polarizability, is hard to oxidize, has a high electronegativity and has vacant orbitals only of high energy. Similarly, a metal ion is classified as a "soft" acid if it is of low charge density, large size and has easily excited outer electrons. A metal ion is a "hard" acid if it is of high positive charge, is small in size and has no easily excited outer electrons. This approach has led to the important generalization (Pearson, 1963) that "hard" acids form strong bonds with "hard" bases whereas "soft" acids prefer to be co-ordinated by "soft" bases. Much less stable complexes are found between a "hard" acid and a "soft" base or a "soft" acid and a "hard" base.

The classification of metal ions in terms of "hard" or "soft" acids is given in Table I. A corresponding grouping of ligands as "hard" or "soft" bases is given in Table II. Ligands containing oxygen as a donor atom tend to be "harder" than those containing nitrogen and these, in turn, are much "harder" than sulphur-containing ligands. One should note that the proton behaves as a very "hard" acid.

The application of the HSAB concept to iron complexes rationalizes many of their observed properties. Whereas Fe^{3+} is seen to be "hard", Fe^{2+} is "borderline". This is consistent with the stronger affinity of iron in its higher oxidation state with oxygen donors. It also means that "hard" donors will favour Fe^{3+} over Fe^{2+} in a redoxing system between the two. The higher oxidation state will be preferred in an aqueous as opposed to a non-aqueous environment. A shift to higher pH will have the same kind of effect. Finally, the extra stability conferred on Fe^{3+} complexes over Fe^{2+} and other divalent transition metal ions as a result of the additional charge increases with the "hardness" of the ligand concerned; likewise,

TABLE I.
METAL IONS AS HARD OR SOFT ACIDS

Hard										
H^+										
Li^+	Be^{2+}									
Na^+	Mg^{2+}	Al^{3+}	Si^{4+}							
K^+	Ca^{2+}	Sc^{3+}	Ti^{4+}	VO^{2+}	Cr^{3+}	Mn^{2+}	Fe^{3+}	Co^{3+}	Ga^{3+}	As^{3+}
Rb^+	Sr^{2+}	Y^{3+}	Zr^{4+}		MoO^{3+}				In^{3+}	
Cs^+	Ba^{2+}	La^{3+}	Hf^{4+}							
			Th^{4+}							
			U^{4+},	UO_2^{2+} and the rare earth ions						
Intermediate										
		Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}				
		Ru^{2+}	Rh^{3+}				Sn^{2+}	Sb^{3+}		
		Os^{2+}	Ir^{3+}				Pb^{2+}	Bi^{3+}		
Soft										
				Cu^+						
			Pd^{2+}	Ag^+		Cd^{2+}			Te^{4+}	
			Pt^{2+},Pt^{4+}	Au^+		Hg^+,Hg^{2+}		Il^+,Il^{3+}		

TABLE II.
LIGANDS AS HARD OR SOFT BASES

Hard									
N_2O	OH^-	RCO_2^-	PO_3^{3-}	SO_4^{2-}	CO_3^{2-}	NO_3^-	ROH	RO^-	R_2O
Intermediate									
NH_3	RNH_2	N_2H_4							
Br^-	N_3^-	NO_2^-	SO_3^{2-}	pyridine aniline					
Soft									
R_2s	RSH	RS^-	SCN^-	$S_2O_3^{2-}$					
R_3P	$(RO)_3P$								
I^-	CN^-								

the differences between complex stability when Fe^{2+} is compared with other first row transition metals in the Irving–Williams sequence ($Mn^{2+} < Fe^{2} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$) increases markedly with the softness of the ligand concerned.

Although the HSAB approach can be made quantitative (Pearson, 1972), it is sufficient to restrict present mathematical considerations of the degree to which a complex will form in solution to a brief thermodynamic outline. In general, successive replacement of the water molecules bound to iron ions in aqueous solution by complexing agents can occur so that a whole series of competitive equilibria arise. As the solvent implicitly occupies sites which are not co-ordinated to other ligands, this can be expressed as follows, charges having been omitted for the sake of generality: