OpenLearn

The Open University

Iron transport and storage

The Open University

Iron transport and storage

OpenLearn | Free learning from The Open University

About this free course

This free course provides a sample of Level 3 study in Science: http://www.open.ac.uk/courses/find/science.

This version of the content may include video, images and interactive content that may not be optimised for your device.

You can experience this free course as it was originally designed on OpenLearn, the home of free learning from The Open University – www.open.edu/openlearn/science-maths-technology/science/chemistry/iron-transport-and-storage/content-section-0

There you'll also be able to track your progress via your activity record, which you can use to demonstrate your learning.

The Open University, Walton Hall, Milton Keynes, MK7 6AA

Intellectual property

Head of Intellectual Property, The Open University
Designed and edited by The Open University

Contents

Introduction

In this course we will see that, despite having a high natural abundance, iron is in very short supply because of the insolubility of its oxides and hydroxides. A result of this is that organisms have developed methods for the uptake, transport and storage of iron. For example, iron storage in mammals, including humans, is achieved by ferritin, which stores iron as a hydrated iron(III) oxide – an example of biomineralisation.

This OpenLearn course provides a sample of Level 3 study in Science.

Learning Outcomes

After studying this course, you should be able to:

- describe some of the biochemical methods by which organisms uptake iron
- describe some of the biochemical processes by which organisms store and transfer iron
- explain why iron is present only in very low concentrations in aqueous solution
- use aspects of iron(III) chemistry to explain the role of macrocyclic ligands in iron uptake and transfer.

1 How do organisms acquire iron?

Metals are an essential part of biological chemistry. Of all the trace elements, iron is the most important, especially as it is present in many essential enzymes and proteins. But how do organisms acquire the iron from their surroundings? Clearly, organisms need to absorb iron biochemically before it can be used in proteins. Also, some method of replacing lost iron quickly is needed: for instance, how is blood replaced once it has been lost through a cut? This prompts the question: what biochemical systems are responsible for the uptake and transport of iron, or, indeed, any metal, within an organism? In this course we shall examine something of what is known about iron uptake, transport and storage in organisms.

We begin by recalling that the iron proteins, myoglobin and haemoglobin, are essential for O_2 transport and storage. The higher structures of these proteins are very precise, and any small changes in their structures lead to inefficient O_2 transport and storage. Indeed, there is a class of human genetic diseases, which affects the structure of haemoglobin specifically. In this class of diseases, called **thalassaemia**, the molecular structure of haemoglobin is distorted; as a result, thalassaemic haemoglobin is inefficient at transporting O_2 (see Box 1).

Box 1 Thalassaemia

Thalassaemia is one of the most common of hereditary (i.e. genetic) human diseases. Thalassaemia patients suffer from similar symptoms to anaemic patients, as essentially both have low counts of healthy red blood cells. The disease is called thalassaemia (Greek: thalassa, sea) because it is very common in countries surrounding the Mediterranean Sea. It is also widespread in Central Africa, India and South-East Asia (Figure 1), Thalassaemia is also known as Cooley's anaemia, after the American physician who first identified it. There are two main types of the disease, thalassaemia-minor and thalassaemia-major. The former usually has symptoms of mild anaemia, whereas the latter is seriously debilitating.

Figure 1 Areas of high incidence of thalassaemia

The treatment for thalassaemia-major is regular blood transfusions, which restore the healthy red-blood cell count to normal levels. However, this leads to problems. The problems are not directly linked to the symptoms of anaemia, which are largely ameliorated by the transfusions, but to the regular intake of large quantities of iron into the body. The human body is capable of excreting a maximum of about 10 mg of iron a day, whereas regular blood transfusions put far more iron into the body. This leads to a condition known as **iron overload** or **haemochromatosis**.

Iron, like all other metals in an organism, has an optimal concentration: too little or too much leads to ill health. For example, one of the symptoms of iron overload is increased susceptibility to bacterial infection. This extra iron can be absorbed by other organisms, particularly bacteria (remember that not only humans require iron to live), which then proliferate and cause infection.

Due to its high mortality rate and widespread occurrence, thalassaemia is the subject of much medical research. It does, however, highlight some important principles for the bioinorganic chemist. Firstly, it is clear that within the healthy human body, biochemical systems carefully control the level of iron so that it is around an optimal value (this control is known as **homeostasis**). Secondly, it appears as if the human body has a means of transporting iron and probably storing it. For example, some iron stores are required in case of sudden loss of iron, say from heavy bleeding. Thirdly, within the body, iron is not made available to other organisms, thus helping to reduce the possibility of bacterial infection. Fourthly, bacteria themselves must have a means of absorbing iron.

In this course we shall examine the principles stated above. To begin with, it will be necessary to study some of the basic chemistry of iron and its coordination complexes. Then we shall look at the biochemical systems involved in iron uptake by bacteria. Finally, we shall examine biochemical systems for transporting and storing iron in humans.

2 Principles of iron chemistry

2.1 The problems of iron uptake

Iron has a high natural abundance. It is the second most abundant metallic element by mass in the Earth's crust (7.1 per cent).

Activity 1

What are the main oxidation states of iron?

Answer

Naturally occurring iron exists primarily in two oxidation states, +2 and +3, but in the presence of O_2 the most stable oxidation state is +3.

The iron concentration in seawater is very low, roughly 5×10^{-11} to 2×10^{-8} mol l^{-1}

Activity 2

If an aqueous pale-green coloured solution of iron(II) nitrate, $Fe(NO_3)_2$, at pH 7 is exposed to air, a brown-coloured precipitate soon forms on standing. The precipitate, which contains iron(III), settles to the bottom of the vessel. What do you think has happened?

Answer

The precipitate is mostly hydrated iron(III) oxide, $Fe_2O_3.nH_2O$ (rust).

So, how is the hydrated iron(III) oxide formed? In aqueous solution, iron(II) will react with O_2 to give iron(III):

$$4Fe^{2+}(aq) + O_2(g) + 4H^+(aq) = 4Fe^{3+}(aq) + 2H_2O(l) \quad (23)$$

The iron(III) is a strong Lewis acid, and will react with water in the following series of hydrolysis reactions to give highly insoluble $Fe(OH)_3$ and $Fe_2O_3.3H_2O$, which appear as a brown-coloured precipitate:

$$Fe^{3+}(aq) + H_2O(l) = [Fe(OH)]^{2+}(aq) + H^+(aq) \quad (24)$$

$$[Fe(OH)]^{2+}(aq) + H_2O(l) = [Fe(OH)_2]^+(aq) + H^+(aq) \quad (25)$$

$$[Fe(OH)_2]^+(aq) + H_2O(l) = Fe(OH)_3(s) + H^+(aq) \quad (26)$$

$$2Fe(OH)_3(s) = Fe_2O_3.3H_2O(s) \quad (27)$$

Notice that the hydrolysis reactions *must* be pH dependent, since a proton is produced in each of the reactions. Therefore, at low pH, say less than pH 1.5, iron(III) is soluble in

water, but at pH 7 insoluble $Fe(OH)_3$ is formed, $Fe(OH)_3$ is profoundly insoluble, with a solubility product, $K_{sp} = 2 \times 10^{-39}$ mol^4 l^{-4}.

Activity 3

What is the concentration of $Fe^{3+}(aq)$ in H_2O at pH 7, assuming that $Fe(OH)_3(s)$ is present?

Answer

We know that the solubility product, K_{sp}, of $Fe(OH)_3 = 2 \times 10^{-39}$ mol^4 l^{-4}. Therefore,

$$[Fe^{3+}][OH^-]^3 = 2 \times 10^{-39} \text{ mol}^4 \text{ l}^{-4}$$

At pH 7: $[H^+] = 10^{-7}$ mol l^{-1} and $[OH^-] = 10^{-7}$ mol l^{-1}. (Remember that we calculate this from $K_w = [H^+][OH^-] = 10^{-14}$ mol^2 l^{-2}.) Therefore,

$$[Fe^{3+}] = \frac{2 \times 10^{-39}}{(10^{-7})^3} = 2 \times 10^{-18} \text{ mol l}^{-1}$$

From the above calculation, we can see that at pH 7, the concentration of $Fe^{3+}(aq)$ is extremely small: 2×10^{-18} mol l^{-1}. The value of 5×10^{-11} to 2×10^{-8} mol l^{-1} for $Fe^{3+}(aq)$ in seawater is much higher than this.

Activity 4

Why do you think the concentration of $Fe^{3+}(aq)$ in seawater is higher than the value you have just calculated?

Answer

This is due to the presence of other ligands that can form soluble iron complexes. For example, chloride, bromide, acetate and nitrate are all potential ligands.

Nevertheless, for such an important bioinorganic element, the concentration of $Fe^{3+}(aq)$ in neutral aqueous solution is very low indeed. This very low concentration is a significant problem when it comes to an organism obtaining iron, since iron in its soluble form is in such short supply. If an organism is to survive, it must have some biochemical means of absorbing the trace amounts of surrounding iron. Also, the organism must prevent iron(III) oxide precipitation once the iron is inside the organism, since this may lead to cell damage. (Although having said this, some organisms, such as pigeons, are known to crystallise iron oxide deliberately within certain parts of their bodies, particularly the brain. These small iron oxide pellets are often magnetic and are thought to act as in-built compasses.)

Activity 5

What are the products of the reaction of a single iron(II) ion with a single molecule of O_2?

Answer

$$Fe^{II} + O_2 = Fe^{III} + O_2^-.$$

The reaction of O_2 with iron(II) gives single-electron oxidation of the iron(II) to iron(III) and the generation of a superoxide radical anion.

This anion is highly toxic to biological systems and must be avoided where possible. Therefore, any free iron(II) is potentially detrimental, acting as a reducing agent for O_2.

Free iron(II) dissolved within an organism is also potentially dangerous. Therefore, the organism must have methods for preventing the formation of free iron(II). (*Note* It is now easy to see why iron-overload patients suffer from a variety of secondary diseases, since the extra iron is not controlled by the normal biochemical systems that deal with iron. There is an increased availability of free iron, which can be chelated by micro-organisms, and an elevated level of iron(II).)

The last property of iron we shall examine is the thermodynamic stability of its coordination complexes.

Activity 6

Would you classify iron(III) as a hard or soft metal?

Answer

Iron(III) is a first-row transition metal in a high oxidation state and so is classified as a hard metal. As such, it will tend to form stable complexes with hard ligands. Typical hard ligands contain oxygen and nitrogen as the coordinating atoms.

Activity 7

Would you expect iron(II) or iron(III) to make a more stable complex with the $edta^{4-}$ anion (Figure 2a)?

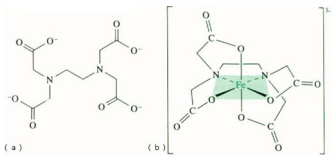


Figure 2 (a) The $edta^{4-}$anion; (b) its coordination mode to iron.

Answer

The hexadentate $edta^{4-}$ ligand coordinates through both O and N. The stability constant of the $[iron(III)–edta]^{3-}$ complex (shown in Figure 2b) is c.10^{25} mol^{-1} l, whereas the analogous stability constant with iron(II) is only c. 10^{14} mol^{-1} l.

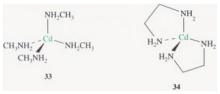
Complexes containing chelate rings are usually more thermodynamically stable than similar complexes without rings; for instance, $\log_{10}$(stability constant) for $[Ni(NH_3)_6]^{2+}$ is 8.61, whereas that for $[Ni(en)_3]^{2+}$ is 18.18. This is known as the **chelate effect**. It is observed for pairs of complexes when the coordinating atom of a monodentale ligand, L, is the same as that of a bidentate ligand, L—L, and there is no steric strain in the chelate ring. It is thought that several factors are involved overall in the chelate effect, but that the most influential is the entropy change in the formation reaction. This can be seen in the experimental data given below in Table 1 for the formation of two four-coordinate cadmium complexes:

$$Cd(aq)^{2+} + nL = [Cd(L)_n]^{2+} \quad (28)$$

$[Cd(CH_3NH_2)_4]^{2+}$, **33**, is coordinated through nitrogen to four monodentate methyl-aminc ligands, whereas $[Cd(en)_2]^{2+}$, **34**, is coordinated through nitrogen to two bidentate ethylcnediamine ligands. We see that the chelated complex is far more stable than the complex with no rings — by a factor of over 10^4 in this case.

Table 1: Stability constants and thennodynamic data at 298.15 K for some cadmium(II) complexes

Complex	Log_{10} (stability constant, *K*)	$\Delta H^{\ominus}_m$ /kJ mol^{-1}	$\Delta G^{\ominus}_m$ /kJ mol^{-1}	$T\Delta S^{\ominus}_m$ /kJ mol^{-1}	$-T\Delta S^{\ominus}_m$ /kJ mol^{-1}
$[Cd(CH_3NH_2)_4]^{2+}$	6.55	-57.32	-37.41	-66.8	19.91
$[Cd(en)_2]^{2+}$	10.62	-56.48	-60.67	14.1	-4.19



It is evident that the enthalpy change for the formation reaction of each complex is very similar, which is to be expected because the atoms involved in each bond, and therefore type of bonding, are similar in each case.

Activity 8

What do you notice about the values for the change in entropy?

Answer

They are very different. The entropy change for the chelated complex is positive, whereas that for the complex with no chelate rings, is negative.

The large difference in entropy changes, $\Delta S^{\ominus}_m$, leads to a big difference in the Gibbs free energy changes for the reactions.

Activity 9

How does this lead to a difference in the stability constants of the two complexes?

Answer

From the relationship $\Delta G^{\ominus}_m = \Delta H^{\ominus}_m - T\Delta S^{\ominus}_m$, we see that a *positive* entropy change will lead to a lower or more negative value for the Gibbs free energy change $\Delta G^{\ominus}_m$, of the reaction. $\Delta G^{\ominus}_m = -2.303RT\log_{10}K$, so the more negative is $\Delta G^{\ominus}_m$, then the larger is *K* and the more stable is the complex.

The leading question now is, why does this happen? The answer seems to be that the dominating factor is the entropy of the ligands. Consider what happens when a hexa-aquo metal ion, M, reacts with a complexing monodentate ligand, L:

$$[M(H_2O)_6]^{n+} + 6L = [ML_6]^{n+} + 6H_2O \quad (29)$$

there is no change in the number of molecules in solution: the six water molecules are displaced by six ligand molecules.

Activity 10

What is the significant difference when the same reaction takes place with bidentate ligands?

Answer

In a similar reaction with bidentate ligands, L–L,

$$[M(H_2O)_6]^{n+} + 3L-L \rightleftharpoons [M(L-L)_3]^{n+} + 6H_2O \qquad (30)$$

there is a net increase of three molecules in solution.

For gas–solid reactions, a greater increase in the number of gas molecules in a reaction leads to a greater entropy change. Something similar happens here, but in solution. The increase in the number of independent molecules in the chelate reaction leads to a more positive $\Delta_r S_m^{\ominus}$.

The chelate effect is usually at a maximum for five- and six-membered rings: smaller rings tend to suffer from strain, and in larger rings the second coordinating atom is no longer very close to the metal.

Macrocyclic ligands, such as porphyrin rings, tend to show the same stabilising effect as polydentate ligands and this is sometimes known as the **macrocyclic effect**.

2.2 Summary of iron chemistry

Try to summarise the main points of iron chemistry for yourself, and then compare it with the list below.

1. From a bioinorganic point of view, we can conclude that despite the high natural abundance of iron, it is in scarce supply in water.
2. The more stable form of iron in oxygenated water is iron(III).
3. The low concentration of Fe^{3+} in water is because of the extreme insolubility of $Fe(OH)_3$.
4. Any iron absorbed by an organism is potentially detrimental in its +2 oxidation state, due to its reaction with O_2 to give superoxide radical anions.
5. Iron(III) is coordinated preferentially by hard ligands, especiallv hard polydentate ligands, which give iron(III) complexes with very high stability constants.

These chemical properties of iron demand that biochemical systems have efficient and effective methods of obtaining and controlling iron. Specifically, they must be able to (i) solubilise and assimilate iron in their local environment, and (ii) protect the iron once it has been absorbed. The rest of this course will describe how this has been achieved by some organisms.

Before moving on, it is worth noting that there is a constant competition for the available iron in the natural world. In many cases the availability of iron is the determining factor in whether an organism can proliferate or not. For example, it is believed that the low concentration of iron in seawater limits the amount of plankton growth.

3 Iron uptake by organisms

3.1 How do organisms take up iron?

Nearly all organisms are able to take up iron. However, only a handful of organisms have had their iron-uptake chemistry studied. The organism that has received most attention (other than human) is a single-cell, prokaryotic bacterium (found in the human large intestine and elsewhere), called *Escherichia coli* (abbreviated to *E.coli*), a high-resolution image of which is shown in Figure 3. The reason that this bacterium has been so thoroughly studied is that it is relatively easy to grow and study colonies of it in the laboratory. The iron-uptake mechanism in *E. coli* is known in a fair degree of detail.

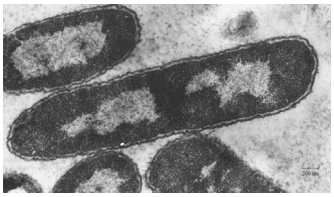


Figure 3 *E. coli* bacterium

There are many harmless strains of the *E. coli* bacterium; the ones found naturally in the human gut are useful because they synthesise several vitamins of the B-complex and vitamin K. However, there are also over 100 pathogenic (i.e. disease-causing) strains of the bacteria. The most infamous is probably *E. coli* O157:H7, which is very virulent. This strain can find its way into the human food chain (from the intestines of cattle where it is thought to originate), and it causes severe food poisoning due to the toxins excreted by the bacteria. The toxins are absorbed from the gut into the bloodstream; damage to the kidneys occurs, which may eventually result in death, particularly for very old or young persons.

E. coli obtains its iron in a remarkable fashion. Each *E. coli* bacterium within a colony, secretes small molecules that are capable of specifically chelating iron. These small molecules are known as **siderophores** (from the Greek for iron carriers; pronounced ‘sid-air-o-fores’). Several types of siderophore are known, each capable of chelating iron in a stable iron–siderophore complex. The structures of some known siderophores are shown in Figure 4.

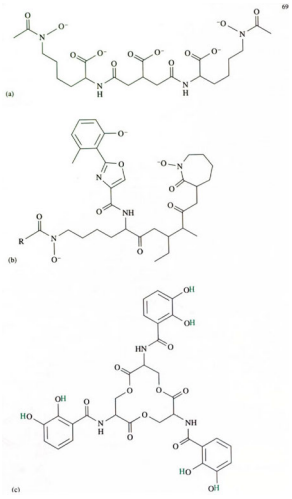


Figure 4 Structures of three siderophores: (a) aerobatin; (b) mycobatin; (c) enterobactin; acidic hydrogens are printed in green

We shall examine the properties of one of the siderophores in more detail below. For all the siderophores, however, their *modus operandi* is to be secreted from the bacterium, to chelate an iron(III) ion selectively in a stable complex, and then to be re-absorbed by a bacterium (not necessarily the original bacterium) as the iron(III)–siderophore complex (see Figure 5 for a schematic representation).

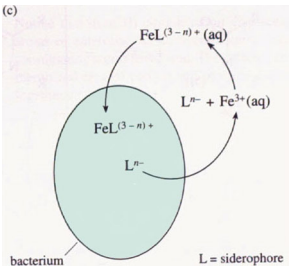


Figure 5 Schematic diagram of iron uptake by a siderophore

Activity 11

Derive an expression for the concentration of an iron(III)–siderophore complex in terms of its stability constant. Explain why an iron(III) siderophore complex needs to have a very high stability constant in order to be biochemically useful.

Answer

By writing the equation for the formation of the complex, we can then derive an expression for the concentration of an iron(III)–siderophore complex in terms of its stability constant:

$$Fe^{3+}(aq) + \text{siderophore}^{n-}(aq) = \text{Fe–siderophore}^{(3-n)+}(aq) \quad (31)$$

$$K_s = \frac{\left[\text{Fe–siderophore}^{(3-n)+}(aq)\right]}{\left[Fe^{3+}(aq)\right]\left[\text{siderophore}^{n-}(aq)\right]} \quad (32)$$

Rearranging gives:

$$\left[\text{Fe–siderophore}^{(3-n)+}(aq)\right] = K_s\left[Fe^{3+}(aq)\right]\left[\text{siderophore}^{n-}(aq)\right] \quad (33)$$

There are two reasons why a high stability constant is required for efficient transport of iron to a bacterium:

Firstly, the value of [iron(III)–siderophore$^{(3-n)+}$(aq)] must be significant; this is because the bacterium will have a much better statistical chance of absorbing the iron(III)–siderophore complex if it is in relatively high concentration.

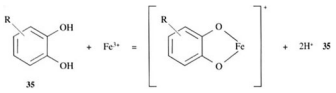
Secondly, knowing that iron(III) is in short supply and its concentration in water at pH 7 is very low, any organism that can competitively chelate the available iron will have a better chance of survival.

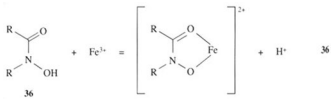
We have shown that the greater the stability constant, the higher will be the concentration of the iron(III)–siderophore complex, thus providing the optimum conditions for the transport of the iron. Let's take a look at the rough values of [Fe^{3+}(aq)] and [siderophore $^{n-}$(aq)]. We know that [Fe^{3+}(aq)] cannot be high and may be as low as 10^{-18} mol l^{-1}, due to the insolubility of many iron(III) compounds. Also, the value of [siderophore $^{n-}$(aq)] cannot be high (that is, probably much less than 10^{-12} mol l^{-1}), since the bacterium can only ever synthesise a small amount of the siderophore. What this all means is that for the value of [Fe–siderophore$^{(3-n)+}$(aq)] to be significant, the value of the stability constant, K_s must be *extremely* high. In other words, for this system to be feasible the equilibrium for the complex formation must lie very heavily to the right.

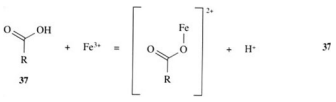
Another requirement of the siderophore ligand is that it must be selective for iron(III), This means that the stability constant for the iron(III)–siderophore complex must be much greater than the stability constants of the siderophore complexes with other metals (including iron(II)). Why is selectivity so important? Firstly, if the siderophore were not selective for iron(III), high concentrations of other metal ions (M^{m+}) would easily displace the iron(III) from any iron(III)–siderophore complex, according to the equation

If this equilibrium lay to the right, then iron could not be obtained in any great amounts by the bacterium. Secondly, the bacterium's biochemical systems for absorbing iron should not be a route for the absorption of toxic metal ions, such as mercury and cadmium.

How does a siderophore achieve this high degree of iron(III) selectivity? The question can be partly answered by examining the structures of the siderophores in Figure 4. We can see that the siderophores are analogous to simpler organic molecules which can coordinate directly to iron(III) ion in a similar fashion, as indicated in reactions 35–37:







Activity 12

Are the coordinating groups of the ligands in reactions 35–37 hard or soft?

Answer

All the groups shown are hard ligands and, as such, form stable complexes with hard metals, like iron(III) and aluminium(III).

The groups in structures **35** and **36**, 1,2-dihydroxybenzene (trivial name catechol, pronounced 'kat-a-kol') and hydroxamic acids, respectively, form particularly stable complexes with iron(III), because they are chelating groups. The chelate 'bite' of these two groups is just about right to form a very stable complex with iron(III).

Of all the siderophores, the one that has received most attention is enterobactin, shown in Figure 4c. The reason for this attention is that enterobactin forms an exceedingly stable complex with iron(III); in fact, it is the most stable, soluble iron(III) complex that is known. The stability constant of fully deprotonated enterobactin with iron(III) is extremely high at about 10^{49} mol^{-1} l!

Note that the definition of stability constant assumes an equilibrium reaction in aqueous solution between a hydrated metal ion and ligand(s), such that we would write enterobactin in its ionised – that is, fully deprotonated – form.

What are the chemical and structural features of enterobactin that give it such a high stability constant with iron(III)? To answer this question we need to examine the structure of enterobactin in more detail. Figure 6 shows the structure of the iron(III)-enterobactin complex.

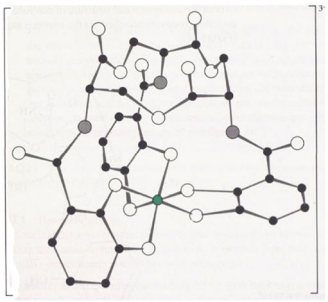


Figure 6 Three-dimensional structure of iron–enterobactin complex; the hydrogen atoms have been omitted for clarity.

Activity 13

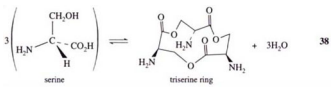
Which type of group found in siderophore model compounds coordinates to the iron in the complex?

Answer

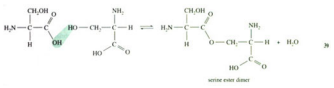
The iron atom is chelated by three, deprotonated catechol (known as catecholate) groups.

Notice that iron(III) complexation displaces the six protons on the catechol oxygen atoms of enterobactin, so, overall, enterobactin is a hexadentate ligand providing six coordinating atoms to the iron. The catechol rings are attached to each other via a twelve-

membered ring of carbon and oxygen atoms. This ring is a serine trimer, condensed together as follows:



Rather than forming the normal peptide C(O)—NH bond between the individual amino acid molecules, the serines are linked via ester bonds, whereby the ester is formed between the —OH of one serine side-chain and the —CO_2H group of another serine:



The result is a twelve-membered ring (known as a triserine ring), with three NH_2 groups pointing away from the ring. These NH_2 groups are all pointing to the *same* side of the imaginary plane formed by the triserine ring (this is because natural serine exists as a single enantiomer). To complete the enterobactin structure, three catechol groups are attached to the NH_2 groups of the triserine ring via amide, C(O)—NH, linkages. The overall three-dimensional structure of enterobactin shows a triserine ring to which three catechol groups are attached via the nitrogen atoms, all linked to the same side of the ring (Figure 7),

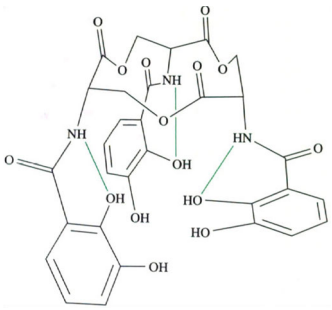
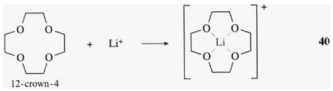


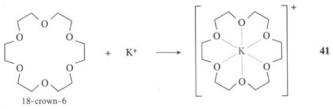
Figure 7 Three-dimensional structure of enterobactin.

Figure 7 also shows that there are other interactions. These interactions are three hydrogen bonds between the NH of a serine group and the oxygen atom of a catechol ring (shown as green lines in Figure 7). This imposes further structural rigidity by preventing each catechol ring from rotating freely, so that not only are the catechol groups all linked to the same side of the triserine ring, but all the calechol oxygen atoms face towards the centre of the ligand.

We see that the free enterobactin ligand is actually rather rigid in its structure, with all six coordinating oxygen atoms of the catechol groups held in position to bind an iron(III) ion (that is, the catechol groups have the same relative positions both *before* and *after* the binding of iron(III)). This rigid arrangement of functional groups before the metal has been chelated is known as **ligand preorganisation**; in other words, the three-dimensional structure of the ligand hardly changes on complex formation. What this means in practice is that a metal ion of a particular size (and charge) forms a particularly stable complex with the preorganised ligand. Iron(III) has the correct size and charge to form a very stable complex with the preorganised enterobactin ligand. and, therefore, is chelated selectively by enterobactin. This is exactly the same phenomenon as selective chelation of alkali metal ions by different sizes of crown ethers. A small crown ligand, like 12-crown-4 (reaction 40), will form stable complexes with a small metal ion., Li^+, whereas a larger

crown, such as 18-crown-6, will form stable complexes with a larger metal ion, K^+ (reaction 41):





Activity 14

Why does preorganisation of a ligand lead to a high stability constant?

Answer

This is a manifestation of the chelate effect. If ΔS for the reaction is positive, then the value of ΔG is more negative, and the stability constant is larger. In a reaction such as complexation of iron(III) with enterobactin, the hexadentate ligand hardly changes its structure, and this will have a larger entropy increase than a reaction to produce a hexacoordinated iron complex from six monodentate ligands. (Remember, however, that ΔS is an overall term for the reaction, and the ligand structure is only one part of it; it also takes into account the entropy effects due to the water molecules surrounding the metal and ligand, etc.)

SAQ 1

Summarise the features of enterobactin that make it selective for iron(III) and that make the iron(III)–enterobactin complex highly stable.

Answer

There are three key points. Firstly, enterobactin is a hexadentate ligand and so the stability of the complex is enhanced by the chelate effect. Secondly, the groups that coordinate to iron(III) are hard ligands. Thirdly, the enterobactin ligand is preorganised and must be exactly the correct size, shape and charge for iron(III) binding.

3.2 Removal of iron

Before leaving enterobactin to look at iron transport and storage in humans, it is worth asking the question: how does *E. coli* remove the iron from such a stable complex as the iron(III)–enterobactin once it has been absorbed?

The answer to this question can be found if we look back to reaction 38. The rigid, three-dimensional structure of the triserine ring of enterobactin is the main reason why enterobactin is such an effective ligand. If the structure of the ring is destroyed, enterobactin loses much of its chelating power. In fact, this is exactly what happens to the iron(III)–enterobactin complex once it has been absorbed by *E. coli*. Enzymes called **esterases** (so-named because they catalyse the decomposition and formation of esters) hydrolyse the triserine ring of enterobactin in the reverse reaction of reaction 38. This

breaks up the triserine ring structure, and the stability constant of the resulting [Fe(catechol)$_3$] complex is much lower than 10^{49} mol^{-1} l. Iron is then relatively easily removed from this complex, so that it can be used in the bacterium cell.

3.3 Summary of Section 3

1. *E. coli* has a remarkable method of obtaining iron from its environment, which involves the use of very powerful iron chelators, called siderophores.
2. One siderophore in particular, enterobactin, forms an extremely stable complex with iron(III).
3. The high stability of this complex is due partly to the rigid, preorganised structure of the ligand, and partly to the iron(III) being the correct size and charge to be chelated effectively by enterobactin.
4. Enzymes called esterases are able to catalyse the hydrolysis of the iron(III)–enterobactin complex and so release iron.

4 Iron transport and storage

4.1 Introduction

As bacteria secrete such powerful chelators into the environment, iron in other organisms must be kept under very close control. Any free iron within an organism is likely to be chelated by a siderophore, which may lead to bacterial infection within the organism In this Section we shall examine the biochemical systems that handle iron within the human body. The two areas we shall study are iron transport and iron storage.

4.2 Iron transport

It is obvious that iron must be transported around the human body. Firstly, it must be transported from the food in the gut to the places where it is required. Mostly, iron is required in the bone marrow, where red blood cells are formed. Red blood cells have a finite lifetime of about only four months, and old cells are destroyed, usually in the spleen. Iron from the destruction of these cells is then transported from the spleen back to the bone marrow to be recycled.

Iron cannot be transported around the body's circulation system as free iron, since it would be susceptible to chelation by siderophores, or may precipitate as iron(III) oxide, or may form iron(II). Therefore, a specific transport protein is required, called **transferrin**. (In fact, a whole class of transferrin-like proteins is involved in iron transport.) Transferrin is a medium-sized protein with a relative molecular mass of about 80 000. The crystal structures of transferrin with and without iron have been obtained, and the overall structure is shown in Figure 8, The structure without any iron (Figure 8a) shows that transferrin can be considered as two very similar polypeptides back-to-back, with each of the polypeptides having a large cleft. The apex of each cleft coordinates one iron atom; each transferrin molecule is therefore capable of transporting two iron atoms. Also, on the binding of iron, there is a significant change in the higher-order structure of the protein, such that the two sides of the cleft corne together and incarcerate the iron atoms (Figure 8b). Both iron atoms are now buried deep within the protein structure. (It is not fully clear why the iron atoms are buried in this way, but it may help in protecting the iron atom from microbial siderophores.)

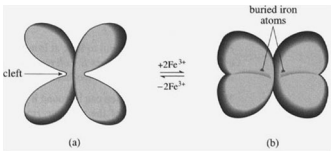


Figure 8 (a) Schematic diagram of transferrin protein; (b) proposed higher-order structure change on complexation of iron(III).

The iron binding site is rich in hard ligands, which are suitable for binding iron(III) in a stable complex (Figure 9a). When the iron atom enters the active site (Figure 9b) it is coordinated by one η^1-aspartyl, one histidyl and two tyrosinate side-chains; a non-protein ligand also coordinates to it. This external ligand is a carbonate, CO_3^{2-}, which is held in place within the protein via hydrogen bonds to the protein backbone.

Activity 15

What is the mode of bonding of the CO_3^{2-} group?

Answer

The carbonate coordinates to the iron in an η^2 fashion; in other words, it is a chelating ligand.

Once the carbonate is held in place, we can see another example of ligand preorganisation, where an octahedral environment of hard/borderline ligands is ready to receive the iron(III) ion. The carbonate binding appears to facilitate the iron binding by the protein and vice versa, and so the system is said to be *synergistic*. It is not clear why this unusual synergistic binding of iron and carbonate occurs in transferrin, but it may have something to do with the way iron is released from transferrin.

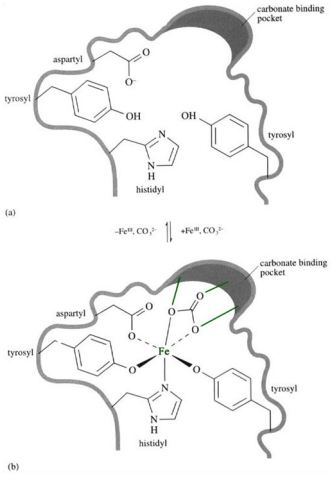


Figure 9 (a) The iron binding site in transferrin; (b) the six-coordinate iron site; the coordination geometry is distorted octahedral. The carbonate is held in place by hydrogen bonds (green lines) to amino acid side-chains inside a small cleft.

Since all the coordinating ligands in transferrin can be considered as hard or 'borderline' ligands, it is no surprise that transferrin forms a very stable complex with iron(III). The stability constant of the Fe(III)–transferrin complex is *c*. 10^{20} mol^{-1} l. This is high enough to protect the iron(III) against the low concentration of any siderophores present. Indeed, the transferrins show mild antibacterial properties, in which their method of operation is to prevent extensive iron chelation by siderophores (see Box 2).

Box 2 Iron in human milk

It has been known for some time that bottle-fed babies are more likely to suffer from gastric infections than breast-fed babies; this may be despite strict hygiene standards. The reason for this probably lies in the availability of iron within the baby's feed. Breast milk is known to contain a transferrin-like protein called **lactoferrin**. The lactoferrin chelates all the iron in the mother's milk, and prevents iron chelation by microbial siderophores. Formula milk, on the other hand, does not contain human lactoferrin, so the iron in the feed is more available for chelation by siderophores secreted by bacteria.

Transferrin also forms relatively stable complexes with other hard metals (Table 2). Current thinking suggests that these other metals are transported by transferrin into cells,

where they are potentially detrimental. One element in particular, aluminium, is of concern because it is used widely in cooking utensils.

Table 2: Stability constants of Metal-transferrin complexes

Metal	Log_{10}(stability constant)
cadmium(II)	5.95
zinc(II)	7.80
aluminium(III)	13.50
iron(III)	22.80

Therefore, we can see from the structure and function of transferrin that the transport of iron within (and without) the body is very carefully managed, so as not to allow any free soluble iron to form. How, then, is iron stored? After all, we must store iron, since we need a reservoir of it for the synthesis of iron-containing proteins, most notably haemoglobin and myoglobin. As with iron transport, the iron storage systems need to ensure that free, soluble iron is not formed.

4.3 Iron storage

In humans, iron is stored mainly in the bone marrow, spleen and liver. About 10 per cent of all the iron in the body is in storage. Two proteins are involved in iron storage; these are called **ferritin** and **haemosiderin** (they also occur in other organisms). We shall only study the better characterised (and simpler!) ferritin.

Each ferritin molecule can store iron up to about 20 per cent of its total mass. This is a very high percentage, considering that less than 0.2 per cent of the total mass of proteins like transferrin and myoglobin is iron. Ferritin is a large protein with a relative molecular mass of 440 000. The crystal structure of ferritin with *no iron* is shown in Figure 10. The overall structure shows that ferritin is a huge, hollow protein, with a wall mostly made up of α-helical peptide chains. The structure is quite symmetrical, being roughly dodecahedral, and is one of the outstanding examples of symmetry in chemistry. The wall contains channels, which lead from the inside to the outside of the hollow ‘sphere’. The channels are rich in amino acids with carboxylate side-chains, which are capable of chelating iron.

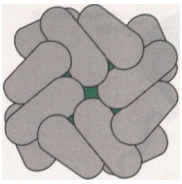


Figure 10 Sub-unit assembly of ferritin. Each sub-unit (shaped like a sausage) is made up of four parallel, α-helical polypeptide chains. The channel at the centre of the structure is clearly visible (dark green area); it lies on a fourfold axis of symmetry.

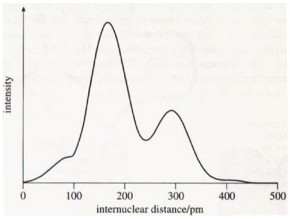


Figure 11 Fe-EXAFS radial distribution plot of iron-containing ferritin. Notice that there are two peaks, the first at 160 pm corresponding to a sphere of oxygen atoms, and the second at 290 pm corresponding to a sphere of iron atoms. (The peak due to the iron atoms is smaller than the peak due to the oxygen atoms; this is not in accord with the relative number of electrons in oxygen and iron atoms, The reasons for this are complex, but involve other factors beside the number of electrons in the intensity of back-scatterring. Also the actual structure of the hydrated iron(III) oxide in ferritin is not 'perfect', in that there are incomplete 'shells' of iron atoms, and poor long-range crystal order.)

The crystal structure of iron-containing ferritin is not known. However, some clues as to its structure have been obtained from EXAFS studies. EXAFS gives information about the direct coordination environment of a particular atom in terms of the number and type of its coordinated atoms (although no angular information is usually available). EXAFS studies on iron-containing ferritin showed that each iron atom is surrounded by an inner shell of six or seven oxygen atoms at a distance of about 160 pm, and by a second shell of seven or eight iron atoms at a distance of about 290 pm (Figure 11). This was a very strange result. How could seven or eight iron atoms be packed around each iron atom? The problem was solved when it was noticed that the EXAFS data were very similar to that of a hydrated iron(III) oxide mineral called ferrihydrite, $5Fe_2O_3.9H_2O$. From this result it was clear that ferritin stored iron partly as a crystalline, hydrated iron(III) oxide. Further studies showed that the inorganic crystalline part was within the hollow sphere of the protein (Figure 12).

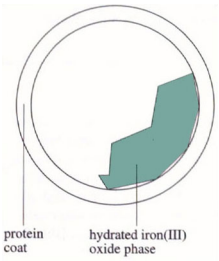


Figure 12 Schematic diagram showing growth of iron(III) oxide within a ferritin macromolecule. The full ferritin contains about 4500 iron atoms.

Therefore, ferritin stores iron as crystalline, hydrated iron(III) oxide within its structure

Activity 16

How will this affect the availability of the iron?

Answer

As the iron(III) oxide is very insoluble, it is unavailable to microbial iron-chelating ligands.

The inorganic iron(III) oxide core is also protected from chelators by the outer protein coat. Moreover, this is a very space-efficient method of storing iron; each ferritin protein macromolecule can store a maximum of 4 500 iron atoms.

Iron is delivered to ferritin (after having been transported by transferrin) where it migrates through the carboxylate-rich channels in the surface of the protein to the interior. The inner side of the protein sphere is also rich in carboxylate residues. It is thought that these carboxylate residues coordinate iron atoms, such that the iron atoms are held in a regular array. This regular array has the correct spacing of iron atoms to encourage the growth of crystals of iron(III) oxide, and in this way an iron(III) oxide phase grows within the ferritin core. The growth of iron(III) oxide in ferritin is an example of biomineralisation.

So it is somewhat ironic that the formation of highly insoluble iron(III) oxides and hydroxides, which causes such a problem in the availability of iron in the environment, is the method by which iron is stored in ferritin!

5 Conclusion

We have seen in this course that, despite having a high natural abundance, iron is in very short supply because of the insolubility of its oxides and hydroxides. A result of this is that organisms have developed methods for the uptake, transport and storage of iron. Bacteria, in particular, secrete very powerful iron chelators known as siderophores. Of all the iron–siderophore complexes, the iron(III)–enterobactin complex has the exceptionally high stability constant of 10^{49} mol^{-1} l.

Other organisms, partly as a defence against siderophores and the need to avoid free iron in solution, have biochemical methods to transport and store iron. The protein most associated with iron transport is transferrin. Iron storage, in mammals, including humans, is achieved by ferritin, which stores iron as a hydrated iron(III) oxide; this is an example of biomineralisation.

The key points of this course are:

1. The concentration of free $Fe^{3+}(aq)$ at pH 7 is very small.
2. Free $Fe^{2+}(aq)$ in an organism is dangerous, because it can react to produce the superoxide radical anion.
3. Iron(III) tends to be coordinated by hard ligands such as those containing oxygen or nitrogen.
4. The chelate effect confers extra stability on iron(III) complexes with polydentate ligands.
5. Siderophores are powerful microbial iron chelators.
6. Enterobactin is a particularly powerful iron chelator It acts as a preorganised hexadentate ligand for iron(III).
7. Esterases catalyse the hydrolysis of the triserine ring in enterobactin, thus enabling iron(III) from the enterobactin complex to be released.
8. Iron transport in mammals is carried out by transferrin, which holds iron(III) in a hexacoordinate site, one of the ligands being η^2-carbonate.
9. Ferritin is an iron storage protein capable of storing up to 4 500 iron atoms per protein macromolecule. The iron is stored as hydrated iron(III) oxide.

SAQ 2

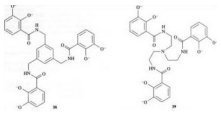
What is a siderophore?

Answer

A siderophore is a microbial iron chelator, whose complex with iron (III) usually has a very high stability constant. Examples include enterobactin, aerobactin and agrobactin.

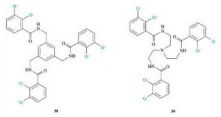
SAQ 3

Compounds **38** and **39** are synthetic siderophores. The stability constant of the iron–**38** complex is 10^{40} mol^{-1} l, whereas the stability constant of iron–**39** is 10^{28} mol^{-1} l. Explain why both stability constants are less than that for iron-enterobactin, and why the stability constant for iron–**38** is greater than that for iron–**39**.



Answer

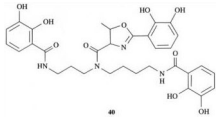
As with enterobactin, both **38** and **39** will coordinate to iron(III) via the six deprotonated oxygens in each structure (highlighted in green below).



The key feature of **38** and **39** that distinguishes them from enterobactin is the replacement of the triserine ring with 1,3,5-trimethylbenzene in **38** and a triethylamine in **39**. The effects are twofold. Firstly, both **38** and **39** are not as rigid (preorganised) as enterobactin. Secondly, both **38** and **39** have different relative spacing of their catechol groups compared to enterobactin. In fact, on chelation of iron(III), the catechol groups in **38** and **39** cannot obtain the most stable spatial arrangement around the iron, because their motion is restricted by the 1,3,5-trimelhylbenzene in **38** and the triethylamine in **39**. Hence the stability constants of the complexes formed by **38** and **39** with iron(III) are lower than for enterobactin. **38** has a slightly higher stability constant with iron(III) than **39** for two reasons. Firstly, **38** is slightly more rigid than **39** and not so much ligand reorganisation is required on complex formation. Secondly, the size of the trimethylbenzene is slightly larger than that of the triethylamine, and the catechol groups in **38** are able to approach the most stable spatial arrangement around the iron(III) ion better than in **39**.

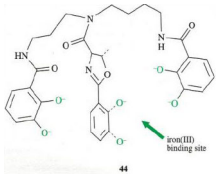
SAQ 4

Structure **40** is the siderophore agrobactin. Sketch the conformation you would expect it to adopt in the complex it forms with iron(III), and indicate the location of the iron(III) binding site.



Answer

Structure **44** shows the expected conformation of agrobactin in its iron(III) complex, where the iron is chelated by the three deprotonated catechol groups, in an analogous fashion to the iron(III) binding with enterobactin.



Keep on learning

Study another free course

There are more than **800 courses on OpenLearn** for you to choose from on a range of subjects.

Find out more about all our free courses.

Take your studies further

Find out more about studying with The Open University by visiting our online prospectus.

If you are new to university study, you may be interested in our Access Courses or Certificates.

What's new from OpenLearn?

Sign up to our newsletter or view a sample.

For reference, full URLs to pages listed above:

OpenLearn – www.open.edu/openlearn/free-courses

Visiting our online prospectus – www.open.ac.uk/courses

Access Courses – www.open.ac.uk/courses/do-it/access

Certificates – www.open.ac.uk/courses/certificates-he

Newsletter – www.open.edu/openlearn/about-openlearn/subscribe-the-openlearn-newsletter

Acknowledgements

The content acknowledged below is Proprietary (see terms and conditions) and is used under licence.

Grateful acknowledgement is made to the following sources for permission to reproduce material in this course:

Course image: Antonis Lamnatos in Flickr made available under Creative Commons Attribution 2.0 Licence.

Figure 3 Courtesy of Biophoto Associates;

Figure 11 From Coordination Chemistry Reviews.

Don't miss out:

If reading this text has inspired you to learn more, you may be interested in joining the millions of people who discover our free learning resources and qualifications by visiting The Open University - www.open.edu/openlearn/free-courses