Iron Use and Storage in the Body:
Ferritin

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Key Concepts:

- Importance of Iron in the Body
- Iron-storage Protein and Control of the Amount of Iron in the Body
- Structure of Ferritin
  - Amino Acids
  - Peptide Subunits
  - 24-Subunit Structure
- Removal of Fe from Ferritin
  - Crystal-lattice Mineral Structure
  - Channels
  - Polar vs. Nonpolar

Related Tutorials:

- *Hemoglobin and the Heme Group: Metal Complexes in the Blood*
- *Energy for the Body: Oxidative Phosphorylation*
- *Treating the Public Water Supply: What Is InYour Water and How Is It Made Safe to Drink?*

Iron in the Body

Of the more than 100 chemical elements known to scientists today, only a relatively small number of these elements are found in the human body. In fact, only 24 different elements are thought to be essential for humans. The largest elemental components of the body, by mass, are oxygen (65%), carbon (18%), hydrogen (10%), and nitrogen (3%). The other elements in the body, such as calcium, phosphorus, iron, and copper, are known to physiologists as **mineral elements** and **trace elements**. Although these elements make up a much smaller percentage of the mass of the body, they are vital to the body's proper functioning. They must be present in the body in the proper amounts, and they must also be available to react with other elements to form critical molecules and participate in important chemical reactions. In this tutorial, we will describe the importance of one essential mineral in the body, **iron**. Although iron comprises only 0.008% of the body's mass (approximately 6 g for a 160-lb (75-kg) adult male), it is necessary for our survival.

Iron complexed with the protein **hemoglobin** is necessary for oxygen transport in the blood. Recall that iron is the central atom of the **heme** group, a metal complex that binds molecular oxygen (O_2) in the lungs and carries it to all of the other cells in the body that need oxygen to perform their activities (e.g., muscle cells). In addition to hemoglobin**,** other important proteins in the body contain heme groups including **myoglobin,** which takes oxygen from hemoglobin and delivers it to muscle cells, and the **cytochromes,** which are important for generating energy. Other proteins, such as those needed for DNA synthesis and cell division, also rely on iron. Furthermore, iron is used to help produce the connective tissues in our body, some of the neurotransmitters in our brain, and to maintain the immune system.

Iron Disorders

Because iron plays such a crucial role in the body, it is important for us to maintain an adequate supply of iron. Our bodies continually lose iron through everyday processes such as urination, defecation, sweating, and sloughing off skin cells. Bleeding contributes to further loss of iron from the body. To compensate for these losses and to maintain an adequate supply of iron, we should consume approximately 18 mg of iron daily. Certain conditions, including heavy bleeding and pregnancy, further increase the requirement for iron consumption. Good dietary sources of iron include red meat, liver, egg yolk, beans, nuts, and fortified cereals.

When the body's supply of available iron is too low, a condition known as **iron deficiency** results. People with iron deficiency cannot produce an adequate amount of hemoglobin to meet their body's oxygen-transport needs. When the deficiency becomes severe such that there are too few circulating red blood cells or the hemoglobin content of these cells is very low, the condition is diagnosed as **iron-deficiency anemia**. The most common symptoms of iron-deficiency anemia are tiredness and weakness due to the inadequate oxygen supply to the body's cells, and paleness in the hands and eyelids due to the decreased levels of oxygenated hemoglobin, which is red-colored. Iron-deficiency anemia can be treated with iron supplements.

It is also possible to have too much iron deposited in the body tissues. This condition is known as **iron overload.** If the iron overload becomes severe (usually when the total amount of iron in the body exceeds 15 g), the condition is diagnosed as **hemochromatosis.** Hemochromatosis can result in serious damage to the body's tissues, including cirrhosis of the liver, heart failure, diabetes, abdominal pain, and arthritis. A recessive genetic mutation can put some people, particularly those of Irish or Celtic descent, at a higher risk for developing hemochromatosis. Treatment for the condition consists of removing blood from the patient to decrease the amount of iron in the body.

Ferritin: The Iron-Storage Protein

How does the body regulate the amount of iron? Fortunately, most of us are able to maintain appropriate levels of available iron in the body, even if our iron consumption does not always exactly match the body's iron loss. This regulation of blood-iron levels is mediated by the protein **ferritin** (Figure 1). Ferritin can release iron if the blood has a low iron concentration, and it can help to store excess iron if the blood and tissues have a high iron concentration. Hence, ferritin functions as a "buffer" against iron deficiency and, to a lesser extent, against iron overload.

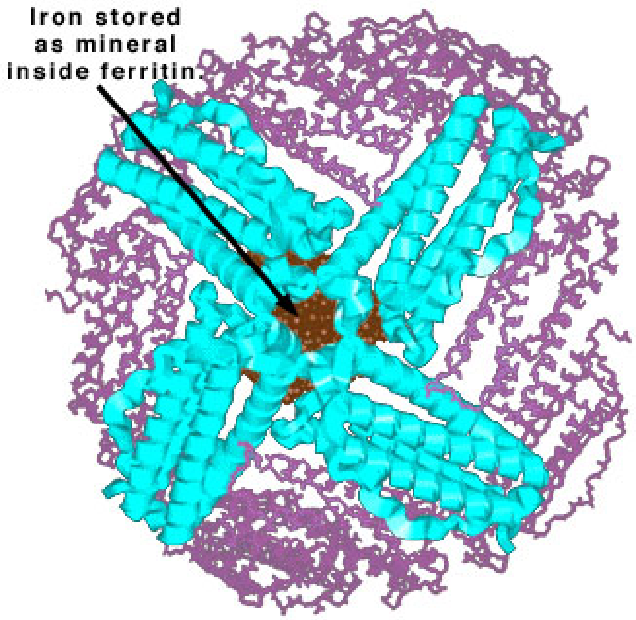


Figure 1[1]: Ferritin

This is a three-dimensional representation of ferritin, the iron-storage protein in the body. Ferritin has a spherical shape, and iron (brown) is stored as a mineral inside the sphere.

Note: This same molecule is shown in another type of representation in Figure 7 below.

Note: To view this molecule interactively, please use Chime, and click on the button below.

View this Molecule Interactively

How does ferritin store iron? Ferritin has the shape of a hollow sphere. Inside the sphere, iron is stored in the Fe(III) oxidation state. It is incorporated in the mineral ferrihydrite, $[FeO(OH)]_8[FeO(H_2PO_4)]$, which is attached to the inner wall of the sphere. To release iron when the body needs it, the iron must be changed from the Fe(III) to the Fe(II) oxidation state. Then, the iron leaves through channels in the spherical structure. To understand how ferritin controls storage and release of iron, we need to first study its structure.

Protein Structure

We will use the different types of *Molecular Representations* to study the structure of ferritin. We will begin at the smallest level of protein structure by using 2D-ChemDraw representations to show how atoms are combined to make **amino acids** and how amino acids come together to form the protein subunits known as **peptides**. We will then examine how the sequence of amino acids determines the shape of the peptide. Finally, we will use the ribbon and CPK representations to show how 24 peptide subunits are combined to make the hollow spherical shape and channels of ferritin. The basic structural features of proteins that you will learn about in this tutorial will provide a foundation for understanding the structure and function of any protein, a tremendously important class of biological molecules.

Amino Acids: The Building Blocks of Proteins

All proteins consist of chains of **amino acids.** An amino acid (Figure 2) is a molecule containing a central carbon atom and three special functional groups: a carboxylic acid group (—COOH), an amino group (—NH_2), and variable **side chain** (generically denoted by "R"). (Note: The "—" in "—COOH" and "—NH_2" indicates a bond to another atom in the rest of the molecule.) There are

[1] The structure of this protein was determined using x-ray crystallography. The structure of the iron core is based on a simplied model compound, and the image was rendered using the Insight II molecular-modeling system from Molecular Simulations, Inc. (see References).

20 different amino acids that can be incorporated into proteins. The side chains of the 20 amino acids have different properties, which in turn give different properties to the amino acids. For instance, side chains may be charged (e.g., glutamate) or electrically neutral (leucine); bulky (tryptophan) or consisting only of a hydrogen atom (glycine).

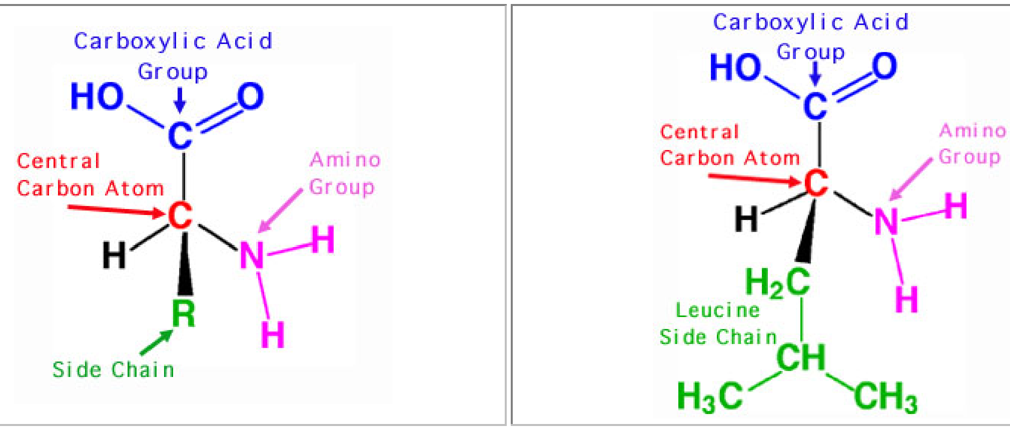


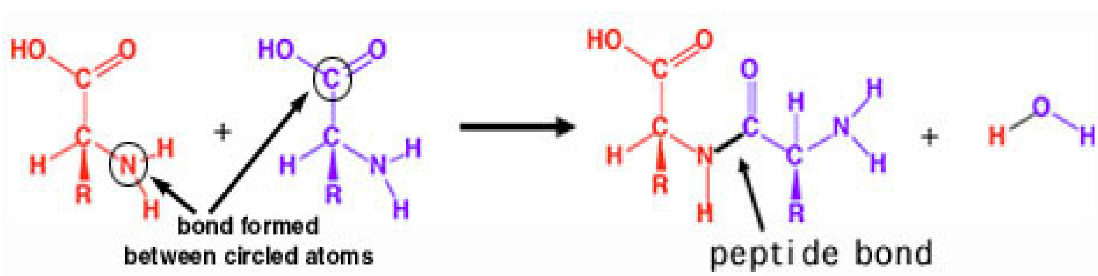
Figure 2

On the left is a two-dimensional ChemDraw model of an amino acid. The carboxylic acid group is shown in blue, the amino group is shown in purple, and the central carbon atom is shown in red. The green "R" represents the side chain, which is different for each of the 20 amino acids.

On the right is a model of leucine, which is one of the twenty amino acids. Note how the figures differ only by the green side chain.

Peptides: Protein Subunits

Amino acids are linked together to form chains known as **peptides.** These links are formed by covalent bonds known as **peptide bonds** between the carbon atom of the carboxylic acid group (—CO_2H) of one amino acid and the nitrogen atom of the amino group (—NH_2) of an <u>adjacent</u> amino acid (Equation 1).



(1)

The peptide bonds in the -CO—NH- units are central to the **backbone** of the peptide chain. Figures 3 and 4 show the three amino acid residues leucine, alanine, and glutamate (Leu-Ala-Glu) that are bound together and form a part of the peptide subunit found in ferritin.

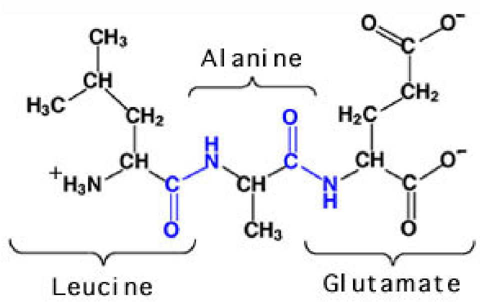


Figure 3

In this figure, the functional groups that form peptide bonds in the amino-acid sequence Leu-Ala-Glu are shown in blue.

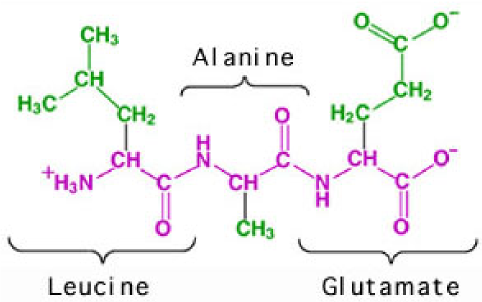


Figure 4

In this figure, the backbone of the amino-acid sequence Leu-Ala-Glu is shown in purple, and the side chains are shown in green.

Peptides may be very long chains of amino acids. There are 184 amino acid residues in each peptide subunit of human ferritin. The side chains of amino acids in a peptide can interact with one another, causing the peptide to fold. The shape of the peptide depends on where the peptide is folded, which in turn depends on the sequence of amino acids in the peptide (i.e., the location of side chains whose properties enable them to interact with other side chains). One common example of folding in a peptide is the alpha helix motif, which is common in many proteins. An alpha helix is formed when there is a regular pattern of side chains that form hydrogen bonds with one another. Figure 5 shows the hydrogen-bonding interactions between amino-acid residues that give rise to the helical structure.

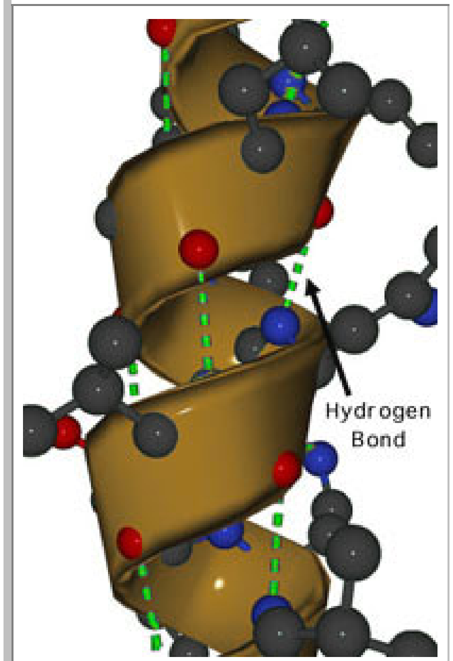


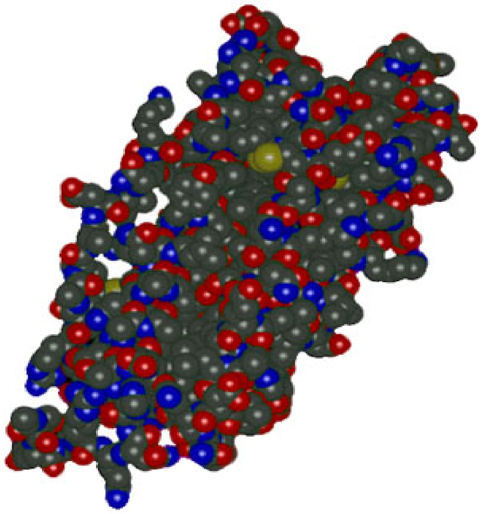
Figure 5

This is a close-up of part of an alpha helix in a peptide chain of ferritin. The helical shape is held together by hydrogen bonds (represented by green dotted lines) between the –NH and –CO functional groups in the backbone. In this figure, the ribbon representation (showing only the trace of the backbone) is superimposed on a ball-and-stick representation, in which the non-hydrogen atoms are shown as spheres, and the bonds are shown as sticks.

Note: The carbon atoms are gray, the nitrogen atoms are blue, and the oxygen atoms are red in this model. Hydrogen atoms are not shown.

View this Molecule Interactively

Below are two representations of the peptide subunit in ferritin. The first representation is a CPK model of the peptide chain, which gives an approximate volume of the subunit. The figure also shows a ribbon representation of the peptide, which is useful for showing the alpha helices in the peptide.



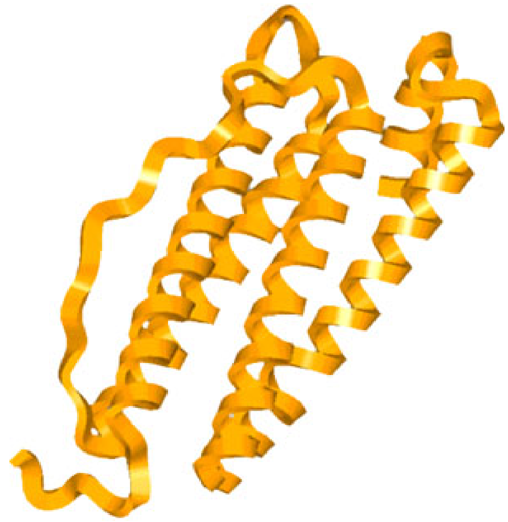


Figure 6

On the left is a CPK (space-filled) representation of a peptide chain in the ferritin protein. All of the heavy (non-hydrogen) atoms are displayed.

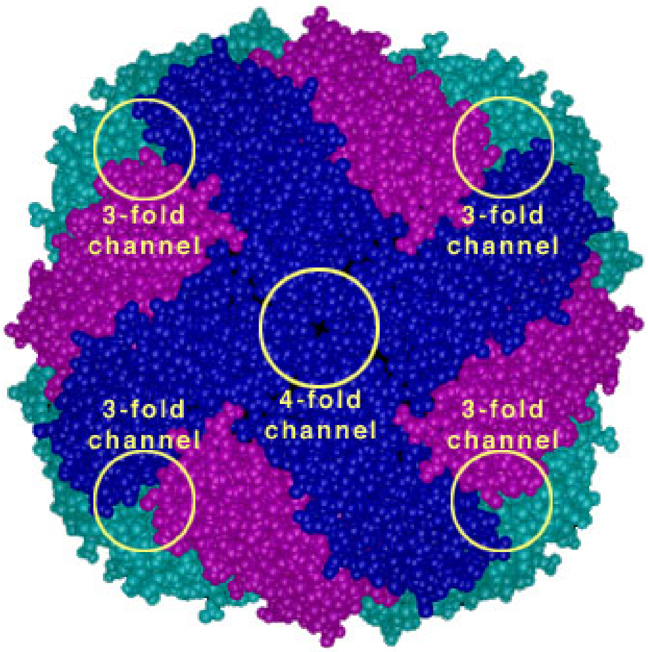
Note: The carbon atoms are gray, the nitrogen atoms are blue, the oxygen atoms are red, and the sulfur atoms are yellow.

On the right is a ribbon representation of the same peptide subunit. The ribbon model traces the backbone of a protein or peptide. This representation does not include the atoms in the side chains of the residues and is often used to represent the three-dimensional structure. Note the bundle of helical segments of the backbone.

Ferritin: Assembly of 24 Peptide Subunits

To make the ferritin protein, 24 identical peptide subunits (Figure 6) are assembled into a **hollow spherical shell** (Figure 7). The sphere that is formed is approximately 80 Angstroms in diameter, and the walls are approximately 10 Angstroms thick. The molecular weight of ferritin (i.e., with all 24 subunits combined) is 474,000 g/mol.

Channels are small holes through which certain ions or molecules can travel. In the ferritin sphere, channels are formed at the intersections of three or four peptide subunits. As we shall see, these channels are critical to ferritin's ability to release iron in a controlled fashion. Two types of channels exist in ferritin. Four-fold channels occur at the intersection of four peptide subunits. Three-fold channels occur at the intersection of three peptide subunits. The two types of channels have different chemical properties, and therefore they perform different functions.



View this Molecule Interactively

Figure 7

This is a molecular model of ferritin in the CPK representation. All of the 24 subunits are identical, but they have been color coded to help illustrate the structure. Dark blue subunits are closest to you, magenta subunits are farther away, and light blue subunits are the farthest away from you. The four subunits colored in dark blue form the walls of a 4-fold channel. The 3-fold channels occur at the intersections of the light blue, dark blue, and magenta-colored subunits. The locations of 3-fold channels are indicated on the figure, but the channels themselves are obscured from this viewing angle.

Note: This figure shows the same view of ferritin as Figure 1 but in a different representation. (Figure 1 uses the ribbon representation for the closest peptide subunits, the stick representation for the other subunits, and the CPK representation for the iron core, which is not shown here.) Compare Figures 1 and 7 to see how these representations provide different information about the structure of ferritin.

Release of Iron from Ferritin

Iron Core

The iron in the ferritin core is stored as Fe(III) in a **crystalline solid** that has the chemical formula $[FeO(OH)]_8[FeO(H_2PO_4)]$. The best model for ferritin's core is the mineral ferrihydrite. A crystalline solid is a three-dimensional structure in which the constituents (i.e., atoms, ions, or molecules) are arranged in a definite repeating pattern. The positions of the constituents' centers are represented by points on a three-dimensional **lattice.** The smallest repeating unit of the lattice is called the **unit cell.** This unit cell is repeated in a specific pattern to form an extended nonmolecular structure (see Figure 8).

In the mineral ferrihydrite, every Fe(III) ion is coordinated to six O(II) ions. However, in ferritin, the mineral core has approximately 10% of the Fe(III) ions coordinated to five O(II) ions and one phosphate group. Most of the phosphate groups that are coordinated to the iron ions lie on the outside of the crystalline structure and are used to bind the mineral to the residues on the inside of the ferritin shell (the protein).

As long as the lattice remains intact, the atoms in the lattice are not soluble because they form part of the lattice's continuous structure. Thus, in order for iron to be released from ferritin, the mineral lattice must be dissolved. This is accomplished by reducing iron from the Fe(III) (ferric) oxidation state to the Fe(II) (ferrous) oxidation state. In the Fe(II) state, iron breaks away from the lattice as the Fe^{2+} ion. The positive charge of the Fe^{2+} ion attracts the electronegative oxygen atoms of water, and a water "cage" forms around the ion, with six water molecules surrounding the ion. Thus, iron becomes soluble as a **hydrated Fe^{2+} ion**, $Fe(H_2O)_6^{2+}$, and it can be released from the ferritin protein via the channels in the spherical shell.

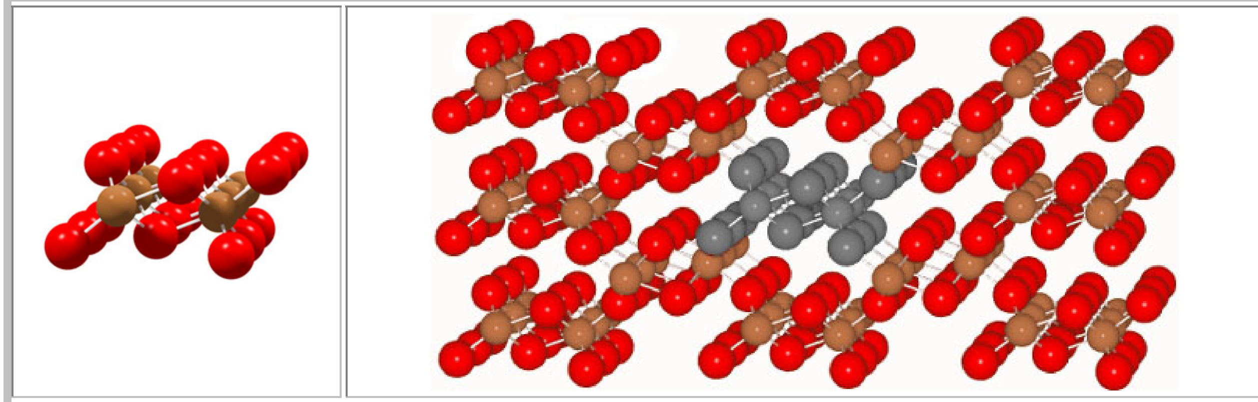


Figure 8[2]

Iron is stored in ferritin as Fe(III) in the mineral $[FeO(OH)]_8[FeO(H_2PO_4)]$. This mineral can be represented by ferrihydrite, FeO(OH) (shown above). Note: the name "ferrihydrite" is used for both $[FeO(OH)]_8[FeO(H_2PO_4)]$ and FeO(OH).

The figure on the left shows the unit cell (the repeating unit) for the ferrihydrite mineral. Fe(III) ions are shown in brown, and O(II) ions are shown in red. Hydrogen atoms are not shown in this figure for simplicity.

The figure on the right shows the crystal-lattice structure of the mineral ferrihydrite. One unit cell is shown in black and white so that it can be recognized easily.

Channels in Ferritin

Once the iron is soluble, how does it leave the ferritin shell? Recall that ferritin has two types of channels in the shell: three-fold and four-fold channels. The soluble Fe^{2+} ion exits through the three-fold channels (Figure 9). These channels are **polar**, which enables the passage of Fe^{2+} ions.

Polarity refers to significant *differences in electronegativity between adjacent atoms* in a molecule. For instance, the hydroxyl (-OH) functional group consists of an oxygen atom, which is highly

[2] The crystal structures were drawn using PowderCell for Windows, and the images were rendered using POV-Ray.

electronegative, covalently bound to a hydrogen atom, which is much less electronegative. The highly electronegative oxygen atom draws the negatively charged electrons in the bond to itself more than the less electronegative hydrogen atom does. Hence, the oxygen atom has a slight negative charge relative to the hydrogen atom. The bond between the oxygen and hydrogen atoms is then said to be polar because it contains a partially charged negative pole (the oxygen) and a partially charged positive pole (the hydrogen). Opposite charges attract one another, so polar molecules interact well with other polar molecules and charged particles. The negative poles attract positive ions or the positive poles of other polar molecules, while the positive poles attract negative ions or the negative poles of other polar molecules.

Some amino acids have side chains that contain polar groups; these amino acids are known as **polar amino acids**. Examination of the amino-acid structure in Figure 2 shows that all amino acids have an amino group and a carboxylic-acid group, both of which are polar. However, these polar groups form part of the backbone and do not contribute to the polarity of an amino-acid residue in a peptide. *Only the side chain determines whether or not the amino acid is considered polar*. The three-fold channel in ferritin (Figure 9) is lined with the polar amino acids aspartate (Asp) and glutamate (Glu) (Figure 10). Because it is lined with polar amino-acid side chains, the three-fold channel is also said to be polar. The channel's polarity allows it to interact favorably with the Fe^{2+} ion and with water. In this interaction, the positive charge of the ion (or the positive pole of water) attracts the negative poles of the side chains (Figure 11). This favorable interaction allows Fe^{2+} to pass through the channel[3].

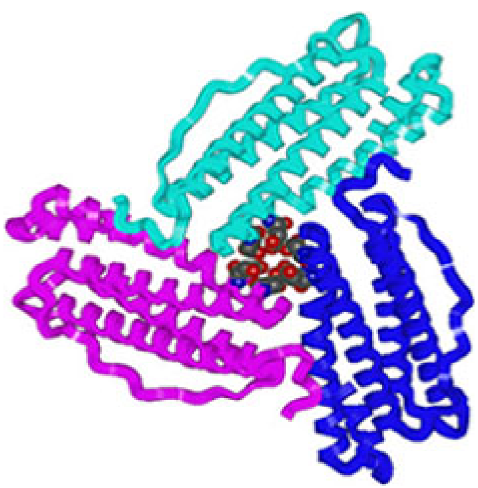


Figure 9

This is a molecular representation of the three-fold (polar) channel in the ferritin protein.

Note: The amino acids that line the channel are shown in the CPK representation, and the remaining portions of the three peptide subunits that form the channel are shown in the ribbon representation.

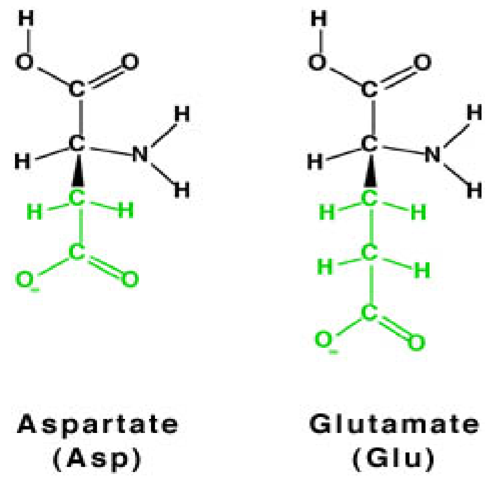


Figure 10

This is a 2D-ChemDraw representation of aspartate (Asp) and glutamate (Glu), the polar amino acids that line the three-fold channels in ferritin. The side chains are shown in green. Recall that only the side-chain groups contribute to the polarity of the residue in a peptide.

Note: To view these amino acids interactively, use Chime, and click on each molecule above.

[3] The hydrated Fe(II) ions, $Fe(H_2O)_6^{2+}$, would be too large to fit through the channel. Most likely, Fe(II) is coordinated to some water molecules and to some of the polar side chains lining the channel as it passes through the ferritin shell. Once outside the channel, the Fe(II) then regains six water molecules and solvated again as $Fe(H_2O)_6^{2+}$.

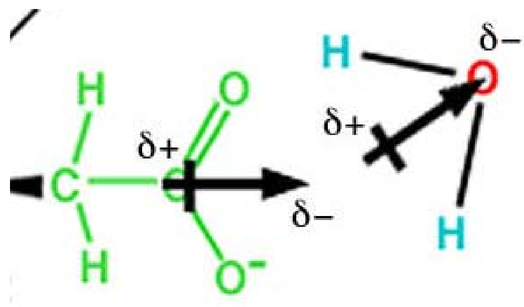


Figure 11

This diagram shows an interaction of a polar water molecule with the polar side chain of aspartate (an amino acid). This figure uses the standard depiction of the direction of polarity: an arrow pointing in the direction of the partial negative charge, with a "+" sign at the pole with a partial positive charge. In addition, the symbols "δ-" and "δ+" may be used to depict the negative and positive poles, respectively.

Now we will examine the four-fold channels in ferritin (Figure 12). These channels are lined with the **nonpolar** amino acid leucine (Figure 13). The side chain of leucine contains only carbon and hydrogen atoms, which have similar electronegativities. Hence, the four-fold channel is considered to be nonpolar. Because it is nonpolar, this channel does not interact favorably with the Fe^{2+} ion, and Fe^{2+} does not leave the ferritin shell through these channels. Rather, it is thought that these channels function as the site of electron transfer, whereby the Fe(III) in the mineral lattice is reduced to Fe(II). However, the mechanism of this electron transfer is not well understood.

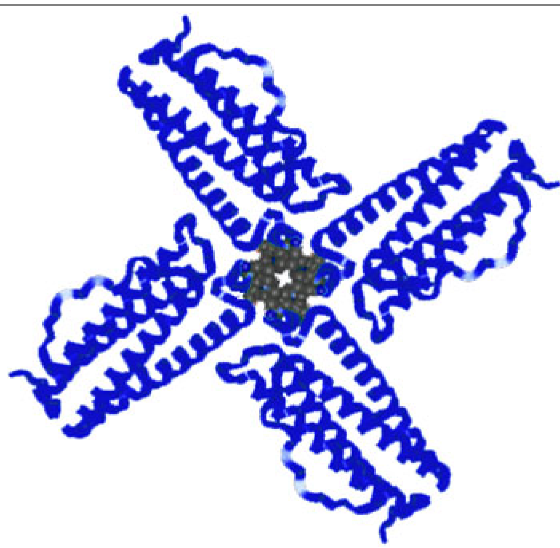


Figure 12

Electrons are transferred via this four fold channel to reduce the Fe(III) in the mineral lattice to Fe(II), thereby rendering the iron soluble so that it can be released from ferritin through the three-fold channel (shown in Figure 9).
Note: The amino acids that line the channel are shown in the CPK representation, and the remaining portions of the four peptide subunits that form the channel are shown in the ribbon representation.

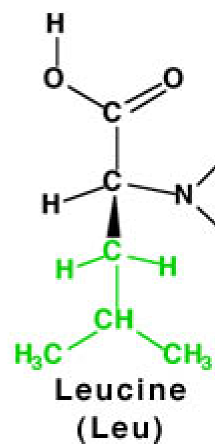


Figure 13

Leucine is the nonpolar amino acid that lines the four-fold channels in ferritin. The side chain is shown in green. Recall that only the side-chain groups contribute to the polarity of the residue in a peptide. To view this amino acid interactively, please use Chime, and click on the molecule.

Conclusion

Iron is an essential trace element that is used to form molecules in the body such as hemoglobin. Ferritin is the protein within the body that stores iron and releases it through channels in a controlled fashion. The unique structure of ferritin forms a spherical shell in which the iron is "stored" as Fe(III) in a crystalline mineral. Ferritin consists of 24 peptide subunits that form two types of channels where these subunits intersect: the 3-fold channel is polar, and the 4-fold channel is nonpolar. The residues that line the channels determine the polarity of the channel. When the Fe(III) in the crystalline mineral is reduced to Fe(II), the iron becomes solvated and ferritin releases the solvated iron, $Fe(H_2O)_6^{2+}$, through the 3-fold polar channel. Hence, ferritin can control the amount of available iron in the body, preventing iron disorders like anemia and iron overload.

The three-dimensional structure of ferritin is crucial to its function within the body. To better understand ferritin's role in the body, we used different types of molecular representations to study ferritin's three-dimensional stucture. Each representation used in this tutorial gives important information about ferritin. Only using these representations in conjunction with one another and with other information about the molecule, can we begin to understand the complex relationship between the protein's structure and its function.

Additional Links:

- The American Hemochromatosis Society provides a wealth of information about iron overload and hemochromatosis, including a new diagnostic test that uses the same procedure for analyzing the iron content in ferritin that you performed in the experiment!
- The Iron Disorders Institute is an organization dedicated to helping people understand the relationship between iron and disease.
- A more advanced Ferritin Molecular-Graphics Tutorial is also available from Washington University.

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