which line the 3-fold and 4-fold axes, through which iron may penetrate to the interior of the molecule, are conserved, as are the putative iron oxidation sites on the internal face of the B-helix.

```
   0                  10                  20                  30
A) S S Q I R Q N Y S T E V F A A V N R L V N L Y L R A S Y T Y
B) x x x x x x x x x x D x x x x x x Y x x x x x x Y x x x x x
C) T x x x x x x x x x E x x x x x x R x x x x H x R x x x x x
D) x x x V x x x x H Q x S x x x I x R Q I x x E x x x x x V x

                  40                  50                  60
A) L S L G F Y F D R D D V A L E G V C H F F R E L A E E K R E
B) x x x x x x x S x x x x x x x x x S x x x x x x x x x x x x
C) x x x x x F x x x x x x x x x x x G x x x x x x x x x x x x
D) x x M S Y Y x D x x x x x x K N F A K Y x L H Q S H x E x x

                  70                  80                  90
A) G A E R L L K M Q N Q R G G R A L F Q D L Q K P S Q D E W G
B) x Y x x x x x x x x x x x x x x x x x x I K x x A E x x x x
C) x A x x x x x L x x E x x x x x x x x x V Q x x S Q x x x x
D) H x x K x M x x x x x x x x x I F L x x I K x x D C x D x E

                 100                 110                 120
A) T T L D A M K A A I V L E K S L N Q A L L D L H A L G S A Q
B) K x P x x x x x x M A x x x K x x x x x x x x x x x x x x R
C) x x L E x x x x x L x x x x N x x x x x x x x x x x x x x Q
D) S G x N x x E C x x H x x x x V x x S x x E x x K x A T D K

                 130                 140                 150
A) A D P H L C D F L E S H F L D E E V K L I K K M G D H L T N
B) T x x x x x x x x x T x x x x x x x x x x x x x x x x x x x
C) A x x x x x x x x x S x x x x K x x x x x x x x x N x x x x
D) N x x x x x x x I x T x T x N E Q x x A x x E L x D x V x x

                 160                 170                 180
A) I Q R L V G S Q A G L G E Y L F E R L T L K H D
B) L H K x G x P E x x x x x x x x x x x x x x x x x
C) x R R W Q x x ‡ x S x x x x x x x x x x x x x x x
D) x x K M G A x E S G x A x x x x D K H x W E T V I M K A K P

R A N F P
```

Fig. 1

Comparison of Amino Acid Sequences of Apoferritins A) Horse spleen, B) Human spleen, C) Rat liver, D) Human H chain. Sequences which are identical with that in the line immediately above are marked x. The human H chain sequence extends 11 residues further than the other sequences, while the rat liver apoferritin has an 8 residue insertion at position 158 (marked as ‡)

The mechanisms involved in iron deposition and mobilisation are assumed to involve oxidation of Fe^{2+} and reduction of hydrolysed (and non-ionic) ferric iron respectively. Our current understanding of these processes will be discussed in the light of comparative sequence studies and the role of ferritins in intracellular iron metabolism will be analysed.

MS6.3 — FR

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THE THREE-DIMENSIONAL STRUCTURE OF APOFERRITIN: A FRAMEWORK CONTROLLING FERRITIN'S IRON STORAGE AND RELEASE

Ferritin is a giant clathrate compound comprising a protein cage encompassing an «iron-core» of the mineral ferrihydrite complexed with phosphate [1]. The space for this mineral is a sphere of *ca.* 80 Å diameter allowing the storage of up to about 4500 Fe^{3+} atoms. The cage, apoferritin, is a nearly spherical protein shell of thickness about 25 Å. This shell is composed of 24 polypeptide chains arranged in 432 symmetry [1] (Fig. 1). Each chain is folded into a roughly cylindrical subunit (*ca.* 25×55 Å), and antiparallel pairs of these subunits form the 12 faces of a rhombic dodecahedron.

The apoferritin subunit is a bundle of 4 long antiparallel helices, A,B,C and D, with a shorter helix, E, lying at about 60° to this bundle. There is also a long strand running the length of the bundle which forms a short stretch of antiparallel β-sheet with a 2-fold related subunit. Subunits are arranged such that one end lies beside a molecular 3-fold axis and the other points towards a 4-fold axis. Around the latter axes four nearly parallel E helices from four subunits are in close contact and form another, shorter bundle, with a small hydrophobic central channel (diameter *ca.* 3-4 Å, length *ca.* 15 Å) which passes through the protein shell. In the L-chains the amino acids lining these channels, twelve leucines from four subunits are con-

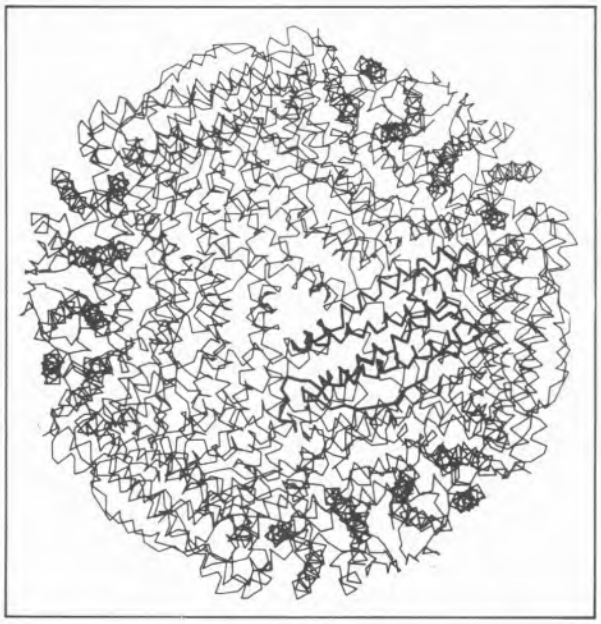

Fig. 1

View of an apoferritin molecule down one of its three-fold axes. A three-fold axis channel can be seen at the centre of the molecule. Six of the molecular two-fold axes lie in the plane of the paper, subunits are drawn as connected α C atoms

served. Channels around the 3-fold axes are shorter (diameter *ca.* 3-4 Å, length *ca.* 8 Å) and hydrophilic in character being lined by three aspartic acid residues towards the inside and three glutamic acid residues towards the outside (Fig. 2). These are conserved in all known sequences. Residues neighbouring these channels are also conserved or conservatively replaced. On the internal surface of the molecule, the rhomb face formed by each pair of subunits is flat except for an intersubunit groove, which contains water molecules. Residues are predominantly hydrophilic, often but not always conserved and many of them are notable for their weak electron density suggesting flexibility. Some of these residues may be in contact with iron-cores in ferritin and may play a role in the initiation of ferrihydrite deposition during ferritin formation.

To provide a storage and reserve function apoferritin must allow the passage of iron into the protein's internal cavity, its deposition inside and the subsequent mobilization of this iron.

How can these processes be related to the three--dimensional structure of apoferritin? Available data consist of kinetic measurements, usually by means of optical absorption spectroscopy, and binding studies of metal probes by X-ray crystallography, ESR, EXAFS or NMR spectroscopy or by equilibrium dialysis. This wealth of data has not yet led to definitive mechanisms due to the complexity of the molecule and of the processes of iron deposition and release. Thus, for example, Zn^{2+}, the most effective known inhibitor of ferritin formation, has been located at four different sites on apoferritin by X-ray crystallography [2].

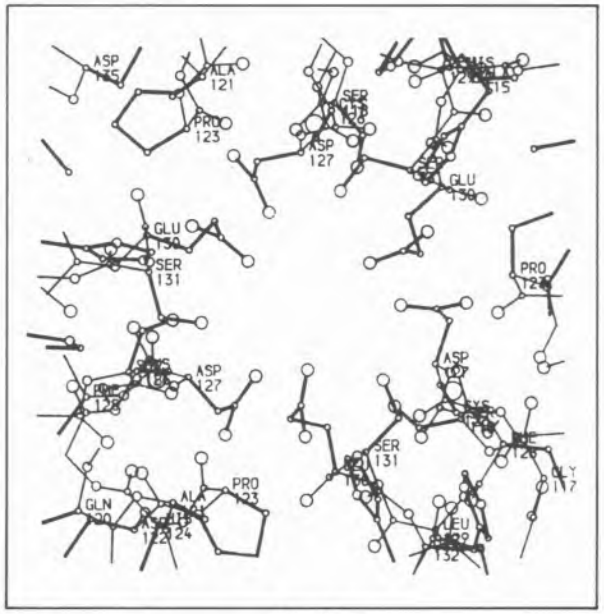

Fig. 2

View of the region round a three-fold channel showing amino acid residues of three subunits related by that axis. Note the three aspartic acid and three glutamic acid residues lining the channels

Iron may also bind at several sites on apoferritin as well as on the iron-core itself. Two metal sites which bind either Zn^{2+} or Cd^{2+} are located in the 3-fold channels, one having three aspartyl groups and the other having three glutamyl groups (and three water molecules) as ligands [2]. In crystals grown from $TbCl_3$ this double site is replaced by a single Tb^{3+} site with all six carboxylate ligands [2]. This indicates flexibility of metal ligand interaction within this intersubunit region which could be a feature important for the channelling of iron into or out of the molecule. Metals are also bound at other positions both inside and outside the molecule. Some of the former may represent sites for initiation of the iron-core.

The three-dimensional structure of the apoferritin molecule and its known metal binding sites will be

presented as a framework for the discussion of mechanisms of iron incorporation into ferritin and mobilization of iron from its iron-core.

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MS6.4 — FR

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THE EFFECT OF HISTIDINE MODIFICATION ON THE IRON BINDING CENTERS OF HUMAN SERUM TRANSFERRIN

The transferrins are a class of iron-binding proteins which include serum transferrin, ovotransferrin from egg white, and lactoferrin found in milk and other fluids. These proteins have molecular weights near 80,000 and reversibly bind two Fe^{3+} ions in separate but structurally similar domains. String models, based on the amino acid sequences of the various transferrins as well as on results from chemical modification studies and spectroscopic data, have been proposed for the iron binding regions of these proteins [1-3]. Likely candidates for the protein ligands in the *N*-terminal domain of human serum transferrin include His--119, His-249, Tyr-185, and Tyr-188. Examination of the portion of the sequence where these groups are clustered reveals several other amino acid residues which may also participate in the uptake and release of iron by the protein. These residues have cationic side chains and include several lysines, arginines and one or two histidines. The present investigation was undertaken to study the effect of histidine modification on the kinetics of iron removal from diferric transferrin by chelating agents.

The modification reaction with ethoxyformic anhydride (EFA) was initially studied. The reaction followed biphasic kinetics consisting of a fast reacting pool of 10 histidines and a slowly reacting pool of 5 histidines.

The iron removal reaction was studied at pH 6.9 in 0.1 M HEPES, 0.02 M $NaHCO_3$ using pyrophosphate (3 mM) as a mediating chelator and desferrioximine B (0.9 mM) as a terminal iron acceptor while monitoring the absorbance decrease at 295 nm. Semilogarithmic plots of the absorbance data showed that the reaction was biphasic, the fast and slow rates corresponding to loss of iron from the *N*-terminal and *C*-terminal binding sites respectively. This assignment was established by conducting urea-PAGE on samples of the reaction mixture at various times. The rate of iron removal from the *C*-terminal monoferric protein was also measured and found to closely correspond to the slower rate of the diferric protein. The two macroscopic pseudo first-order rate constants were obtained by curve fitting the absorbance data to the sum of two exponentials. Under the conditions of the experiment, iron was removed from the *N*-terminal site at a rate approximately three times faster than from the *C*-terminal site.
The four microscopic rate constants were obtained from nonlinear least-squares curve fitting of the time dependence of the concentrations of the four transferrin species, *i.e.* [Tf], [Fe_NTf], [TfFe_C], and [FeTfFe], to the general equations for sequential iron removal from the diferric protein by two parallel pathways. The microscopic constants were