The location of antigenic sites on ferritin molecules

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Immunoreactivities of peptides purified after cleavage of human liver apoferritin are reported and discussed in relation to the known 3-dimensional and primary structures of homologous apoferritins. These studies point to 3 antigenic sites occupying continuous inter-helical regions of the polypeptide chains which lie on the surface of the apoferritin molecule. Other antigenic regions may encompass amino acids remote in the primary structure or belonging to different subunits.

Ferritin *Amino acid sequence* *Dot immunobinding assay* *Antigenic site* *3-dimensional structure*

1. INTRODUCTION

The iron-storage protein, ferritin, is a widely distributed protein composed of 24 subunits [1]. Tissue ferritin levels are responsive to iron and this is generally reflected in its concentration in serum. Elevated tissue and serum ferritin levels may also occur in some disease states including some forms of malignancy. Immunoassay is widely used to monitor these levels [2]. However, such assays are complicated by the presence in ferritin of two subunits, known as H and L; the H subunit being more prevalent in heart and the L in liver and spleen [3]. Heart and liver or spleen ferritins differ immunologically and, indeed, ferritins from a single tissue can be fractionated into species varying in their immunoreactivities in parallel with their subunit content [4]. Cancer ferritins are usually H-rich but some investigators have suggested they may be abnormal, possibly representing carcino-foetal types [5].

Sequences corresponding to two distinct amino acid subunits in human spleen [6] and liver [7,8] and in rat liver ferritin [9] have recently been determined either as amino acid or as cDNA sequences. Homologies between the L subunit sequences of horse [10], human and rat (~85%) are greater than those between the two human liver sequences (presumed H and L, 57%) [7,11].

The 3-dimensional structures of apoferritins from liver and spleen of horse, human and rat are homologous [12]. That of horse spleen has been refined at 2.8 Å resolution [1]. Examination of this structure, which is a symmetrical arrangement of 24 subunits of high helix content, suggests some of the antigenic determinants may be located in inter-helical regions on the molecular surface.

Our studies aim to locate and characterise antigenic sites on the surface of the known structure of the ferritin molecule in order to understand the immunological differences between various tissue ferritins and also between the two subunits (H and L). We compare immunoreactivities of peptides produced by enzymic or chemical cleavage during the primary sequence determination of human liver apoferritin [7], to a variety of antisera. They will be discussed in relation to the known 3-dimensional structure of the intact ferritin molecule and to sequence differences between human H and L subunits.

2. MATERIALS AND METHODS

Ferritin was prepared from human liver and spleen, horse spleen and rat liver and purified essentially as described in [13]. Antisera to human and horse spleen and to rat liver ferritin were obtained as in [14]. Two monoclonal antibodies to

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human liver ferritin were a gift from Dr J. Gauldie. Human liver ferritin was reduced to apoferritin and peptides were isolated from it as described in [7]. Chemicals for immunological detection of peptides were purchased from Sigma (Poole, Dorset). Nitrocellulose membranes and the colour development reagent for the peroxidase reaction were obtained from Bio-Rad (Caxton Way, Watford). The dot immunobinding assay was carried out initially according to [15] or in later assays following the method of [16] with alkaline phosphatase conjugated second antibodies.

3. RESULTS AND DISCUSSION

Fig.1 shows typical results indicating the reactivities as, shown by the dot immunobinding assay, of human liver peptides towards unfractionated antisera raised to human spleen, horse spleen, or rat liver ferritins and to two anti-human liver ferritin monoclonal antibodies. Immunoreactivities of the unfractionated ferritins are also shown. It is striking that there is extensive interspecies cross-reactivity and that this applies both to the reactivities of the human liver peptides towards the polyclonal antibodies and to the three ferritin preparations towards the human liver monoclonals. One peptide (sequence 145–174) shows marked reactivity towards both monoclonals and polyclonals. Three other peptides, 83–91, 121–140 and 76–82 towards polyclonal antisera give some reaction and several other peptides react but to a lesser extent. A few peptides (46–53, 53–59 and 97–100) show little or no reactivity. The complete amino acid sequence of human liver apoferritin (presumed L subunit) is shown in fig.2 and is compared with sequences of horse spleen and rat liver L subunit and human liver H subunit. Fig.3 gives relative antigenicities of human liver peptides to homologous polyclonal antiserum.

Fig.4 shows a ribbon diagram of an apoferritin subunit and a diagrammatic representation of the

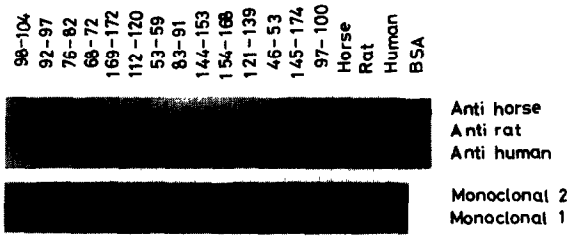

Fig.1. Dot immunobinding assay of peptides, from human liver apoferritin, horse spleen, rat liver and human liver apoferritins and bovine serum albumin (BSA), with antisera to horse spleen ferritin, rat liver ferritin, human spleen ferritin and two monoclonal antibodies to human liver ferritins. Peptides are numbered as in the amino acid sequence.

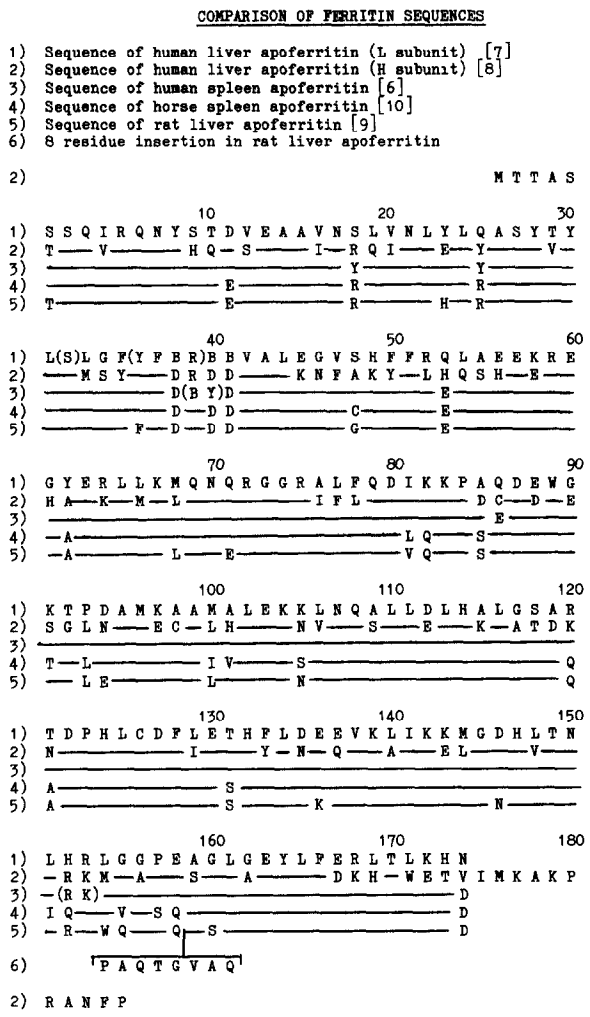

Fig.2. Comparison of ferritin sequences. Sequences displayed are: 1, Human liver apoferritin (L subunit) [7]; 2, human liver apoferritin (H subunit) [8]; 3, human spleen apoferritin [6]; 4, horse spleen apoferritin [10]; 5, rat liver apoferritin [9]; 6, 8-residue insertion in rat liver apoferritin [9].

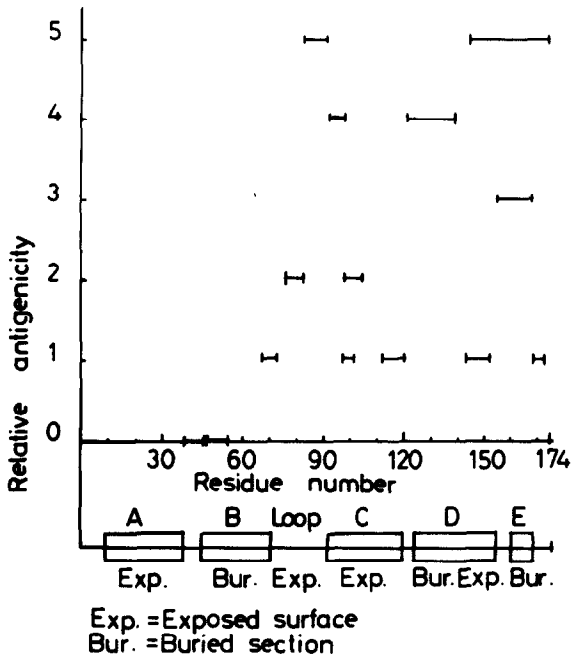

Fig.3. Schematic representation of positions of helices and antigenic reactivities of peptides from human liver apoferritin against antiserum to human spleen ferritin. Antigenic reactivities, as seen in the dot immunobinding assay, are graded on a scale of 0–5.

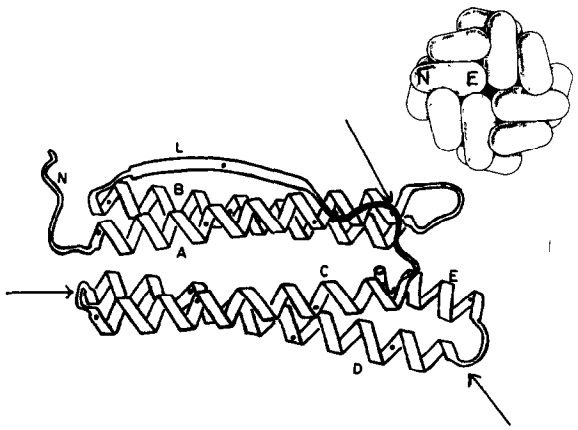

Fig.4. Ribbon diagram of one of the apoferritin subunits and diagrammatic representation of the 24-subunit quaternary structure, both viewed from the outside of the protein shell. Immunoreactive regions are indicated by the arrows. Every 10th residue is indicated on the ribbon diagram by a dot.

24-subunit quaternary structure both viewed from the outside of the apoferritin shell. From these it can be seen that peptides found to be markedly immunoreactive contain regions (indicated by arrows) which are both non-helical and situated on the outside of the molecule. Thus peptide 145–174 encompasses the DE turn between helices D and E (although it includes regions within D and E which are buried); peptide 83–91 runs from the C-terminal end of a short region of β-ribbon in the BC loop to the beginning of helix C entirely on the outside of the molecule (although some of its side chains are directed away from the surface); 76–82 is also on the external BC loop in the section making a short region of anti-parallel pleated sheet and 121–140 begins at the CD turn, although it proceeds into a region of the D helix which is buried. These regions, particularly 83–91 and 151–159 are those that might be expected to contain antigenic determinants on the basis of the known structure and the finding in other proteins of such determinants within surface non-helical loops [17]. It is of interest to note that the three non-helical regions are situated near symmetry axes in the quaternary structure. Of peptides showing little or no reactivity, 46–53 and 53–59, both from the B helix, are completely buried and 97–100 is very short. The peptides showing modest reactivity include 144–153 and 154–174 and the lowered activity of these peptides as compared with 145–174 may mean that the integrity of the region 151–159 must be preserved for full activity. Of the other weakly reacting peptides, 98–104 is within the C helix with 4 of its side chains (Ala, Val, Leu and Lys) facing outwards from the surface and 92–97, also within C, has 3 outward facing side chains (Leu, Asp and Lys) and again 112–120, also in the C-helix, has 6 external side chains (Asp, Ala, Leu, Ser, Ala and Gln). Peptides 68–72 and 169–172 (both showing very weak reactivity) are buried, however. The fact that they are found to react at all, may be due to disruption of the structure either as a result of mixing with adjuvant or after injection into the rabbit, so that antibodies are raised to these normally buried regions.

Cross-reactivities of the peptides and whole molecules with heterologous antisera deserve comment. Peptides 83–91 from L sequences are very similar with 85 Ala or Ser, 86 either Gln or Glu and 91 either Lys or Thr, the other residues (including

Asp 87 and Glu 88) are identical. 121–140 and 76–82 are also largely homologous. However, the marked cross-reactivity of 145–174 is interesting and perhaps surprising in view of the findings that the L-subunit of rat liver ferritin contains an insertion of 8 residues following residue 158 and there are apparently several other substitutions in this region. The reactivity of the two human monoclonals is somewhat less towards the rat ferritin than towards the human. Although we have no data on immunoreactivities of human L-peptides to H-specific monoclonals, sequence comparisons may be instructive. A considerable number of the amino acid substitutions, although by no means all, fall on the outside of the molecule and some of these may be antigenic determinants. It is particularly noteworthy that several substitutions are obvious in peptide 83–91, this region showing much less homology in human H and L subunits than is found in the L subunits of the three species compared. This strengthens the assignment of this region as one containing antigenic determinants, and one which may be responsible for some of the differences in reactivities of L- and H-rich ferritin molecules. Other such regions could include, for example, 92–97 where there are 4 changes including Pro, Leu and Lys, Glu. It should also be borne in mind that here we have only examined continuous regions of sequence and that many of the antigenic regions may be 'conformational' and comprise amino acids remote in sequence or belonging to neighbouring subunits. Indeed, this is suggested by further studies with fractionated antisera which have provided populations reacting with whole molecules but with much diminished activity towards the peptides. However, the structure is a rather compact arrangement and if flexibility is important in antibody recognition then the continuous sequences we have pin-pointed seem to belong to regions most able to meet this criterion. We hope that this and subsequent studies will provide the basis for an understanding of the specificity of ferritin antibody recognition.

ACKNOWLEDGEMENTS

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