conformation. Further interpretation must await results at higher resolution, but it is interesting to note that in glutathione reductase a conformation of the NADP+ which was more compatible with the usual NAD+ conformation found in the dehydrogenases was observed (Zappe et al., 1977).

Some interesting results have been obtained when crystals of 6-phosphogluconate dehydrogenase have been transferred to potassium tartrate or sodium citrate supporting media. A transition takes place to a closely related form, with the same space group, but with cell dimensions $a = 7.19\, \text{nm}$, $b = 14.68\, \text{nm}$, $c = 10.06\, \text{nm}$. The intensity pattern changes markedly; at 0.6 nm the mean fractional isomorphous difference is 39% (in $F$), compared with 10% for a heavy-atom derivative. Crystals of this type are sometimes found in $(\text{NH}_4)_2\text{SO}_4$ and increase of $(\text{NH}_4)_2\text{SO}_4$ concentration beyond 90% saturation tends to induce the change. A 0.6 nm difference map between the forms shows large positive and negative features, suggesting either a major conformational change or a bulk movement of the subunits. Rotation function calculations do not indicate a bulk rotation. There is some evidence linking the change to movements in the active site; sulphate ions probably bind in this region and crystals of the platinum derivative are liable to transform spontaneously.


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X-Ray Analysis of Thyroid Hormone Binding to Prealbumin

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Human prealbumin is a plasma protein that is known to interact with the thyroid hormones (Robbins & Rall, 1960) and with retinol-binding protein (Kanai et al., 1968), the carrier protein for vitamin A. Preliminary X-ray studies of the crystalline protein (Blake et al., 1971) showed that the prealbumin molecule is a symmetrical tetramer of 55000 mol.wt., exhibiting 222 symmetry. Extension of the X-ray studies to 0.25 nm resolution (Blake et al., 1974) revealed that the subunits had an extensive $\beta$-sheet structure, and were compactly arranged around a well-defined channel. The primary structure of human prealbumin has been reported independently in the form of a tentative overlap of a complete set of peptides (Gonzalez, 1972; Gonzalez & Offord, 1973) and as a complete amino acid sequence (Kanda et al., 1974). These data were incorporated in the X-ray model determined at 0.25 nm resolution, to be used as the basis for an extensive Fourier refinement against a complete set of X-ray data to 0.18 nm resolution (Blake et al., 1978).

The protein has two hormone-binding sites whose association constants (Ferguson et al., 1975) for L-thyroxine are $1.05 \times 10^8 \text{M}^{-1}$ and $9.55 \times 10^5 \text{M}^{-1}$; tri-iodo-L-thyronine also binds at these sites about an order of magnitude less strongly. The X-ray results indicate that these two sites are structurally identical and are deeply buried in the narrow cylindrical channel that runs through the centre of the protein molecule. We have also discovered that part of the surface of the prealbumin molecule is complementary in size and shape to the stable B-form of double-helical DNA (Blake & Oatley, 1977). This putative DNA-binding site, which has the form of a deep semicylindrical groove equipped with a pair of helically disposed arms, is constructed almost entirely
from a symmetry related pair of $\beta$-sheets. Although in principle the great diversity of protein structure allows binding sites for any ligand to be constructed, no detailed model of an interaction between native double-helical DNA and a globular protein had previously been proposed. The site we observe is almost entirely composed of regular protein structure, and therefore provides a general model for protein–DNA interactions, especially as the mode of binding that is indicated incorporates many of the features that have been proposed for such interactions (von Hippel & McGhee, 1972).

X-ray analysis

A preliminary description of the tertiary and quaternary structure of the prealbumin molecule determined from a 0.25 nm-resolution Fourier map phased with three isomorphous derivatives was reported earlier (Blake et al., 1974). Following this preliminary analysis the complete amino acid sequence of prealbumin has been determined (Kanda et al., 1974), and readily incorporated into the X-ray model. This model has now been partially refined against 0.18 nm-resolution X-ray data, the practical limit of the diffraction pattern, to give an overall R-factor of 28% for the 24000 unique reflections.

The prealbumin monomer is a classic $\beta$-barrel molecule, with eight $\beta$-strands organized into two four-stranded sheets. The whole molecule is a tetramer whose subunits are arranged tetrahedrally. The $\beta$-sheets represent the major points of subunit interaction: monomers associate into dimers by antiparallel hydrogen-bond interactions between equivalent sheets, giving the dimers two eight-stranded sheets; the dimers associate in the whole molecule by opposing equivalent eight-stranded sheets face-to-face in the centre of the molecule. The prealbumin molecule shown in Fig. 1

![Diagram of prealbumin molecule](image)

**Fig. 1. Stereo drawing of the $\alpha$-carbon positions of the prealbumin tetramer looking down the molecular x-axis**

The hormone-binding sites are located in the channel which runs horizontally through the molecule parallel to the z-axis. The proposed DNA-binding sites are located at the top and bottom of the Figure, so that the DNA is located with its helix axis parallel to x-axis.

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is therefore a very simple structure composed of four eight-stranded β-sheets, an 'inner' pair sandwiched between an 'outer' pair. A remarkable feature of the structure is that the inner sheets are not in contact, but form, or are separated by, an open channel that runs right through the molecule.

**Thyroxine-binding site**

The central channel that contains the thyroxine-binding sites can be seen in Fig. 1 running horizontally through the centre of the molecule. The surface of the channel is tightly organized from the polypeptide chains of the inner, four stranded β-sheet of each of the four subunits, and takes the form of a 16-stranded β-cylinder about 5 nm long, and 0.8 nm in diameter. The molecular 222 symmetry divides the channel into two symmetry related halves clearly showing that the two binding sites are identical. In addition, one of the molecular twofold axes coincides with the channel axis, showing that each site itself has twofold symmetry. The chemistry of the channel is characterized by three elements arranged linearly along it, which reading from the centre are: (1) a hydrophilic patch formed by the hydroxys of the serine-117 and threonine-119 pairs of residues; (2) a hydrophobic patch formed by the methyl groups of leucine-17, threonine-106, alanine-108, leucine-110, threonine-119 and valine-121 pairs; (3) a group of charged residues including the paired side chains of lysine-15, glutamate-54 and histidine-56. The absence of any of the molecule's 48 hydrophobic aromatic residues from that channel is both clear and striking.

The interaction of prealbumin with L-thyroxine and tri-iodo-L-thyronine has been examined by calculating difference electron-density maps of the protein–hormone complexes, initially in projection at 0.3 nm resolution, and subsequently at 0.19 nm resolution in three dimensions for L-thyroxine. Each set of maps shows a number of discrete peaks that almost certainly represent the electron-dense iodine substituents of the hormone, but have rather little indication of the hormone carbon skeleton. Nevertheless, because of the very tight constraints imposed on the conformations of the hormones by the bulky iodines, adequate molecular models can be built from knowledge of the positions of the iodine atoms alone.

The projection maps of both L-thyroxine and tri-iodo-L-thyronine binding, calculated in two perpendicular directions (looking down, and across, the central channel), could be interpreted, with the aid of certain packing considerations, in terms of very similar binding modes for the two hormones (Blake & Oatley, 1977). The binding mode of L-thyroxine, shown in Fig. 2, allows each of the characteristic substituents of the hormone to enjoy a favourable protein environment. Each of the 3'- and 5'-iodine atoms lies between and in contact with the side chains of leucine-17 and leucine-110, and each of the 3- and 5-iodine atoms fits into a pocket lined with the methyl groups of threonine-106, alanine-108, and valine-121 and the β- and γ-methylenes of lysine-15. These positions are also consistent with the interaction of the 4'-hydroxy group, via a water molecule, with the hydroxy groups of the serine-117 and threonine-119 pairs of residues, and with the interaction of the hormone's α-carboxylate and α-amino groups with lysine-15 and glutamine-54 respectively.

The three-dimensional map of tri-iodo-L-thyronine binding at 0.19 nm is fully consistent with the binding of L-thyroxine deduced from the projection maps. The presence of at least two peaks corresponding to the single 3'-iodine of tri-iodo-L-thyronine indicates clearly that this hormone is bound as a mixture of at least two conformations, with the single iodine bound alternatively at the sites of the two iodine-binding sites of L-thyroxine.

**DNA-binding site**

Our attention was drawn to the possibility of a DNA-binding site on prealbumin by the remarkable cylindrical–helical depression in the outer surface of the molecule, shown in Fig. 1. The site is formed by the 'outer' β-sheets of two symmetry related subunits. The normal right-handed twist of the sheets coupled with the symmetry induced by the molecular twofold axis parallel to the y-axis gives rise naturally to a concave cylindrical
The main chain is shown in light line and the side chains which point into the site are shown in medium line. The position of the L-thyroxine molecule, shown in heavy line, was derived by fitting the co-ordinates of Cody (1974) to the iodine positions indicated in the projection maps.

The extent to which this site is complementary to double-helical DNA was explored by model building, using an interactive computer display system (Barry & North, 1971). The co-ordinates used were those of the main chain atoms of prealbumin from the latest stage of refinement, and a standard set for all the atoms of the B-form of the DNA double helix (Langridge et al., 1960). These co-ordinates were maintained throughout the fitting, which therefore corresponded to the mating of two rigid molecules.

This view corresponds to that from the top of Fig. 1. The main chain atoms of the outer β-sheet are shown in light line with the hydrogen bonds in broken line. The DNA is drawn in heavy line. The twofold axis parallel to the y-axis is positioned at the centre of the diagram.
The final fit of the DNA to prealbumin obtained from the computer display is shown in Fig. 3. Its characteristics are as follows:

1. The twofold axis of symmetry of the protein coincides with the twofold axis of symmetry in the DNA.
2. The DNA double helix lies in the pronounced cylindrical depression in the protein molecule with its helix axis parallel to the axis of the depression.
3. The pair of helical arms of the protein fit closely into the wide groove of the DNA double helix. This is the most striking feature of the fit because it could allow the arms to interact with the DNA bases whose edges are exposed in the wide groove. We therefore consider that the helical arms can act as 'reading heads' that probe the DNA for a compatible base sequence. The twofold symmetry relation of the arms would necessarily imply that such compatible base sequences must be palindromic. However, Fig. 3 shows that this is not necessarily true for the two base pairs at the very centre of the binding site, which are in fact more distant from the protein surface than the base pairs on either side. This relieves the requirement for an exact palindrome at the centre of the interaction site as has been observed for the lac operator (Gilbert & Maxam, 1973) and the Haemophilus endonuclease specificity site (Kelly & Smith, 1970).

The thyroid-hormone nuclear receptor is a protein that combines both thyroid hormone and DNA-binding function, and thus it would appear that the most likely explanation of the observation of a structural complementarity to DNA in prealbumin is that both it and the receptor have evolved from an ancestral protein whose structure encompassed both hormone- and DNA-binding sites.

We acknowledge the Medical Research Council for their financial support of this project and for the provision of a postdoctoral research assistantship to S. J. O. We also thank the Science Research Council for the provision of a research studentship to J. M. B. We are grateful to Dr. J. Robbins of National Institutes of Health, Washington, and Dr. E. C. Jorgensen of the University of California for useful discussions on hormone binding.