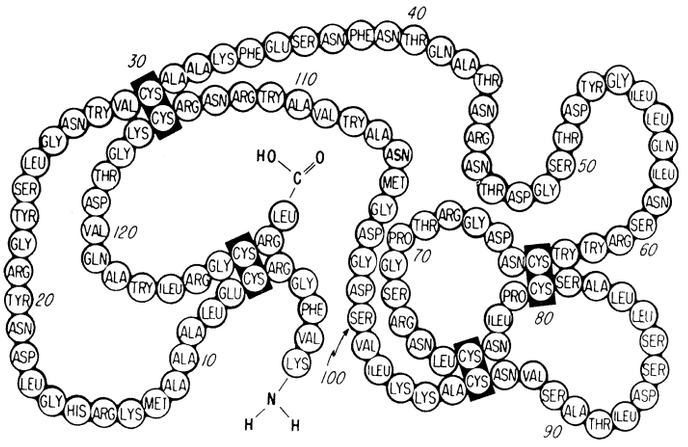

Fig. 26.1.3.10. The amino-acid sequence of hen egg-white lysozyme (Canfield & Liu, 1965).

shown in Fig. 26.1.3.9. The strongest feature in this part of the map corresponded to a disulfide bridge (C), in which the two sulfur atoms lie one above the other in the direction of the *c* axis. The first challenge was to identify this bridge. It is clearly connected to a helical region of the molecule (A), which ran in the direction from top right to bottom left of the diagram, with the main-chain carbonyl groups pointing in this direction. Consequently, this helix ran from its amino terminus on the right to its carboxyl terminus in the centre of the map. The map was quite clear enough to count the α-carbons from the cysteine residue that forms part of the disulfide bridge, and it was immediately apparent that the fourth residue from the cysteine towards the carboxyl terminus is an aromatic residue, probably phenylalanine. Inspection of the amino-acid sequence in Fig. 26.1.3.10 showed that only one pair of residues satisfied this condition, Cys30 and Phe34.

Given this start, interpretation of the map and the construction of a molecular model were relatively straightforward. The model was constructed in a metal frame, the top and bottom of which consisted of sheets of blockboard. The *a* and *b* axes were drawn parallel to the diagonals of these boards to cover the coordinate ranges, respectively, $x = -1/4$ to $+1/4$ and $y = 0$ to $+1/2$ to a scale of 2 cm to 1 Å. This was the scale of the brass models, constructed by Cambridge Repetition Engineers Ltd, which were used to build the model. The height of the frame covered the full extent of the *c* axis. The heavy-atom coordinates and the computer programs were both based on the wrong-handed space group $P4_12_12$. It was not until the anomalous scattering from the heavy atoms was incorporated that the correct space group $P4_32_12$ was assigned. The Fourier-map sheets were actually stacked the opposite way round and a left-handed system of axes was used for the model. In retrospect, this should have been put right at once, but the system was not easy to change.

Holes were drilled in the top and the base boards on the grid defined by the *a* and *b* axes, and these were used to support an array of brass rods parallel to the *c* axis to which the model components could be attached. The model building was carried out by two subgroups, CCFB and VRS in one and ACTN and DCP in the other, so that work could go on continuously throughout each day. The method employed was to examine the map density corresponding to the next amino-acid residue to be located and to mark the positions of the constituent atoms with small washers or nuts. The coordinates of these atoms were then read from the map (making use of the superimposed grid and estimating the *z* coordinates from the extent to which adjacent *z* sections contributed to the density). These

Table 26.1.3.3. *Discrepancies in amino-acid sequences (excluding Asp/Asn)*

	Residue						
Reference	40	41	42	58	59	92	93
Canfield & Liu (1965)	Thr	Gln	Ala	Ile	Asn	Val	Asn
Jollès *et al.* (1964)	Gln	Ala	Thr	Asn	Ile	Asn	Val

coordinates were then located in the model by means of the coordinate grids drawn on the base and top boards and by the use of a plumb line marked with the *z* coordinates. At this stage it was usually possible to fix a model of the amino-acid residue in place in the model frame with remarkably little trouble, though, of course, fine adjustment was necessary as the model grew.

This bout of model building began towards the end of February 1965 and proceeded quite rapidly. The main difficulties arose from the fact that the two amino-acid sequences that were available did not agree in every respect. Eleven amino-acid residues were identified differently by Jollès (Jollès *et al.*, 1964) and by Canfield (Canfield & Liu, 1965). Four of the discrepancies involved Asn and Asp, which cannot be distinguished in the electron-density map with any degree of certainty. The remaining discrepancies are shown in Table 26.1.3.3.

Inspection of the residues 40, 41, 42, 92 and 93 showed quite clearly that the shapes in the electron-density map fitted the Canfield side chains. Our initial conclusion, however, was that the electron densities corresponding to residues 58 and 59 were more consistent with the sequence proposed by Jollès than that published by Canfield. Accordingly, in our first detailed description of the

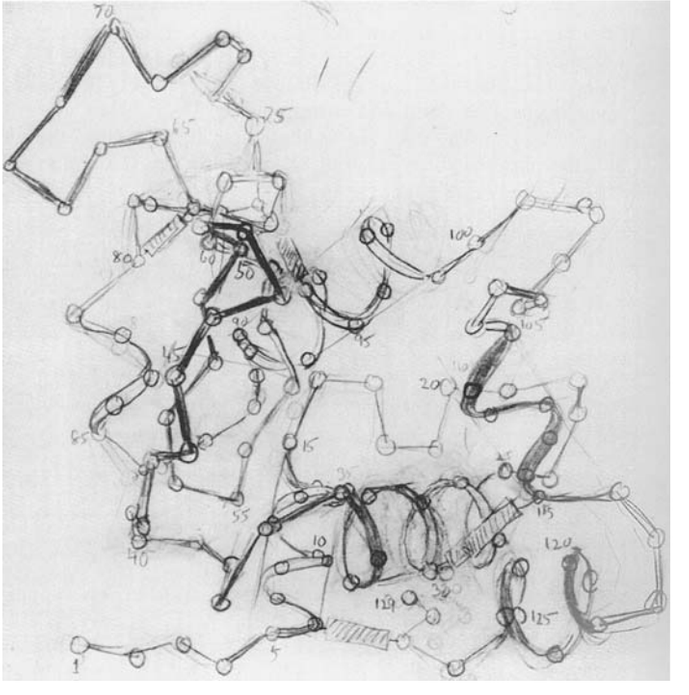

Fig. 26.1.3.11. Schematic drawing of the main-chain conformation of lysozyme. The drawing was made from observations of the molecular model by Sir Lawrence Bragg and later prepared for publication by Mrs S. J. Cole.

references