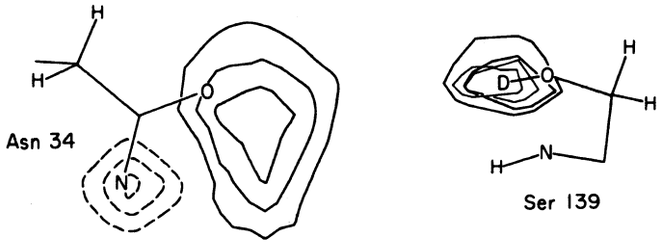


(*a*) (*b*)

Fig. 19.1.5.1. Difference map of Asn34 in the trypsin structure. (*a*) In a protein X-ray analysis, the difference in scattering intensity between O and NH_2 is much too small to be detected. In contrast, the neutron-scattering magnitudes of oxygen and nitrogen (5.8 f versus 9.4 f) are quite dissimilar, and there is additional scattering at the nitrogen site from the two bound deuterium atoms. The resulting differential is over 350%, quite large enough to be detected for well ordered side chains. The nitrogen and oxygen positions shown are from the X-ray model. The difference density indicates that the orientation of the nitrogen and oxygen atoms is incorrect and should be rotated by 180° around the C_β–C_γ bond. (*b*) Difference map for Ser139. On well ordered hydroxyl side chains, the orientation of deuterium atoms can sometimes be assigned.

in the Fourier map from the true positions and, coupled with their short bond lengths, complicates the interpretation of the results. Additionally, it has been shown that small errors in positional and thermal parameters of the parent atoms can further complicate the identification of hydrogen-atom positions (Kossiakoff & Spencer, 1981).

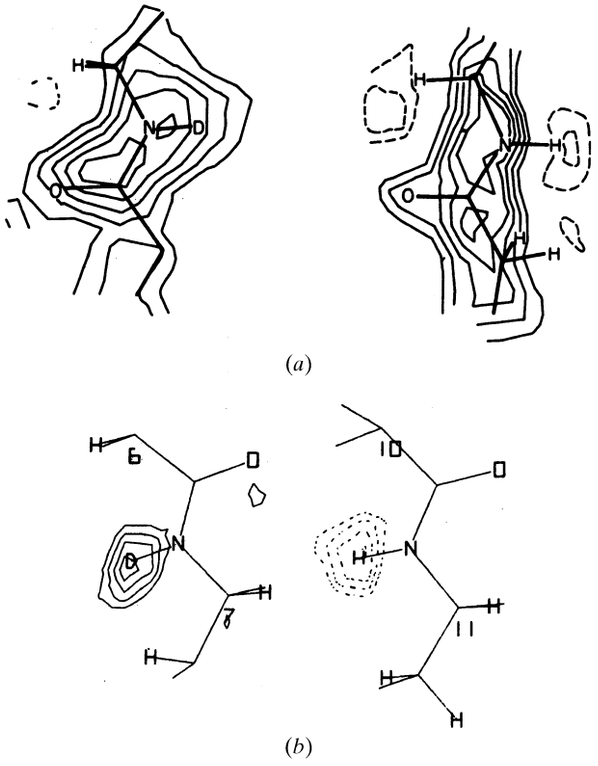
19.1.6. Refinement

The methodologies employed to refine neutron data are essentially the same as those used in most X-ray studies. These include real-space (Hanson & Schoenborn, 1981; Norvell & Schoenborn, 1976; Schoenborn & Diamond, 1976), reciprocal-space (Bentley & Mason, 1980; Phillips, 1984; Wlodawer & Hendrickson, 1982; Wlodawer & Sjolin, 1981) and restrained difference-map refinement (Kossiakoff & Spencer, 1980; 1981). A joint refinement technique in which the neutron and X-ray data are refined simultaneously has been developed (Wlodawer & Hendrickson, 1982). In addition to the normal difficulties encountered in the refinement of any protein structure, there are several that are peculiar to the neutron-diffraction technique. These special problems arise from the close proximity of hydrogen atoms to their parent atoms, coupled with the effects of the negative scattering length of the hydrogen atoms. Potential problems exist when the difference density generated from positional errors of one atom overlaps an adjacent atom site. The situation is further complicated by the fact that, because of its negative scattering length, an error in a hydrogen-atom position is minimized by moving the atom down the gradient, that is, in the opposite direction to that required for correcting parent-atom positions. To evaluate the extent of this problem in refinement, a test was devised using a 2.2 Å data set (Kossiakoff & Spencer, 1981). The coordinates of the protein trypsin were perturbed by a varying, but known, amount from their ideal positions. It was determined that, in general, convergence towards the true coordinate could be obtained when the coordinate errors were less than 0.3 Å; however, if the parent atom (an atom with one or more hydrogens attached to it) was displaced by more than 0.6 Å from its correct position, the effect of neighbouring hydrogens rendered the calculated shifts inaccurate. The results of this study support the observations of other investigators that it is absolutely crucial that the starting phasing model be of high quality, because the range of convergence of a neutron analysis is relatively small.

19.1.7. D_2O – H_2O solvent difference maps

D_2O – H_2O solvent difference maps provide an unbiased method for identifying water molecules and exchangeable hydrogens (Kossiakoff *et al.*, 1992). For several years, the large difference in the scattering characteristics of neutrons by H_2O compared to D_2O has been effectively exploited by using density matching and exchange labelling in small-angle neutron-scattering experiments. This difference can likewise be exploited in neutron protein crystallography to determine the detailed structural characteristics of protein hydration through the calculation of solvent difference maps (Shpungin & Kossiakoff, 1986; Kossiakoff *et al.*, 1992). In practice, such maps are obtained by comparing the changes in diffracted intensities between two sets of data – one obtained from a crystal having H_2O as the major solvent constituent, and a second where D_2O is the solvent medium. To a good approximation, the protein-atom contributions to the scattering intensities in both data sets are equal and cancel, but since H_2O and D_2O have very different scattering properties, their differences are accentuated to reveal an accurate and nearly unbiased representation of the solvent structure.

The features of a solvent difference map of this type are not as affected by errors in the phasing model as conventional difference Fourier maps. In addition, there are refinement procedures that can be applied to them that lead to significant enhancement in signal/noise discrimination. The basic feature of the method is a set of density-modification steps based on the fact that a considerable amount of information about the density distribution of the crystallographic unit cell is known. For instance, it is known that



(*a*)

(*b*)

Fig. 19.1.8.1. Sections of neutron density maps taken in the plane of the peptide group. (*a*) $2F_o - F_c$ maps showing an example of an exchanged and unexchanged amide peptide group. (*b*) $D_2O - H_2O$ difference density map showing the same.

references