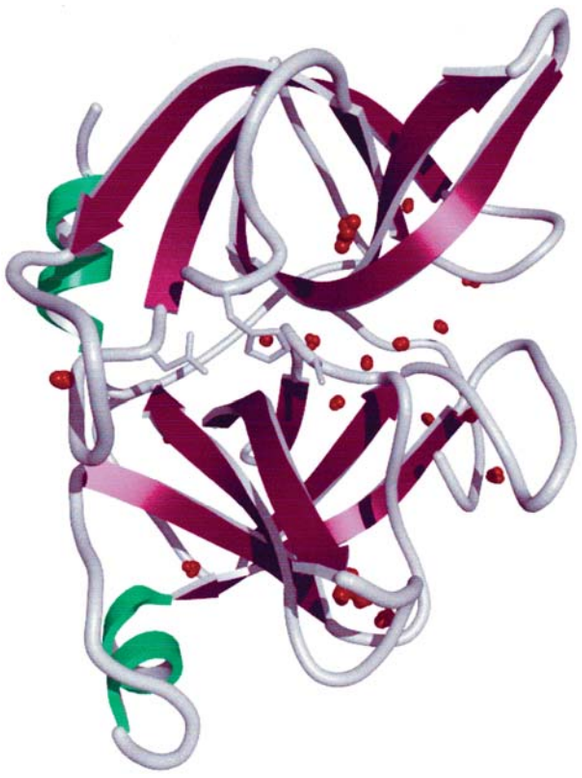

Fig. 23.4.4.4. Crystal structure of porcine pancreatic elastase represented as a ribbon diagram using *MOLSCRIPT* (Kraulis, 1991). The two α-helices are shown in green, the β-sheets are in purple and the coils are in grey. Elastase contains 240 amino-acid residues, and is composed of two β-barrel domains. The catalytic triad (Asp108, His60 and Ser203) is shown explicitly. The buried crystallographic water molecules found in 11 superimposed elastase structures solved in a variety of solvents are shown in red.

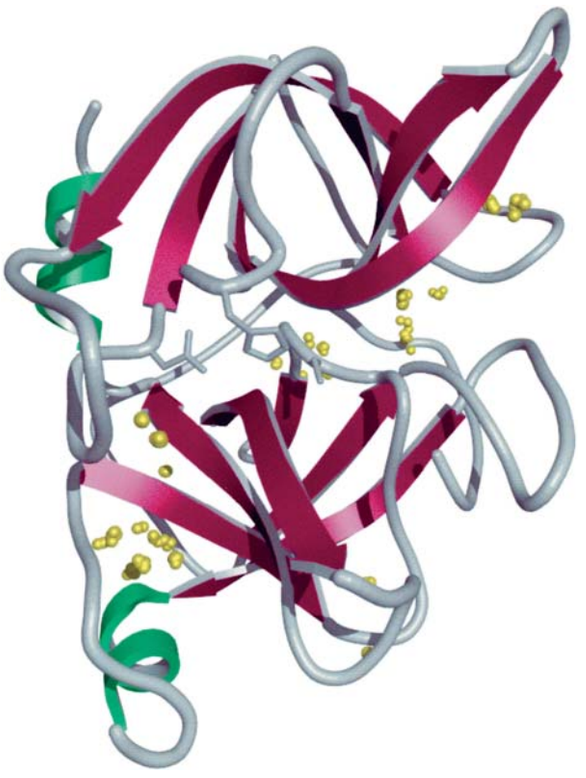

Fig. 23.4.4.5. Elastase structure represented as in Fig. 23.4.4.4. The crystallographic water molecules found in channels in 11 superimposed elastase structures solved in a variety of solvents are shown in yellow.

understanding of the functional roles played by structurally conserved water molecules as discussed above and in the following sections.

The 29 water-binding sites classified as channel contain water molecules that make hydrogen bonds with at least two other water molecules within a protein groove. The analysis of a high-resolution crystal structure of elastase (1.65 Å) revealed seven channels with a total of 32 water-binding sites (Meyer *et al.*, 1988). All of these channels were also identified in the analysis of the 11 structures in Table 23.4.4.1 (Bellamacina *et al.*, 1999). In addition, two other channels were observed. The locations of the nine elastase channels identified by the new criteria are shown in Fig. 23.4.4.5. Channels are often found in areas associated with buried water molecules, namely, at the crevice between the two domains and sandwiched between secondary-structure elements, where they lead from the surface of the protein to a buried water molecule. Fig. 23.4.4.5 also shows that the Cα superposition of the protein structures leads to a spread of water molecules within the channels. In any given structure, only two or three water molecules may be present, but the precise location and interaction with protein atoms vary so that when taken together the collection of structures gives a sense of flow inside the channels.

Of the remaining 374 water-molecule sites present within the 11 elastase structures included in this study, 56 were classified as crystal-contact sites and 318 as surface sites. Crystal-contact sites were considered to be occupied by water molecules that are within 4.0 Å of a symmetry-related protein molecule in the crystal. Fig. 23.4.4.6 shows the position of all the water molecules found to occupy these sites. The relatively large number of crystal-contact water-binding sites is a result of the somewhat broad criterion used to select them. Many of these sites are not within hydrogen-bonding distance from the nearby protein molecule, and most are not well conserved from structure to structure. Only eight of the 56 sites are occupied in the majority of the structures, and four of these make good multiple hydrogen bonds with two symmetry-related protein molecules in the crystal. These four water molecules seem to be structurally significant in the formation of the crystal contacts.

Surface water molecules were taken to be those that interact with side-chain protein atoms on the surface or make no more than two hydrogen-bonds with backbone atoms. When the 11 structures are superimposed, the surface water molecules occupying a given site are not tightly clustered. Furthermore, there is flexibility in the interactions between these water molecules and the nearby protein atoms. For example, it is often the case that all water molecules within a surface site make two or three hydrogen bonds to protein atoms, but only one of them is conserved in all of the structures where the water molecule is present at the site. Fig. 23.4.4.7 illustrates the position of all of the surface water-binding sites. Although over half of these sites are occupied in at least two of the 11 structures, a good proportion of them (178) are found in only one of the structures considered.

While crystal-contact and surface water sites were classified separately, it is important to point out that, with the exception of the four crystal-contact water-binding sites mentioned above, the crystal-contact sites exhibit very much the same traits as the surface water sites. The difference is that in the latter case, the 'surface' is provided by a single protein molecule, while in the former the interaction between two symmetry-related protein molecules constitutes the surface with which the water molecules interact.

Of the 318 surface water molecules, 21 are in the active site. The active-site water molecules were selected to be those within 4 Å of any atom belonging to either the trifluoroacetyl-Lys-Phe-*p*-

references