Volume 213, number 2, 423-427 FEB 04534 March 1987

The hydration of protein secondary structures

D.J. Barlow and P.L. Poole*

Departments of Pharmacy/Pharmacology, King's College London (KQC) Chelsea Campus, Manresa Road, London SW3 6LX and **Department of Biology & Biochemistry, North East London Polytechnic, Romford Road, London E15 4LZ, England*

Received 10 December 1986; revised version received 15 January 1987

The hydration of the main-chain carbonyl (CO) groups in proteins have been studied using infra-red spectroscopy, and computer-graphics analysis of high resolution protein crystal structures. The IR measurements indicate that the strength of water binding to the CO groups is lower in β-sheet proteins compared with α-helical ones. Analysis of the protein crystal structures shows that this is due primarily to differences in the geometry of water-CO group interactions in the two types of secondary structure.

Secondary structure; Protein hydration; Peptide group; Hydrogen bonding

1. INTRODUCTION

The interaction between protein and solvent has been subject to considerable research. Many techniques such as calorimetry, infra-red, Raman and NMR spectroscopy, have been used to provide 'general' information on water-protein systems – with most of the studies concerned with the differences between bulk water and the water of hydration (reviews [1,2]). Very few of these experiments have addressed the problem of why one protein may have different hydration properties to another.

More 'specific' details of protein-water interactions have been furnished by analysis of the crystal environments of proteins, as determined by X-ray diffraction methods. Various authors have looked in detail at the interaction between water and a particular protein (e.g. human lysozyme [3]), and Baker and Hubbard [4] have recently conducted a systematic analysis for several proteins.

In our own work on protein hydration [5], we have used the information provided by infrared spectroscopic techniques, in conjunction with X-ray crystallographic data. We have used these techniques to analyse the interactions between water and a protein's carboxylate groups, and have demonstrated how the hydration of a protein can be influenced by the extent of ion-pairing between its surface charged groups. The experiments showed that the strength of water binding to COO groups were lower in proteins with large numbers of ion-pairs.

In the same work [5] we also noted that there was a significant (albeit smaller) variation in the strength of water binding to a protein's peptide CO groups, which appeared to be correlated with the predominant type of secondary structure present: the strengths of water binding to the peptide CO groups in β-sheet proteins were lower than those for proteins composed mainly of α-helices.

Correspondence address: D.J. Barlow, Depts of Pharmacy/Pharmacology, King's College London (KQC), Chelsea Campus, Manresa Road, London SW3 6LX, England

2. EXPERIMENTAL

The infra-red experiments were carried out as described in [5]. Briefly, these experiments involve increasing the temperature of hydrated protein films, and following the resultant changes in specific infra-red bands caused by the dehydration

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

of particular atomic groups. From the absorbance changes we derive an apparent equilibrium constant (K_{app}) for the reaction,

$$\text{hydrated protein atom group} \xrightarrow{K_{app}} \text{protein atom group} + \text{water}$$

at temperature T. A van 't Hoff's plot (of $\ln(K_{app})$ vs $1/T$) then gives an apparent ΔH for the reaction (ΔH_{app}), which is a measure of the interaction strength between water and the protein atom group considered.

In the analysis of protein crystal structures, the atomic coordinates for proteins were taken from the Cambridge Protein Databank [6]. Contact areas for protein atoms were calculated according to Lee and Richards [7].

The inspection of the protein structures on an Evans & Sutherland PS II colour computer graphics system was carried out using the program FRODO [8].

Further details of the methods and calculations used are described in the following section.

3. RESULTS AND DISCUSSION

The ΔH_{app} values for water binding to peptide CO groups have been determined for 12 proteins, and these results are summarised in table 1.

We note that for 6 of the 7 proteins, which are based upon anti-parallel β-sheets, the ΔH_{app} values for the peptide CO groups lie in the range 14.1–15.8 kcal·mol^{-1}. (The anomalous behaviour of the β-sheet protein pepsin is most likely due to its unusual ionic nature (see [5]). This indicates that the hydration of peptide CO groups may also be affected by the distribution of charged residues.) The remaining 5 proteins, which contain appreciable amounts of α-helix, have significantly higher ΔH_{app} values: in the range 17.5–18.7 kcal·mol^{-1}. These results confirm our earlier observation that the interaction between

Table 1

The relationship between protein secondary structure, peptide CO group accessibility, and the ΔH_{app} values for water-peptide CO group interactions

Protein	Percent sheet	Percent helix	ΔH_{app} (kcal·mol^{-1})	Mean contact area of carbonyl O (Å^2)	
				Sheet	Helix
γ-Crystallin II	47	3	14.3 ± 0.7	0.7 (1.3)	–
γ-Crystallin III[a]	–	–	14.7 ± 0.7	–	–
γ-Crystallin IV[a]	–	–	14.1 ± 0.7	–	–
Immunoglobulin	49	3	15.1 ± 0.8	–	–
Trypsin	36	8	15.8 ± 0.8	1.1 (1.9)	1.4 (2.2)
Superoxide dismutase	42	0	15.7 ± 0.8	–	–
Carboxypeptidase	17	35	18.3 ± 0.9	0.5 (1.5)	1.1 (2.3)
Lysozyme	11	31	17.5 ± 0.9	1.0 (1.9)	0.8 (1.6)
α-Lactalbumin	11	31	18.5 ± 0.9	–	–
Cytochrome c	2	41	18.7 ± 0.9	–	0.5 (1.2)
δ-Crystallin[b]	<9	80	17.9 ± 0.9	–	–
Dihydrofolate reductase	33	25	–	0.7 (1.7)	0.9 (1.4)
Pepsin	48	9	18.1 ± 0.9	–	–
			Mean	0.8	0.9

[a] 2° structure unknown, but protein has a high sequence homology with γ-crystallin II [12]

[b] 2° structure unknown but circular dichroism indicates low β-sheet content [13–14]

Standard deviations on the mean CO oxygen atom contact areas are shown in brackets

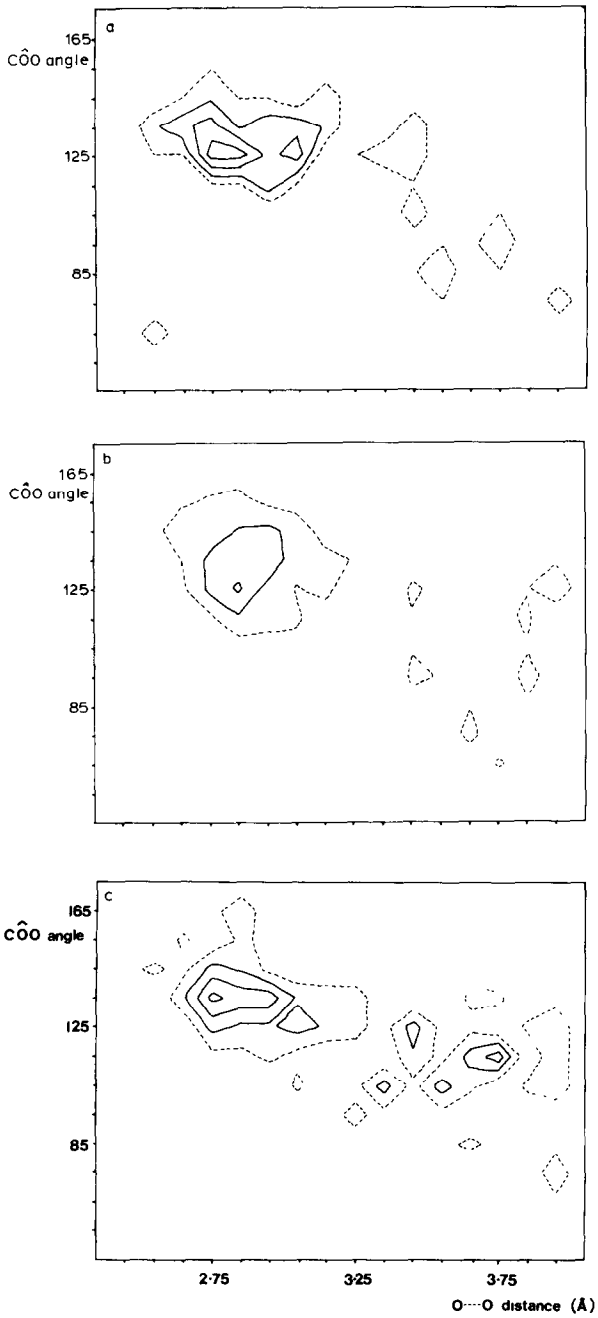

Fig.1. Contour plots showing the distribution of hydrogen bond geometries for the interactions between water and peptide CO groups in proteins. COO angles are shown in degrees and O-O distances in Å. (a) Data for the 114 water-CO group interactions, for the CO groups of residues involved in α-helices. (b) Data for the 453 water-CO group interactions, for the CO groups of residues involved in turn/coil regions. (c) Data for the 164 water-CO group interactions, for the CO groups of residues involved in β-sheets. Each plot is produced by contouring the corresponding scatter plot according to the percentage of interactions with a given hydrogen bond geometry. Contour lines in the range 1–4% are drawn at intervals of 1%, with the 1% contours represented by dashed lines.

water and a protein's peptide CO groups depends upon its secondary structural content.

To appreciate why this should be so, we considered how the environments of the CO groups might differ between helical and β-sheet proteins. From calculations made by Richards and Richmond [9], it appeared that the phenomenon could be explained simply in terms of a lower solvent accessibility for β-sheet compared to α-helix CO groups (the contact areas being, respectively, 0.1 and 0.7 Å^2, for the CO oxygen atoms). However these values were calculated for idealised secondary structures and are unreliable as estimates of the CO accessibilities in proteins. To obtain more realistic estimates therefore, we used the atomic co-ordinates for a number of protein crystal structures [6], and calculated the mean contact areas for the peptide carbonyl oxygen atoms in α-helical and β-sheet residues (see table 1).

We find that the ranges of contact areas are very similar for α-helix and β-sheet (0.49–1.4 Å^2 vs 0.53–1.06 Å^2), and also that there is a high standard deviation for each protein, which reflects a wide variation in the contact areas for peptide CO groups between individual helices/strands. We conclude that there is little difference in the solvent accessibility for peptide CO groups in helices as opposed to β-sheets, and that given the thermal motion of the proteins in solution, this difference (0.94 Å^2 vs 0.77 Å^2) may be insignificant. Thus, the different values of ΔH_{app} for β-sheet and helical proteins cannot be accounted for simply in terms of differences in the average accessibilities of these groups.

Since the interaction between water and a protein's peptide CO groups may also be weakened by poor hydrogen bond geometry, we have investigated this possibility by studying the interaction between water and the CO groups in high resolution protein crystal structures. For each of the peptide CO groups in each of 7 proteins (see

table 1), a search was made for all water molecules lying within 4 Å. The O-O distance and COO angle were then recorded for each interaction, and the results were tabulated separately for the peptide CO groups of residues in α-helix, β-sheet and coil/turn regions. The 3 separate sets of data were first summarised as plots of O-O distance vs COO angle, and these scatter plots were then contoured according to the density of points falling within each section of the plots (fig.1), in order to allow a direct comparison of the hydrogen bond geometries for the different classes of water-CO group interaction.

In the contour plot for α-helix CO groups (fig.1a), there is a clustering of points in the region centred about 2.8 Å (O-O separation), 125° (COO angle). This is the only significant 'peak' in the plot and corresponds to CO-water hydrogen bonds with close to optimum geometry. (Small molecule studies [10] give the optimum hydrogen bond geometry as 2.8 Å, 126°). A similar 'peak' is seen in the contour plot for coil/turn CO groups (fig.1b), although the contours here are more widely spaced, indicating a greater variation in the hydrogen bond geometries for these interactions.

However, in the plot for the peptide CO groups of β-sheet residues (fig.1c) there are 2 regions: one, equivalent to that seen in the α-helix and coil/turn plots, and a second centred around 3.3–3.7 Å, 115°. The density of points falling in this latter region indicates that a significant proportion of the water molecules, that are close to the peptide CO groups of β-sheet residues, lies beyond the distance commonly allowed for a hydrogen bond. However, an O-O distance of ~3.6 Å is considered appropriate for a non-bonded contact or Van der Waals interaction [11].

A detailed study of these hydration patterns, using computer graphics, shows that the water molecules that are in Van der Waals contact with the CO groups of β-sheet residues, are generally hydrogen bonded to other parts of the protein main-chain, or to neighbouring side-chain groups (see fig.2a). This is not the case, however, in α-

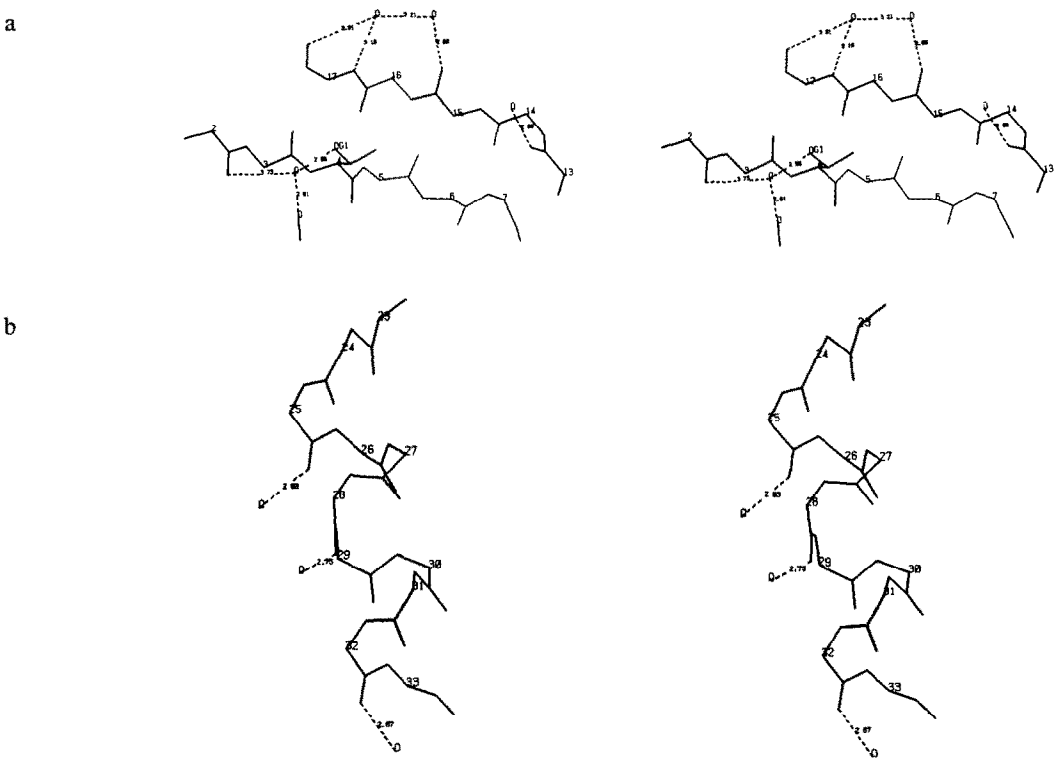

Fig.2. Stereo-views showing the interactions between water and main-chain CO groups in (a) 2 β-strands of γ-crystallin II, and (b) an α-helix from dihydrofolate reductase.

helices, where the water molecules are organised as a spine along one side of the structure, each molecule forming a 'good' hydrogen bond with a CO group, even when hydrogen bonded to a side-chain (fig.2b).

In conclusion, therefore, we find that the strength of water binding to the peptide CO groups of β-sheet proteins, is lower than in proteins composed mainly of α-helices, and that this is caused principally by differences in the geometry of the water-CO group interactions.

In our earlier work [5] we suggested that the unusual hydration properties of certain eye-lens proteins – the γ-crystallins might be a function of their high ion-pair contact and compacted β-sheet structure. The results presented here confirm the second of these hypotheses, and reinforce the view that water-protein interactions play an important part in biological systems.

ACKNOWLEDGEMENTS

This research was carried out at Birkbeck College, using the facilities of the Department of Crystallography. Both P.L.P. and D.J.B. acknowledge the support of the SERC, and would like to thank Birkbeck photographic unit for producing the photographs.

REFERENCES

[1] Finney, J.L. and Poole, P.L. (1984) Comments Mol. Cell. Biophys. 2, 129–151.
[2] Finney, J.L. and Poole, P.L. (1985) Proc. Int. Symp. Biomol. Struct. Interactions Suppl. J. Biosci. 8, 23–35.
[3] Artymiuk, P.J. and Blake, C.C.F. (1981) J. Mol. Biol. 152, 737–762.
[4] Baker, E.N. and Hubbard, R.E. (1984) Prog. Biophys. Mol. Biol. 44, 97–179.
[5] Poole, P.L. and Barlow, D.J. (1986) Biopolymers 25, 317–335.
[6] Bernstein, F.C., Koetzle, T.F., Williams, E.J.B., Meyer, E.J. jr, Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542.
[7] Lee, B. and Richards, F.M. (1971) J. Mol. Biol. 55, 379–400.
[8] Jones, T.A. (1978) J. Appl. Crystallogr. 11, 268–272.
[9] Richards, F.M. and Richmond, T. (1978) in: Molecular Interactions and Activity of Proteins (Porter, R. and Fitzsimons, D.W. eds) CIBA Foundation Symposium 60, Excerpta Medica, Amsterdam, Oxford.
[10] Ramakrishnan, C. and Prasad, N. (1971) Int. J. Protein Res. 3, 209–231.
[11] Quiocho, F.A. (1986) Annu. Rev. Biochem. 55, 287–315.
[12] Wistow, G., Slingsby, C., Blundell, T., Driessen, H., De Jong, W. and Bloemendal, H. (1981)
[13] Horintz, J. and Piatigorsky, J. (1980) Biochim. Biophys. Acta 624, 21–29.
[14] Narebor, M. and Slingsby, C. (1985) Exp. Eye Research 40, 273–283.