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Nuclear magnetic resonance studies of the hydration of proteins and DNA

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Abstract. The behaviour of water in the presence of proteins and DNA as elucidated by nuclear magnetic resonance is reviewed. The picture that emerges is that in dilute solution only those water molecules in the interior of the biopolymers or in clefts have their motions substantially affected. In concentrated systems the situation is more complicated because many more motions have to be considered, but there is no evidence of special effects due to the biopolymers being present. The case of nonfreezing water in protein solutions is considered, and it is suggested that this is not evidence for 'bound' water but simply due to the effects of the inhibition of protein precipitation.

Key words. Water; protein; DNA; hydration; NMR.

Introduction

The interactions of biopolymers with water have been studied by nuclear magnetic resonance (NMR) for 45 years. The first reports [1–3], on proton NMR, demonstrated the widening of the water resonance in the presence of DNA and in biological materials. At the time interpretation of the data was based on the current ideas about water biopolymer interactions. These tended to view the water surrounding proteins, and by implication other biopolymers, as highly structured and icelike [4]. Although this view was modified, there was a continuing tendency to regard the water around proteins and DNA as bound in some way. This view derived from two sets of observations. The first of these was that when a protein solution was cooled below the freezing point of water, some water remained unfrozen even down to very low temperatures [5]. The second of these was that the relaxation times of protons, deuterons or ^{17}O in water were all enhanced by the presence of biopolymers [6]. The implication of the enhancement was taken to be that the correlation times for motion of the water were increased.

The origins of the nonfreezing water in biopolymer solutions continue to be controversial, but recent developments in the use of high-field, high-resolution NMR and ^{17}O NMR relaxometry have offered a self-consistent explanation of the enhancement of water relaxation by biopolymers. As a consequence of this a new view of the dynamics of water in contact with biopolymers has arisen. This is reviewed in the next section.

The dynamics of water biopolymer interactions

The advent of very high field (600 MHz) proton NMR in the late 1980s made possible studies of the hydration of proteins that were much more detailed than before. Wüthrich and co-workers [7–10] exploited this opportunity in a detailed study of the hydration of bovine pancreatic trypsin inhibitor (BPTI) and other proteins in dilute solution. The first experiments on BPTI [7, 11] were only able to distinguish water protons with residence times of greater than 0.3 ns. Only four such waters were observed; these corresponded to four water molecules that had been observed crystallographically. The remaining water molecules were deduced to have residence times at the protein of less than 0.3 ns. Such a conclusion puts very severe limits on the possible binding of water to proteins and questions the whole concept of significant amounts of bound water. In further sophistication of the experiment [8–13], data on the faster-moving water molecules became available. These results suggested that the surface water associated with

BPTI had lifetimes in contact with the protein of 100–300 ps. However, the interpretation of the data was strongly dependent on the model chosen for the motions of the water molecules (for a discussion of the technicalities see [11]). Even though it was not possible to extract precise figures for the lifetimes of the water molecules, the findings did confirm the observation that only those molecules buried in the structure could be said to have any long-lived interaction with the protein.

An additional finding was that the water was generally associated with the protein surface and that the water molecules observed in the crystal structure as being associated with the surface showed no special behaviour. In effect the observation of surface waters in the crystal was fortuitous and had no significance for the behaviour of hydration water in solution.

In a further refinement, a detailed model calculation was carried on BPTI in aqueous solution and compared with experimental results [14]. Good consistency was found, and thus it was reasonable to assume that the residence times calculated represented realistic values. Apart from the four internal water molecules, it was found that the average residence time was 39 ps for water molecules near to the backbone and 24 ps for water molecules near to the side chains. The shortest residence times (19 ps) were found near charged atoms, whereas near to nonpolar and polar side chains the residence times were 25 and 36 ps, respectively. There was no correlation between the residence time of the water molecules and the type of secondary structure that the amino acid residue was in.

Similar experiments have been carried out in DNA solutions [15, 16]. The results suggest that the water associated with the major groove has residence times similar to those of surface waters in proteins. However, in the minor groove the residence times are similar to those observed in the buried waters of proteins. These water molecules correspond the spine of water observed in the minor groove in X-ray diffraction experiments.

In a more recent paper [17] Wüthrich et al. have reviewed the methods for using high-resolution NMR to obtain information about hydration of macromolecules and have presented results for the hydration of a protein DNA complex from both NMR measurements and model calculations. The complex consists of the homeodomain of *Antennapedia* bound to the major groove of DNA. One water molecule located at the DNA-protein interface has a residence time of between 400 and 1000 ps.

Whilst much data has been accumulated by high-resolution methods, precise values for correlation and residence times have not been forthcoming. NMR relaxometry offers a route to more precise data but needs a good theoretical model with which to work. Relaxometry measurements do not contain specific information about chemical species but measure the NMR relaxation times of nuclei. The relaxation times may then be related to correlation times for motion provided a useful model for motion can be found. Following the high-resolution proton NMR work, it might be thought that the most obvious nucleus for relaxometry studies would be protons. They are very abundant and are easy to measure. Unfortunately, exchange processes between the exchangeable protons of proteins and the water almost totally dominate the relaxation behaviour of protons [11], and thus useful information about dynamics is lost. A way of avoiding this is to use water enriched in ^{17}O and to observe this nucleus.

Stimulated by the results obtained by Wüthrich and co-workers, Denisov and Halle [18] used ^{17}O relaxometry as a way of obtaining detailed dynamic data. They studied BPTI, which has four internal water molecules and consists of 58 amino acid residues, and ubiquitin, which consists of 76 residues and has no internal water molecules. In BPTI they observed a large dispersion of relaxation but a much smaller one in ubiquitin. From this they inferred that the internal water molecules were responsible for the dispersion and that these had residence times inside the protein between 6 ns and 4 μs. For surface water molecules the rotational correlation times were of the order of 20 ps compared with typical values of 3 ps in bulk water at the same temperature. A more recent study [19] has confirmed these results.

The combination of ^{17}O and ^{2}H relaxometry has proved to be particularly effective in the analysis of protein hydration dynamics [20]. Although ^{2}H relaxometry suffers from some of the problems of proton relaxometry, the effects of exchange can be taken account of and useful information extracted. In a study of five proteins Denisov and Halle [20] concluded that a number of types of hydration water could be observed. These are water in cavities in the protein, water in clefts in the protein, water bound to metals in the protein and surface water. These are illustrated in figure 1 following the scheme of Denisov and Halle. Typically all these types of water, with the exception of the surface water, potentially have long correlation times. In the case of cleft water only water which has low accessibility and is hydrogen-bonded is considered to be likely to have long correlation times.

The general picture that emerges from both high-resolution NMR and relaxometry is that there is no evidence that any hydration water can be said to icelike and that only a very few water molecules per protein can be said to be bound in any sense. This is a far cry from the views of the early investigators, but some questions still remain to be answered by the current models. Two of the most pertinent are:

1) The results discussed above are obtained in dilute solutions. Do the deductions also apply to situations in which the protein in highly concentrated, as it is in organs such as muscle and tendon?

2) Why, if there is no bound water, is the phenomenon of nonfreezing water observed?

Water in concentrated biopolymer systems

A good starting place for the consideration of more concentrated systems is to consider what happens when polymers in a dilute solution are brought more closely together. This is illustrated in figure 2. In low dilutions the polymer units are far apart and there is bulk water between the polymers. As they move closer together the water becomes restricted between polymer units, and finally, at low water concentrations water is either in a monolayer form or trapped between polymer units. The effects of water trapping in pores and exchange phenomena in the NMR of inhomogeneous systems has been considered in detail by Hills [21].

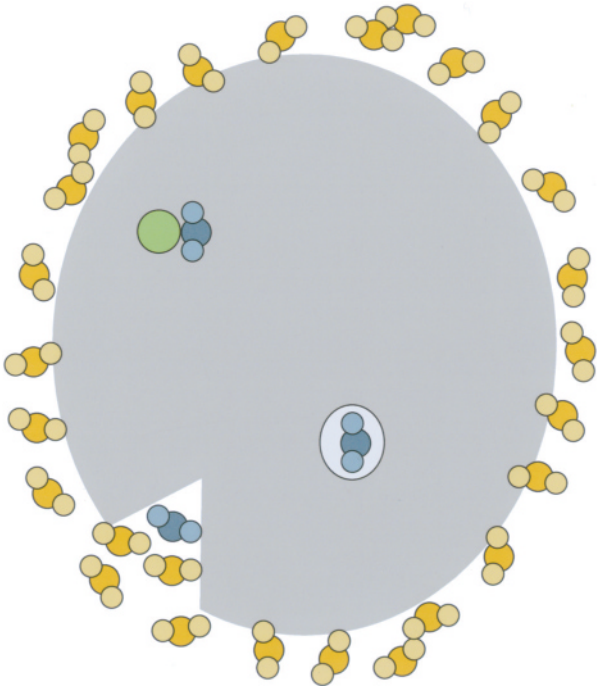

Figure 1. The possible hydration sites for a protein (after Denisov and Halle [20]) The water molecules in light blue are those with short correlation times. Those in dark blue are those with long correlation times. The three sites represented are a cleft site, an internal site and a site bound to a metal ion (shown in green).

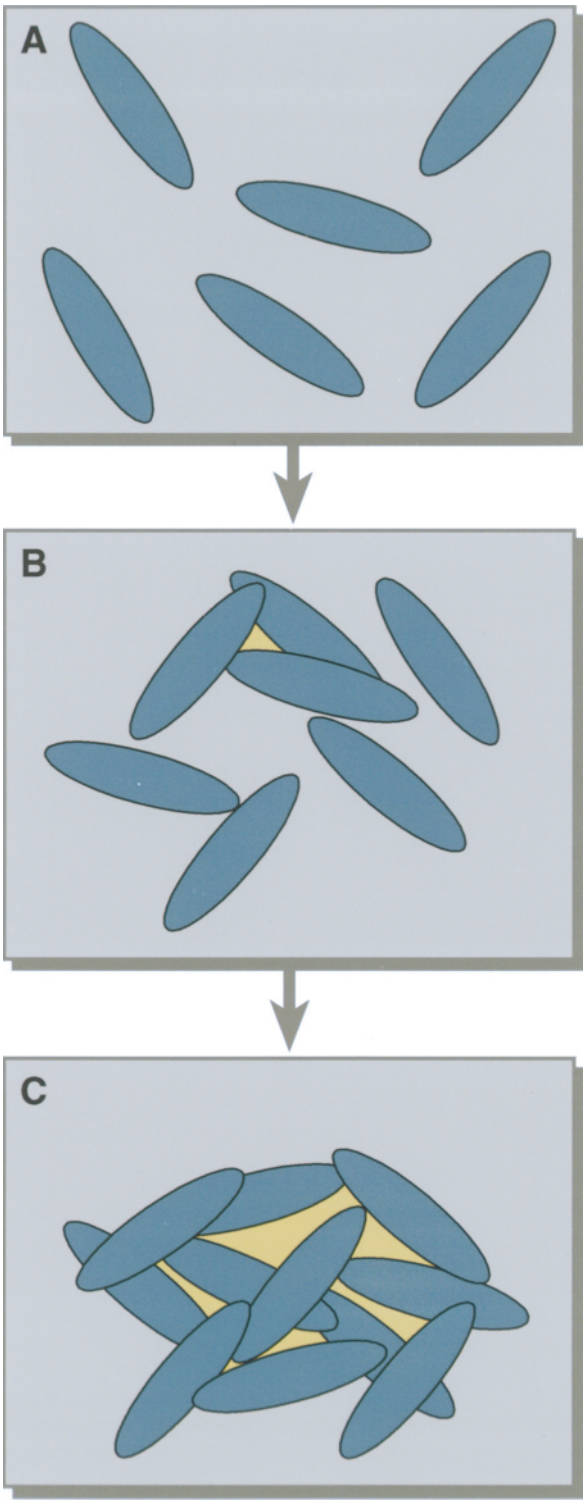

Figure 2. The effects of increasing concentration in a biopolymer solution. *(A)* The dilute case: there are no interactions between the polymers, and most of the water is in the bulk state. *(B)* The more concentrated case: some interaction and some trapped water, but most of the water is still in the bulk state. *(C)* The low water case: all of the water is either trapped or forms a monolayer around the protein.

In the case where the polymers exhibit local ordering, as may be the case with cylindrical polymers such as DNA or in ordered tissue such as muscle, there may be local anisotropy in the motion of the water as shown in figure 3. Under these circumstances the full description of the motion of the water becomes very difficult. The number of possible correlation times is illustrated in figure 3 [11, 22]. In the large spaces between the ordered regions there will be regions with properties similar to bulk water; these have a diffusion coefficient D_B, a rotational correlation time τ_B and a proton exchange rate τ_{EB}. Inside the ordered regions the water will rotate anisotropically with a correlation time τ_R and with a diffusion coefficient D_I. On the time scale of τ_I the water molecules will experience a variety of orientations of the ordered regions. The relative time scales of τ_I and the time scale for relaxation due static interactions resulting from anisotropic motion will determine the NMR line shape (for a further discussion see [11]). Water may also exchange between the ordered and bulk regions in exchange time τ_{ES}. Finally, there may be water bound in protein interiors or clefts characterised by a correlation time τ_P.

Whilst it is true that, as the biopolymer concentration increases the correlation times for water tend to reduce, even at water contents of the order of less than 30% the reduction in diffusion coefficient is only about one order of magnitude [23]. There is thus no real evidence that the effects of biopolymers on water, with the exception of internal waters, are any more than a physical restriction.

Nonfreezing water

Given that the evidence points towards a relatively nonspecific interaction between water and proteins and DNA, it seems surprising that these materials seem to have such a strong effect on the freezing point of water. The first systematic observers of this effect [24] felt that the observed effects must be due to a change in the

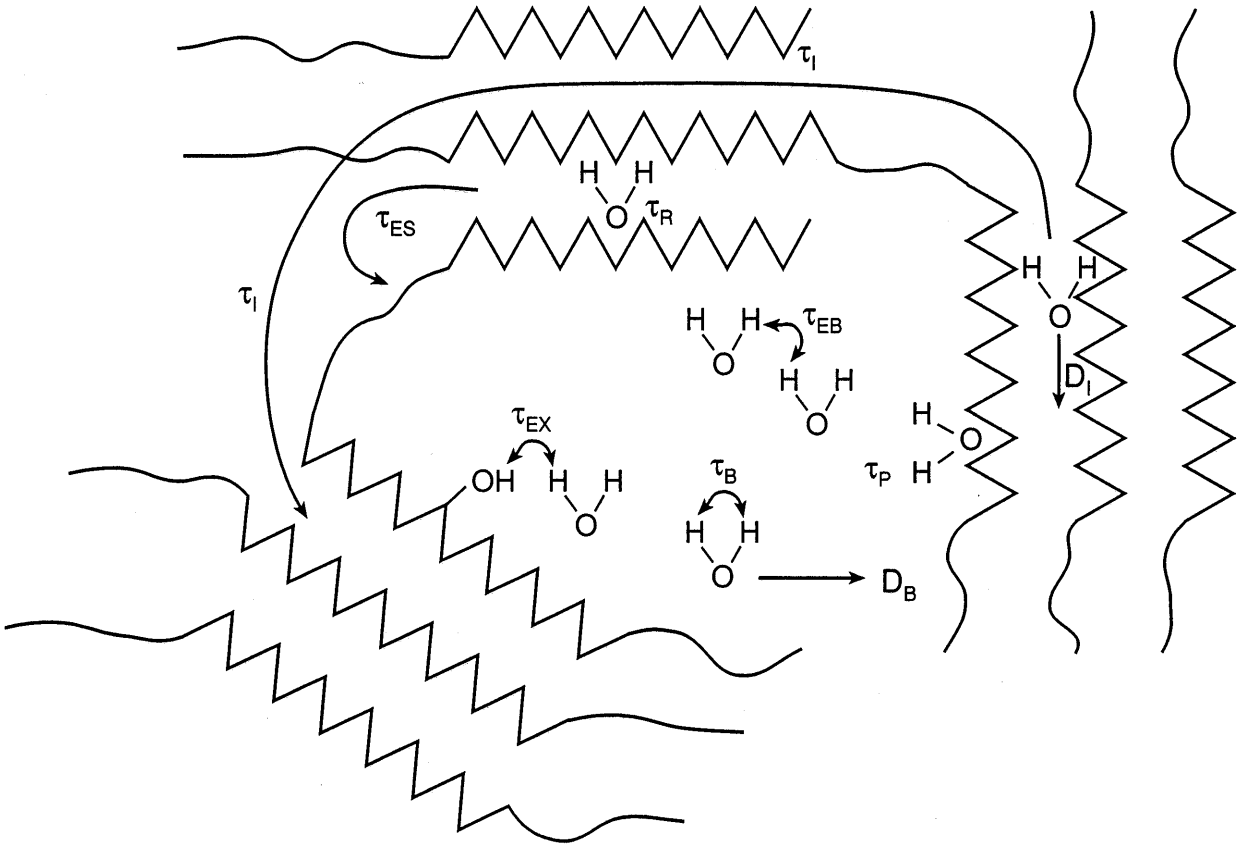

Figure 3. The motions that have to be considered in order to describe the dynamics of water protons in a locally ordered system. The details are given in the text.

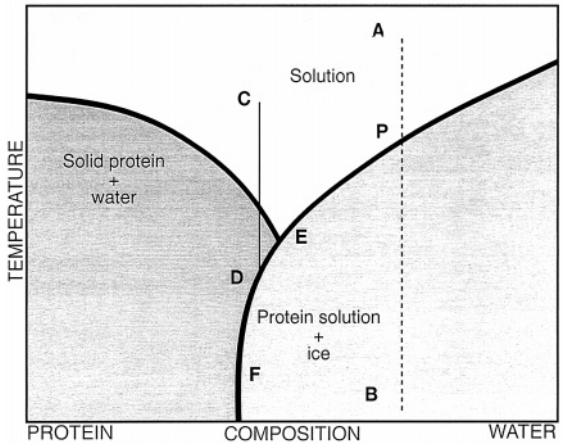

Figure 4. The phase diagram for a protein-water system. For explanation see text.

thermodynamic properties of water due to the presence of protein and that this must represent 'bound' water. The values obtained are remarkably consistent and are generally about 0.3 g of nonfreezing water per gram of protein [24].

Other arguments about the nonfreezing effect have centred on the formation of glassy states [25]; however, there is no credible evidence to support this contention [26]. A possible answer lies in the fact that cooled solutions of polymers are rarely at equilibrium. If they were, it would be easy to crystallise proteins. As the solution is cooled, there is a tendency to precipitate, but this is very often frustrated by entanglements or the difficulty of forming crystals in an increasingly viscous solution.

The situation is depicted in the phase diagram in figure 4. Consider a protein solution at point A in the diagram: as the temperature is lowered, the solution will remain homogeneous until the point P is reached; at this point ice will begin to form and the composition will follow the freezing point curve. At the eutectic point E it would be expected that protein would precipitate and at lower temperatures only solid protein and ice would exist. However, since the precipitation of the protein is inhibited, a nonequilibrium situation exists, and the ice continues to form. As the temperature decreases, therefore, the protein/water ratio increases as indicated by the line EF. The effect of this increase is to reduce the water activity coefficient, which in turn reduces the water vapour pressure. When the water vapour pressure is the same as the ice vapour pressure, there is no thermodynamic driving force for the formation of ice and hence no more ice is formed. The point at which this is likely to happen is when the activity is about 0.8, a value readily reached and consistent with the protein water ratios observed [21, 26]. The phenomenon of nonfreezing water can thus be explained in simple thermodynamic terms by a process that is readily understood. No appeal to binding of water or some special biopolymer/water interaction is required.

When the protein concentration is high, what happens depends on the starting points of temperature and concentration. At point C cooling will not result in precipitation of the protein at the freezing point curve, but solution will continue to exist with an unchanged composition until point D is reached. Here ice will be formed and the composition will follow the line DF with a mixture of ice and nonfreezing water. If the starting point is inside the freezing point curve in a situation where solid protein is added to water, or the composition is reached by the absorption of water vapour, solid protein and water will coexist, and homogeneous solution will not be formed.

Conclusions

The interactions of proteins and DNA with water are similar in kind to those of other aqueous solutes and are explicable readily in straightforward terms. This does not imply that solvation is not an important force in determining the behaviour of biopolymers. Solvation is important, but the nature of the interaction is generally nonspecific and with no special effect on the water dynamics or structure.

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1 Jacobson B, Anderson W. A. and Arnold T. (1954) A proton magnetic resonance study of the hydration of deoxyribonucleic acid. Nature **173:** 772–773
2 Hazelwood C. F., Nichols B. L. and Chamberlain N. F. (1969) Evidence for the existence of a minimum of two phases of ordered water in skeletal muscle. Nature **222:** 747–750
3 Chapman G. and Mclauchlan K. A. (1967) Oriented water in the sciatic nerve of rabbit. Nature **215:** 391–392
4 Klotz I. M. (1958) Protein hydration and behaviour. Science **128:** 815–821
5 Kuntz I. D., Brassfield T. S., Law G. D. and Purcell G. V. (1969) Hydration of macromolecules. Science **163:** 1329–1331
6 Mathur-de Vre R. (1979) The NMR studies of water in biological systems. Prog. Biophys. Mol. Biol. **35:** 103–134
7 Otting G. and Wüthrich K. (1989) Studies of protein hydration in aqueous solution by direct observation of individual protein bound water molecules. J. Am. Chem. Soc. **111:** 1871–1875
8 Otting G., Leipinsh E and Wüthrich K. (1991) Proton exchange with internal water molecules in the protein BPTI in aqueous solution. J. Am. Chem. Soc. **113:** 4363–4364
9 Otting G., Leipinsh E., Farmer B. T. and Wüthrich K. (1991) Protein hydration studied with homonuclear 3D ^{1}H NMR experiments. J. Biomolecular NMR **1:** 205–209

10 Wüthrich K and Otting G. (1992) Studies of protein hydration using in aqueous solution by high resolution nuclear magnetic resonance spectroscopy. Int. J. Quantum Chem. **42:** 1553–1561
11 Belton P. S. (1994) NMR studies of protein hydration. Prog. Biophys. Mol. Biol. **61:** 61–79
12 Kriwacki R. W., Hill R. B., Flanagan J. M., Caradonna J. P. and Prestgard J. H. (1993) New NMR methods for the characterisation of bound waters in macromolecules. J. Am Chem. Soc. **115:** 8907–8911
13 Otting G., Leipinsh E and Wüthrich K. (1991) Protein hydration in aqueous solution. Science **254:** 974–980
14 Brunne R. M., Leipinsh E., Otting G., Wüthrich K. and van Gunsteren W. F. (1993) Hydration of Proteins a comparison of experimental residence times of water molecules. J. Mol. Biol. **231:** 1040–1048
15 Kubinec M. G. and Wemmer D. E. (1992) NMR evidence for DNA bound water in solution. J. Am Chem. Soc. **114:** 8739–8740
16 Otting G., Leipinsh E. and Wüthrich K. (1992) NMR observation of individual molecules of hydration water bound to DNA duplexes: direct evidence for a spine of hydration water present in aqueous solution. Nucleic Acids Res. **20:** 6549–6553
17 Wüthrich K., Billeter M., Güntert P., Luginbühl P., Riek R. and Wider G. (1996) NMR studies of the hydration of biological macromolecules. Faraday Discuss. **103:** 245–253
18 Denisov V. P. and Halle B. (1994) Dynamics of the internal and external hydration of globular proteins. J. Am. Chem. Soc. **116:** 10324–10325
19 Denisov V. P. and Halle B. (1995) Protein hydration dynamics in aqueous solution: a comparison of bovine pancreatic trypsin inhibitor and ubiquitin by oxygen-17 spin relaxation dispersion. J. Mol. Biol. **245:** 682–967
20 Denisov V. P. and Halle B. (1996) Protein hydration dynamics in aqueous solution. Faraday Discuss. **103:** 227–244
21 Hills B. P. (1998) Magnetic Resonance Imaging in Food Science, Wiley, New York.
22 Belton P. S. (1997) NMR and the mobility of water in polysaccharides. Int. J. Biol. Macromol. **21:** 81–88
23 Kotitschke K., Kimmich R., Rommel E. and Parak F. (1990) NMR study of diffusion in protein hydration shells. Prog. Coll. Polymer Sci. **83:** 211–215
24 Kuntz I. D. and Kauzmann W. (1974) Hydration of proteins and polypeptides. Adv. Protein Chem. **28:** 239–345
25 Franks F. (1986) Unfrozen water: yes unfreezable water: hardly; bound water certainly not. Cryoletters **7:** 207–208
26 Belton, P. S. (1991) The physical state of water in foods. In: Food Freezing: Today and Tomorrow, pp. 1–13, Bald W. B. (ed.), Springer, London.