Short Note

EXPERIMENTAL CONFIRMATION, FROM MODEL STUDIES, OF A KEY PREDICTION OF THE POLARIZED MULTILAYER THEORY OF CELL WATER

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According to a corollary of the association-induction hypothesis[1] the bulk of cell water exists in a state of polarized multilayers.[2–4] That state is attributed to water interaction with a matrix of alternating negative CO (N) and positive NH (P) sites on certain extended protein chains widely present in living cells. Such a matrix of alternating N and P sites is termed an NP-NP-NP system.

In this approach, the polarized multilayers of cell water can partially exclude certain solutes, including hydrated ions such as Na^+, sugars, and free amino acids.[2,3,5] For proper function of the polypeptide chains as well as their models of synthetic polymers, direct exposure of the N and P sites to the water molecules is essential. The formation of intramolecular H-bonds as in the α-helical conformation, or the formation of intermolecular H-bonds as in the β-pleated sheet conformation, annuls this inherent capability of the protein chains to bring about the long-range ordering of water and the consequent reduction of solubility of that water for the multiatomic or de *facto* multiatomic solutes mentioned above.

Recent experimental studies in our laboratory have led to the conclusion that water in concentrated solutions of the native globular proteins investigated indeed does not exclude, or excludes only weakly, solutes such as Na salts, sucrose, and amino acids. But the findings confirmed, on the other hand, that a concentrated sol or gel of gelatin—because it possesses considerable proline and hydroxyproline and thus an indisposition to form a-helical folds—offers a substantial portion of its NH-CO groups for direct interaction with and polarization of deep layers of water molecules. These layers then show pronounced ability to exclude Na+ and other solutes, thus confirming the theoretical prediction.

The theory that the gelatin-water system owes its solute exclusion property to the inability of gelatin to form intramolecular hydrogen bonds was further confirmed by an extensive series of studies of synthetic polymers and denatured proteins. Studies of the synthetic polymers additionally revealed that of the N and P sites of a protein NP-NP-NP system, it is the carbonyl oxygen that plays the dominant role. Polymers containing oxygen in the carbonyl form as well as in various other forms

are capable of exercising long-range ordering effect of water molecules even when the alternating sites on the chains are not positively charged but are merely vacant.

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REFERENCES

1. G. N. Ling, ***A Physical Theory of the Living State: The Association-Induction Hypothesis,*** Blaisdell, Waltham, Massachusetts, 1962.
2. ———, "The physical state of water in living cell and model systems," ***Ann.*** NY ***Acad. Sci.,*** *125,* 401 (1965).
3. ———, "Hydration of macromolecules," in ***Water and Aqueous Solutions, A.*** Horne, Ed., Wiley-Interscience, New York, 1972, pp. 201–213.
4. ———, "Water structure at the water-polymer interface," in ***Water Structure at the Water-Polymer Interface,*** H. H. Jellinek, Ed., Plenum, New York, 1972, pp. 4–13.
5. G. N. Ling, C. Miller and M. M. Ochsenfeld. "The physical state of solutes and water in living cells according to the association-induction hypothesis," ***Ann.*** NY ***Acad. Sci., 204,*** 6 (1973).
6. G. N. Ling and W. Negendank, "The physical state of water in frog muscles," ***Physiol. Chem. Phys., 2,*** 15 (1970).

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