Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta 1749 (2005) 214 – 233

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

http://www.elsevier.com/locate/bba

Invited Review

Temperature excursion infrared (TEIR) spectroscopy used to study hydrogen bonding between water and biomolecules

Jane M. Vanderkooi*, Jennifer L. Dashnau, Bogumil Zelent

Johnson Research Foundation, Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6059, USA

Received 6 October 2004; received in revised form 15 February 2005; accepted 9 March 2005
Available online 8 April 2005

Abstract

Water is a highly polar molecule that is capable of making four H-bonding linkages. Stability and specificity of folding of water-soluble protein macromolecules are determined by the interplay between water and functional groups of the protein. Yet, under some conditions, water can be replaced with sugar or other polar protic molecules with retention of protein structure. Infrared (IR) spectroscopy allows one to probe groups on the protein that interact with solvent, whether the solvent is water, sugar or glycerol. The basis of the measurement is that IR spectral lines of functional groups involved in H-bonding show characteristic spectral shifts with temperature excursion, reflecting the dipolar nature of the group and its ability to H-bond. For groups involved in H-bonding to water, the stretching mode absorption bands shift to lower frequency, whereas bending mode absorption bands shift to higher frequency as temperature decreases. The results indicate increasing H-bonding and decreasing entropy occurring as a function of temperature, even at cryogenic temperatures. The frequencies of the amide group modes are temperature dependent, showing that as temperature decreases, the amide group H-bonds to water strengthen. These results are relevant to protein stability as a function of temperature. The influence of solvent relaxation is demonstrated for tryptophan fluorescence over the same temperature range where the solvent was examined by infrared spectroscopy.

Keywords: H-bonding; Amide; Isotope; Water

1. Introduction

Questions about water interactions with macromolecules are many. What is the special nature of water that allows it to be fluid at physiological temperatures? How do water molecules bond to each other? How do they bond to macromolecules? How can water – a fluid – be required to stabilize the structure of macromolecules?

To address these questions, we need to consider the forces involved between non-covalently bonded molecules. To study molecular interactions, infrared (IR) techniques are increasingly being utilized as versatile tools. IR absorption measures frequencies of light on the order of 10^{13} s^{-1}. The resolution of this technique is essentially atomic since vibrations of atomic groups are seen. The popularity of IR spectroscopy is rising due to a variety of factors including: (1) development of rapid kinetic techniques to monitor relaxation of vibrational levels; (2) ability to synthesize peptides and proteins with known structure and with isotopic labeling; and (3) development of computational methods for spectral analysis. In this review, we emphasize a fourth property of IR: that IR spectral lines are temperature dependent. We call the analysis of IR spectra measured at different temperatures the temperature excursion infrared (TEIR) technique. We summarize work on the nature of water in commonly used cryo-solvents, namely glycerol and sugar matrices. Water interactions with the protein amide group and other protein groups are also summarized.

Forces that determine structure also affect the vibrational and optical spectra in characteristic ways. It follows that by examining the spectra, we can begin to understand the nature and strength of forces that determine macromolecular

---

* Corresponding author. Tel.: +1 215 898 8783.
E-mail address: vanderko@mail.med.upenn.edu (J.M. Vanderkooi).

1570-9639/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2005.03.008

structure. TEIR techniques will be described, but water has been studied by an arsenal of techniques. Chaplin has posted a highly useful Web site on water with comprehensive referencing [1].

2. Water

On the periodic table, H is the first element and oxygen is the eighth. The electron of H is in the 1s orbital and oxygen distributes its 8 electrons in orbitals as follows: 2(1s), 2(2s), 4(2p). The s orbitals are spherical, while the p orbitals can be described by:

$$p_x = \sin\theta\cos\theta; \quad p_y = \sin\theta\sin\theta; \quad p_z = \cos\theta. \qquad (1)$$

In H_2O, the two hydrogen atoms supply their 1s electrons to fill the 2p orbitals of O. The orbitals hybridize, and the 4 bonding orbitals arrange in an approximate tetrahedral shape around the O. Two of the lobes contain the positively charged hydrogen atoms, and the other two lobes contain the two lone pair electrons, producing high electron density on these lobes. The electrons produce large electrostatic effects, depicted in a contour map in Fig. 1.

The arrangement of electrons in water makes for a very strongly dipolar molecule. The Mullikan population of charge on each H shown in Fig. 1 is +0.42035 e and the O has a charge of −0.8407 e. An electron charge corresponds to 1.6022×10^{-19} C. The distance between O and H is calculated to be 0.943 Angstrom. Using the H−O−H bond angle equal to 105°, the calculated dipole moment μ=2.3 D (1 Debye (D)=3.336×10^{-30} C×m). From experimental measurement, gaseous water has an electric dipole moment of μ=1.8 D [2].

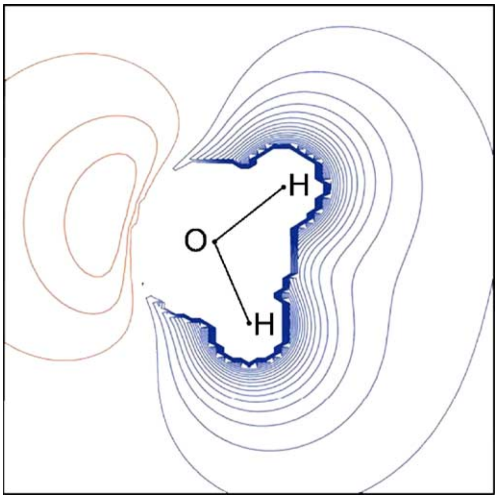

Fig. 1. The map was made following these procedures: (1) geometry optimization of water molecule using HF/6-31G* level of theory in Gaussian98. (2) Calculation of atomic charges in Gaussian98 using HF/6-31G* GFINPUT IOP(6/7=3) pop=full geom=check guess=check. (3) Visualization of electrostatic map in MOLDEN. The electrostatic map has two color contours. The blue contours (2–20) represent positive values with increments of 0.025. The red contours (21–23) represent negative values with the same increments. The 0.000 contour is not shown. The HOH angle is 103.742° and the O−H distance is 0.943 A. The calculated charge on O was −0.8407 and on each H was +0.42035.

3. Types of bonding

A discussion of bonding between molecules can be found in Israelachvili's book [3] and on the web site of Austin [4]. Dipolar interactions and hydrogen bonding are the major bonding forces determining water–water interactions and water interactions with other compounds. Two other bonding forces, Coulombic and van der Waals, have less influence on water–water interactions, but they also affect spectra of groups in macromolecules in characteristic ways.

3.1. Dipolar interaction

Based solely upon charges, the water in condensed phases would arrange so that two H's of the neighboring water would be associated with the lone pair electrons of the O.

The interaction energy, $w(r)$, between fixed dipolar residues is:

$$w(r) = \mu_1\mu_2\left[2\cos\theta_1\cos\theta_2 - \sin\theta_1\sin\theta_2\cos\phi\right]/4\pi\varepsilon_o r^3) \qquad (2)$$

where μ is the electric dipole moment (C m), the sin and cos terms are geometric factors expressing the relative orientation of dipole 1 and 2, ε is the dielectric permittivity of free space and r is the center-to-center dipole distance. The interaction energy falls off rapidly with distance. The distance dependence predicts that nearest neighboring groups have the largest effects on any group. The ordering by the dipolar effect is approximately equal to kT. Statistically, the water molecules have many possibilities for arrangement. Therefore, water has a large amount of entropy that increases as temperature increases.

If defects that disrupt the attraction patterns of water due to dipolar interactions are put in the lattice, entropy can decrease. This is the origin of the hydrophobic effect, and it is the reason why hydrocarbons do not dissolve in water. The hydrophobic effect is accepted as a major basis for protein stability [5]. This effect reduces the possibility of interactions between water molecules making it energetically unfavorable for a non-polar group to be in water. The entropy decrease thus raises the net-free energy of the system. Stable, water-soluble proteins have their hydrophobic groups buried inside.

3.2. "The name's Bond. Hydrogen Bond"

The second type of interaction that we consider is hydrogen bonding (H-bonding). Hydrogen bonds keep