

Hydrogen/deuterium isotope effects in water and aqueous solutions of organic molecules and proteins



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ABSTRACT

It is pointed out that hydrogen/deuterium substitution, frequently used in neutron scattering studies of the structure and dynamics of hydrogenous samples, can have significant effects on structure, phase behavior and protein stability. The effects must be kept in mind in the interpretation of such experiments. In suitable cases, these effects can be mitigated by an appropriate choice of experimental conditions.

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1. Introduction

Hydrogen/deuterium (H/D) isotope substitution is frequently used to extract partial structure information from neutron diffraction measurements. The intensity measured in a single diffraction measurement, after appropriate corrections, can be written

$$I(Q) = |\bar{b}|^2 S(Q) = \sum_{ab} c_a c_b \bar{b}_a \bar{b}_b S_{ab}(Q) \quad (1)$$

where \bar{b}_a is the coherent scattering length for element a and $S(Q)$ and $S_{ab}(Q)$ are, respectively, the average structure factor and partial structure factor for the element pair (a, b) . The partial structure factors are in turn related to the partial pair correlation functions $g_{ab}(r)$ that convey detailed structural information related to the physics and chemistry of the liquid:

$$S_{ab}(Q) = 4\pi\rho_0 \int_0^\infty r^2 [g_{ab}(r) - 1] \frac{\sin Qr}{Qr} dr \quad (2)$$

The partial structure factors $S_{ab}(Q)$ are obtained from measurements on samples of different isotopic composition (e.g., with H_2O , D_2O and mixtures of the two) on the assumption that only the scattering lengths \bar{b}_a change, not the $S_{ab}(Q)$. For an n -component system, $n(n+1)/2$ or more independent measurements are carried out. An example of partial structure factors of water obtained by Soper [1] with this method is shown in Fig. 1.

H/D substitution is also used to separate the dynamical behavior of specific components in aqueous solutions. The scattering

then comes principally from the components with natural hydrogen and that from the deuterated or partially deuterated components is either neglected or accounted for in some manner. A typical example is the work of Saboungi and collaborators on glucose solutions, in which natural glucose in D_2O was measured to determine the dynamics of the sugar molecules [2], while selectively deuterated glucose in H_2O was measured to obtain the water dynamics [3]. In neutron spin-echo measurements on hydrogenous materials, it is often advantageous to use deuterated specimens to avoid the negative contribution to the scattering from the spin-incoherent scattering from ^1H [4]. Furthermore, in a variety of spectroscopic techniques for protein structure determination, it is standard procedure to dissolve the protein in D_2O instead of H_2O [5]. It is recognized of course that the dynamics of a particular component will be modified by the change of total mass, e.g., the unavoidable partial deuteration of the sugar molecules in the first measurement just alluded to. Nevertheless, there is an underlying assumption that the deuterated system is essentially the same as the one being investigated, and that computer simulations of systems containing only natural hydrogen can be compared directly with experimental data.

The aim of this short paper is to point out that H/D substitution can have significant effects on structure, phase behavior and protein stability. These effects should be kept in mind in the interpretation of such experiments.

2. Effects on structure

The structural differences between H_2O and D_2O have been studied by several groups with X-ray diffraction [6–8]. Differences

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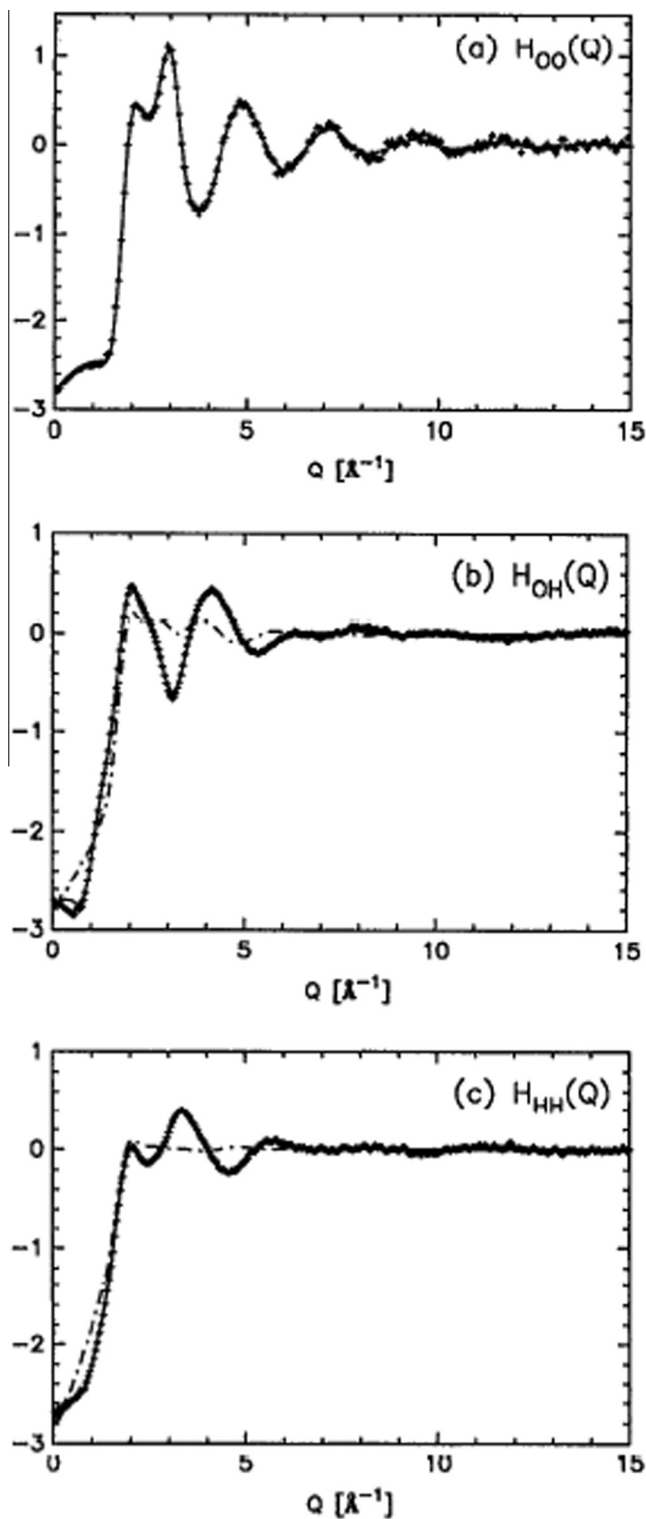


Fig. 1. Partial structure factors of water obtained by neutron diffraction with H/D isotope substitution. From Soper [1].

in thermodynamic properties such as density and melting point are, of course, well known (Fig. 2), and must be taken into account in structural comparisons. Badyal et al. [7] measured differences in diffraction patterns between H₂O and D₂O at the same temperatures and densities, corresponding to the four points shown in Fig. 2. The differences, shown in Fig. 3 for the equal-temperature measurements, are relatively small after correcting for the density

difference of the two samples, yet they are nevertheless real and experimentally reproducible.

Fig. 4 shows the results for the isothermal difference structure factor

$$\Delta_T D_X(Q) = D_{X,H_2O}(Q) - D_{X,D_2O}(Q) : T = 14.5^\circ\text{C}$$

where $D_X(Q) = [S(Q) - 1]$. Broad, asymmetric peaks are evident at $\sim 2.3 \text{ \AA}^{-1}$, roughly covering the region between the two first peaks in the total $D_X(Q)$, and 3.7 \AA^{-1} , where $D_X(Q)$ has a local minimum. In order to compare the measured quantum effects with thermal effects produced by a temperature increase, the figure shows the isochoric temperature differential (ITD) corresponding to a 5.5°C temperature increase in D₂O, obtained from the measurements of Bosio et al. [9], as a dashed lines. The general similarity of the two results confirms the idea that the larger quantum effects in H₂O can be represented to some extent by an additional degree of thermal disorder. However, significant discrepancies remain, with the main peak in $\Delta_T D_X(Q)$ shifted to lower Q relative to the ITD.

In the Fourier transform of the structure factors to real space (not shown), the nearest-neighbor O–O distance at 2.85 \AA falls on the leading edge of the main peak in $\Delta_T g_X(r)$, indicating an outward shift on the nearest-neighbor O–O distance in H₂O compared with D₂O. The effective compression of the oxygen framework in going from H to D can be rationalized in terms of a decrease in spatial delocalization of D relative to H, an effect which is of an intrinsic quantum-mechanical nature [10]. Results from neutron diffraction measurements [8,11] indicate that the intramolecular O–H distance is also longer in H₂O than D₂O, although there is a disagreement in the two studies about the magnitude of the shift and also about the difference in the length of the O–H hydrogen bond.

Equivalent data for the isochoric difference structure factor

$$\Delta_\rho D_X(Q) = D_{X,H_2O}(Q; 21.0^\circ\text{C}) - D_{X,D_2O}(Q; 34.5^\circ\text{C})$$

are shown in Fig. 5. The isochoric results exhibit some significant differences relative to the isothermal case. The most obvious is an increase in amplitude by a factor of three (cf. ordinate scales of Figs. 4 and 5). Taking account of the 13.5°C difference in the measurement temperatures for H₂O and D₂O, together with the rough equivalence of the isothermal difference to an ITD of 5.5°C , one could expect the isochoric difference to be approximated by a 19°C ITD. Fig. 5 compares the isochoric difference data with the ITD's corresponding to a 19°C temperature increase in D₂O, obtained from the measurements reported in Ref. [9]. The agreement is quite good and the phase shifts are less pronounced than in the equivalent comparison for the isothermal case. Also shown in Fig. 5 is the 'temperature corrected' $\Delta_\rho D_X(Q)$ obtained by subtracting the ITD's corresponding to the 19°C temperature difference from the measured isochoric differences. These data are similar in phase to the ITD itself.

3. Effects on phase behavior

Phase transitions and critical phenomena can be expected to be particularly sensitive to the details of molecular interactions. Of particular interest is the topic of re-entrant phase transitions in multicomponent liquid mixtures [12], characterized by a closed-loop coexistence curve. The first careful determination of a closed-loop phase diagram was made by Cox [13], who studied the 3-methylpyridine + D₂O binary system (3MP/HW). Fig. 6 shows a rendering of his results. He also showed how the loop size ΔT of this curve can be shrunk by replacing D₂O by H₂O, disappearing altogether with complete replacement.

Maira-Vidal et al. [14] performed small-angle neutron scattering (SANS) and quasielastic neutron scattering (QENS) measurements of this system along the two dotted lines shown in Fig. 6.

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