Mean Square Fluctuations of Hydrogen Atoms and Water-Biopolymer Interactions in Hydrated Saccharides

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ABSTRACT We have used the elastic neutron scattering technique to investigate the dynamics of the two main saccharidic components of starch: amylose and amylopectin. The measurements were carried out in the temperature range of 20 to 320 K and at different hydration levels from the dry state up to 0.47 g saccharide/g D_2O. In the dry samples, the atomic dynamics is harmonic up to approximately 300 K. In the hydrated samples a "glass-like" transition leading to an anharmonic dynamics is observed. The onset of the anharmonicity occurs at temperatures that increase from ~180 K to 260 K upon decreasing hydration from 0.5 to 0.1 g saccharide/g D_2O. This behavior is qualitatively similar to that observed in hydrated globular proteins, but quantitative differences are present. Assuming a simple asymmetric double-well potential model, the temperature and hydration dependence of the transition have been described in terms of few physical parameters.

INTRODUCTION

Carbohydrates play an important role in a wide range of biological processes. Their role may be structural (cellulose in the cell walls of plants, chitin in the outer skeleton of crustaceans, peptidoglycans in the bacteria cell wall), or functional (they are present in the corneal tissue, in the vitreous humor, and in the synovial fluid). Glucose is the primary energy source for living cells, and starch and glycogen are its storage forms for plants and animals, respectively.

In this work we focus on the two main components of starch: amylose and amylopectin. Amylose constitutes 20–30% of total starch. It is a linear polymer of glucose (from 600 to 6000 units) in which the D-glucose residues are connected through (1→4) α-D-glucosidic linkages. Amylopectin, the other component, (70–80% of total starch), has the same primary structure, but (1→6) branching bonds are also present in the average proportion of 1 (1→6) every 12–15 (1→4) linkages. The average unbranched chain length of amylopectin ranges between 15 and 30 units, and the degree of polymerization is of the order of 10^5 to 10^6 glucose units (Zobel, 1988; O'Sullivan et al., 1999).

Structural studies have shown that the conformation of native crystalline amylose in the granules of cereals (A form), and in the tubers (B form) consists of left-handed double helices with intertwined parallel chains (Wu and Sarko, 1978; Imberty et al., 1988), and with an alternating distribution of hydrophilic and hydrophobic regions over the entire surface (Immel, 1995). Several authors have evidenced the role of water molecules in establishing the three-dimensional organization of starch (Slade et al., 1993; Rindlav et al., 1997). A variety of complex structures from ordered fiber packing at moderate hydration level to dilute gels can be formed, depending on the degree of water association. Water may act also as a plasticizer inducing some degree of alignment of the saccharidic units in starch (French, 1998). At low hydration levels up to a water content of ~30% a considerable ordering of water around the saccharidic helices occurs, whereas above 33%, there is some evidence of the presence of more mobile and "freezable" water (Lechert, 1981). Such behavior implies that water comes into play not only in establishing the structural features of these polysaccharides, but also in determining their dynamics at the molecular level. Moreover, recent developments in food science focus on the role of hydration in food processing, indicating that water is in a dynamically constrained glassy state, rather than in an equilibrium/thermodynamic phase. Concepts and models developed in the field of synthetic polymer science are currently used in food polymer science, leading to new insights and advances beyond the traditional concepts involving water activity and chain mobility (Kulik et al., 1997). This approach requires a deeper insight on the dynamical properties, and particularly on the processes around the glass transition that play an important role in the stabilization of food systems.

Spectroscopic studies providing information on water-saccharide interactions are of interest not only for application-oriented investigations but also for basic biophysical studies. Studies on the local dynamics of polysaccharidic chains are interesting for a comparison with other biopolymers such as polypeptides. It is well known that the atomic dynamics of globular proteins can be described in terms of models originally developed for amorphous systems (Frauenfelder et al., 1985). It has been shown that hydration in proteins above ~200 K induces a dynamic transition, similar to the glass transition in disordered systems, from a simple harmonic dynamics to an anharmonic behavior (Parak et al., 1988; Doster et al., 1989, 1999; Andreani et al., 1995; Filabozzi et al., 1995).

Received for publication 6 February 2001 and in final form 18 April 2001.
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0006-3495/01/08/1190/05 $2.00

Neutron inelastic and elastic scattering are very suitable techniques to investigate this kind of dynamical transitions because they provide information on the atomic motions in the space (1–10 Å) and time (0.1–100 ps) scales relevant for these processes. In this work, we report results of elastic neutron scattering investigations on the temperature and hydration dependence of the atomic dynamics in amylose and amylopectin.

MATERIALS AND METHODS

Amylose (type III, from potato) and amylopectin (from potato) were purchased from Sigma-Aldrich Biochemicals Inc. (St. Louis, MO). The powders were first dried upon heating to 90°C in inert atmosphere, and then rehydrated in saturated D_2O atmosphere. The conditioning cycle was then repeated to ensure full H/D substitution of all the exchangeable hydrogens, and also the complete removal of any residual preparation butanol. Then a quantity corresponding to 0.3 g of dry polysaccharide was kept in a saturated D_2O atmosphere until the required hydration level was reached. The samples were sealed in a vacuum-tight aluminum cell with 0.5-mm aluminum windows suitable for the neutron experiments. The investigated hydrations, h (g. D_2O/g polysaccharide) were 0, 0.12, and 0.47 for amylose, and 0, 0.15, 0.32, and 0.40 for amylopectin.

Because of the large n-p incoherent cross-section of protons (80.26 barn) with respect to that of deuterium (2.05 barn), the neutron signal measured is essentially attributable to the nonexchangeable protons of the saccharidic chains. The one attributable to exchangeable hydrogens and to the water hydrogens is <4% of the total.

Elastic neutron scattering

The incoherent elastic neutron scattering scans were performed using the thermal backscattering spectrometer IN13 at the Institut Laue-Langevin (Grenoble). IN13 makes use of thermal neutrons selected by the (442) Bragg reflection of a CaF_2 monochromator ($\lambda = 2.23$ Å, $E = 16.45$ meV). The beam scattered from the sample is reflected in backscattering geometry by a set of CaF_2 analyzers and then finally collected by an array of 35 He^3 detectors.

On IN13 quasielastic neutron scattering spectra are obtained by varying the monochromator temperature with respect to that of the analyzers, whereas in the elastic scan configuration, the temperature of the monochromator is kept equal to that of the analyzer crystals. The measured elastic scattering intensity ($I_{el} \equiv S(Q,\omega = 0)$), in the present case, corresponds to a window of ± 4 μeV of accepted tolerance approximating $\omega = 0$ (8 μeV being the instrumental energy resolution [FWHM]). Because of the relatively high energy of the neutrons selected by the monochromator, IN13 can access quite a large range of momentum transfer, Q ($Q = 4\pi \sin\theta/\lambda$, 2θ being the scattering angle), i.e., from 0.3 to ~5.5 $Å^{-1}$. This makes IN13 an ideal instrument to investigate the anharmonic behavior of the mean square atomic displacement that is usually observed in hydrated biopolymers.

Data analysis

The elastic scattering intensities were corrected for empty cell contribution and normalized to a vanadium standard to compensate for detector efficiency. Fig. 1 shows, in a three-dimensional representation, the Q and temperature dependence of the elastic intensity curves measured for amylose at $h = 0.47$. It can be seen that the $\ln(I_{el})$ curves are linear versus Q^2 at the lower temperatures, whereas a nonlinear behavior is observed above 190 K with a change in slope around $Q^2 \sim 10$ $Å^{-2}$. This behavior is reminiscent of the one observed in amorphous solids on approaching the

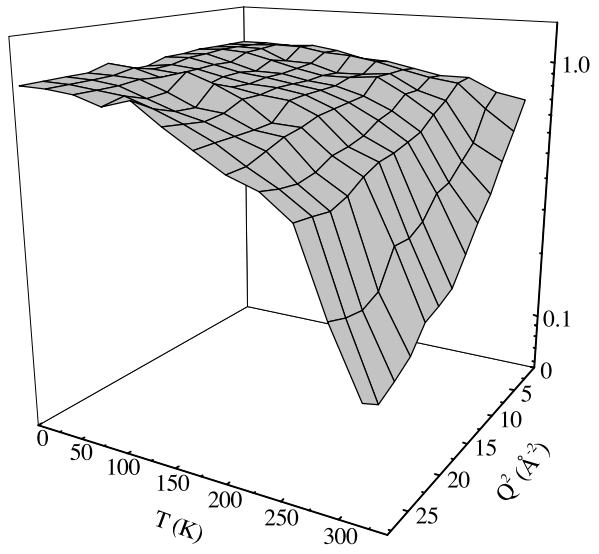

FIGURE 1 Temperature- and Q-dependence of the normalized elastic scattering intensities for amylose at hydration $h = 0.47$. The onset of the kinetic transition is visible as a marked decrease of I_{el} vs. T, as well as a nonlinear dependence of lnI_{el} upon Q^2.

glass transition temperature, T_G, as well as in various hydrated polypeptides. In hydrated globular proteins, it has been attributed to the onset of conformational motions of the side chains of the polypeptide scaffolding (Doster et al., 1989; Andreani et al., 1995; Filabozzi et al., 1995). It can be described in terms of a model involving an asymmetric double-well conformational potential (Stoeckli et al., 1986; Doster et al., 1989). This model is a simplification of a more realistic one that should consider a manifold of conformational minima. However, in the case of globular proteins, the model has been able to describe quantitatively the main features of the temperature-dependent dynamic transitions observed. We have adopted the same simplified model with the aim of comparing the dynamic behavior of quite different biopolymeric structures. In this frame the elastic scattering intensity I_{el} can then be written as:

$$I_{el} \equiv S(Q, \omega = 0) = e^{-Q^2\langle u_G^2\rangle} \times \left[1 - 2p_1p_2\left(1 - \frac{\sin(Qd)}{Qd}\right)\right] \quad (1)$$

where $\langle u_G^2\rangle$ represents the sum of all the quasiharmonic (Gaussian) contribution to the total atomic displacement; $p1$ and $p2$, are the occupation probabilities for the two minima; and d is the "distance" between them. The Gaussian mean square displacement $\langle u_G^2\rangle$ was mainly determined from the data in the harmonic regime, and its temperature dependence was described in terms of a Debye model:

$$\langle u_G^2\rangle = \left(\frac{\hbar^2}{36M}\right)\left(\frac{1}{k_BT_D}\right)\left[1 + 4\left(\frac{T}{T_D}\right)^2\int_0^{(T_D/T} \frac{x}{e^x - 1}\,dx\right] \quad (2)$$

here the Debye temperature, T_D, and the oscillator mass M are the fit-parameters.

Using standard χ^2 minimization routines, the data were fitted according to the following steps: 1) The curves in the harmonic regime (T<150 K) were fitted to Eq. 2; in this way, the Debye temperature T_D and the zero-point mean square displacement, $\langle u_0^2\rangle = (\hbar^2/36M)(1/k_BT_D)$ were deduced. It turns out that the harmonic contribution is hydration-independent for both amylose and amylopectin. It was then kept fixed in the subsequent

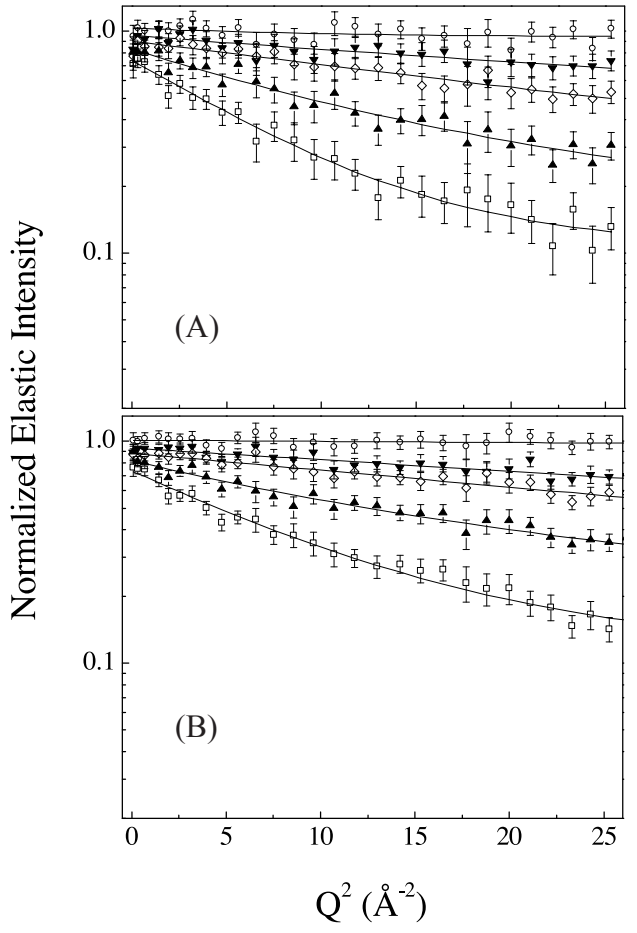

FIGURE 2 Elastic scattering intensity vs. Q^2 for amylose (*a*) and amylopectin (*b*) at hydration $h = 0.47$ and 0.32, respectively, and at some selected temperatures. In *A* and *B*, T = 45, 175, 225, 275, and 315 K (*top* to *bottom*).

analysis. 2) The I_{el} vs. Q^2 curves were fitted to Eq. 1 having d, and the product $p1 \times p2$ as free fit parameters. It came out that the distance d between the conformational minima was very weakly dependent upon hydration and temperature, and an average temperature- and hydration-independent value for $d = 1.0$ Å was then adopted. 3) The data were then fitted with this additional constraint to obtain the temperature dependence of the occupation probabilities.

RESULTS AND DISCUSSION

The Q dependence of the elastic scattering intensities for some selected temperatures are shown in Fig. 2, for amylose and amylopectin, together with the fit to Eq. 1 (continuous curves) as described in the previous section. We may remark that the adopted model is able to reproduce correctly the Q dependence above and below the onset of the anharmonic dynamics. From these fits we have deduced the total mean square displacement of hydrogens $\langle u_h^2 \rangle$ that is given by the initial slopes of the curves in Fig. 2, i.e., from Eq. 1:

$$\langle u_h^2 \rangle = \langle u_G^2 \rangle + \frac{p_1 p_2 d^2}{3} \qquad (3)$$

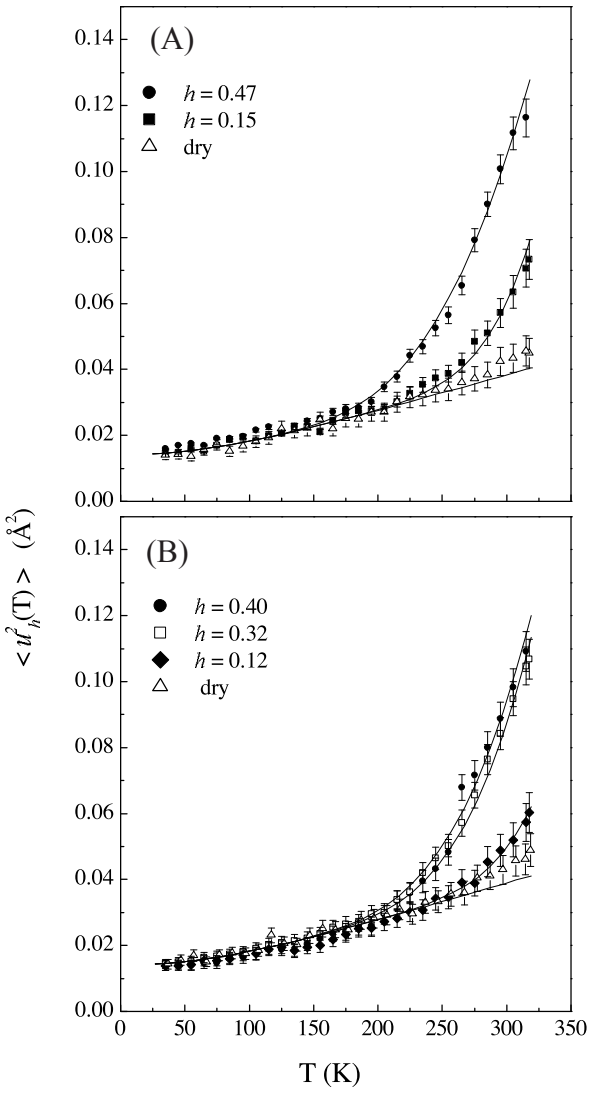

FIGURE 3 Temperature dependence of the mean square displacement of nonexchangeable hydrogens for amylose (*A*) and amylopectin (*B*), at different hydration levels.

The temperature dependence of $\langle u_h^2 \rangle$ for amylose and amylopectin at different hydration levels are reported in Fig. 3. As already noted, a common hydration-independent behavior of the mean square atomic displacements is observed in the low temperature range where only harmonic motions are present (i.e., when $\langle u_h^2 \rangle \equiv \langle u_G^2 \rangle$). From Fig. 3 it also appears that, even in the dry samples, small deviations from the harmonic Debye behavior appear above room temperature. Although this "intrinsic" anharmonicity is not driven by water-biopolymer interactions, it is somewhat reminiscent of the well known interactions in solids at high temperatures that are associated with higher-order terms (cubic, quartic) in the potential. It is worth noticing that deviations from the harmonic behavior have also been evidenced in dry metmyoglobin from neutron scattering (Doster et al., 1999) and Mössbauer absorption (Parak et al., 1988) experiments. In

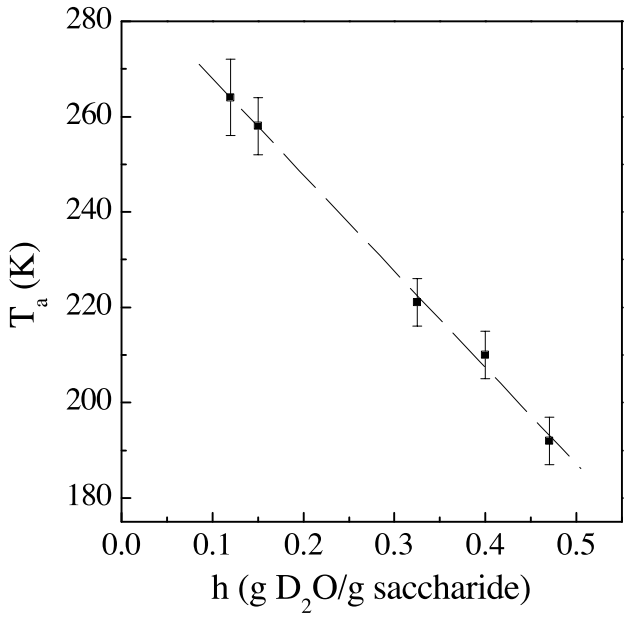

FIGURE 4 Hydration dependence of the activation temperature, T_a, defined in the text as the temperature at which $(\langle u_h^2\rangle - \langle u_G^2\rangle)/\langle u_G^2\rangle = 5\%$.

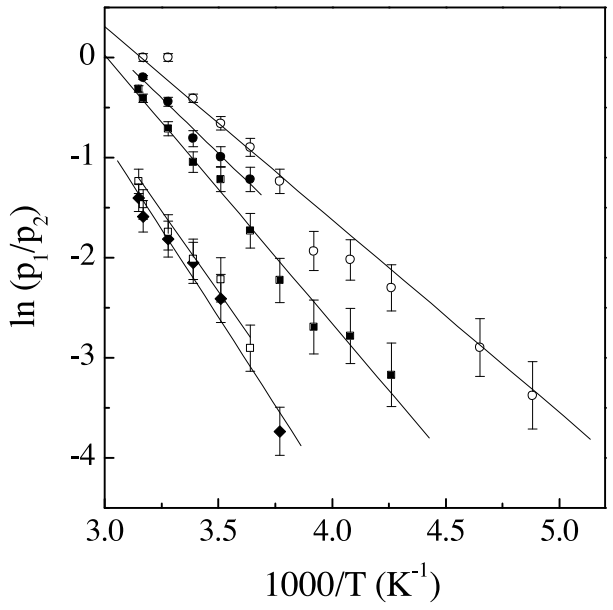

FIGURE 5 van't Hoff plot of the occupation probabilities p_1 and p_2. For amylose: (□) $h = 0.15$, (○) $h = 0.47$. For amylopectin: (◆) $h = 0.12$, (■) $h = 0.32$, (●) $h = 0.40$. The straight lines are fits to $\ln(p_2/p_1) = -\Delta H/RT + \Delta S/R$.

these cases they seem to occur at T ≥ 180 K, i.e., close to the Debye temperature that is estimated to be ~200 K for metmyoglobin. From the fit of the mean square displacement in the low temperature region, we obtain $T_D = 445$ K, although a first evidence of anharmonic behavior begins to appear above 300 K.

In hydrated biopolymers a further hydration-dependent, dynamic transition occurs, leading thus to an enhanced anharmonicity with respect to the dry case. In both amylose and amylopectin, we find that the temperature at which this anharmonic contribution is observed depends upon the hydration level of the sample. To quantify this behavior we have defined a conventional temperature (T_a) for the activation of the hydration-dependent anharmonic behavior as the temperature at which the ratio $\Delta u^2/u^2 = (\langle u_h^2\rangle - \langle u_G^2\rangle)/\langle u_G^2\rangle = 5\%$. The temperature variation of T_a is reported in Fig. 4, and it shows an almost linear dependence upon hydration. The fact that T_a decreases with increasing hydration indicates an overall softening of the dynamics of the saccharides; this can be related to the known "plasticizing" effect of water in starch (Kulik et al., 1997). In the frame of the adopted potential model, we expect to observe a marked variation of the model parameters upon temperature and hydration.

A van't Hoff plot of the occupation probabilities *p1* and *p2*(Fig. 5) allows us to quantify their temperature variation and to evaluate the energy asymmetry of the double well, ΔH, as well as the entropy, ΔS, involved in the kinetic transition. The values of ΔH and $\Delta S/R$, as obtained from the fit shown in Fig. 5, are reported in Table 1: a marked, almost linear decrease with increasing hydration occurs for ΔH, and a smaller one for $\Delta S/R$. A comparison with similar data obtained in metmyoglobin (Doster et al., 1989) evidences the higher stiffness of the double-helical polysaccharidic structure with respect to a protein globule. In the hydrated samples, we obtained ΔH values in the range 15 ÷ 20 kJ mol^{-1}, and values of ΔS/R of ~6 to 7, the analogous values for metmyoglobin at a comparable hydration level being 12 kJ mol^{-1} and 3, respectively.

At a given temperature, both amylose and amylopectin show a similar increase of the total mean square hydrogen displacement $\langle u_h^2\rangle$ with increasing hydration, h, at least up to ~0.3. In the case of amylopectin at $h = 0.32$ and 0.4, we observe nearly the same value for $\langle u_h^2\rangle$. This might indicate that a saturation value has almost been reached; however, more data points in this hydration region are needed to confirm this behavior. The presence of different hydration regimes in these saccharides would not be surprising. Indeed, from diffraction studies of ordered amylose fibers, it turns out that the double helices are arranged around a

TABLE 1 Energy asymmetry, ΔH, and entropy $\Delta S/R$ for amylose and amylopectin

Sample	h	ΔH (kJ/mol)	$\Delta S/R$
Amylopectin	0.12	29 ± 2	9.8 ± 1.0
Amylose	0.15	27 ± 2	9.0 ± 1.0
Amylopectin	0.32	22 ± 1	8.1 ± 0.4
Amylopectin	0.40	18 ± 1	6.8 ± 0.4
Amylose	0.47	16 ± 1	6.1 ± 0.4

Derived from data in Fig. 5 at different hydration levels h ($h = g$ D_2O/g saccharide) investigated.

channel that at $h = 0.27$ is fully filled with well localized and spatially ordered water molecules. These are located on sixfold helices following the crystalline symmetry with 36 water molecules in the unit cell (Imberty et al., 1988). We note that because $h = 0.27$ corresponds to the almost full hydration of this channel, it is not surprising that up to $h \sim 0.3$ the hydration process affects in the same way the dynamics of the hydrogens in both amylose and amylopectin. At $h > 0.3$ the helices are fully solvated, and upon further hydration more "mobile" water molecules are added. These are located outside the channels and are less tightly associated to the saccharidic helices.

CONCLUSIONS

A first result that clearly emerges from this study is the presence in hydrated starch polysaccharides of a dynamic glass-like transition. Therefore, this feature is common not only to globular proteins but also to other classes of biomolecules, irrespective of the details of their structural arrangement. The fact that biopolymers with considerably different structural arrangement display this solvent-induced harmonic-to-anharmonic transition points to existence of a common molecular mechanism driving the transition. It can be brought back to the presence of a sufficiently extended network of hydrogen (H—) bonds between water molecules and the hydrophilic sites of the biomolecule. The fluctuations of this H-bond network drive the conformational transitions of the whole molecular assembly; therefore, the topology and the energetics of this fluctuating network are the key elements in tuning the observed dynamics.

The strong dependence of the transition temperature upon hydration in amylose and amylopectin reflects the known plasticizing role of water in polysaccharides. Water-saccharide interactions determine and stabilize the packing of the saccharide helices, and, at the same time, strongly influence the energy landscape of atomic motions. The overall higher stiffness of the double helical packing of the investigated polysaccharides is reflected in the relatively high value of the Debye temperature with respect to that of proteins.

A two-state model with an asymmetric potential well can describe with sufficient accuracy the elastic scattering results. It provides a simple way to characterize the geometry and the energetics of the anharmonic atomic displacements in terms of few physical parameters: the jump-distance d and the energy parameters, ΔH and ΔS. The evidence that a relatively simple two-state model is able to reproduce with sufficient accuracy the dynamics of a complex macromolecule-water system is consistent with the hypothesis that the continuous breaking and formation of H-bonds might be the leading mechanism of the observed phenomena. In this view the two states of the potential well can be associated to open and closed H-bonds states (Doster and Settles, 1999). The temperature and hydration dependence of the dynamics is therefore determined by the variations in the population of broken and closed H-bonds and in the associated probabilities.

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