The Temperature Dependence of Internal Molecular Motions in Hydrated and Dry α-Amylase: The Role of Hydration Water in the Dynamical Transition of Proteins

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ABSTRACT Internal molecular motions of proteins are strongly affected by environmental conditions, like temperature and hydration. As known from numerous studies, the dynamical behavior of hydrated proteins on the picosecond time scale is characterized by vibrational motions in the low-temperature regime and by an onset of stochastic large-amplitude fluctuations at a transition temperature of 180–230 K. The present study reports on the temperature dependence of internal molecular motions as measured with incoherent neutron scattering from the globular water-soluble protein α-amylase and from a protein-lipid complex of rhodopsin in disk membranes. Samples of α-amylase have been measured in a hydrated and dehydrated state. In contrast to the hydrated sample, which exhibits a pronounced dynamical transition near 200 K, the dehydrated α-amylase does not show an appreciable proportion of stochastic large-amplitude fluctuations and no dynamical transition in the measured temperature range of 140–300 K. The obtained results, which are compared to the dynamical behavior of protein-lipid complexes, are discussed with respect to the influence of hydration on the dynamical transition and in the framework of the glass transition.

INTRODUCTION

Internal molecular motions, which give many biological molecules the conformational flexibility required for their function, have been studied in the past with various experimental and theoretical techniques. In particular, faster equilibrium fluctuations (time scale 10^{-7} to 10^{-14} s) are very sensitive to the level of hydration and show a pronounced temperature dependence. In many studies of hydrated proteins the temperature dependence of internal flexibility has been examined by Mössbauer spectroscopy (Parak and Knapp, 1982), optical spectroscopy (Nocek et al., 1991; Di Pace et al., 1992), calorimetric measurements (Miyazaki et al., 1993), x-ray crystallography (Frauenfelder et al., 1979; Rasmussen et al., 1992), infrared spectroscopy (Demel et al., 1997), neutron spectroscopy (Doster et al., 1989), and molecular dynamic simulations (Steinbach and Brooks, 1996). All of these studies revealed qualitatively that the internal flexibility of the proteins is drastically increased above 170–230 K as compared to temperatures below this transition temperature (T_d). Because of phenomenological similarities of the dynamical behavior between proteins and glass-forming liquids, these observations have been related to the well-known dynamical glass transition (see, for example, Iben et al., 1989; Green et al., 1994). Some of the above-mentioned techniques were used to analyze the "averaged mean square displacements" of the motions as a function of temperature. The motions occurring above the transition temperature were found to be anharmonic "large-amplitude," low-frequency motions. Furthermore, these high-temperature motions (above T_d) seem to be more stochastic than vibrational (see, for example, Fitter et al., 1997). The most important feature of the high-temperature internal flexibility is that the stochastic "large-amplitude" motions allow the protein to perform dynamical transitions between conformational substates (Frauenfelder et al., 1979). In the case of many proteins (e.g., enzymes, receptors, ion pumps) it is obvious that the conformational flexibility of the whole protein or parts of it is essential for function. If the flexibility is reduced at low hydration levels or at low temperatures, the protein function is hampered or even disabled (Rupley and Careri, 1991; Rasmussen et al., 1992; Fitter et al., 1998a). The kind of solvent (e.g., water, ethylene glycol, trehalose, etc.) and the amount of solvent molecules play an important role in the internal molecular motions of the protein and affect the characteristics (e.g., transition temperature) of the dynamical transition (Iben et al., 1989; Hagen et al., 1995; Demel et al., 1997; Cordone et al., 1998). Because the above-mentioned solvent substances and water/cosolvent mixtures themselves are glass-forming liquids (Sokolov et al., 1995; Hofer et al., 1989; Crowe et al., 1996), with their individual transition temperatures, the temperature-dependent interaction of solvent and protein is complex. The question arose whether the measured dynamical transition is an inherent property of the protein (Nocek et al., 1991; Parak and Frauenfelder, 1993), or whether the protein dynamics is dominated by solvent properties and therefore is a "slave to the solvent" (Iben et al., 1989; Ansari et al., 1995). The controversial debate demonstrates that there is still a demand for further investigations elucidating the physical and chemical details of the dynamical properties of biological macromolecules.

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The incoherent neutron scattering, which makes use of a large incoherent cross section of hydrogen nuclei (~40 times larger than the cross section of other elements), is a technique well suited to the study of all internal molecular motions on the picosecond time scale. In the present work the temperature dependence of internal molecular motions in a water-soluble globular protein (α-amylase) and in a protein-lipid complex (bovine disk membranes) has been investigated. α-Amylase is an enzyme that catalyzes the hydrolysis of linkages in starch components, and the disk membrane contains an important membrane protein, rhodopsin, which functions as a photoreceptor. As in previous studies on purple membranes (Fitter et al., 1996, 1997), the effects of temperature and hydration on the dynamical behavior of the biological macromolecules were examined by using the concept of quasielastic incoherent structure factors (see, for example, Lechner, 1994) to quantify the internal flexibility in different time domains. In particular, the influence of hydration on the dynamical transition and the underlying mechanisms of the transition are the focus of this study. In addition, the dynamical behavior was related to the solvent properties as known from recent diffraction measurements.

MATERIALS AND METHODS

Sample preparation

The enzyme α-amylase from *B. amyloliquefaciens* was purchased as a lyophilized powder (Boehringer Mannheim GmbH). Protein (230 mg) (molecular mass 58,403 Da) was dissolved in 2 ml D_2O (D_2O purity 99.8%). This suspension was deposited on a surface of aluminum plates (thickness 0.2 mm, diameter 50 mm) and subsequently dried under controlled conditions in a dessicator. Finally, one sample has been dried by exposing it for 2 days to silica gel ($h = 0.05$ g solvent/g protein), and a second sample has been rehydrated using vapor exchange over saturated K_2SO_4 solution (corresponding to 98% relative humidity at 20°C), leading to hydration of $h = 0.4$.

The composition of the disk membrane is dominated by the high density of the integral membrane protein rhodopsin (molecular mass 40,000 Da) and the high content of lipids (~60 mol lipids/mol rhodopsin). The preparation of disk membrane (DM) from rod outer segments of the bovine retina is described in previous publications (Wilden and Kühn, 1982; Fitter et al., 1998b). Disk membranes (250 mg) were spread as hydrated (also with D_2O) membrane stacks on the surface of aluminum plates. Finally the samples were rehydrated at 98% relative humidity, corresponding to $h = 0.5$.

The hydration level of all samples was controlled by the determination of the sample weight. For neutron scattering measurements the samples were sealed in a circular slab-shaped aluminum container.

Incoherent neutron scattering

In the case of incoherent neutron scattering, the predominant part of the measured signal is due to nonexchangeable hydrogen atoms. Many of the charged and polar polypeptide side groups as well as the polar lipid headgroups (for the disk membranes) contain exchangeable protons, which are replaced by deuterons if the samples are hydrated with D_2O. Nevertheless, most of the protons are not exchangeable, and, from a practical point of view relevant to the incoherent neutron scattering, these protons are distributed nearly homogeneously in the sample. For this reason we have to consider the same individual incoherent cross section for all hydrogen atoms (79.91 barns), which "monitor" the general dynamical properties of the sample as a probe structure (for more details see Bee, 1988; Lechner, 1994).

The neutron scattering experiments have been carried out using the time-of-flight (TOF) spectrometer IN6 (Institute Laue-Langevin, Grenoble). The TOF spectra were measured using an incident wavelength of 5.1 Å, with an elastic energy resolution of 80 μeV (FWHM), within an angular range of $10.3° \le \phi \le 113.5°$. All samples, including vanadium standard and empty can, were measured with a sample orientation angle of $\alpha = 135°$ with respect to the incident neutron beam direction. The measurements have been performed at temperatures between 150 and 300 K, with cooling and heating rates of ~25 K/h. The TOF spectra were corrected, normalized, grouped, and transformed to the energy transfer scale by using adapted standard routines. Because of poor counting statistics (some spectra were measured for only 5 min), the data of individual measurements have been added up into only one group with $\phi = 55.0°$. Relatively large transmission values of $T(90°) \approx 0.93$–0.95 made it possible to perform a preliminary data analysis without a correction for multiple scattering.

As already demonstrated in a previous work (Fitter et al., 1997), a sum of an elastic term and two Lorentzians fits the measured spectra satisfyingly. Therefore, it is appropriate to write the theoretical incoherent scattering function in the form

$$S_{\text{theor}}(\vec{Q}, \omega) = e^{-\langle u^2\rangle Q^2} \cdot \left[A_o(\vec{Q}) \cdot \delta(\omega) + \sum_{n=1}^{2} A_n(\vec{Q}) \cdot L_n(H_n, \omega) \right] \quad (1)$$

The scattered intensity was separated into this elastic $\delta(\omega)$-shaped component and quasielastic Lorentzian-shaped contributions $L_n(H_n, \omega)$, parameterized by the width $H_n = (\tau_n)^{-1}$ (τ_n are the corresponding correlation times) and the quasielastic incoherent structure factors A_n. The amplitude of the elastic component is given by the elastic incoherent structure factor (EISF) A_0. In this approach, using data that have been summed up in only one group (elastic Q-value of 1.1 $Å^{-1}$), the structure factors A_n and the linewidths H_n are used as phenomenological parameters. These parameters describe general properties of the internal motions occurring in the protein (protein-lipid complex, respectively) without using a specific model that implies more details about the geometry of the motions. The theoretical scattering function was fitted to the measured scattering function by using the following relation:

$$S_{\text{meas}}(\vec{Q}, \omega) = F \cdot e^{-\hbar\omega/2k_BT} \cdot [S_{\text{theor}}(\vec{Q}, \omega) \otimes S_{\text{res}}(\vec{Q}, \omega)], \quad (2)$$

where a convolution with the resolution function $S_{\text{res}}(Q,\omega)$ (obtained from vanadium measurements) and factors, such as the normalization factor F and the detailed balance factor $\exp(-\hbar\omega/2k_BT)$ have been applied. The fits have been applied to data within an energy transfer range from −1.7 to 5.0 meV. More methodical details are described elsewhere (Lechner, 1994; Fitter et al., 1996, 1997).

RESULTS AND DISCUSSION

Temperature dependence of quasielastic scattering

In analogy to previous studies on purple membranes (Fitter et al., 1997), a fit with two Lorentzians was also reasonable for the data measured with samples of the α-amylase (see Fig. 1 *a*) and with disk membrane samples. A sufficient fit with two Lorentzians was not only applicable for spectra measured at room temperature, but also for the low temperature measurements. In the case of complex biomolecules, every Lorentzian represents a broad distribution of many

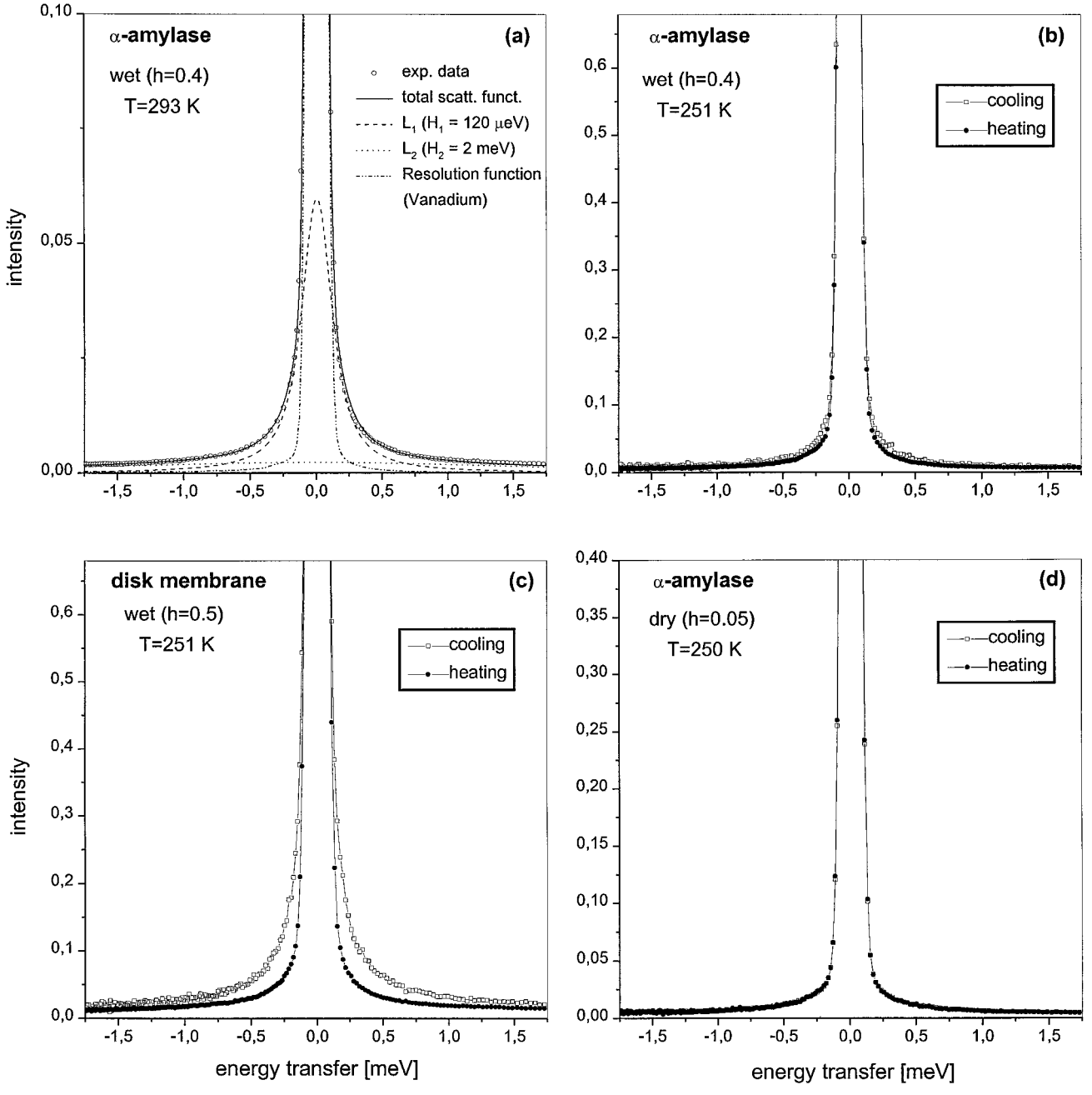

FIGURE 1 Time-of-flight spectra of α-amylase and disk membranes. The fits have been applied to data ranging from −1.7 to 5.0 meV, but for clarity, the spectra are shown here in a restricted energy transfer range (±1.5 meV). The largest intensity values (*top of the frame*) correspond to 10% of the elastic peak intensity in each case. (*a*) This figure illustrates the quality of the applied fitting procedure. The total scattering function, as composed of an elastic and two quasielastic components, fits the experimental data. (*b–d*) These figures show spectra as measured once during the heating process, and again during the cooling process at a temperature of $T \approx 250$ K.

Lorentzians (related to many different correlation times of manifold motions occurring in a protein). As a consequence of this, there is a correlation between the fit parameters (structure factors A_n and linewidths H_n). Therefore, an independent determination of the temperature dependence of A_n and H_n is not unambiguous. Nevertheless, preferably two rather different linewidths were obtained from the fits. These fits revealed for each sample between 140 and 300 K a narrow spectral component with $H_1 = 100–150$ μeV (HWHM) and a broader component with $H_2 = 1.5–2.4$ meV. For practical reasons, in the subsequent fitting procedure fixed values of $H_1 = 120$ μeV and $H_2 = 2$ meV have been used for all samples and all temperatures, and we studied only the temperature dependence of the quasielastic structure factors (A_n). Without going into the details of temperature dependence of the correlation times and the amplitudes, a general temperature-dependent "internal flexibility" of the biological macromolecule is studied by the

quasielastic structure factors measured as a function of temperature (see Fitter et al., 1997, for a discussion of this approach). Applying this procedure, all spectra are characterized by two components: motions with correlation times of a few picoseconds (τ_1 = 5.5 ps; "slow", with τ [s] = (H [meV] · 1.519 · 10^{12})$^{-1}$) and faster motions within a few tenths of a picosecond (τ_2 = 0.3 ps; "fast"). The temperature dependence of the obtained incoherent structure factors is shown in Fig. 2. The spectra of all samples have been measured in sequence, from room temperature to low temperatures (cooling) and from low temperatures back to room temperature (heating). The analysis revealed the following results:

1. Dry α-amylase shows a component of the "slower" motions (A_1) that is very small (~2% of the total scattering) at all temperatures (Fig. 2 *a*). The faster motions, represented by A_2, are characterized by a smooth linear increase with the temperature. In contrast to this, the wet sample of α-amylase exhibits a considerable contribution of "slower" motions at room temperature, which vanishes below a transition temperature T_d of ~200–230 K (Fig. 2 *b*). The amplitude of the faster component in the wet sample in-

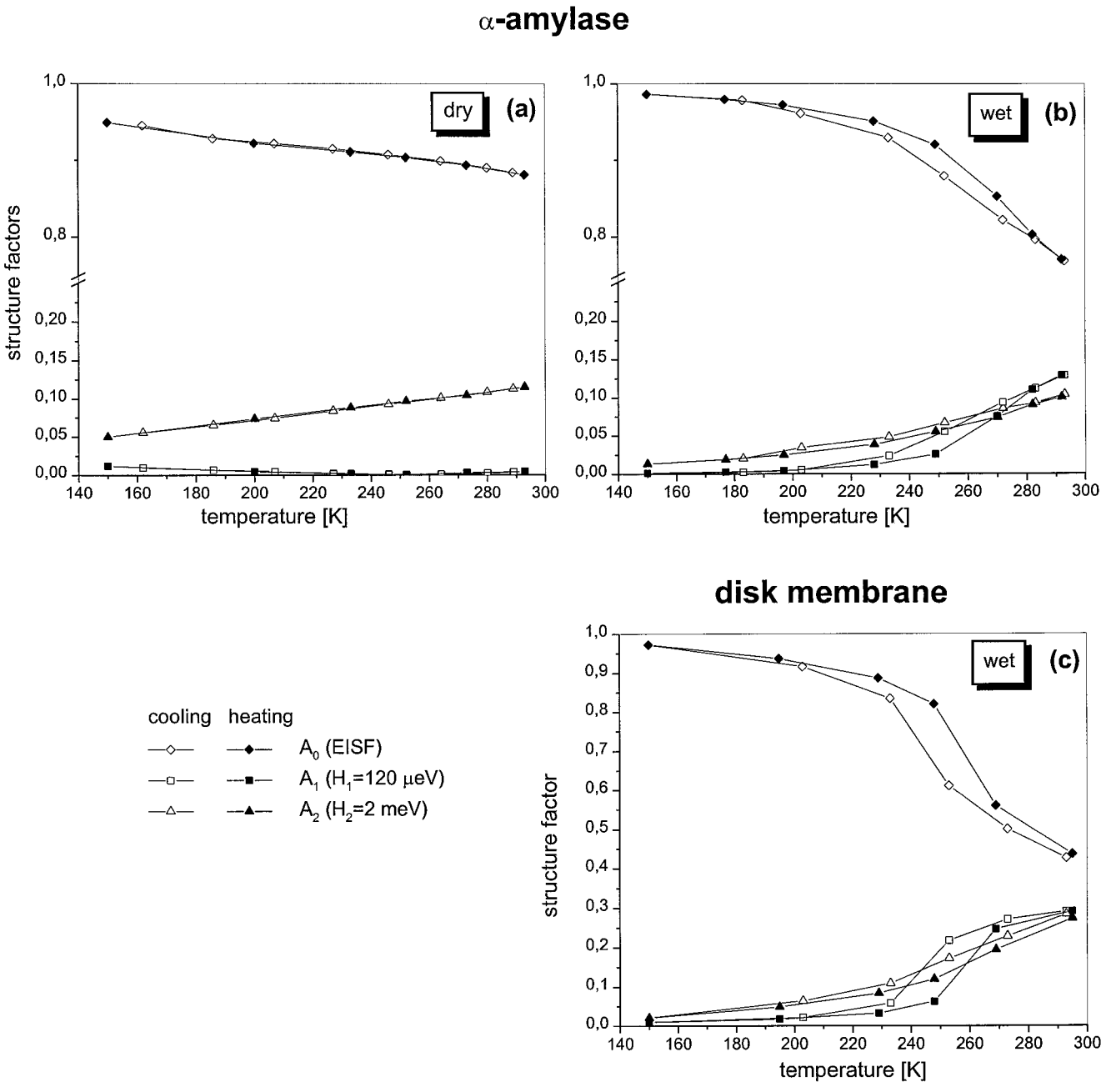

FIGURE 2 Incoherent structure factors as a function of temperature. The sum of these structure factors is equal to unity. The statistical error of the structure factors is approximately ±0.015, which is about the size of the symbols.

creases smoothly (and linearly) at temperatures below 200 K, but also shows a slightly stronger increase with a kind of transition at 200 K and therefore a weak deviation from the linear behavior of the low-temperature range. Below 270 K, the faster component in wet samples has significantly smaller A_2 values as compared to dry samples.

2. A dynamical transition, as a deviation from the linear low-temperature behavior related to an additional increase in A_1 or A_2 at the transition temperature T_d, is observed in both components of the wet samples (Fig. 2 *b* and *c*) but is not observed in any component of the dry sample (Fig. 2 *a*).

3. The dry sample does not show any difference between data that have been measured during cooling or heating (Figs. 1 *d* and 2). For wet samples we find for both components that spectra measured during cooling exhibit significant larger quasielastic incoherent structure factors as compared to spectra measured during heating (Figs. 1, *b* and *c*, and 2). In the latter case the cooling-heating cycle is characterized by a hysteresis.

4. The measurements of wet disk membranes revealed results qualitatively similar to those for wet α-amylase. In the case of disk membranes one observes, perhaps because of a slightly higher hydration level of $h = 0.5$, larger quasielastic components and a more pronounced hysteresis (Figs. 1, *b* and *c*, and 2, *b* and *c*). In particular, the fast quasielastic component A_2 is clearly larger in the disk membrane as compared to the α-amylase.

It is known from previous studies on purple membranes (Lechner et al., 1998) and disk membranes (Fitter et al., 1998b) that at moderate hydration levels of $h \approx 0.3–0.5$ part of the solvent starts to crystallize at ~265 K. A similar behavior has been observed for numerous globular water-soluble proteins (see, for example, Finney and Poole, 1984; Doster et al., 1986; Rupley and Careri, 1991). For the membrane samples it was shown that the freezing of solvent leads to partial dehydration of the protein-lipid complex. Upon reheating of the sample, the frozen solvent melts at a higher temperature of ~275 K. Therefore, the sample is not fully rehydrated until this temperature is reached. The process of freezing and melting of hydration water is characterized by a hysteresis, which is observed if one measures the lamellar cell parameters as a function of temperature (see Lechner at al., 1998). In the present work, a hysteresis in the temperature dependence of the incoherent structure factors is visible in wet samples, which is similar and related to the hysteresis of lamellar cell parameters. With respect to the data presented here, the crystallization of solvent was really proved only in the disk membranes, but it is very likely that the process of freezing and melting of solvent also occurs in the moderately hydrated powder sample of α-amylase. In contrast to hydrated samples, the few remaining solvent molecules in dehydrated samples (in the case of purple membrane with $h \leq 0.24$) are bound rather tightly to the surface of the protein (protein/lipid complex, respectively), and therefore crystallization of solvent is not observed (Lechner et al., 1998; Fitter et al., 1998b). As a consequence of this we do not obtain a hysteresis in the temperature dependence of the structure factors in the dry α-amylase. The comparison of the dry and the wet α-amylase emphasized that the few water molecules in the dry α-amylase ($h = 0.05$ corresponds to 146 water molecules per protein molecule) are not sufficient to allow the protein dynamical transitions between conformational substates. The observed motions remain predominately vibrational, and slower stochastic picosecond motions do not occur to an appreciable extent, even at high temperatures (up to $T = 300$ K). The amplitude of A_2, which is related to faster motions, does not show any transition and has significantly larger values (for $T < 270$ K) as compared to A_2 values in the wet sample. The reduced vibrational component in wet as compared to dry samples at low temperatures is certainly related to solvent or solvent properties: either the partially crystallized solvent inhibits the vibrational motions, or the solvent is still able (even at low temperatures) to damp those vibrational motions. In the case of the latter, the damped motions appear to be too slow (with $\tau > 8$ ps, which is not resolved by the given energy resolution of $\Delta E = 80$ μeV) to occur in the quasielastic contribution (A_1). A very similar behavior of low-temperature vibrational motions is visible in data obtained from wet and dry hemoglobin (Martel et al., 1991). In the hydrated α-amylase (with $h = 0.4$, with ~1170 water molecules per protein molecule), the amount of solvent allows the onset of stochastic large amplitude motions at the transition temperature. According to the experimental results, there is evidence for the fact that the component representing the faster motions (A_2) includes mainly vibrational motions with a linear temperature dependence and a small contribution of stochastic motions characterized by a transition. Although in dry samples A_2 covers the same time regime as compared to wet samples, a stochastic contribution seems to be absent in the case of dry samples. Previous studies on dehydrated globular water-soluble proteins did not reveal a consistent picture of the temperature dependence of the internal molecular motions. For example, a study using Mössbauer absorption spectroscopy on α-chymotrypsin (to which iron labels were firmly bound) reports on air-dried samples that did not show a dynamical transition between 90 and 270 K (Parak, 1986). Another Mössbauer study on freeze-dried myoglobin revealed a small but well-resolved transition at 200 K (Parak and Frauenfelder, 1993). Molecular dynamics simulations (MDSs) investigating the effect of different solvent concentrations on myoglobin produced a dynamical transition, even without hydration water, and suggest that water is not required for the dynamical transition to occur in the protein (Steinbach et al., 1991; Smith, 1991). In contrast to the above-cited experimental and theoretical (MDS) observations made in the case of myoglobin, the results of the present work support the idea that a minimum of water molecules are a prerequisite for a dynamical transition (at least for a transition between 150 and 300 K).

Low-frequency excitations

Low-frequency vibrational modes in proteins have been measured previously by Raman spectroscopy (Painter et al., 1982) and neutron spectroscopy (Bartunik et al., 1982; Martel et al., 1991). Based on normal mode analyses (Go et al., 1983) and molecular dynamics simulations (see, for example, Smith, 1991), these modes have been attributed to global, collective, damped vibrations involving molecular subunits (protein domains, secondary structure elements). As a prominent feature in many proteins, a broadened peak was observed in energy regime between 2 and 4 meV (20–30 cm^{-1}) (Painter et al., 1982) (1 meV is equivalent to 8.006 cm^{-1}). A similar peak was often observed for many other (also anorganic) glass-forming systems and is known as the boson peak in this field of research. Such a boson peak, centered at ~3.8 meV (150 K), is also visible in the spectrum of α-amylase and shifts to lower frequencies with increasing temperature (Fig. 3 *b*). At high temperatures the peak is strongly superimposed by the quasielastic scattering. In the dry sample a weak boson peak is observed, which is centered at ~2.5 meV and seems not fully vanish at higher temperatures (Fig. 3 *a*). In comparison to α-amylase samples, the spectra of disk membranes show a boson peak with a different shape and a much broader maximum, which includes intensity in the range from 0.5 to 7 meV (Fig. 3 *d*). These low-frequency excitations seem to include at least two maxima, which might be related to the more complex composition of the several different biological macromolecules in the disk membrane, as compared to only one water-soluble protein. As shown in a study on hydrated hemoglobin, an additional peak near 7 meV might also be due to the presence of solvent (Martel et al., 1991). Frozen aqueous ingredients (H_2O and D_2O) contribute to this energy regime with a broad peak. On the other hand, the above-cited study was performed with much larger hydration levels of $h = 1$, which enhanced the influence of solvent-related modes.

Further previous scattering studies on the water-soluble protein myoglobin (Cusack and Doster, 1990; Diehl et al., 1997) and on the protein-lipid complex of bacteriorhodopsin in purple membranes (Ferrand et al., 1993; Fitter, unpublished results) revealed peaks with very similar shapes as compared to α-amylase (asymmetrical circular shaped peak with a high-frequency tail), but with different maximum positions at 3.1 meV (myoglobin) and 2.4 meV (purple membranes) in wet samples. These results indicate that the general feature of low-frequency excitations is present in all biological macromolecules, but these molecules exhibit their individual frequency distributions, characterized by their own peak positions and/or shapes of the peak. The level of hydration seems to influence the vibrations related to the boson peak, because a shift of the peak position in wet samples to lower frequencies in dry samples is observed for the α-amylase (Fig. 3 *c*). A similar shift of the peak position was found for myoglobin (Diehl et al., 1997). Based on molecular dynamics simulations performed with myoglobin at low temperatures and at different levels of hydration, the hydration-dependent peak shift was related to the number and the strength of protein-water hydrogen bonds (Steinbach et al. 1991). In this picture, dehydration leads to smaller force constants, which lower the peak frequency and increase the vibrational amplitude (Diehl et al., 1997). Another approach concerning the origin of the boson peak led to an interpretation in terms of acoustic modes (see, for example, Painter et al., 1982). It has been shown for a small globular protein (phycocyanin) that collective modes in dry and wet samples are characterized by a different speed of propagation (Bellissent-Funel et al., 1989). Consistent with the ratio between the sound velocity in dry (~2, 260 m/s) and wet (~3, 300 m/s) phycocyanin samples, the ratio between the boson peak frequencies of dry (2.5 meV) and wet (3.8 meV) α-amylase samples is very similar.

Dynamical glass transition

The dynamical coupling of the water solvent to the biological macromolecule is dominated by two categories of water characterized by different interactions. The interaction with charged and polar groups evokes an influence of solvent on the protein dynamics through electrostatic and dipole-dipole interactions. Results from molecular dynamic simulations on globular proteins suggested that electrostatic protein-water interactions reduce the barriers between conformational substates (Steinbach and Brooks, 1994; Norin et al., 1994). Spectroscopic studies on lysozyme revealed, on the other hand, that the hydration of charged polypeptide side groups has little effect on the protein internal flexibility (Shah and Ludescher, 1993). A second category of water molecules forms hydrogen bonds with carbonyl and with N-H groups of the backbone and with several polypeptide side chains. At sufficient hydration, an extensive hydrogen-bonded network is formed with solvent molecules and specific sites of the protein structure (see, for example, Kuntz and Kauzmann, 1974; Finney et al., 1980; Bizzarri et al., 1995). In this picture the breaking and forming of alternative hydrogen bonds is a prerequisite for stochastic large-amplitude motions (e.g., side-chain reorientations). Hydrogen bond lifetimes from 0.1 ps to a few ps (see, for example, Benigno et al., 1996) support the assumption that the hydrogen bond-breaking dynamics is the main contribution to the internal molecular flexibility, as observed in the present work. A very small contribution of (slow) stochastic motions in dry α-amylase, accompanied by the absence of a dynamical transition and of a hysteresis in the temperature dependence of the incoherent structure factors, demonstrates that the transition must be related directly to the solvent and solvent properties. (Note that obtained incoherent structure factors are predominately due to scattering from the protein (at least 95%), and scattering from the solvent (D_2O) can be neglected (see, for example, Fitter et al., 1997). Therefore, in the present study, we do not observe the dynamics of the solvent itself, but the effect of

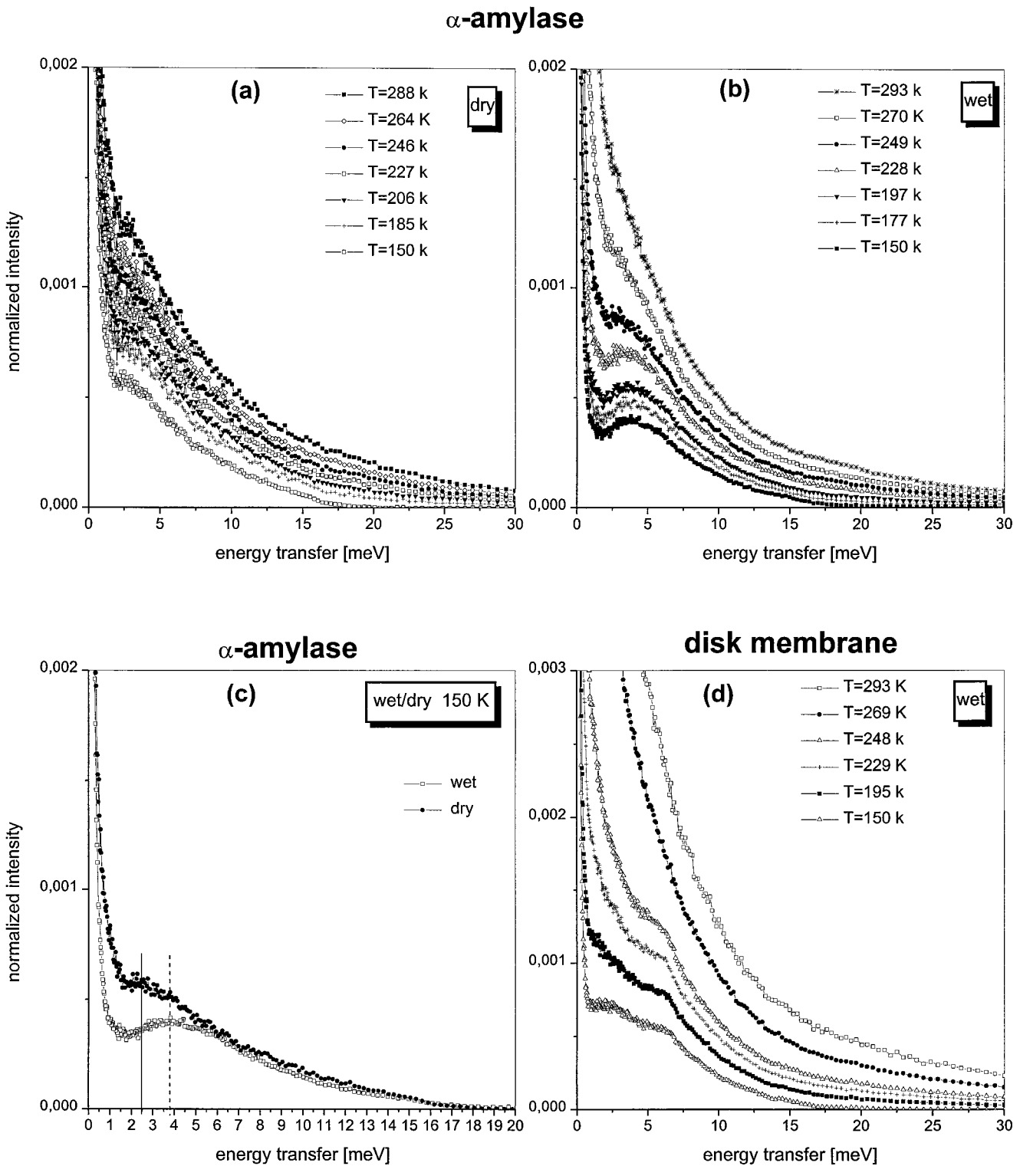

FIGURE 3 The features of low-frequency excitations (boson peak) are shown in the time-of-flight spectra measured at different temperatures.

solvent on the protein dynamics.) In the observed temperature range (150–300 K) and, in particular, at the functionally relevant physiological temperatures, only the entity of protein and sufficient solvent (i.e., hydrated protein) exhibits the functionally required dynamical properties. Previous studies revealed that both the sole protein and the pure solvent (water in our case) on their own seem to be glass-forming systems. Calorimetric investigations with water-soluble globular proteins encountered transition temperatures far above room temperature ($T_g \approx 190°C$) in

anhydrous proteins. The transition temperature decreases with increasing water content in the sample (Sochava, 1997). In the case of pure water, a transition temperature (cross-over temperature) was found at $T_c \approx 220$ K (Sokolov et al., 1995). In myoglobin crystals the (bound) hydration water is characterized by a transition temperature between 180 and 220 K (Doster et al., 1986; Daniels et al., 1996). Beside proteins, several other hydrated organic structures, such as DNA (H. Grimm, personal communication), cyclodextrin (Steiner et al., 1991), and (even less complex) anorganic compounds like CsH_3O_2 (Lechner et al., 1991), revealed a dynamical transition at a temperature range $T =$ 180–230. The structures of all of these mentioned complexes are characterized by hydrogen-bonded networks. These hydrogen-bonded networks, with hydrogen bonds of (slightly) different bond strengths and lifetimes, seem to determine the transition temperature near 200 K. It looks like this transition near 200 K is not a (unique) property of proteins, but is determined by the solvent.

Compared to the water-soluble α-amylase, the protein-lipid complex of the purple membrane shows a slightly larger contribution of slow stochastic motions (~7% of the total scattering) in the dry state at room temperature (Fitter et al., 1997). It is supposed that these motions are related to hydrophobic side groups of the membrane protein and to the (flexible) hydrophobic chains of the lipids. Whereas in water-soluble proteins the hydrophobic side groups are covered in a more or less rigid core, in transmembrane proteins, such as rhodopsin and bacteriorhodopsin, many hydrophobic side groups are exposed to that part of the "protein surface" that represents the protein/lipid boundary. A temperature dependence of the dynamical behavior in dried ($h \leq 0.05$) protein-lipid complexes might be different from that of globular proteins, if the moving groups in the dry protein-lipid complex are affected mainly by van der Waals interactions. Nevertheless, a rather small amount of solvent is sufficient for a dynamical transition to occur in a protein-lipid complex. Partially hydrated ($h = 0.18$) purple membranes exhibit a well-resolved dynamical transition near 200 K, very similar to that of the fully hydrated sample (Fitter et al., 1997). Up to now, incoherent neutron scattering studies on proteins (monitoring the "overall flexibility" on the picosecond time scale) did not reveal results, whereas, depending on the hydration level, the transition temperature is shifted to significantly different temperatures as compared to those found at $h = 0.3$–0.4.

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