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LETTER

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The "Protein Dynamical Transition" Does Not Require the Protein Polypeptide Chain

Giorgio Schirò,[†] Chiara Caronna,[‡] Francesca Natali,[§] M. Marek Koza,[∥] and Antonio Cupane*[,†]

[†]Department of Physics, University of Palermo, Via Archirafi 36, I-90123 Palermo, Italy

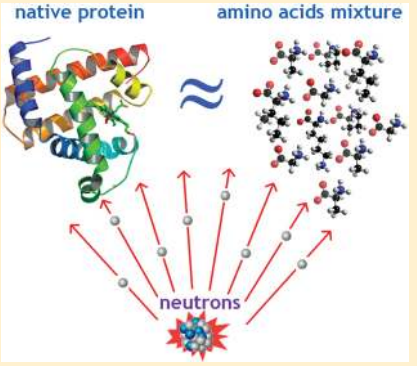
[‡]SLAC National Accelerator Laboratory, Menlo Park, California 94025, United States

[§]CNR-IOM, c/o ILL, 6 Rue Jules Horowitz, BP 156-38042 Grenoble, France

[∥]ILL, 6 Rue Jules Horowitz, BP 156-38042 Grenoble, France

Supporting Information

ABSTRACT: We give experimental evidence that the main features of protein dynamics revealed by neutron scattering, i.e., the "protein dynamical transition" and the "boson peak", do not need the protein polypeptide chain. We show that a rapid increase of hydrogen atoms fluctuations at about 220 K, analogous to the one observed in hydrated myoglobin powders, is also observed in a hydrated amino acids mixture with the chemical composition of myoglobin but lacking the polypeptide chain; in agreement with the protein behavior, the transition is abolished in the dry mixture. Further, an excess of low-frequency vibrational modes around 3 meV, typically observed in protein powders, is also observed in our mixture. Our results confirm that the dynamical transition is a water-driven onset and indicate that it mainly involves the amino acid side chains. Taking together the present data and recent results on the dynamics of a protein in denatured conformation and on the activity of dehydrated proteins, it can be concluded that the "protein dynamical transition" is neither a necessary nor a sufficient condition for active protein conformation and function.



SECTION: Biophysical Chemistry

Protein dynamics is characterized by molecular motions occurring on a very large time-scale ranging from femtoseconds (vibrations) to seconds (long-range molecular diffusion). Within this broad interval, motions occurring in the pico- to nanoseconds time scale are of particular interest and biological relevance since they cover the transition region from "discrete" local excitations of small molecular subunits to slower processes involving cooperative motions of larger parts of the macromolecular assembly.[1] This time window is exactly covered by neutron scattering techniques, in view of the typical instrumental energy resolution of neutron spectrometers.

Relevant information on protein dynamics has been obtained by investigating the temperature dependence of the mean square displacements (msd's) of relevant protein atoms. In fact, neutron scattering[2] and a variety of techniques, including Mössbauer spectroscopy,[3] optical spectroscopy[4] and molecular dynamics (MD) simulations,[5,6] evidenced a steep increase of atomic msd occurring in the temperature range of 180−220 K and marking a harmonic to anharmonic transition upon increasing temperature. It is now widely accepted that protein dynamics is actually characterized by two anharmonic onsets:

The first one occurs in the 100−150 K region, does not depend on the hydration level of the protein, and is largely attributable to methyl group rotations entering the time scale accessible by the instrumental resolution.[7−9]

The second one occurs at ∼220 K and is observed only in samples hydrated above a critical threshold (typically ∼0.2 g of water/g of protein). This second onset is known as the "protein dynamical transition". It is strongly coupled with solvent dynamics since it is suppressed in dry proteins and enhanced as hydration increases; moreover it can be substantially reduced when the proteins are embedded in confining matrices.[10−12] Although its occurrence is clearly established, the physical origin of the protein dynamical transition is still a matter of discussion: in particular, it is highly debated whether it corresponds to a kind of "glass transition" occurring in the system or to a resolution effect due to thermally activated motions entering the finite time window covered by the spectrometer.[13,14]

The second onset of anharmonic dynamics is deemed necessary for enzyme activity and protein function;[15] however, the above statement has been questioned, since counterexamples of residual enzymatic activity in the absence of dynamical transition have been reported;[16,17] moreover, the dynamical transition has also been observed in denatured protein samples and in short synthetic peptides with neutron scattering,[18,19] terahertz spectroscopy,[20] and NMR.[21] The presence of a dynamical transition occurring at

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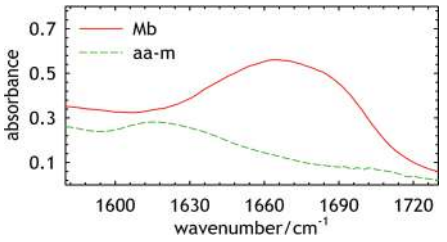

Figure 1. Absorption spectra in the region of the Amide I′ band measured by diffuse reflectance FT-IR spectroscopy at room temperature for powder samples of aa-m and Mb. The curves are vertically translated for clarity. The experimental procedures for diffuse reflectance FT-IR measurements are given in the SI.

the same temperature region has also been observed in DNA[22] and transfer RNA (tRNA),[23] suggesting that it is not a peculiar feature of peptide polymers.

It is also evident that, in proteins, both backbone and side-chains contribute to the dynamical transition; indeed, studies on homomeric polypeptides[8] showed that the dynamical transition is observed even in polyglycine (which has only backbone H) and therefore also occurs in the absence of side chains and involves backbone fluctuations. However, some relevant questions remained unaddressed, in particular: (1) Can the dynamical transition be observed even in the absence of backbone? (2) Is the dynamical transition really relevant to the onset of biological function? (3) What are the relative contributions of the polypeptide chain backbone and of the amino acid side chains to protein dynamics?

In this work we address these open questions. To this aim we study with both elastic and inelastic neutron scattering the dynamics of a mixture of amino acids (and hemin) powders (from here on called aa-m). The peculiarity of the aa-m is the absence of peptide bonds, and hence of the polypeptide chain, while its molecular composition is chosen so as to reproduce that of a real protein, myoglobin (Mb). We compare this non biological system with the corresponding native protein, at two different hydration levels $h = 0$ and $h = 0.2$ ($h = [\mathrm{g\,D_2O}]/[\mathrm{g\ protein}]$). The rationale of looking at the $h = 0.2$ hydration level is twofold: (1) it is sufficiently low as to guarantee that the scattering signal is safely attributable to the incoherent signal of nonexchangeable H atoms of peptide systems,[24] whereas the D_2O signal is negligible; and (2) it is high enough to allow the activation of the large scale fluctuations responsible for the dynamical transition.

The absence of peptide chain in the aa-m was directly checked with diffuse reflectance Fourier transform infrared (FT-IR) spectroscopy. In Figure 1 we show the infrared spectra of aa-m and Mb in the region of the Amide I′ band (1600−1700 $\mathrm{cm^{-1}}$), whose position and shape are sensitive to the secondary structure content of a protein.[25] The absence of the typical Amide I′ band in aa-m (evident by comparison with the Mb spectrum) demonstrates the absence of peptide bonds and polypeptide chains; the weaker band at ∼1620 $\mathrm{cm^{-1}}$ is usually observed in D_2O hydrated single amino acids and is related to the ionized $-COO^-$ carboxyl group.[26,27]

In Figure 2, panels a and b, the msd of aa-m and Mb are shown, at $h = 0$ and $h = 0.2$, obtained by elastic incoherent neutron scattering experiments performed at a backscattering spectrometer with a resolution of 8 μeV full width at half-maximum (fwhm). The procedure to calculate msd values from the elastic dynamic structure factor is described in the Supporting Information (SI). It is evident that the dependence on temperature and hydration is qualitatively the same in the two different systems.

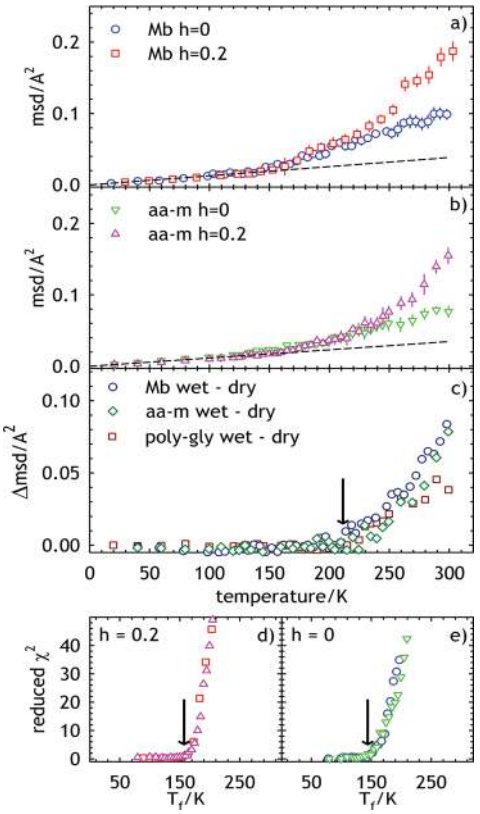

Figure 2. Panels a and b: total msd as a function of temperature obtained by analyzing the elastically scattered intensity $S(q,T,\omega = 0)$ using the Gaussian approximation (for details on data analysis see the SI); dashed lines are linear fits in the harmonic temperature region. Panel c: msd differences (Δmsd) between hydrated and dry samples (data relative to polyglycine are taken from ref 28). Panels d and e: reduced χ^2 values obtained from fitting the msd curves with a linear function up to a given temperature T_f, as a function of T_f; the arrows mark the temperatures at which breaks in the temperature dependence are observed.

Indeed, only one anharmonic onset is observed at ∼150 K for both dry samples, while a second onset at ∼220 K (dynamical transition) is observed in both hydrated samples. In order to estimate the harmonic-to-anharmonic transition temperature for the first onset in a more quantitative way, we calculated the reduced χ^2 values obtained by fitting the msd versus T data with a linear dependence from lowest temperature up to a given temperature T_f. The results of such procedure are shown in Figure 2, panels d and e, and confirm the close similarity between aa-m and Mb. To evaluate the dynamical transition temperature, we subtracted the msd of the dry samples, where the dynamical transition is not present, from those of the hydrated ones. The resulting curves are reported in Figure 2, panel c), compared with analogous data relative to polyglycine,[28] and shows the transition temperature to be at ∼220 K irrespective of the system. The data in Figure 2 unambiguously show that aa-m and Mb have identical transition temperatures: 150 and 220 K for the first and second onset, respectively.

As far as the msd amplitudes are concerned, a direct comparison between the two samples at both hydration levels is reported in Figure 3. The msd differences between Mb and aa-m (Δmsd) are highlighted in the lower panel of Figure 3: at temperatures higher than 150 K, msd's relative to Mb are larger than those relative to aa-m; above 220 K, a further Δmsd increase is observed

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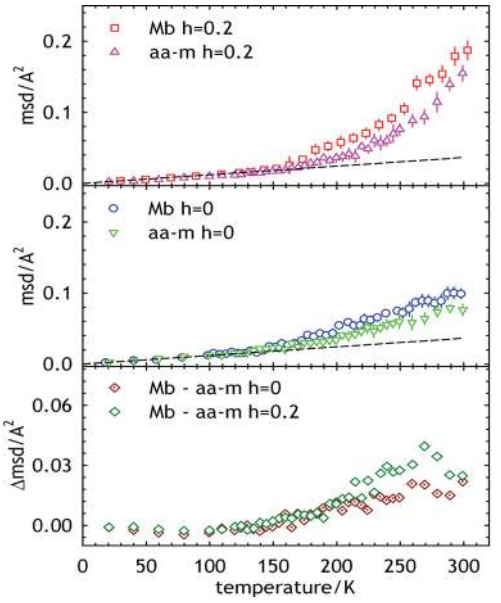

Figure 3. Total msd as a function of temperature at the two hydration levels investigated (upper panels) and msd differences between Mb and aa-m (Δmsd; lower panel). Dashed lines are linear fits in the harmonic temperature region.

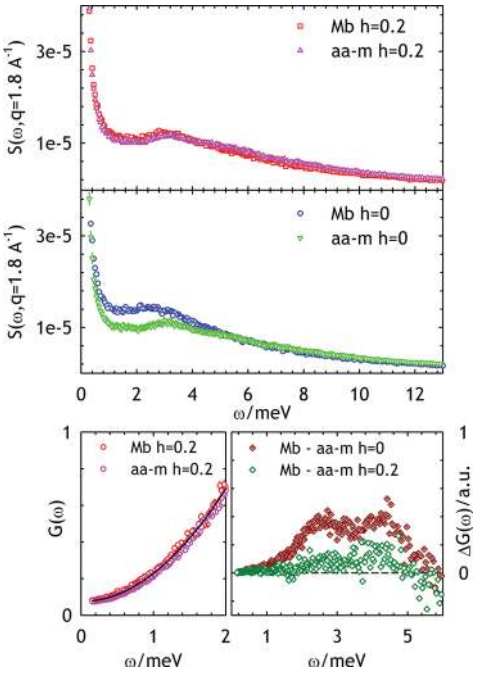

Figure 4. Upper panels: dynamic structure factor $S(q,\omega)$ of D_2O-hydrated and dry powders of Mb and aa-m at T = 150 K. Note that no quasi-elastic broadening is observed. Lower panels: (left) density of vibrational states $G(\omega)$ at low energies; the solid line is a fitting with a ω^2 law; (right) $\Delta G(\omega)$, i.e., differences between Mb and aa-m density of vibrational states, for dry and D_2O-hydrated samples; the black dashed line indicates the zero difference.

for the hydrated samples. The above effects are small (~0.04 Å^2 at room temperature) but systematic and outside the experimental error (see upper panels of Figure 3).

From the data reported in Figures 2 and 3 we draw the following conclusions:

Both anharmonic onsets are observed in the hydrated aa-m sample (i.e., in the absence of any polypeptide chain) and occur at the same transition temperature as in the real protein. While for the low temperature onset (attributed to methyl group rotations) this is an expected result, for the dynamical transition this is a new and relevant result since it implies that the "protein dynamical transition" does not require the protein polypeptide chain. This indicates that caution should be used when stressing any functional relevance of the dynamical transition. Indeed, aa-m is a system with the very same molecular groups composition of a real protein, but without any biological function. The fact that the "protein dynamical transition" did not need the protein secondary and tertiary structure had already been shown by neutron scattering and terahertz spectroscopy experiments on denatured proteins;[18,20] the present results clearly show that the "protein" (i.e., a polypeptide chain) is not even needed. If combined with recent results on the enzymatic activity of dry proteins,[17] we can conclude that the "protein dynamical transition" is neither a necessary nor a sufficient condition for the biological function to take place.

The close similarity of the dynamical transition temperature in structurally different hydrated systems such as polyglycine (pure backbone contribution), aa-m (pure side-chains contribution), and Mb (backbone and side-chains contributions) confirms that hydration water plays the main role in this process. This conclusion holds true whether the dynamical transition is a real glass-like transition or it is linked to a resolution effect. An analysis of the hydrated-dry difference msd in terms of Arrhenius plots and activation enthalpies, as suggested by Frauenfelder and co-workers[29] can be found in the SI.

The dynamical transition mainly involves hydration water coupled fluctuations of the amino acids side chains, as shown by comparing the msd amplitudes for the aa-m and the Mb. Small backbone-specific contributions can be detected for both anharmonic onsets. For the msd related to the dynamical transition, these contributions amount to about 0.04 Å^2 at room temperature, in good agreement with what is measured for polyglycine.[8] However, it should be considered that differences between the Mb and aa-m samples related to the side-chains environment, the amount of surface covered by water, the extent of polar/apolar interactions, and the presence of "free" carboxylic and amino groups, could also influence the amplitude of fluctuations.

In order to further investigate the role of the polypeptide chain on protein dynamics, we performed inelastic incoherent neutron scattering experiments at a time-of-flight spectrometer with a resolution of 70 μeV fwhm. Inelastic neutron scattering revealed a broad band occurring in the energy range of 1.5−3.5 meV in protein spectra (the so-called "boson peak", already observed in glass forming systems), corresponding to an excess in the low frequency vibrational density of states over the Debye level. Although a number of studies has been performed,[30−34] the origin of the boson peak is not yet well understood. In Figure 4 we report the dynamic structure factors, $S(\omega)$, obtained from normalized inelastic scans at 150 K and momentum transfer q = 1.8 Å^{-1} (details on the normalization and binning of inelastic spectra are given in the SI). Note that no quasi-elastic broadening is observed and that the spectra scale very well with the Bose factor[30] in the temperature range 100−180 K (data not shown). It is evident that, in hydrated samples, the "boson peak" is identical

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in aa-m and Mb (this result had been previously anticipated,[35] but the data were never shown). This means that the distribution of vibrational modes is qualitatively the same in the two systems, and excludes the idea that the low frequency vibrations responsible for the boson peak in proteins depend on the conformation and packing of the peptide chain, or on the chemical linkage between amino acids. The low-frequency harmonic dynamics in hydrated protein samples is then dominated by vibrational motions of amino acid side-chains more than by collective motions of the polypeptide chain.

The dry Mb system shows an increased intensity at low frequency as compared to the hydrated case; this effect had already been reported[36] and assigned to strong protein-water H-bonds, which depress the librational motions of the polar side chains. Interestingly, this is not observed for aa-m samples that show almost no hydration dependence. To highlight differences between Mb and aa-m, we calculated the density of vibrational states $G(\omega)$, and, in the lower panels of Figure 4, we report $G(\omega)$ at low energies (where a ω^2 dependence is observed, as expected for harmonic solids) for Mb and aa-m at $h = 0.2$ and the differences between Mb and aa-m at $h = 0$ and $h = 0.2$, $\Delta G(\omega)$ (details on the calculation of $G(\omega)$ from $S(\omega,q)$ are given in the SI). A protein-specific excess of vibrational states in the frequency range 1−5 mev (8−40 cm^{-1}) is evident in the dry samples and is almost suppressed in the hydrated ones. This indicates that the low-frequency scattering excess observed in dry Mb has to be related to backbone-specific vibrational motions, damped in the presence of hydration water, more than to librational motions of the polar side chains. On the other hand, the absence of hydration effect in the aa-m sample indicates that the interaction of carboxylic groups with hydration water molecules does not play a relevant role in determining the side chain harmonic dynamics.

In conclusion, the reported results show that:

1 the anharmonic onset at ∼150 K (in both dry and hydrated samples) and the dynamical transition (in the hydrated samples) at ∼220 K are also observed for the amino acid mixture: the "protein dynamical transition" is related mainly to fluctuations of hydrated amino acid side chains and does not require the polypeptide chain. However:

2 backbone specific contributions to protein dynamics can be detected. In fact:

2a in the anharmonic regime, the amplitude of mean square fluctuations is enhanced in the presence of the polypeptide chain; the effect amounts to ∼0.04 $Å^2$ at room temperature, i.e., to about 20% of the total msd;

2b in the harmonic regime, a low frequency excess is observed in the inelastic spectrum of dry Mb sample, probably related to backbone specific low frequency vibrations damped in the presence of hydration water.

3 Our experimental evidence supports the idea that, independently of the model proposed to explain the phenomenon, water not only sustains but also drives the onset of dynamical transition, and any structural differences in different peptide systems only determine the amplitude of the related fluctuations.

The present results call for other relevant points to be addressed in future work, concerning mainly the role of interaction between hydration water and different polar and apolar side chains and the effects of side chain size or charge on the dynamical transition and boson peak. Further studies on homomeric polypeptide chains and single amino acids are currently under way to address these crucial issues.

EXPERIMENTAL METHODS

Amino acids powders were purchased from Sigma-Aldrich (St. Louis, MO) and hydrated with D_2O Euriso-Top, purity 99.96%. Details on the sample preparation are given in the SI. The infrared spectra were collected with a Jasco FT-IR-410 spectrometer equipped with a Pike diffuse reflectance accessory. Elastic neutron scattering temperature scans (20−300 K) were performed at the thermal backscattering spectrometer IN13 (Institut Laue-Langevin, Grenoble, France) with an incident wavelength $\lambda = 2.23$ Å and an energy resolution of 8 μeV fwhm. Inelastic neutron scattering experiments were performed at the time-focusing time-of-flight spectrometer IN6 (Institut Laue-Langevin, Grenoble, France) with an incident wavelength $\lambda = 5.1$ Å and an energy resolution of 70 μeV fwhm. Further details on the instrumental equipments and an extended discussion on the data analysis are given in the SI.

ASSOCIATED CONTENT

Supporting Information. Details on samples preparation; experimental procedures for infrared and neutron scattering measurements; elastic and inelastic neutron scattering data reduction and analysis; analysis of hydrated-dry Δmsd in terms of the Arrhenius law. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*E-mail: cupane@fisica.unipa.it.

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