Eur Biophys J (2008) 37:543–549
DOI 10.1007/s00249-007-0249-9

ORIGINAL PAPER

Hydration dependent dynamics in sol–gel encapsulated myoglobin

Giorgio Schirò · Michele Sclafani · Francesca Natali · Antonio Cupane

Received: 27 September 2007 / Revised: 15 November 2007 / Accepted: 20 November 2007 / Published online: 1 February 2008

Abstract In this work we study the effect of hydration on the dynamics of a protein in confined geometry, i.e. encapsulated in a porous silica matrix. Using elastic neutron scattering we investigate the temperature dependence of the mean square displacements of non-exchangeable hydrogen atoms of sol–gel encapsulated met-myoglobin. The study is extended to samples at 0.2, 0.3 and 0.5 g water/g protein fractions and comparison is made with met-myoglobin powders at the same average hydration and with a dry powder sample. Elastic data are analysed using a model of dynamical heterogeneity to take into account deviations of elastic intensity from gaussian behaviour in a large momentum transfer range and reveal a specific, model independent, effect of sol–gel confinement on protein dynamics, consisting mainly in a reduction of large-scale motions that are activated at temperatures larger than ~230 K. Surprisingly, the effect of confinement depends markedly on hydration and has a maximum at about 35% water/protein fraction corresponding to full first shell hydration. The presence of hydration-dependent MSD also in encapsulated met-Mb strongly supports the idea that the effect of sol–gel confinement on protein dynamics involves a modification of the structural/dynamical properties of the co-encapsulated solvent more than direct protein–matrix interactions.

Keywords Protein dynamics · Silica hydrogels · Dynamics in confinement · Elastic neutron scattering · Hydration shell · Protein mean square displacements

Advanced neutron scattering and complementary techniques to study biological systems. Contributions from the meetings, "Neutrons in Biology", STFC Rutherford Appleton Laboratory, Didcot, UK, 11–13 July and "Proteins At Work 2007", Perugia, Italy, 28–30 May 2007.

G. Schirò · M. Sclafani · A. Cupane (✉)
Department of Physical and Astronomical Sciences, CNISM and University of Palermo, Via Archirafi, 36, 90123 Palermo, Italy
e-mail: cupane@fisica.unipa.it

F. Natali
INFM-CNR and CRS-SOFT, c/o OGG, 6 Jules Horowitz, BP 156, 38043 Grenoble, France

Introduction

It is well known that in vivo biological systems (e.g. proteins and other biomolecules inside the cell) are characterized by conditions of crowding and geometrical confinement (Minton 2001; Ellis and Minton 2003). It has been suggested that this physical state can show different properties with respect to the low-concentration solution systems often used in biophysical studies: indeed several works (see, e.g. Minton 2001 and references therein) showed that inside the cell chemical equilibrium and chemical reactivity are perturbed by non-specific solute–solute interactions and excluded volume effects.

On the other hand, it is generally recognised that proteins at room temperature are fluctuating objects and that an in depth knowledge of their dynamics, as well as of their structure, is required to understand the functional behaviour. In recent years several studies, both experimental (Fenimore et al. 2003, 2004; Paciaroni et al. 2002) and simulative (Tarek and Tobias 2002; Tournier et al. 2003), have indicated the existence of a strong coupling between solvent and protein dynamics; in particular the well known "dynamical transition" from harmonic to anharmonic protein motions that occurs in the temperature region around 200–250 K and is generally related to the onset of biochemical activity, has been shown to depend upon the

protein hydration state (Doster et al. 1989; Rasmussen et al. 1992; Ferrand et al. 1993; Paciaroni et al. 2002; Tournier et al. 2003; Caliskan et al. 2006).

Studies on the dynamic properties of proteins in confined geometry are far less abundant, although it has been suggested that confinement plays an important role in the dynamics of biomolecules (Massari et al. 2006; Schirò et al. 2007; Schirò and Cupane 2007). Moreover, in confinement, protein–solvent coupling may be even stronger than in solution; as an example, a recent study on the large-scale quaternary transition in hemoglobin confined in a rigid silica matrix (Schirò and Cupane 2007) showed that relaxation rates are slowed down in confinement, the extent of this effect being markedly related to the viscosity of the co-encapsulated solvent. These observations highlight the biological relevance of investigations on the dynamic properties of proteins and solvents in conditions of confinement and crowding, hydration level and temperature being the essential parameters to be varied (Pieper et al. 2007).

Aim of this study is therefore to investigate the dynamical properties of myoglobin at various hydrations and in conditions of geometrical confinement; in order to better clarify the role of confinement in a solid matrix the results are compared with the "classical" hydrated powder system, at the same average hydration levels.

Dynamical properties are investigated through the temperature dependence of mean square displacements (MSD) of the non-exchangeable protein hydrogen atoms, measured with elastic neutron scattering. Confinement conditions are realized by sol–gel encapsulation: through the hydrolysis/polycondensation of an alcoxide precursor one obtains a porous and disordered silica matrix, in which water and proteins can be trapped (Ellerby et al. 1992). In our sol–gel protocol, the protein + hydration shell acts as a template directing the formation of the silica cages and network: this provides the encapsulated proteins with ample surrounding space to accommodate solvent. Pore size estimates in analogous sol–gel silica matrices (measured from N_2 desorption isotherms via the Barret-Joyner-Halenda (BJH) approach) give a dominant contribution of "mesopores" with $\approx 35 \div 45$ Å BJH pore diameter (Wei et al. 2001), thus indicating that at most one protein molecule (plus solvent) is present in one matrix pore. Encapsulated proteins are characterized by spectral properties almost indistinguishable from the solution conditions. They retain their functionality and show enhanced conformational stability and resistance against denaturation (Ping et al. 2003; Fiandaca et al. 2004); moreover their hydration level can be easily varied, so that these systems are almost ideal candidates to study the interplay between protein and hydration water dynamics in confinement conditions.

Materials and methods

Samples

Lyophilised horse Mb (Sigma Aldrich) was dissolved at room temperature in 0.1 M K-phosphate buffer pH 7. A fourfold molar excess of potassium ferricyanide, $C_6FeK_3N_6$, was added to obtain the ferric (met) state of the protein; after equilibration, the excess potassium ferricyanide was removed by prolonged dialysis against a 0.1 M K-phosphate buffer solution at pH 7, in the cold. The protein solution was then deuterated with repeated H_2O–D_2O (Euriso-Top, purity 99.97%) exchanges following a dilution/concentration procedure previously described (Caronna et al. 2005), thus obtaining a final D_2O percentage greater than 99%. The final protein concentration was $\sim$27% by weight.

Protein encapsulation in silica hydrogels was performed using a protocol already described (Schirò et al. 2007). A solution containing TMOS (60% v/v), D_2O (38% v/v) and HCl 0.04 M (2% v/v) was sonicated for 20 min in an ice bath and mixed in a 1:1 proportion (in volume) and at 7°C with the above met-Mb/D_2O solution. A gel about 1 mm thick was formed in about 1 min; after gelification, it was left to age in a controlled atmosphere of N_2/D_2O. The hydration levels were determined from the observed mass change on drying: hydration level is here defined as $h = $ [g D_2O]/[g protein]. met-Mb hydrated powders were prepared with the following procedure: a solution of horse Mb in D_2O (concentration 50 mg/ml) was held at room temperature for approximately 24 h, centrifuged for 20 min at 10°C and subsequently re-lyophilised; the resulting powder was held for about 30 h under vacuum at 45°C and considered our dry ($h = 0$) sample. We are aware of the fact that the above procedure is unable to remove the water tightly bound to charged groups on the protein surface (Rupley et al. 1983; Rupley and Careri 1991) and amounting to $\approx$2% w/w. The powder was then held in a controlled N_2/D_2O atmosphere in order to obtain the desired hydration levels that were estimated by measuring the powder mass change.

Elastic neutron scattering

Incoherent elastic neutron scattering measurements as a function of temperature were performed on the thermal ($\lambda = 2.23$ Å) high-energy resolution backscattering spectrometer IN13 (Institut Laue-Langevin, Grenoble, France) that is characterized by a very large momentum transfer range ($0.2 < Q < 4.9$ Å^{-1}) with a good and nearly Q-independent energy resolution. IN13, therefore, allows to access the space and time windows of 1–6 Å and 0.1 ns,

respectively. In our experiments the energy resolution was fixed to 8 μeV. The neutron beam scattered from the sample was reflected in almost perfect backscattering geometry by CaF2 analysers to be finally collected by 35 ^{3}He detectors in the region $1.1 < Q < 4.9$ Å^{-1} and by a position sensitive detector (PSD) in the low Q region ($0.2 < Q < 0.8$ Å^{-1}). The elastic energy value ($\Delta\omega = 0$) was kept fixed within 3 μeV of accepted tolerance.

The elastic scattering intensities ($I_{el}(Q) \equiv S(Q, \omega = 0)$), suitably corrected for the empty sample holder contributions, were normalised with respect to the lowest temperature measurement to compensate for spurious background contributions and detector efficiency. No Bragg peaks were observed due to crystalline water at any of the measured temperature points. In all the experiments, the sample thickness was suitably chosen to minimize the neutron absorption from the sample, thus avoiding correction from multiple scattering contributions. A transmission of about 88% was guaranteed using 1 mm thick Al flat sample holder.

Data analysis

The experimental data consist of the normalized incoherent elastic intensity $S_{inc}(Q, \omega = 0)$ at different temperatures. In the low Q region the Q-dependence of $S_{inc}(Q, \omega = 0)$ is given, in the frame of the gaussian approximation by: $S(Q, \omega = 0) = I_0 e^{-(\langle \Delta u^2 \rangle Q^2/6)}$, where I_0 is a constant and $\langle \Delta u^2 \rangle = \langle u_T^2 - u_{20\,K}^2 \rangle$ is the normalized mean-square amplitude of the hydrogen atoms atomic displacements (MSD). The gaussian approximation is valid for Q values that satisfy the condition $Q^2 \langle \Delta u^2 \rangle \approx 2$ (Doster et al. 1989; Natali et al. 2000). However, as clearly shown from the data in Fig. 1, for our samples a deviation from a gaussian behaviour, particularly evident at high temperatures, is observed in the high Q region. Two possible models to take into account this behaviour have been proposed:

1. In the first model the non-gaussianity is attributed to the heterogeneity of hydrogen atoms MSD, resulting in a superposition of gaussian forms with different widths; a simplified version of this description has been introduced by considering a bimodal distribution (Nakagawa et al. 2004; Roh et al. 2005; Doster and Settles 2005).
2. In the second model hydrogen atoms are considered all equivalent and can fluctuate in an anharmonic potential between two sites of different energy, whose minima are separated by a distance d [asymmetric double well model, originally proposed by Doster et al. (1989)].

In a previous work (Schirò et al. 2007) we used both models to fit data relative to myoglobin encapsulated in silica hydrogel and we showed that statistical control parameters resulting from the fitting procedures are unable to discriminate between them; however, the results obtained and the effects of encapsulation observed were largely model independent. In the present work we have verified that both models describe equally well the Q-dependence of elastic scattering function at all temperatures and for all the hydration levels explored. In the following we will report only the analysis obtained with the dynamical heterogeneity approach. For the sake of clarity we recall that the model assumes the validity of the gaussian approximation but postulates the existence of a distribution function of hydrogen atoms MSD, $f(\langle \Delta u^2 \rangle)$, considered to be bimodal of the form:

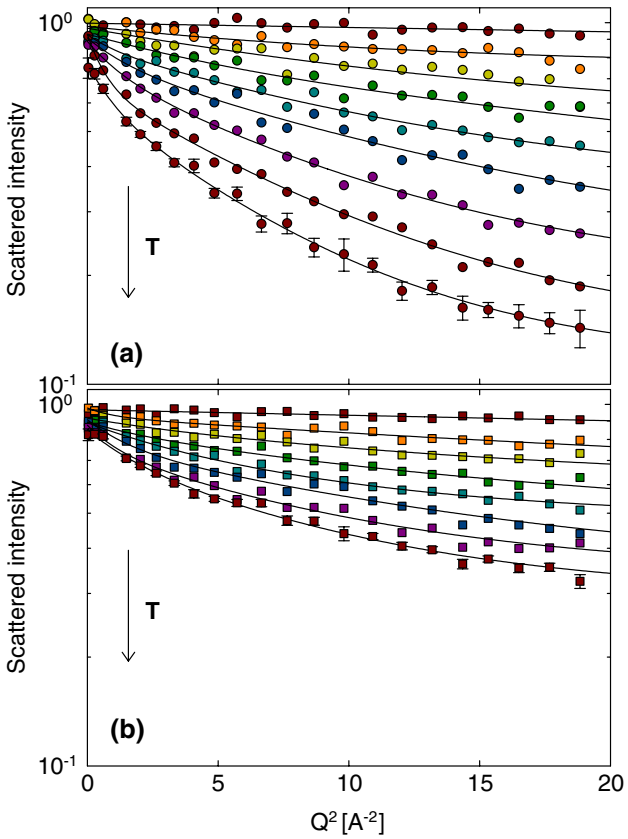

Fig. 1 Normalized elastic intensities vs. Q^2 at various temperatures for samples at $h = 0.3$. Panel **a** met-Mb hydrated powder; panel **b** met-Mb encapsulated in silica gel. Data sets refer to few selected temperatures from 50 to 290 K. The *continuous lines* represent fittings of the experimental data in terms of the dynamical heterogeneity model [Eq. (2) in the text]. For figure clarity, *error bars* are reported only for the data at high temperature

$$f(\langle \Delta u^2 \rangle) = a_1 \delta(\langle \Delta u^2 \rangle - \langle \Delta u^2 \rangle_1) + a_2 \delta(\langle \Delta u^2 \rangle - \langle \Delta u^2 \rangle_2) \quad (1)$$

where $\delta(x)$ is the delta function and a_1 and $a_2 = 1 - a_1$ are the population fractions. Using Eq. (1) the scattering function becomes:

$$S(Q, \omega = 0) = \int_0^\infty \left[a_1 \delta(\langle \Delta u^2 \rangle - \langle \Delta u^2 \rangle_1) + a_2 \delta(\langle \Delta u^2 \rangle - \langle \Delta u^2 \rangle_2) \right] e^{-\frac{\langle \Delta u^2 \rangle}{6} Q^2} d\langle \Delta u^2 \rangle$$
$$= a_1 e^{-\frac{\langle \Delta u^2 \rangle_1}{6} Q^2} + a_2 e^{-\frac{\langle \Delta u^2 \rangle_2}{6} Q^2}. \quad (2)$$

As already discussed (Schirò et al. 2007), the choice of a simplified bimodal distribution is supported by several recent observations indicating that hydrogen dynamics in proteins arise from two main contributions, related to methyl group hydrogens and to all other non exchangeable hydrogens, respectively (Roh et al. 2005; Doster and Settles 2005).

Results and discussion

The normalized elastic intensities as a function of Q^2 relative to the sample at a representative hydration level ($h = 0.3$) are reported in Fig. 1 for some selected temperatures. Data in panel (a) refer to Mb powder and those in panel (b) to encapsulated Mb. The main effect of confinement in silica gel, evident from a first inspection of data reported in Fig. 1, is a reduction of dynamics of non-exchangeable hydrogens of myoglobin; this can be inferred from the much lower decrease of elastic intensity as a function of Q^2 for the encapsulated protein, mainly at high temperatures. The continuous lines are fittings of the experimental data obtained with the bimodal distribution (Eq. 2): as discussed in Schirò et al. 2007 parameters a_1 and a_2 have been set at 0.29 and 0.71, respectively at all the temperatures explored, since the fraction of methyl hydrogens over the total non-exchangeable hydrogens is, in horse Mb, 0.29. A behaviour analogous to that reported in Fig. 1 is observed at all the hydration levels investigated.

MSD are reported in Fig. 2 as a function of temperature for metMb in silica hydrogel (right panels) and for metMb powder (left panels); data relative to a "dry" powder sample are also reported, for comparison. Data relative to methyl and non-methyl hydrogens are reported in the upper and lower panels, respectively. Note that MSD are plotted on an absolute scale, i.e. by adding to the normalized values the contributions of zero point vibrations, assumed to be ≈ 0.01 and ≈ 0.003 Å^2 for methyl and non-methyl hydrogen atoms, respectively (Doster 2006). The continuous lines correspond to the purely harmonic contribution obtained by fitting of the low temperature trend ($T < 130$ K) in terms of the relation $\langle x^2/2 \rangle = A \coth[h\nu/2k_B T]$; parameters values are: $A = 1.0 \times 10^{-2}$ and 1.3×10^{-3} Å^2 for methyl and non-methyl hydrogens respectively, and $\nu = 30$ cm^{-1}.

At all hydrations and for both the powder and silica systems, two activations of anharmonicity are observed. The first one occurs at 100–150 K and has been attributed to the onset of methyl group rotations (Roh et al. 2005; Doster and Settles 2005; Colmenero et al. 2005); it is clearly independent of hydration and is present also in dry powder.[1] Coherently, no relevant deviations from harmonicity are observed for the MSD of "non-methyl" groups in the region 100–150 K. The second one occurs at about 200–250 K and can be identified with the well known "dynamical transition", already observed in several biological systems in the same temperature range; it strongly depends upon hydration and is not observed in the dry powder sample. The fact that the dynamical transition in hydrated protein powders is solvent coupled, being absent in dry samples and approaching saturation at high hydrations, was already well established; conversely, it is a new and intriguing result that a rather strong dependence of MSD behaviour on hydration level is observed also in the encapsulated samples (Fig. 2, panels c, d). This suggests that the effect of confinement on protein motions is mediated by the hydration shell.

A more detailed comparison between metMb in silica gel and metMb powder at different hydrations is presented in Fig. 3. It is evident that at all the investigated hydration levels, the first onset of anharmonicity is unaffected by protein encapsulation while the solvent-coupled dynamical activation above 230 K is clearly reduced by protein encapsulation in silica gel. The "confinement effect" brought about by sol–gel encapsulation on protein dynamics can be quantified by defining the quantity:

$$F = \frac{\langle \Delta x^2/2 \rangle_{\text{hydrated powder}} - \langle \Delta x^2/2 \rangle_{\text{hydrated gel}}}{\langle \Delta x^2/2 \rangle_{\text{hydrated powder}} - \langle \Delta x^2/2 \rangle_{\text{dry sample}}} \quad (3)$$

whose physical meaning is simply the fraction of large amplitude anharmonic motions that is absent in the sol–gel

[1] The activation of methyl group rotation even in "dry" powder may be put in relation with the presence, in our dry sample, of residual water molecules tightly bound to charged groups on the protein surface (see "Materials and Methods" section). These water molecules, although being translationally restricted, can perform rotational motion and therefore, by exchanging hydrogen bonds with the protein surface, activate the methyl group rotations (Doster et al. 1986; Tarek and Tobias 2000, 2002; Giuffrida et al. 2006).

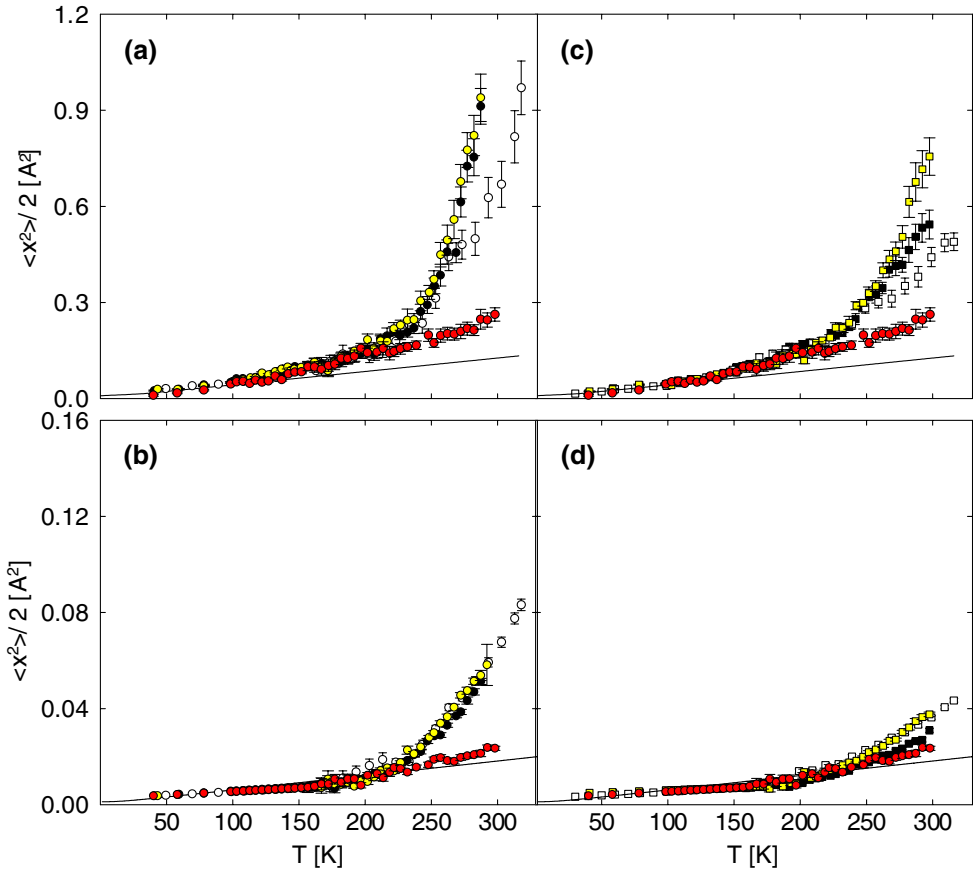

Fig. 2 Temperature dependence of the MSD obtained using the dynamical heterogeneity model. Panels **a**, **b** MSD in met-Mb powder for methyl and non-methyl hydrogens, respectively. Panels **c**, **d** same as panels **a**, **b**, for met-Mb in silica gel. *Red symbols*: $h = 0$; *white symbols*: $h = 0.2$; *black symbols*: $h = 0.3$; *yellow symbols*: $h = 0.5$. *Continuous lines* represent the harmonic contributions to the MSD (see text)

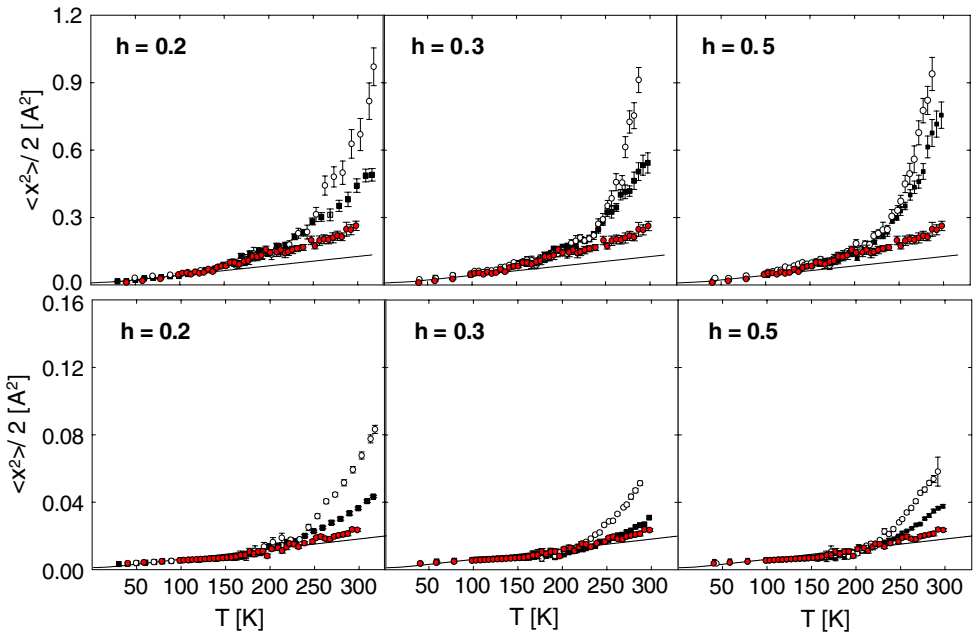

Fig. 3 Comparison between MSD in met-Mb powder (*white circles*) and met-Mb in silica gel (*black squares*) at different hydration levels. Upper panels: methyl hydrogens contribution. Lower panels: non-methyl hydrogens contribution. Data relative to dry powder sample (*red circles*) are superimposed for comparison. Harmonic contributions to the MSD are reported as *continuous lines*

encapsulated sample due to the confinement effect. The quantity F calculated at $T = 290$ K is reported in Fig. 4 as a function of hydration. As it can be seen, sol–gel encapsulation of met-Mb is able to hinder the anharmonic MSD of non-exchangeable hydrogens by more than 50%; interestingly the stabilizing effect seems to have a maximum around 35% hydration, i.e. at a value corresponding to a full hydration shell (Lounnas and Pettitt 1994; Doster et al. 1986).

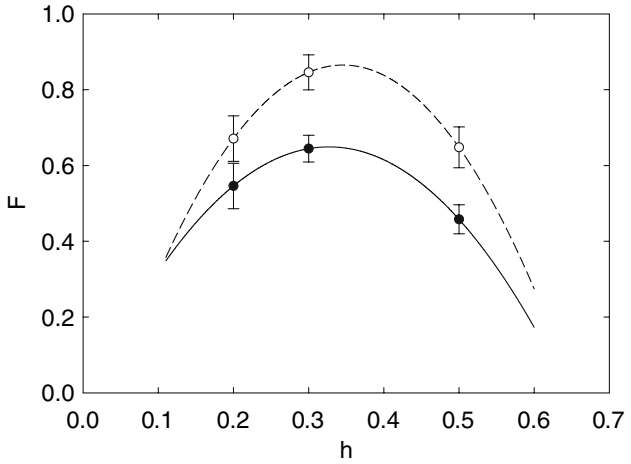

Fig. 4 Percentage of "confinement effect" as defined by Eq. (3) in the text, for methyl hydrogens (*full circles*) and non-methyl hydrogens (*empty circles*); $T = 290$ K. *Lines* are guides to the eye

We can try now to rationalize the above effects in terms of protein–solvent interactions and of solvent dynamics in confinement. It has been proposed (Fenimore et al. 2004; Iben et al. 1989; Tarek and Tobias 2002; Cornicchi et al. 2006; Giuffrida et al. 2006) that a cooperative and properly structured hydrogen bond network is responsible for the correlation between protein conformational fluctuations and the dynamics of surrounding water molecules. Moreover, there is experimental evidence (Bergman and Swenson 2000; Swenson et al. 2006) that water in hard confinement is characterized by a strong perturbation of hydrogen bond network, mainly due to the absence of a long-range bonding propagation, leading to partial (or even complete) disruption of cooperative water molecules motions and, depending on the hydration level, to the disappearance of the collective α-relaxation (Cerveny et al. 2004). On the other hand, it has been proposed that large-scale, functionally relevant, protein motions are governed ("slaved", in the terminology of Fenimore et al. 2003) by the α relaxation in the solvent. It is therefore reasonable to relate the reduction of the hydration dependent protein MSD observed at high temperature ($T > 230$ K) in sol–gel encapsulated myoglobin to a perturbation of solvent collective dynamics due to confinement. Coherently, recent quasi-elastic neutron scattering data (Schirò et al. 2007) showed that the diffusion coefficient of D_2O confined inside the pores of a silica matrix is reduced by at least one order of magnitude with respect to a bulk sample. The fact that the MSD reduction is more pronounced at hydration values near 35% (see Fig. 4) suggests that solvent molecules in the first hydration shell are mostly involved in this effect.

One may wonder why solvent dynamics (and, in turn, the solvent-coupled protein motions) should be so different in the two systems, silica gel and hydrated powder, since also in hydrated powder solvent is likely to be confined in a nanometer or sub-nanometer geometry. A tentative explanation could consider the recent terahertz spectroscopy data suggesting that the biological relevance of water–protein interaction resides in the coherence between correlated water motions and protein fluctuations (Heugen et al. 2006). Within this scenario, one could think that while in hydrated powders the solvent shell around one protein is confined by other fluctuating protein/solvent molecules, inside the silica matrix solvent layers on the surface of myoglobin are confined by the rigid walls of the matrix pores that are not able to preserve the coherent fluctuations of the hydrogen-bonded solvent network.

Conclusions

The main results reported in this work may be summarized as follows:

(a) The large amplitude protein motions that become activated above the dynamical transition temperature (∼230 K) are solvent dependent; this fact was well known for hydrated powders, but is here reported for the first time for a protein in hard confinement.
(b) The confinement effect, i.e. the reduction of the above mentioned anharmonic protein motions observed in sol–gel encapsulated confined samples, depends on the average protein hydration.

The reported data can be rationalized in terms of protein–solvent interactions and of solvent dynamics in confinement, and suggest that the confinement effect is related to a perturbation of solvent collective dynamics (α relaxation) due to confinement more than to direct protein–matrix interactions.

Acknowledgments We are indebted to Prof. I. Pucci-Minafra (Dept. DOSAC, University of Palermo) for use of the lyophilizer available at her laboratory. We are also indebted to Prof. L. Cordone and to Drs. M. Levantino and C. Caronna for discussions. This work has been supported by Italian MIUR (grant PRIN 2005, N° 2005027011).

References

Bergman R, Swenson J (2000) Dynamics of supercooled water in confined geometry. Nature 408:283–286
Caliskan G, Briber RM, Thirumalai D, Garcia-Sakai V, Woodson SA, Sokolov AP (2006) Dynamic transition in tRNA is solvent induced. J Am Chem Soc 128:32–33
Caronna C, Natali F, Cupane A (2005) Incoherent elastic and quasi-elastic neutron scattering investigation of hemoglobin dynamics. Biophys Chem 116:219–225

Cerveny S, Schwartz GA, Bergman R, Swenson J (2004) Glass transition and relaxation processes in supercooled water. Phys Rev Lett 93:245702

Colmenero J, Moreno AJ, Alegria A (2005) Neutron scattering investigations on methyl group dynamics in polymers. Prog Polym Sci 30:1147–1184

Cornicchi E, Marconi M, Onori G, Paciaroni A (2006) Controlling the protein dynamical transition with sugar-based bioprotectant matrices: a neutron scattering study. Biophys J 91:289–297

Doster W (2006) Brownian oscillator analysis of molecular motions in biomolecules. In: Fitter J, Gutberlet T, Katsaras J (eds) Neutron scattering in biology, methods and applications. Springer, Berlin, Heidelberg, New York, pp 461–483

Doster W, Settles M (2005) Protein–water displacement distributions. Biochim Biophys Acta 1749:173–186

Doster W, Bachleitner A, Dunau R, Hiebl M, Lüscher E (1986) Thermal properties of water in myoglobin crystals and solutions at subzero temperatures. Biophys J 50:213–219

Doster W, Cusack S, Petry W (1989) Dynamical transition of myoglobin revealed by inelastic neutron scattering. Nature 337:754–756

Ellerby LM, Nishida CR, Nishida F, Yamanaka SA, Dunn B, Valentine JS, Zink JI (1992) Encapsulation of proteins in transparent porous silicate-glasses prepared by the sol–gel method. Science 255:1113–1115

Ellis RJ, Minton AP (2003) Join the crowd. Nature 425:27–28

Fenimore PW, Frauenfelder H, McMahon BH, Parak FG (2003) Slaving: solvent fluctuations dominate protein dynamics and functions. Proc Natl Acad Sci USA 99:16047–16051

Fenimore PW, Frauenfelder H, McMahon BH, Young RD (2004) Bulk-solvent and hydration-shell fluctuations, similar to α- and β-fluctuations in glasses, control protein motions and functions. Proc Natl Acad Sci USA 101:14408–14413

Ferrand M, Dianoux AJ, Petry W, Zaccai G (1993) Thermal motions and function of bacteriorhodopsin in purple membranes—effects of temperature and hydration studied by neutron-scattering. Proc Natl Acad Sci USA 90:9668–9672

Fiandaca G, Vitrano E, Cupane A (2004) Ferricytochrome *c* encapsulated in silica nanoparticles: structural stability and functional properties. Biopolymers 74:55–59

Giuffrida S, Cottone G, Cordone L (2006) Role of solvent on protein–matrix coupling in mbco embedded in water–saccharide systems: a Fourier transform infrared spectroscopy study. Biophys J 91:968–980

Heugen U, Schwaab G, Bründermann E, Heyden M, Yu X, Leitner DM, Havenith M (2006) Solute-induced retardation of water dynamics probed directly by terahertz spectroscopy. PNAS 103:12301–12306

Iben IET, Braunstein D, Doster W, Frauenfelder H, Hong MK, Johnson JB, Luck S, Ormos P, Schulte A, Steinbach PJ, Xie AH, Young RD (1989) Glassy behavior of a protein. Phys Rev Lett 62:1916–1919

Lounnas V, Pettitt BM (1994) A connected-cluster of hydration around myoglobin: correlation between molecular dynamics simulations and experiment. Proteins 18:133–147

Massari AM, Finkelstein IJ, Fayer MD (2006) Dynamics of proteins encapsulated in silica sol–gel glasses studied with IR vibrational echo spectroscopy. J Am Chem Soc 128:3990–3997

Minton AP (2001) The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. J Biol Chem 276:10577–10580

Nakagawa H, Kamikubo H, Tsukushi I, Kanaya T, Kataoka M (2004) Protein dynamical heterogeneity derived from neutron incoherent elastic scattering. J Physical Soc Japan 73:491–494

Natali F, Gliozzi A, Rolandi R, Relini A, Cavatorta P, Deriu A, Riccio P, Fasano A, (2000) Myelin basic protein reduces molecular motions in DMPA, an elastic neutron scattering study. Physica B 301:145–149

Paciaroni A, Cinelli S, Onori G (2002) Effect of the environment on the protein dynamical transition: a neutron scattering study. Biophys J 83:1157–1164

Pieper J, Hauss T, Buchsteiner A, Baczynski K, Adamiak K, Lechner RE, Renger G (2007) Temperature- and hydration-dependent protein dynamics in photosystem II of green plants studied by quasielastic neutron scattering. Biochemistry 46:11398–11409

Ping G, Yuan JM, Vallieres M, Dong H, Sun Z, Wei Y, Li FY, Lin SH (2003) Effects of confinement on protein folding and protein stability. J Chem Phys 118:8042–8048

Rasmussen BF, Stock AM, Ringe D, Petsko GA (1992) Crystalline ribonuclease A loses function below the dynamical transition at 220 K. Nature 357:423–424

Roh JH, Novikov VN, Gregory RB, Curtis JE, Chowdhuri Z, Sokolov AP (2005) Onsets of anharmonicity in protein dynamics. Phys Rev Lett 95:038101

Rupley JA, Careri G (1991) Protein hydration and function. Adv Protein Chem 41:37–172

Rupley JA, Gratton E, Careri G (1983) Water and globular proteins. Trends Biochem Sci 8:18–22

Schirò G, Cupane A (2007) Quaternary relaxations in sol–gel encapsulated hemoglobin studied via NIR and UV spectroscopy. Biochemistry 46:11568–11576

Schirò G, Sclafani M, Caronna C, Natali F, Plazanet M, Cupane A (2007) Dynamics of myoglobin in confinement: an elastic and quasi-elastic neutron scattering study. Chem Phys. doi: 10.1016/j.chemphys.2007.07.045 (in press)

Swenson J, Jansson H, Bergman R (2006) Relaxation processes in supercooled confined water and implications for protein dynamics. Phys Rev Lett 96:247802

Tarek M, Tobias DJ (2000) The dynamics of protein hydration water: a quantitative comparison of molecular dynamics simulations and neutron scattering experiments. Biophys J 79:3244–3257

Tarek M, Tobias DJ (2002) Role of protein–water hydrogen bond dynamics in the protein dynamical transition. Phys Rev Lett 88:138101

Tournier AL, Xu J, Smith JC (2003) Translational hydration water dynamics drives the protein glass transition. Biophys J 85:1871–1875

Wei Y, Xu JG, Feng QW, Lin MD, Dong H, Zhang WJ, Wang C (2001) A novel method for enzyme immobilization: direct encapsulation of acid phosphatase in nanoporous silica host materials. J Nanosci Nanotechnol 1:83–93