Spectroscopy 24 (2010) 143–147
DOI 10.3233/SPE-2010-0421
IOS Press

Dielectric properties of myoglobin at 10 GHz by microwave cavity perturbation measurements

M. Bonura *, G. Schirò and A. Cupane
CNISM and Dipartimento di Scienze Fisiche ed Astronomiche, Università di Palermo, Palermo, Italy

Abstract. We report on the temperature dependence, at microwave (mw) frequency, of the imaginary part of the dielectric constant (ε'') in myoglobin powder samples with different hydration levels (h). The measurements have been performed by the cavity perturbation technique, in the range of temperature 80–345 K. The sample is located inside a glass capillary along the axis of a cylindrical copper cavity, resonating in the TE_{011} mode at 9.6 GHz, where the mw electric field has a node. By measuring the variation of the quality factor of the resonant cavity, one can extract the imaginary part of the dielectric constant. At temperatures higher than 230 K we observe an evident increase of the dielectric losses with increasing temperature; the effect scales almost linearly with hydration, indicating that it must be attributed to a relaxation of water in the hydration shell of the protein. Furthermore, at $h \geqslant 0.18$, we observe a clear peak in the ε'' vs. T curve, that shifts towards lower temperatures upon increasing hydration; this shows that the activation enthalpy of the hydration water relaxation decreases with hydration. More in general, our data show that the technique of microwave cavity perturbation allows one to study the dynamics of water molecules in the hydration shell of proteins and to extend information obtained with dielectric techniques to the mw frequencies.

Keywords: Dielectric constant, microwave properties, water dynamics, hydrated proteins

1. Introduction

The physiological function of proteins is strongly correlated to their interaction with the surrounding environment. In particular, water molecules play a key role in determining both the static structure and the dynamics of proteins. Water influences protein relaxations, allows them to associate and dissociate, enables proton transfer and facilitates a large number of biochemical processes [5,7,9,14,15]. Interaction with the protein surface also changes the dynamics of water molecules: structural water is tightly bound to the protein and it is almost impossible to remove it without damaging the protein, while water in the first hydration shell exhibits slower relaxations with respect to bound water. There is a debate in the recent literature on how far from the protein surface this effect is propagated [4,8].

It is well known that free water molecules show a dielectric relaxation, due to rotational motions, at room temperature at $\sim$10 GHz [3]; at the same time, it is expected that bound water exhibits relaxation rates slower than free-water's one. Consequently, investigating the mw response of proteins may allow one to extract important information on the dynamics of water molecules and consequently, on their interaction with biological matter.

*Corresponding author: M. Bonura, CNISM and Dipartimento di Scienze Fisiche ed Astronomiche, Università di Palermo, via Archirafi 36, I-90123, Palermo, Italy. E-mail: marco.bonura@fisica.unipa.it.

In this paper we report a preliminary experimental study of the dielectric energy losses at mw frequencies in hydrated powders of myoglobin (Mb). Measurements have been performed by the cavity perturbation technique using a copper cavity, of cylindrical shape with golden plated walls, tuned in the TE_{011} mode at 9.6 GHz. In fact, it has been shown that in the TE_{011} mode one can measure the dielectric properties of a sample even though it is placed on a pure microwave magnetic field with zero electric field [16]. The method has important advantages over E-field cavity perturbation measurements, especially for high dielectric constant (water containing samples); moreover, since the working frequency is inside the region of dispersion of water molecules, the experimental technique is expected to enable one to investigate the dynamics of water in the hydration shell of proteins. This investigation complements and extends previous studies from our group on the dynamics of protein and hydration water in hydrated myoglobin powders performed with combined elastic neutron scattering and dielectric spectroscopy experiments [10–12].

A previous investigation by Singh et al., as early as 1981, reported on the dielectric properties of water adsorbed in metmyoglobin crystals (400 water molecules per protein) measured by cavity perturbation technique [13]. Our approach is different: besides the use of H-field measurements, the novelty of our experiment is that we investigate protein powders at different hydration levels spanning from 80 to 450 water molecules per protein, in the temperature range 80–345 K. The aim is to investigate the effect of increasing hydration levels, in the absence of any constraint provided by crystal packing forces. We observe a relaxation that can be clearly attributed to hydration water molecules; the presence of a well defined peak at high hydration levels enables to determine the activation enthalpy of the relaxation process that is on the order of 20 kJ/mol and decreases with increasing hydration.

2. Experimental technique and samples

Lyophilized Mb from equine skeletal muscle was purchased from Sigma-Aldrich and used without further purification. Mb is in the oxidized form (met) and essentially salt-free with a purity greater than 95%. In principle, the presence of salt in the sample could give a spurious contribution of direct conductivity. However, due to the small amount ($<5\%$) of impurities and the high value of frequency used, these effects are expected to be negligible. A nominally "zero hydration" sample was obtained by heating at 47°C for 24 h under vacuum. Samples at various hydrations were obtained by putting aliquots of the "zero hydration" sample in a closed container in the presence of deionized water. The hydration level of the sample ($h \equiv$ gr[water]/gr["dry" protein]) was estimated by weighting the sample; when the required hydration value was reached, NMR quality glass capillaries were filled with the hydrated powder and sealed. With this procedure we have prepared four samples with $h = 0.1$, $h = 0.18$, $h = 0.3$, $h = 0.5$. Another capillary has been filled with the "zero hydration" sample; in the following we will refer to this sample as the "dry" sample, even if we do not exclude that structural water is still present.

The imaginary part of the dielectric constant at mw frequency has been measured by the cavity-perturbation technique [6]. A copper cavity, of cylindrical shape with golden-plated walls, is tuned in the TE_{011} mode resonating at 9.6 GHz; the sample is located along the axis of the cavity, where the mw electric field has a node. The cavity is placed inside a cryostat which allows to operate in the temperature range 2.2–350 K. Further details of the apparatus are reported elsewhere [1]. As demonstrated by Zhai et al., by measuring the quality factor of the resonant cavity one can measure the dielectric properties of

the sample even though it is placed in a pure mw magnetic field [16]. In fact, the imaginary part of the dielectric constant of the sample is given by

$$\varepsilon'' = \Gamma(1/Q_L - 1/Q_U), \tag{1}$$

where Q_L is the quality factor of the cavity loaded with the sample, Q_U that of the empty cavity and Γ the geometry factor of the sample, which depends both on the sample properties and on the resonant mode. The resonant curve of the cavity is revealed by an Hp-8719D Network Analyzer; the quality factor is obtained by fitting the resonant curve with a Lorentzian law.

In order to obtain the temperature dependence of ε'', both Q_L and Q_U have been measured in the range 80–345 K. The measurement procedure has been optimized in order to achieve thermalization between the sample and the cavity. Initially, the cavity is cooled down to liquid-nitrogen temperature, in He atmosphere; then the temperature is let to increase up to room temperature, without any external heating both for the cavity and for the sample. Measurements at higher temperatures were performed by heating the cavity at 0.2 K/min by means of a Lakeshore temperature controller, up to 345 K. The entire 80–345 K temperature scan was performed in about 48 h.

3. Experimental results and discussion

Figure 1 reports the imaginary part of the dielectric constant, ε'', at the fixed frequency $\omega/2\pi = 9.6$ GHz as a function of temperature for the samples investigated. As can be seen, an increase of ε'' is observed at temperatures higher than $\sim$230 K; moreover, at hydrations higher than 18%, a clear peak is observed, suggesting that we are detecting a relaxation in the samples. The raw data in Fig. 1 indicate the following facts:

(1) The signal intensity scales almost linearly with hydration and is present even in the "dry" sample. Since the frequency of 9.6 GHz is within the range of expected water relaxations, we attribute the measured signal to relaxation(s) of water molecules in the hydration shell of the protein. The fact that the signal is present also in the "zero hydration" sample indicates that our drying procedure is not able to remove all the water molecules and that some tightly bound "structural" waters (on the order of 2–3% [9]) are still present.

(2) The peak temperature, as reported in Table 1, increases with decreasing hydration, even below one full protein hydration shell ($h \sim 0.35$, for myoglobin) and are shifted to temperatures outside the range investigated at hydrations below $h = 0.18$. This suggests that the activation energy of the relaxation of water molecules in the hydration shell increases with decreasing hydration.

A more quantitative analysis of the data can be performed by assuming that we are observing a relaxation process characterized by a single relaxation time (Debye approximation)[1] whose temperature dependence is given by the Arrhenius law. In this case:

$$\varepsilon''(\omega, T) = \Delta\varepsilon\left[(\omega\tau_0 \mathrm{e}^{E/RT})/(1 + (\omega\tau_0 \mathrm{e}^{E/RT})^2)\right], \tag{2}$$

where ω is the resonance frequency of the cavity, $\Delta\varepsilon$, E and τ_0 are the dielectric strength, the activation energy and the pre-exponential of the relaxation process, respectively. A fit of the data at $h = 0.3$ in terms

[1]The conductivity term of the type σ/ω has been shown to be very small in our samples at the investigated frequency [10] and has therefore been neglected.

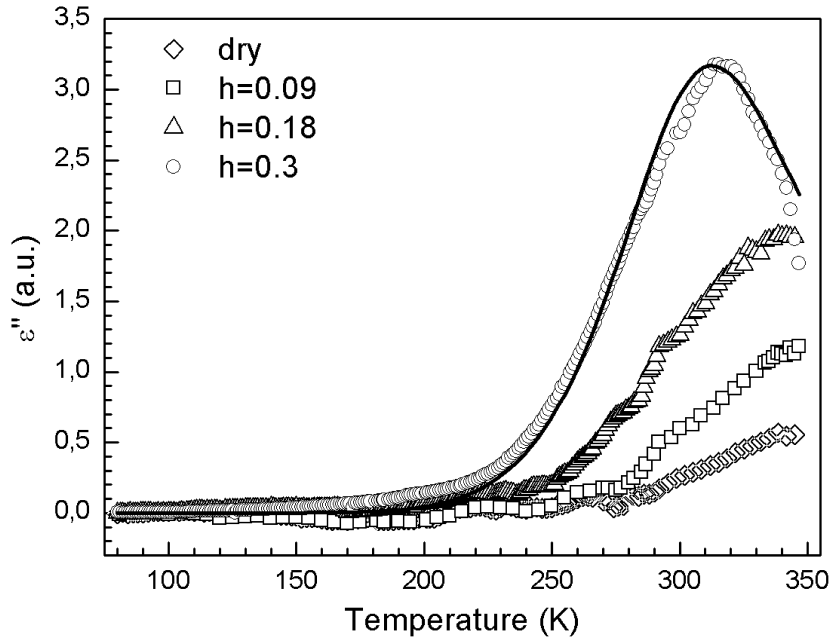

Fig. 1. Temperature dependence of the imaginary part of the dielectric constant (ε'') for myoglobin powder with different hydration levels. Measurements have been performed at fixed frequency $\omega/2\pi = 9.6$ GHz. Open symbols indicate the experimental results; for the sample with $h = 0.3$ it is also reported the best-fit curve obtained as described in the text.

Table 1
Values of the temperature at which the peak in $\varepsilon''(T)$ curve, obtained at $\omega/2\pi = 9.6$ GHz, is observed, for different hydration values. For $h < 0.18$ no peak has been observed in the range of temperatures investigated

h	Dry	0.09	0.18	0.3	0.5
$T_{\max}$ (K)	–	–	340	316	311

of Eq. (2) is shown in Fig. 1 as a continuous line and gives $\tau_0 \approx 1.5 \times 10^{-14}$ s and $E \approx 23000$ J/mol. As can be seen, although Eq. (2) is able to reproduce the main features of the data, misfits are still present; they can be likely traced to the presence of a distribution of activation energy for the relaxation process [2]. A more detailed data analysis is currently under way and will be published elsewhere.

From Eq. (2), the temperature at which $\varepsilon''(\omega, T)$ has a maximum can be easily calculated as:

$$T_{\max} = E[-\ln(\omega\tau_0)/R], \tag{3}$$

showing that in our samples the activation energy for rotational relaxation of water molecules decreases with increasing hydration.

We note also that, being $\omega\tau = 1$ at the $\varepsilon''(\omega, T)$ peak, data in Fig. 1 imply that at $T \approx 310$ K the relaxation time of water molecules in the hydration shell of myoglobin at $h = 0.3$–0.5 (corresponding to 300–450 water molecules per protein, i.e. almost to one full hydration layer) is around 15 ps. This is, as expected, slower than the relaxation time in bulk water ($\tau \approx 4$ ps) but, surprisingly, faster than the relaxation time of water adsorbed in metmyoglobin crystals at ~400 water molecules per protein ($\tau \approx 80$ ps, [13]). Consistently, Singh et al. have not reported about a peak in the ε'' vs. T curves, while

in our experiment a clear peak is observed even at 270 water molecules per protein ($h = 0.3$). This suggests that crystal packing forces are effective in slowing down motions not only of the protein but also of the hydration shell and calls for caution when discussing data obtained on crystals. To the best of our knowledge, a thorough study comparing dielectric relaxation of hydration water in protein powders and crystals has not been carried out, yet.

Acknowledgements

The authors are very glad to thank M. Li Vigni and A. Agliolo Gallitto for their interest in this work and G. Napoli for technical assistance.

References

[1] M. Bonura, A. Agliolo Gallitto, M. Li Vigni and G.A. Ummarino, Field-induced suppression of the π-band superconductivity and magnetic hysteresis in the microwave surface resistance of MgB_2 at temperatures near T_C, *Superconductor Science and Technology* **22** (2009), 055010–055019.
[2] M. Brecht, B. Klosgen, C. Reichle and K.D. Kramer, Distribution function in the description of relaxation phenomena, *Molecular Physics* **96**(2) (1999), 149–160.
[3] R. Buchner, J. Barthel and J. Stauber, The dielectric relaxation of water between 0°C and 35°C, *Chemical Physics Letters* **306** (1999), 57–63.
[4] S. Ebbinghaus, S.J. Kim, M. Heyden, X. Yu, U. Heugen, M. Gruebele, D.M. Leitner and M. Havenith, An extended dynamical hydration shell around proteins, *Proceedings of the National Academy of Sciences of the United States of America* **104**(52) (2007), 20749–20752.
[5] P.W. Fenimore, H. Frauenfelder, B.H. McMahon and F.G. Parak, Slaving: Solvent fluctuations dominate protein dynamics and functions, *Proceedings of the National Academy of Sciences of the United States of America* **99**(25) (2002), 16047–16051.
[6] S. Li, C. Akyel and R.G. Bosisio, Precise calculations and measurements on the complex dielectric constant of lossy materials using TM_{010} cavity perturbation techniques, *IEEE Transactions on Microwave Theory and Techniques* **29**(10) (1981), 1041–1048.
[7] G. Otting, E. Liepinsh and K. Wuthrich, Protein hydration in aqueous solution, *Science* **41** (1991), 974–980.
[8] S.K. Pal, J. Peon and A.H. Zewail, Biological water at the protein surface: Dynamical solvation probed directly with femtosecond resolution, *Proceedings of the National Academy of Sciences of the United States of America* **99**(4) (2002), 1763–1768.
[9] J.A. Rupley and G. Careri, Protein hydration and function, *Advances in Protein Chemistry* **41** (2009), 37–172.
[10] G. Schirò, A. Cupane, E. Vitrano and F. Bruni, Dielectric relaxations in confined hydrated myoglobin, *Journal of Physical Chemistry B* **113**(28) (2009), 9606–9613.
[11] G. Schirò, M. Sclafani, C. Caronna, F. Natali, M. Plazanet and A. Cupane, Dynamics of myoglobin in confinement: An elastic and quasi-elastic neutron scattering study, *Chemical Physics* **345**(2,3) (2008), 259–266.
[12] G. Schirò, M. Sclafani, F. Natali and A. Cupane, Hydration dependent dynamics in sol–gel encapsulated myoglobin, *European Biophysics Journal with Biophysics Letters* **37**(5) (2008), 543–549.
[13] G.P. Singh, F. Parak, S. Hunklinger and K. Dransfeld, Role of adsorbed water in the dynamics of metmyoglobin, *Physical Review Letters* **47** (1981), 685–688.
[14] H.J. Steinhoff, B. Kramm, G. Hess, C. Owerdieck and A. Redhardt, Rotational and translational water diffusion in the hemoglobin hydration shell: Dielectric and proton nuclear relaxation measurements, *Biophysical Journal* **65** (1993), 1486–1495.
[15] S.N. Timasheff, Protein hydration, thermodynamic binding, and preferential hydration, *Biochemistry* **41** (1991), 13473–13482.
[16] Z. Zhai, C. Kusko, N. Hakim, S. Sridhar, A. Revcolevschi and A. Vietkine, Precision microwave dielectric and magnetic susceptibility measurements of correlated electronic materials using superconducting cavities, *Review of Scientific Instruments* **71** (2000), 3151–3160.