The Role of the Hydration Shell of Proteins

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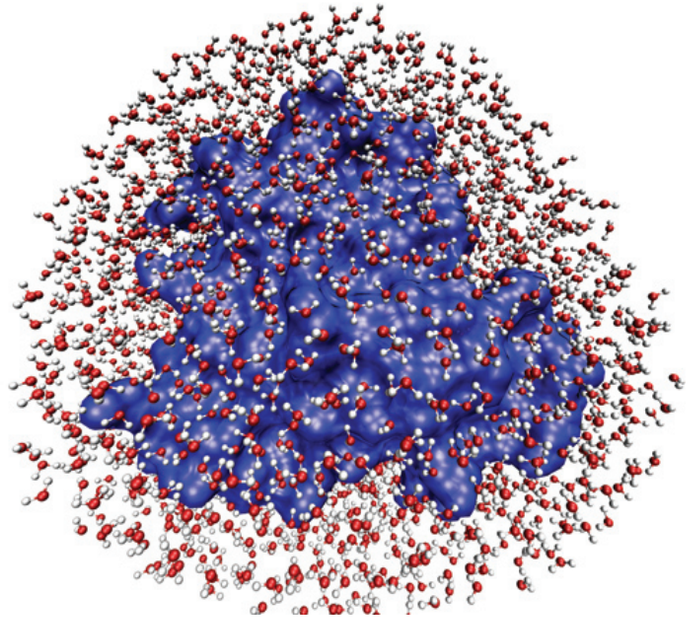

Fig. 1. The hydrated protein myoglobin (blue surface) with 1911 water molecules (red and white sticks-and-balls), the approximate number needed for optimal function (h = 2). The water forms a shell about 5 Å thick around the protein.

Proteins perform most of the functions necessary for life. A quantitative understanding of how proteins work and how their functions can be controlled is essential for many of the missions of LANL such as biodefense. Water is also essential for life and for the workings of proteins, but a real understanding of why water is crucial has been lacking. Our research now provides at least some of the answers to this fundamental query. Proteins are surrounded by a hydration shell of one to two layers of water (Fig. 1). This water is crucial for the functioning of proteins—dehydrated proteins do not work. Despite a very large number of publications, the workings of the hydration shell have not been clarified [1]. We believe that we understand the role of the hydration shell and how it controls internal protein motions that are involved in function [2]. Our model is based on insights from the physics of glass-forming liquids, new experiments, and the concept of a hierarchically organized energy landscape. Glass-forming liquids exhibit two major types of fluctuations, α and β [3]. The α-fluctuations represent structural changes that affect large-scale motions of the protein, but do not depend on hydration. Here we are concerned with the β-fluctuations in the hydration shell. β-fluctuations depend on hydration and occur even if the environment is rigid. We assert that their role is the driving of internal protein motions. To prove this claim, we have measured the β-fluctuations in the hydration shell and compared them with internal fluctuations determined with the Mössbauer effect and incoherent neutron scattering. The result is unambiguous: internal motions are slaved to the β-fluctuations in the hydration shell.

The experiments that led to the understanding are in principle simple. Myoglobin (Mb), an oxygen storage protein, is used as a model system. Experiments performed a long time ago showed that carbon monoxide moves through the interior of hydrated Mb even if Mb is embedded in a solid [4]. However, it was not clear what permitted the motion. Much later we guessed that it could be β-fluctuations in the hydration shell [5]. To test this claim, we use dielectric relaxation to measure the β-fluctuations. To ensure that the fluctuations originate in the hydration shell, Mb at various hydration levels is embedded in solid poly(vinyl) alcohol. The temperature dependence of the relaxation rate coefficient, $k_\beta(T)$, for various hydration levels is shown in Fig. 2a. A typical spectrum is shown in Fig. 2b. The internal protein fluctuations are determined using published data on the Mössbauer effect of ^{57}Fe in the heme group of Mb [6]. A fraction f(T) of γ-rays emitted by ^{57}Fe in Mb suffers no energy loss. f(T) is controlled by the fluctuations experienced by the ^{57}Fe nuclei. In the simplest approximation, $f(T) = 1$ if the fluctuations are slower than the lifetime $\tau_{Mö} = 140$ns of the nuclear level that emits the γ-rays, and $f(T) = 0$ if the fluctuations are faster. The crucial feature now is the inhomogeneity of protein samples. The broad spectrum

shown in Fig. 2b implies that each protein has a unique fluctuation frequency υ and that the spectrum represents the probability of finding proteins with a given frequency υ. Proteins with circular frequencies $\omega = 2\pi\upsilon < 1/\tau_{M\ddot{o}}$ emit γ-rays without energy loss. The line at $k_{M\ddot{o}} = 1/\tau_{M\ddot{o}}$ in Fig. 2b demarcates the two areas—the red area to the left labeled a_β gives the fraction of recoilless emitters. The test of our hypothesis is now clear. The β fluctuations in the hydration shell drive the internal motions if $f(T) = a_\beta(T)$. We have determined $a_\beta(T)$ and Parak and collaborators have measured f(T). The two functions are plotted in Fig. 3. The close agreement proves that the β-fluctuations in the hydration shell indeed drive the internal protein motions. The result suggests a large number of new experiments.

This work has been done in collaboration with Izabela R. Stroe, Jan Swenson, Helén Jansson, Robert D. Young and Albert Migliori.

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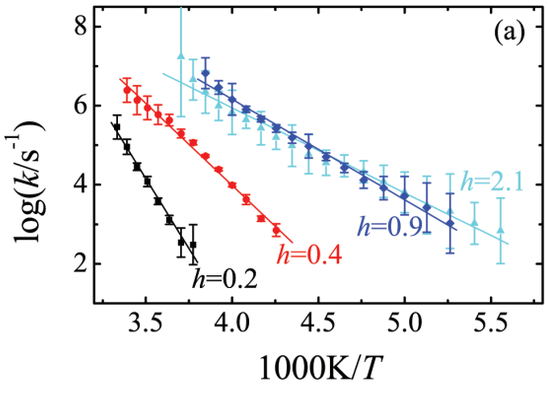
Funding Acknowledgments
LANL Directed Research and Development Program



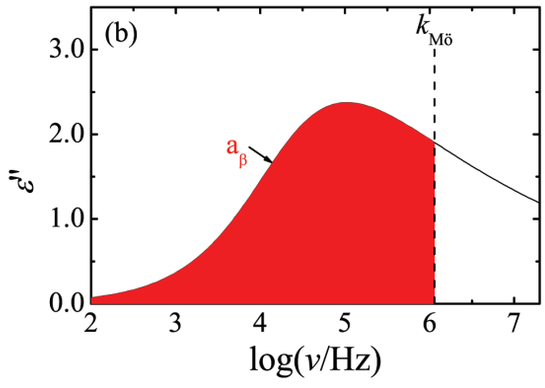


Fig. 2. Hydration-relaxation processes in myoglobin. (a) An Arrhenius plot of the β-processes for Mb embedded in PVA for various values of the hydration h. (b) The smoothed dielectric relaxation spectrum of the β-relaxation in Mb at 265K. The vertical line denoted by $k_{M\ddot{o}}$ gives the dephasing rate corresponding to $\tau_{M\ddot{o}} = 140$ ns, the mean Mössbauer lifetime of ^{57}Fe. The fractional area to the left of $k_{M\ddot{o}}$ is denoted by a_β.

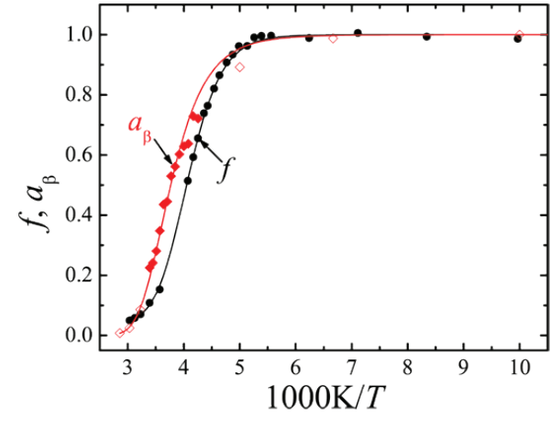


Fig. 3. The temperature dependence of the dielectric relaxation area $a_\beta(T)$ and the recoilless fraction $f(T)$ of the Mössbauer effect. The similar temperature dependence between the two independently measured functions demonstrates that the internal protein motions, characterized by $f(T)$, are determined by the hydration shell, characterized by $a_\beta(T)$. Note that $f(T)$ is difficult to measure at low temperature because small uncertainties in protein vibrational motions change $f(T)$ strongly. It is also difficult to measure at high temperature because the elastic component becomes very small.