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Bulk Modulus of a Protein Active-Site Mimic

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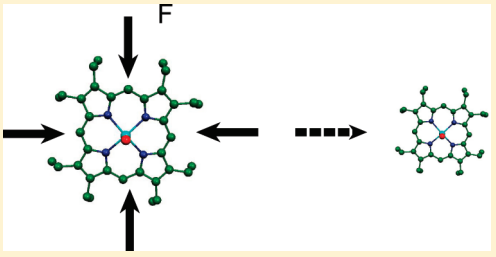
ABSTRACT: Flexibility is an important property of porphyrins, both natural and synthetic. We applied two synchrotron-based techniques, nuclear resonance vibrational spectroscopy and inelastic X-ray scattering, to quantify this property by measuring the bulk modulus of a protein active-site mimic [chloro(octaethylporphyrinato)iron(III)] and the resilience of the iron environment. Their values are 6.95 ± 0.24 GPa and 15.4 ± 0.5 N/m, respectively.



1. INTRODUCTION

The active site in many proteins is represented by the heme (iron porphyrins), with functions such as oxygen binding and transport (myoglobin, hemoglobin) and electron transport (cytochromes).[1] Porphyrins synthesized in the laboratory, on the other hand, are used as mimics of the active site in heme proteins[2,3] and enzymes,[4,5] and as building blocks for enzyme mimics,[6,7] heme proteins,[8] macromolecules,[9] and light-harvesting systems.[10−12]

The flexibility of a porphyrin plays a significant role in a variety of processes, both natural and artificial. For example, the ability of a protein to function is due in large part to the flexibility of its heme,[13] while the structure−function relationship of porphyrin-based molecular wires[14] and the functionality of host−guest systems used to build enzyme mimics[15] are affected by the flexibility. A convenient way to quantify this property is to measure the bulk modulus, a task that, to the best of our knowledge, has not yet been accomplished.

Here, we combine two synchrotron-based techniques, nuclear resonance vibrational spectroscopy (NRVS) and inelastic X-ray scattering (IXS), to measure the bulk modulus of chloro(octaethylporphyrinato)iron(III) Fe(OEP)Cl (Figure 1). We recently applied the same method for cytochrome *c*, an electron-carrying heme protein, also involved in apoptosis.[16] In addition, we extract the resilience of the iron environment from the NRVS results.

Almost all organisms require iron to live.[17] This transition metal is often located at the center of the proteins' prosthetic group, the heme. While Fe(OEP) resembles the heme of many proteins, Fe(OEP)Cl in particular is a good model for chloride binding to the active site of peroxidases.[18−21]

2. EXPERIMENTAL SECTION

2.1. Materials. ^{57}Fe-enriched Fe(OEP)Cl for NRVS and IXS measurements was purchased from Midcentury Chemicals and synthesized as described in ref 22, respectively.

2.2. Nuclear Resonance Vibrational Spectroscopy. NRVS produces the complete vibrational spectrum of a probe nucleus.[23] It has a high degree of selectivity by targeting a single atom (^{57}Fe in the present study) in a complex molecule and ignoring the motions of all the other atoms.

The experiment was carried out at sector 3-ID-D of the Advanced Photon Source at Argonne National Laboratory. The spectrum consists of a central resonance, due to the recoilless excitation of the ^{57}Fe nuclear excited state at E_0 = 14.4125 keV, and a series of side bands corresponding to creation or annihilation of vibrational quanta, displaced from the recoilless absorption by an energy $\pm E$. Normalization based on Lipkin's sum rules[24] produces a signal equal to the excitation probability. The program PHOENIX,[25] working under the assumptions that samples are isotropic and harmonic, subtracts the background and the resolution function, and removes temperature factors and multiphonon contributions to yield the Fe-weighted vibrational density of states (VDOS) $D(\overline{\nu})$ ($\overline{\nu} = E/hc$). VDOS defines the vibrational properties at all temperatures for a harmonic system[26] and is normalized according to $\int D(\overline{\nu})\, d\overline{\nu} = 3$.[27] The experimental energy resolution was 0.85 meV ($\approx$7 cm^{-1}). The temperature, determined from the detailed balance factor in the excitation probability spectrum, was 87 K.

2.3. Inelastic X-ray Scattering. IXS yields information about collective motions of atoms via the dynamic structure factor $S(Q,E)$, where Q is the momentum transfer and E is the transferred energy.[28]

The experiment was performed at sector 3-ID-C of the Advanced Photon Source at Argonne National Laboratory, with an incident X-ray beam energy of 21.657 keV and an experimental

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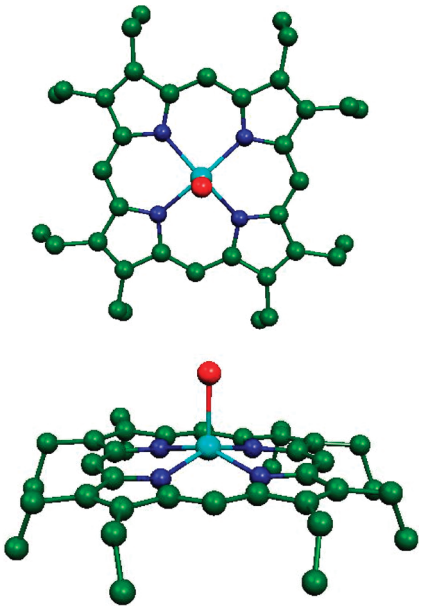

Figure 1. Two views of the structure of Fe(OEP)Cl,[40] generated with the program MOLEKEL.[59] Color scheme: cyan = iron, blue = nitrogen, green = carbon, red = chloride. Hydrogen atoms have been omitted for clarity.

energy resolution of 2.3 meV. The measured signal, consisting of a strong elastic peak and a weak inelastic part in the form of two symmetric shoulders, is proportional to $S(Q,E)$ convoluted with the experimental resolution function. The chosen model for $S(Q,E)$ is the sum of a central Lorentzian, a damped harmonic oscillator function for the inelastic part (with the detailed balance term factored in), and a background term (B):[29]

$$S(Q,E) = \frac{1}{\pi}\frac{I_C\Gamma_C}{E^2+{\Gamma_C}^2} + \frac{E}{1-\exp\left(\frac{-E}{k_BT}\right)}\frac{1}{\pi}\frac{4I\Gamma\Omega}{(E^2-\Omega^2)^2+4\Gamma^2E^2}+B \qquad (1)$$

where I_C and Γ_C are the intensity and the width of the elastic line, respectively; and I, Γ, and Ω are the intensity, width, and energy of the inelastic excitation, respectively. These terms are all Q-dependent. The static structure factor $S(Q)$ was measured with a sodium iodide detector. The experiment was carried out at different temperatures (298, 250, 200, 150, 100, and 50 K).

3. RESULTS AND DISCUSSION

During the past decade, NRVS has become a powerful tool for probing the iron vibrational dynamics in model compounds, including porphyrins,[30] Fe−H(D)$_6$ molecules,[31] and Fe−S clusters.[32] The complete Fe VDOS of Fe(OEP)Cl is shown in Figure 2a. A normal-mode analysis of the vibrational dynamics for this model compound was published earlier.[33] The dominant feature in the 200−300 cm^{-1} frequency range corresponds to the stretching of the Fe−N_{pyr} bonds,[27] while the strong peak at 356 cm^{-1} is due to the stretching of the Fe−Cl bond.[27,34,35]

The low-frequency region of a NRVS spectrum corresponds to collective modes, rather than just to those involving iron−

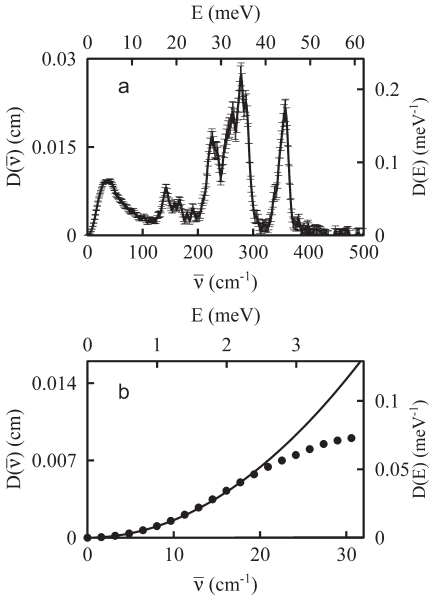

Figure 2. NRVS results. (a) Complete Fe VDOS spectrum. (b) Low-frequency part of Fe VDOS (circles), from which the Debye sound velocity is extracted (eq 2, continuous line). The error bars due to statistics are smaller than the size of the symbols.

ligand bonds.[36−38] We calculated the Debye sound velocity v_D from the Fe VDOS at low frequencies (up to 20 cm^{-1}, Figure 2b) according to:[36]

$$v_D = \left(\frac{4\pi m_{Fe}c^3}{\rho}\frac{\bar{\nu}^2}{D(\bar{\nu})}\right)^{1/3} \qquad (2)$$

where m_{Fe} is the mass of the iron atom and ρ = 1337 kg/m^3 is the density (an average based on two values reported in the literature[39,40]). We obtained a value of 1145 ± 11 m/s. For a similar model compound but lacking the chloride ligand Fe(OEP), v_D = 1204 m/s.[41]

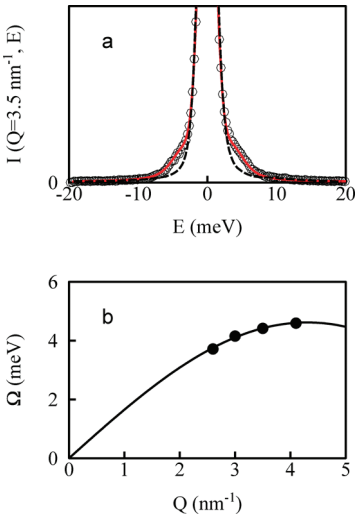

Figure 3. IXS results. (a) Measured signal at T = 298 K and Q = 3.5 nm^{-1} (open circles), the fit (red line), and the experimental resolution function (dashed line). The error bars due to statistics are smaller than the size of the symbols. (b) Dispersion curve (eq 3).

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Figure 3a shows the IXS spectrum at T = 298 K and Q = 3.5 nm^{-1}, including the fit. We extracted the longitudinal sound velocity v_L from the dispersion of the inelastic excitation[42] (Figure 3b):

$$\Omega = \frac{2v_L\hbar Q_{max}}{\pi}\sin\left(\frac{\pi}{2}\frac{Q}{Q_{max}}\right) \quad (3)$$

with the following fitting parameters: v_L = 2562 m/s and Q_{max} = 4.3 nm^{-1}. Like in cytochrome c,[16] the intensity of the inelastic peak decreases considerably with decreasing temperature, but its position remains essentially the same. On the basis of this observation, we consider v_L to be approximately independent of temperature.

The Debye sound velocity is related to v_L and v_T (transverse sound velocity) according to:[43]

$$\frac{1}{{v_D}^3} = \frac{1}{3}\frac{1}{{v_L}^3} + \frac{2}{3}\frac{1}{{v_T}^3} \quad (4)$$

which yields v_T = 1011 ± 10 m/s. We calculated the adiabatic bulk modulus (K_S = 6.95 ± 0.24 GPa) from:

$$K_S = \rho\left({v_L}^2 - \frac{4}{3}{v_T}^2\right) \quad (5)$$

The value of the bulk modulus, being a measure of the molecule flexibility, is strongly affected by the molecular structure. For example, two-ring molecules are more rigid than the individual rings,[44] a trend that is confirmed by our results: the K_S value for the four-pyrrole porphyrin investigated here is higher than that of the individual pyrroles (Table 1). Similarly, for linear hydrocarbons, the isothermal compressibility decreases with the increase in the number of atoms (e.g., 1.69 GPa^{-1} for 1-hexene, 1.3 GPa^{-1} for 1-octene[45]).

Table 1. Bulk Modulus of Some Cyclic Molecules (the Number of Aromatic Rings Is Shown in Parentheses) and of the Heme in Modified Proteins

	K_S (GPa)	reference
benzene (1)	1.4	44
pyridine (1)	1.9	44
pyrrole (1)	2	44
quinoline (2)	2.6	44
tetralin (2)	2	44
Fe(OEP)Cl (4)	6.95 ± 0.24	this work
horseradish peroxidase[a]	10	46
myoglobin[b]	14.3	47
cytochrome c[c]	20	48

[a] Mesoporphyrin IX-substituted. [b] Protoporphyrin IX-substituted. [c] Zn-substituted.

Since Fe(OEP)Cl is a model for the active site in heme proteins, its bulk modulus can be compared to that of the heme environment in (modified) proteins, which was measured with the pressure-tuning burning hole technique at cryogenic temperatures.[46−48] In proteins, K_S ranges between 10 and 20 GPa (Table 1), which are higher values than for Fe(OEP)Cl but of the same order of magnitude. We note that the lower compressibility of the active site in (Zn-substituted) cytochrome c[48] compared to that of (protoporphyrin IX-substituted) myoglobin[47] is in qualitative agreement with their functional roles, which require a higher flexibility for the ligand-binding myoglobin than for the electron-carrying cytochrome c.

Another useful parameter for quantifying the flexibility is the resilience k_r, which describes the temperature dependence of atomic fluctuations.[49] The resilience of the iron environment in heme proteins (Table 2) was measured with NRVS and predicted from molecular dynamics simulations,[50] but can also be extracted from Mössbauer spectroscopy results.[36,51] We obtained k_r (15.4 ± 0.5 N/m) from the Fe VDOS of Fe(OEP)Cl (Figure 2a), as described previously:[50]

$$k_r = \frac{3m_{Fe}}{\int_0^\infty \omega^{-2}D(\omega)\,d\omega} \quad (6)$$

in which $\omega = 2\pi c\bar{\nu}$.

Table 2. Resilience of the Iron Environment in a Heme Mimic and in Heme Proteins

	k_r (N/m)	reference
Fe(OEP)Cl	15.4 ± 0.5	this work
deoxymyoglobin	20−21	36, 50
Fe(III) cytochrome c	27−28	51, 50

Our results (Tables 1 and 2) confirm quantitatively the expectation that the porphyrin is more flexible than the protein hemes, whose mobilities are limited by the bonds connecting them to the apoproteins.

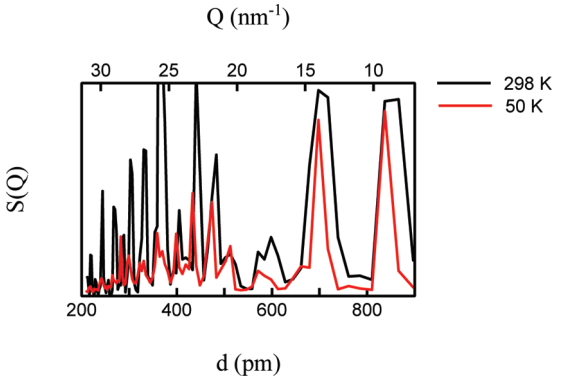

Figure 4. Static structure factor at T = 298 K and T = 50 K in units of momentum transfer Q and interatomic distances d.

Knowing K_S, one can calculate the isothermal compressibility β_T, which is directly related to volume fluctuations[52] and to the mean atomic displacements,[53] from:

$$\beta_T = \frac{1}{K_S} + \frac{T\alpha^2}{\rho c_p} \quad (7)$$

where T, ρ, c_p, and α are the temperature, density, isobaric specific heat capacity, and coefficient of thermal expansion, respectively. To the best of our knowledge, the value of c_p for Fe(OEP)Cl has not been measured, but calorimetric measurements on related porphyrins yielded an average value of ≈350 J/kg/K at 87 K.[54] On the other hand, we are not aware of any report on the value of α, but we estimated it from the dependence of the static structure factor $S(Q)$ on temperature (Figure 4). The sharp features in $S(Q)$ are related to the interatomic distances d

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according to $Q = 2\pi/d$. The position of the peak around 700 pm, which corresponds roughly to the size of the porphyrin core, changes from 699 pm at 50 K to 704 pm at 298 K, thus yielding a linear thermal expansion coefficient of approximately 2.8×10^{-5} K^{-1} (approximately the same value is obtained from the shifts of other peaks). Therefore, the second term in the right-hand side of eq 7 can be neglected (i.e., $\beta_T \approx 1/K_S$).

4. CONCLUSIONS

We measured the bulk modulus of a heme protein active site mimic [Fe(OEP)Cl] and the resilience of its iron environment with two synchrotron-based techniques (NRVS and IXS). Not unexpectedly, this four-tetrapyrrolic molecule is more rigid than the individual pyrrole rings, but more flexible than the actual protein hemes. Owing to the extreme tunability of NRVS, the method presented in this study is suitable for model compounds with structures far more complex than Fe(OEP)Cl (e.g., dendritic iron porphyrins,[55] artificial enzymes[56]) and for other naturally occurring and synthetic metallic cyclic tetrapyrroles in which flexibility may play an important role.[57,58]

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