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Molecular dynamics in cytochrome *c* oxidase Mössbauer spectra deconvolution

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ABSTRACT

In this work low temperature molecular dynamics simulations of cytochrome *c* oxidase are used to predict an experimentally observable, namely Mössbauer spectra width. Predicted lineshapes are used to model Lorentzian doublets, with which published cytochrome *c* oxidase Mössbauer spectra were simulated. Molecular dynamics imposed constraints to spectral lineshapes permit to obtain useful information, like the presence of multiple chemical species in the binuclear center of cytochrome *c* oxidase. Moreover, a benchmark of quality for molecular dynamic simulations can be obtained. Despite the overwhelming importance of dynamics in electron–proton transfer systems, limited work has been devoted to unravel how much realistic are molecular dynamics simulations results. In this work, molecular dynamics based predictions are found to be in good agreement with published experimental spectra, showing that we can confidently rely on actual simulations. Molecular dynamics based deconvolution of Mössbauer spectra will lead to a renewed interest for application of this approach in bioenergetics.

1. Introduction

Proteins carry out a wide variety of functions in cells: they play structural roles, catalyze almost all reactions in the organism, are involved in transport functions and have regulatory functions. Due to such a multiplicity of tasks, proteins are characterized by diversity. In order to carry out their roles, proteins must have some peculiar physical properties [1]. First, they must have a particular structure. The definite distribution of their amino acids allows for specific interactions with other molecules and creates particular physical and chemical properties at the active sites [2]. Second, these structures must be dynamic: for example, the stereochemical structure of some active sites is determined by specific interactions [3], and dynamic changes respond to regulatory stimuli [4]. The interplay between specific structures and dynamic changes is extremely important for protein function.

One of the most important function of proteins concerns electron transfer reactions [5]. Three principal parameters are required to describe electron transfer in protein systems: driving force, reorganization energy, and distance [5]. Marcus and Sutin [6] described the behavior observed in many electron transfer systems in which the electron (tunneling) rate has a Gaussian dependence on driving force, increasing from low driving force to a maximum rate when the driving force matches the reorganization energy parameter and then falling again as the driving force increases above the optimum. Equations that capture exponential distance dependence and Gaussian free energy dependence of electron transfer rate have been proposed [7]. These equations, based on simple square barrier tunneling models, appear adequate for predicting electron transfer rates in many protein systems within an order of magnitude. Using ruthenated protein model the electron transfer data have been examined according to a metal to metal metric or metal to macro cycle metric showing a two order of magnitude falling off below the electron transfer rate per Å, consistent with the square barrier model [5]. But no general consensus about this mechanism has been attained. Different views, based on pathway interference or on natural selection of the protein tunneling medium, have been proposed [8,9]. Most authors consider that the protein medium between donor and acceptor could have been selected to increase or decrease any particular electron transfer reaction. In model systems, the more covalently is the medium linked between donor and acceptor, the lower the electron tunneling barrier appears and the faster the electron transfer rate is [7]. Some theoretical approaches attempt to find the best combination of covalent bonds and through space gaps [8,10], others consider the packing density of the protein medium as an indicator for the effective barrier height [11]. In order to discriminate among these different views of the electron transfer process, a better estimate of the three above mentioned parameters is needed.

The distance metrics between donor and acceptor are of overwhelming importance. Molecular dynamics derived distances between the donor–acceptor couples are nowadays used to obtain better estimate of the electron transfer rates in protein systems [5,12]. One of the best studied electron transfer protein is cytochrome *c* oxidase [13–15]. Cytochrome *c* oxidase is the terminal

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enzyme in the aerobic respiratory chains. The enzyme catalyzes electron transfer to molecular oxygen. The Cu_A copper binuclear center represents the entry site of electron from cytochrome *c*. The subsequent electron transfer reaction occurs in two steps: from Cu_A to heme a and further to the heme a_3–Cu_B binuclear center, in which the oxygen reduction to water takes place. Proton transfer reactions concomitant to electron transfer create and maintain a transmembrane protonmotive force, which is used for the ATP synthesis. The central importance of cytochrome *c* oxidase for bioenergetics reactions has stimulated an enormous amount of studies on the structure [16–18], electron transfer and proton transfer reactions [13–15]. In a series of works the influence of thermal motion of cytochrome *c* oxidase in electron transfer as well as in proton transfer reactions has been explored [19–21].

Due to the central functional role of the protein dynamics, an important aspect is provided by comparison of molecular dynamics simulations with protein motion experimental data. Some experimental techniques have been used to study protein dynamics and forces involved in protein thermal motion [22]. For example, techniques like fluorescence labeling, spin labeling or NMR can be used to investigate the time scale of fluctuation processes. The most important techniques furnishing data on protein dynamics are high resolution X-ray diffraction, neutron diffraction and Mössbauer spectroscopy [23]. Mössbauer spectroscopy is particularly interesting in heme containing proteins. The Mössbauer resonant nucleus (i.e. the ^{57}Fe in heme containing proteins) probes the motion of its immediate neighborhood. This spectroscopy senses all dynamic processes faster than the half time of Mössbauer nucleus, which is in the order of 10^{-7} s. Protein motions with time resolution in 10^{-7}–10^{-9} s range are probed by Mössbauer spectroscopy, whereas position fluctuations of Mössbauer nucleus much slower than the half time are ignored by this spectroscopy. Some papers are available in the literature in which Mössbauer spectroscopy has been used to study cytochrome *c* oxidase [24–26]. These works were particularly committed to elucidate electronic and magnetic states of iron ion in the enzyme.

Aim of this study is to provide a simple molecular dynamics based prediction of cytochrome *c* oxidase Mössbauer spectral lineshape, and to compare it with published experimental spectra. Such an analysis can offer useful insight about the correspondence between *in silico* predicted protein motions and experimental available information. Moreover, this molecular dynamics – aided spectral deconvolution can provide a practical basis for a detailed analysis of cytochrome *c* oxidase Mössbauer spectra, allowing detection of different chemical species.

2. Methods

Coordinates for the *Paracoccus denitrificans* cytochrome *c* oxidase subunit I and II have been obtained from the PDB entry 3HB3 [27]. The *psfgen* VMD [28] plug-in was utilized to build the readable file with added hydrogen atoms. All the reported molecular dynamics simulations have been performed by using the program NAMD2 [29], with the CHARMM22 all atom force field with the CMAP correction [30,31]. Charges parameterization of the redox metal centers were accordingly to [32]. The TP3 M model for water molecules has been used [30,31], an analog of TIP3P [33] . Langevin dynamics was utilized to maintain a constant temperature, with the damping coefficient set to 5 ps^{-1}, unless differently stated. An integration time step of 2 fs was assumed throughout all simulations, unless differently indicated. In all cases a cut-off of 12 Å (switching function starting at 10 Å) for van der Waals interactions was assumed. The non-bonded pair list parameter was fixed to 13.5 Å and rewritten every 20 fs. Molecular dynamics simulations were carried out on an Intel Pentium M-based computer; every ns simulation required about 60 h of single CPU time.

Overall structural changes of the protein were monitored by computing root mean-square deviations (RMSD) over the entire trajectories using VMD [28]. The crystallographic structure served as the reference point. The atom–atom and the center of mass–atom distances were calculated using the VMD Tcl interface. Spectral reconstructions are performed essentially as reported in [34–38]. Briefly, the lineshape of Mössbauer spectra $I(\omega)$ has been calculated as

$$I(\omega) = \frac{\sigma_0 \Gamma}{2} \int_{-\omega}^{+\omega} \exp\left(-i\omega t - \frac{\Gamma|t|}{2\hbar} - \frac{k_B T k^2}{\alpha\gamma}(1 - \exp(-\alpha|t|))\right) dt \quad (1)$$

where σ_0 was the resonance absorption cross section, Γ the natural line width by the pseudo-uncertainty principle, t the time and T the absolute temperature. The parameters α and γ were calculated from position autocorrelation function and from Gaussian distribution of heme iron atom displacement. Calculations of position autocorrelation functions and Fast Fourier Transform were done with Scilab [39]. A more detailed description is reported in Supplementary Methods.

Complex lineshape spectra were fitted as linear combinations of Lorentzian doublets using Gnuplot software package [40]. The analytical expression of Lorentzian doublet was

$$I(v) = -A \frac{\frac{W}{2}}{(|v - \delta| - \frac{J}{2})^2 + \frac{W^2}{4}} \quad (2)$$

where δ was the isomer shift, J the distance between the doublet peaks, corresponding to the ΔE_Q in Mössbauer spectroscopy and W the half-height width of the peak. The last parameter was obtained from molecular dynamics derived spectral lineshape and was considered to be constant in the fitting procedure.

3. Results and discussion

In order to obtain molecular dynamics simulations of low temperature thermal noise of cytochrome *c* oxidase the 3HB3 structure of *P. denitrificans* enzyme has been used [27]. We chose this particular entry in the PDB because it is one of the best with respect to the crystallographic resolution, in which numerous water molecules are clearly resolved, as well as because it comprises only two subunits of the enzyme. Moreover, due to the striking similarity of mitochondrial-like cytochrome *c* oxidases [41], simulation results can be considered indicative of heme–copper oxidases behavior. Simulations have been performed at 5 K; a so low temperature was chosen because we are interested in matching molecular dynamics with lineshape of published cytochrome *c* oxidase Mössbauer spectra. Available spectra, at the best of our knowledge, have been performed at 4.2 K. We have chosen also to perform *in vacuo* simulations to avoid time consuming calculations and due to the extremely low temperature in which we are interested in. At this temperature proteins (and solvents) are expected to behave much more as solid-like structures trapped in a single conformational substate.

Using a Langevin damping parameter of 5 ps^{-1}, which is usually considered a good default value, after minimization and dynamic equilibration, a stable RMSD was reached (0.9451 ± 0.0002 Å), and then simulations were run at least for 1.8 ns. For each simulation the displacement of heme iron atom relative to cytochrome *c* oxidase as a whole was calculated. The iron displacement is limited to perpendicular motion respect to the heme plane. This unidirectional motion for heme a iron atom is reported in Fig. 1, left panel. The relative displacement distribution, reported in Fig. 1, right

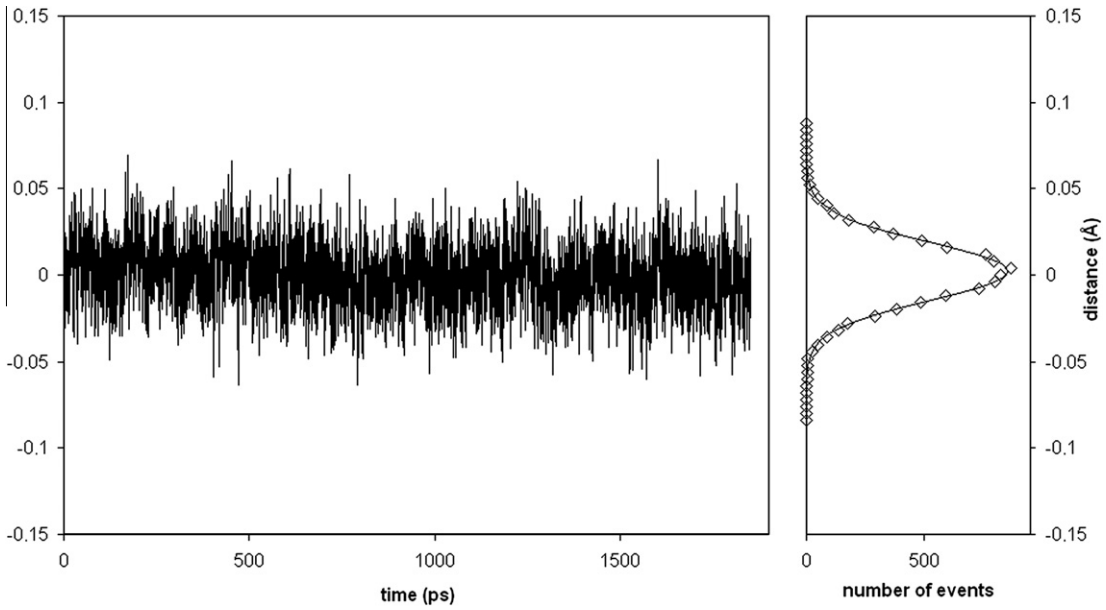

Fig. 1. Displacement of the heme a iron atom. Left panel: the displacement of heme iron atom relative to the protein as a whole is plotted as a function of time. Time is in ps and displacement in Å. Right panel: distribution of iron atom; the data extracted from simulation are shown as diamonds and the Gaussian fit of the data is shown as a solid black line.

panel, is best fitted by a Gaussian. Similar findings are obtained for the heme a_3 iron atom (not shown). Based on these data, we assume that heme iron is in a harmonic well potential for subsequent analysis. The Boltzmann displacement distribution of a particle in a harmonic oscillator potential well is expected to be Gaussian. The apparent spring constants that best match the obtained Gaussian distributions for heme a and for heme a_3 iron atoms have been calculated and are reported in Table 1.

From the iron atoms displacement, the position autocorrelation function can be calculated. In Fig. 2 position autocorrelation function data for heme a iron atom are reported. The position autocorrelation falls off to near zero values in roughly 10 ps. Similar results have been obtained for position autocorrelation function data for heme a_3 iron atom (not shown). Since position autocorrelation function is an important feature in determining the lineshape of Mössbauer spectra [35,36,38], the influence of some simulation parameter on the auto-correlated atom motions has been explored. We tested the influence of shorter timesteps and of shorter rewriting times of the non-bonded pair list interactions without significant differences with respect to the reported simulation (not shown). The influence of Langevin parameter on the position autocorrelation has been particularly explored. Simulations using very low, down to zero, values of Langevin damping parameter have been performed but are not reported because we are unable to obtain stable ones over 500–1000 ps. On the other hand, simulations with values of Langevin damping parameter higher than 5 ps^{-1} have also been performed but are not considered further in this work because unrealistically low RMSD distributions were obtained. Beside these observations, by using different values for Langevin damping parameter, no significantly slower (or faster) decay of position autocorrelation functions could be obtained.

Table 1
Harmonic oscillators features from thermal noise simulation at 5 K.

	σ (Å)	f (pN/Å)	ω_0^2 (ps^{-2})	b (ps^{-1})	$W_{1/2}$ (MHz)
Heme a	1727×10^{-2}	2.32×10^3	245	118	1.22 ± 0.02
Heme a_3	2770×10^{-2}	1.80×10^3	189	80	1.23 ± 0.02

Following the algebra reported above, for the motion of a Brownian overdamped oscillator, the position autocorrelation function could be analytically described (see Supplementary Methods). This equation has been used to fit the position autocorrelation function data, in order to assess the parameters reported in Table 1. These parameters have been used to calculate the lineshape of Mössbauer spectra (see Supplementary Methods for details). The predicted width of Mössbauer spectra of both heme a and heme a_3 is extremely narrow, near to the natural line width expected for a Mössbauer resonant atom (see Table 1). Published low temperature Mössbauer spectra show larger widths. For example, for the ascorbate reduced bovine cytochrome *c* oxidase in [25] a half-height width of 2.4 MHz and 3.2 MHz for heme a and heme a_3 irons atoms, respectively, could be estimated. Even if this work is focused on the *P. denitrificans* enzyme, similarity in structure and function of the two enzymes leads to consider that no such larger deviation can be expected. Therefore some alternative explanations to account for the discrepancies between predicted and observed lineshape width are needed. As pointed out by the same authors of [25], there are several possibility to account for Mössbauer width, larger than expected. Ruling out the possibility that this broadening is due to the presence of unresolved magnetic hyperfine interactions, the preferred explanation is that one of iron – containing redox centers, namely the heme a_3, can be present in, at least, three different states. It is well known that, in the resting enzyme, heme a_3–Cu_B binuclear center could harbor different ligands, deriving from the enzyme cycle or from the preparation being medium used. For example, the presence of hydroxyl or water ligands coming from the catalytic cycle, or the presence of chloride ions has to be considered [42,43]. In order to compare the calculated spectra with the published experimental ones, a set of artificial points has been constructed. This set has been generated considering the lineshape of the reduced bovine cytochrome *c* oxidase spectra [25]. The artificial set has been fitted with a series of Lorentzian doublets whose half-height width has been fixed to 1.2 MHz, i.e. similar to the half-height width of the calculated spectra (see Supplementary Methods for details). Up to five different Lorentzian doublets has been allowed to be used for the fitting. Best results have been obtained with a linear combination of four

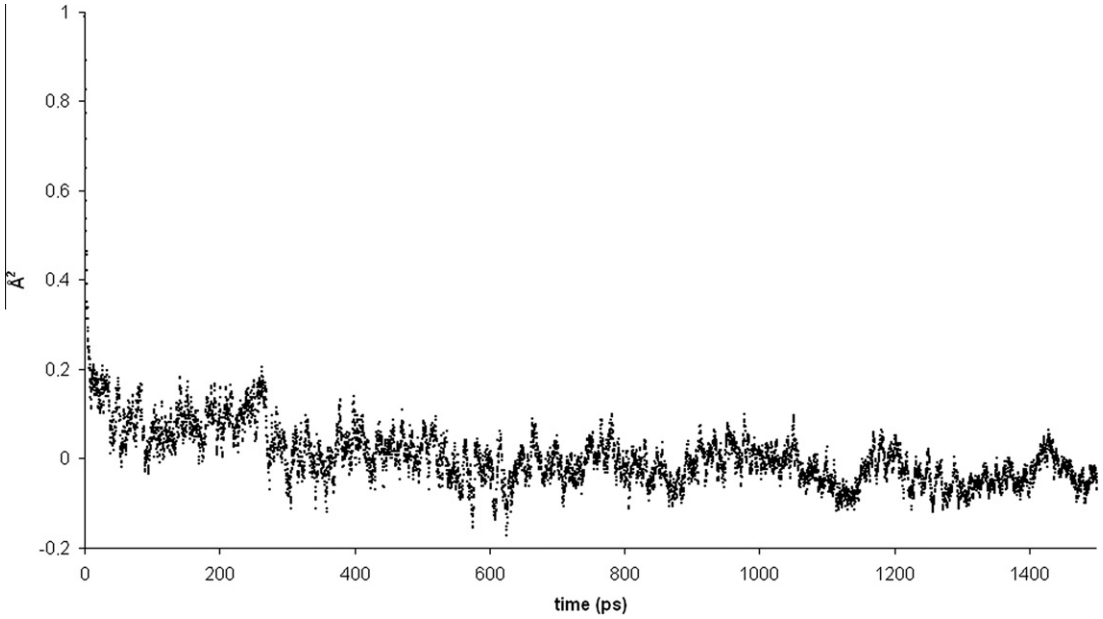

Fig. 2. Position autocorrelation function data. The reported data are relative to the heme a iron atom displacement reported in Fig. 1.

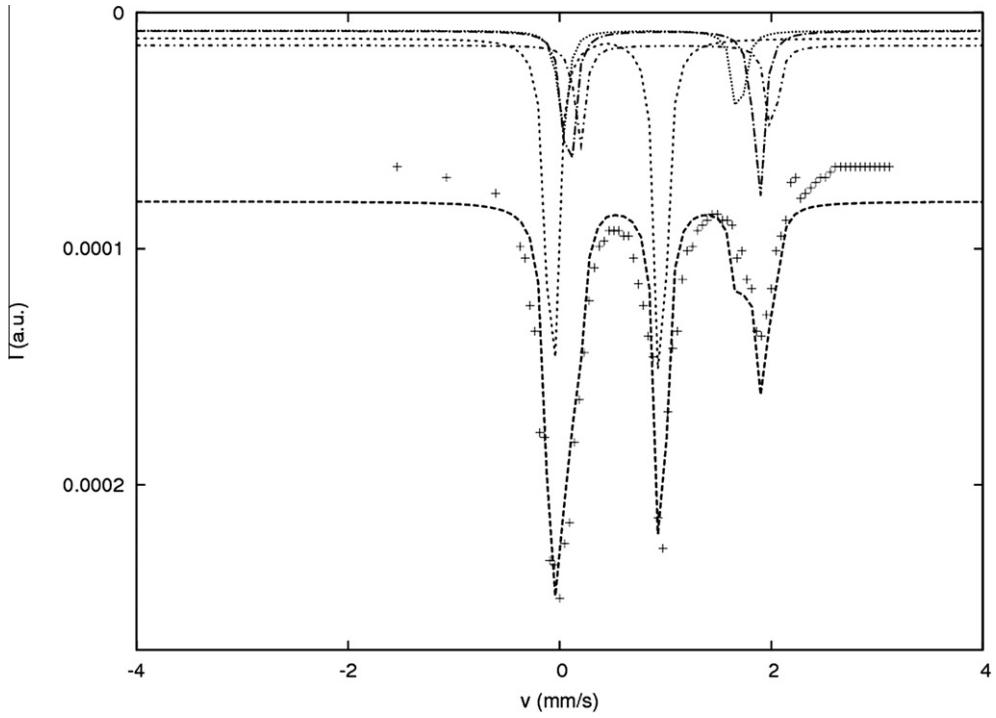

Fig. 3. Fitting of data-set points representing a cytochrome *c* oxidase Mössbauer spectrum with a four Lorentzian doublets linear combination. Data-set points have been constructed in order to respect the lineshape features of spectra reported in [25]. The 0.443 (mm/s) centered Lorentzian doublet, attributed to Fe-*a* component (see Table 2), is drawn as dashed line; the other dotted-dashed lines represent Fe-a_3 contributions (as detailed in Table 2). Dashed solid line represents the linear combination of Lorentzian doublets.

different Lorentzian doublets, as reported in Fig. 3 and in Table 2. The lineshape of the spectra reported in [25] could be explained by considering two groups of Lorentzian doublets: the first, composed by only one doublet accounting for the 53% of the signal, which is attributable to the heme a iron atom; the second group, composed of three Lorentzian doublets which cumulatively account for the 47% of the signal, is attributed to three differently resonant forms of the heme a_3 iron atom.

Table 2
Best fitting parameters of experimental data.

	Fitted data				Experimental data	
	%	δ (mm/s)		ΔE_Q (mm/s)	δ (mm/s)	ΔE_Q (mm/s)
Fe-a	53	0443 ± 0005		1033 ± 0012	0.43 ± 0.04	1.03 ± 0.07
	47	13	0.86 ± 0.02	1.66 ± 0.04	–	–
Fe-a_3		22	0.98 ± 0.02	1.80 ± 0.02	0.93 ± 0.10	1.85 ± 0.10
		12	1.10 ± 0.02	1.82 ± 0.04	–	–

4. Conclusions

Even if no more detailed speculations will be performed about the nature of these three different doublets, these data clearly demonstrate that the broadening of low temperature experimental cytochrome *c* oxidase Mössbauer spectra could be obtained from the lineshapes predicted by molecular dynamics simulations. On the other hand, one could infer that atom motions obtained by molecular dynamics simulations, at least in the conditions used in this work, are satisfactorily realistic even if the simulations have been done *in vacuo* and using only a subset of cytochrome *c* oxidase subunits. This work suggests that these motions could be confidently used in order to gain further insight on the dynamics dependence of the electron transfer phenomenon. Moreover, the possibility to use relatively simple models able to predict the lineshape of Mössbauer spectra and to compare them with the experimental ones could stimulates a revival of this powerful technique in the bioenergetics field.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.11.140.

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