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Determining the Conformational Change that Accompanies Donor-Acceptor Distance Fluctuations: An Umbrella Sampling Analysis

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Abstract

The response of a protein to variation in a specific coordinate can provide insights into the role of the overall architecture in the structural change. Given that the calculated potential of mean force governing the fluctuation of an electron transfer donor-acceptor distance in the NADH:Flavin oxidoreductase (Fre)/FAD complex was shown to agree with experiment, an analysis of the structural response of the rest of the protein to that distance change was made. Significant displacements are found throughout much of the protein and the coupling pathway resulting in the structural changes was determined. A covariance analysis based on the quasiharmonic modes of the unperturbed protein was used to provide information concerning how the residues motions are correlated. It is found that of the three regions identified as moving together in an NMR study, two undergo significant structural changes when the electron donor-acceptor distance is varied and the third does not.

Introduction

It is now accepted that conformational dynamics is essential to bimolecular function^{1,2}. Recent developments in real time studies of single molecules have provided much new information concerning enzyme dynamics. Of particular interest are distance sensitive probes based on fluorescence energy transfer (FRET) or electron transfer (ET), which have been used to study the time dependence of conformational fluctuations^{3,4}. One single-molecule study made use of fluorescence quenching by electron transfer from a Tyr (Tyr³⁵) to the isoalloxazin ring of FAD complexed with the enzyme NAD(P)H: flavin oxidoreductase (Fre)⁴. The fluctuations in the electron transfer rate were interpreted in terms of distance fluctuations between the donor and acceptor, based on the exponential distance dependence of the electron transfer rate⁵. A stretched exponential decay of the distance autocorrelation function was observed⁴ and a number of models were developed to interpret the results^{6,7}. Molecular dynamics simulations of the protein by itself and in the presence of perturbing potentials provided additional insights into the origin of the observed stretched exponential⁸. The potential of mean force for the fluctuating donor-acceptor distance was calculated and found to be in agreement with that estimated from electron transfer

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Supporting Information Available: Twelve correlated groups are identified as moving together during the equilibrium conformational fluctuation, through a cross-correlation analysis of the unperturbed system. This material is available free of charge via the Internet at <http://pubs.acs.org>.

experiments. Interestingly, the calculated autocorrelation function of the distance fluctuations has a simple exponential behavior at low temperatures and stretched exponential behavior at higher temperatures on the femtosecond to nanosecond time scales. This indicated that the calculated dynamic disorder arose from a wide range of trapping times in potential wells on the protein energy landscape and suggested a corresponding origin for the stretched exponential behavior observed experimentally on longer time scales.

Because only one coordinate is studied, the single-molecule experiments did not provide information concerning possible correlations between the fluctuations of the rest of the protein and that of the observed distance. Vallurupalli and Kay used relaxation dispersion NMR ensemble measurements to study the millisecond dynamics of the same complex with the purpose of finding structural fluctuations that could be related to the single-molecule measurements⁹. Both ¹⁵N and ¹³C spin relaxation rates throughout the Fre-FAD system were measured; they probe motions of the backbone ¹⁵N and of methyl groups ¹³C in the sidechains of Ile, Val, and Leu. Specifically, the NMR measurements provide information on exchange processes on the ms time scale that alter the chemical shift. Although the actual nature of the motions involved could not be determined, it is of interest that the residues studied fall into three groups in terms of the estimated exchange rates and their temperature dependence; the latter yielded activation enthalpies on the order of 15 kcal/mol. Two of the groups involve residues that are close to either Tyr35 or FAD. The authors concluded that they observed “three distinct motional processes that can account, in part, for the observed distance fluctuations measured in single molecule studies”, although the experiments do not provide any direct evidence for such a conclusion; i.e., that the motional processes are related to the observed fluctuations in the electron transfer distances.

To obtain further insights into the protein motions that are coupled to the observed Tyr³⁵-isoalloxazine distance fluctuations, we extended the previous analysis of the distance fluctuation⁸. Recognizing that ns simulations, *per se*, can only provide information on that time scale, we had performed potential of mean force (pmf) calculations to map out the one-dimensional free energy surface corresponding to the distance between the donor and acceptor moieties. As previously reported, the calculated PMF surface over a range of center-to-center distances between 6.6 Å and 9.2 Å agreed well with that estimated from the experiments⁸. This result suggested to us that we could usefully examine how the average structure of the protein changed as a function of the position of the umbrella potential to determine the structural changes in the protein that accompany the distance fluctuation.

Methods

Details of the simulations are given in ref.⁸. Briefly, nine 1ns umbrella sampling MD simulations were performed. The umbrella potentials were applied between the centers of the phenyl ring of Tyr³⁵ and the FAD isoalloxazine ring with the minima of the potentials at 6.6, 6.9, 7.2, 7.4, 8.1, 8.3, 8.6, 8.9 and 9.2 Å. The average distance between Tyr³⁵ and the isoalloxazine observed in the simulation in the absence of an umbrella potential is 7.8 Å, while the distance in the x-ray structure of Fre/Riboflavin is 7.5 Å¹⁰, suggesting that meaningful structures are being studied.

To complement the molecular dynamics results, we used the free simulation, 1 ns in length, to evaluate the cross-correlation or normalized covariance matrix element c_{ij} for residues i and j ; $c_{ij} = \langle \delta x_i \delta x_j \rangle / (\langle \delta x_i^2 \rangle \langle \delta x_j^2 \rangle)^{1/2}$, where δx_i and δx_j are the deviations of the spatial coordinates x_i and δ_j from their mean value and the $\langle \rangle$ represent averages over the simulation¹¹.

Results and Discussions

Movements of Residues

To explore the structural changes that accompany the Tyr³⁵/isoalloxazine distance change, we examined the average structures obtained from the 1 ns sampling trajectories with umbrella potentials applied at the different distances. A time period of 1 ns appears to be sufficient to obtain the average structures since the changes are relatively small and the potential of mean force has converged (see Ref. ⁸). Fig. 1a shows the average backbone displacements in the presence of the umbrella potentials relative to the average structure obtained in the simulation of the unperturbed system. The backbone RMS fluctuations observed in the simulation without an applied potential are shown in Fig. 1b for comparison. Clearly, the amplitudes of displacements in Fig. 1a, which are as large as several Angstroms, are significantly greater than the equilibrium fluctuations (typically about 1 Å). This demonstrates that the displacements are a response to the perturbation of the protein by the umbrella potential. In Fig. 1c we show the difference between the average structures for the umbrella potential centered at 6.6 Å (a distance smaller than that in the average unperturbed structure) and centered at 9.2 Å (a distance larger than that in the average structure). The figure makes clear that the same residues are involved in the largest changes (Fig. 1a), which suggests that the magnitude of the response of the protein to the perturbation is encoded in the structure. However, these residues move in significantly different directions. We note also that the residues whose positions are significantly altered are not limited to those that are close to where the umbrella potential is applied; e.g. large displacements occur for Trp¹⁷⁴ which is ~25 Å from Tyr³⁵ and the isoalloxazine ring.

The displacements in response to the umbrella potentials are projected on the average free dynamics structure in Fig. 2; see caption. We show only the limiting structure results for clarity; i.e., those for the umbrella potentials at 6.6 Å and 9.2 Å.

Comparison with NMR Results

To compare with the NMR analysis, we show in Fig. 3a, adapted from the Fig. 3 of Vallurupalli and Kay ⁹, the groups of residues sharing similar dynamics; see Introduction. Figure 3b presents the results obtained from the cross-correlation analysis of the unperturbed system. Each color corresponds to a group of sequential residues that move together (see SI and the figure caption for details of the method used). There are twelve correlated groups obtained by the present method. The off-diagonal elements in the correlation matrix show that both sequential and nonsequential groups of residues are correlated in some cases. Residues in group 1 identified by NMR (green in Fig. 3a) correspond to the green helix in Fig. 3b; residues in group 2 (red in Fig. 3a) correspond to part of the extended red strand in Fig. 3b; and residues in group 3 (blue in Fig. 3a) are found in the blue helix in Fig. 3b, but also in parts of separate groups shown as dark blue and purple in the figure. Figure 3c shows the correlated groups of residues that have displacements larger than 2 Å. The red group of residues is missing because its displacements are no larger than the normal RMS fluctuations. Since the NMR experiments do not measure the magnitude of the displacement, the present analysis suggests the movements of the green and blue residues are major components of the global protein conformational response, while the red group is not.

Propagation of the Perturbation

To examine how the perturbation due to the umbrella potential is propagated, we show first the displacement of Tyr³⁵ and the FAD isoalloxazine (see Fig. 4). The sidechain of Tyr³⁵ moves significantly on compression but very little on expansion due to the caging of the surrounding protein residues. The entire cofactor undergoes significant displacements in the

presence of the umbrella potential. The tail of the cofactor moves in the same way on compression as on expansion, but the isoalloxazine ring undergoes a large displacement on expansion and moves less on compression. Figure 2 shows that the FAD-isoalloxazine cofactor extends from near the middle of the protein all the way to the lower portion and suggests that the cofactor displacement is likely to be the major source of the protein response.

The carbonyl groups and the amide hydrogen at the edge of the isoalloxazine ring form hydrogen bonds, respectively, to the amide hydrogen and the hydroxyl group of Ser¹¹⁵; these hydrogen bonds exist in the structures with all the applied potentials. The rotation of the isoalloxazine around this anchor point, which is near the bottom of the ring, appears to be the primary origin of the effect of the umbrella potential on the protein (as shown in Figs. 4 and 5). This rotation makes a methyl group on the FAD isoalloxazine ring collide with the Phe²⁰³ sidechain. At the same time, Phe²³¹ moves closer to fill the space left by FAD isoalloxazine ring rotation. The collision between FAD and Phe²⁰³ pushes the helix from Glu²⁰⁴ to Ser²¹⁵ (gray and blue helices, Fig.5), and introduces a small tilt in the helices. Through the sidechain repulsive interaction between Phe²¹³ and Leu¹⁸⁶, as shown in Fig.5, this movement propagates down to the adjacent helix and the rest parts of the protein. The long yellow arrow schematically represents the path of the conformational change. On the left side of the protein, the rotation of FAD isoalloxazine ring drags the FAD ribose chain with it. The ribose CH₂ group, directly attached to the isoalloxazine ring, collides with the Ala⁶⁶ sidechain and pushes the flexible loop of residues 66–72 (gray and blue loop in Fig.5) down as indicated by the short yellow arrow. Such propagation paths account for the displacement map given in Fig. 2, where roughly only the bottom half of the protein is significantly affected by the umbrella potentials.

We also see more detailed agreement between the simulation results and the NMR observations (9). Relaxation-dispersions for residues Leu¹⁸⁶, Phe²³¹ and Phe²¹³ were observed in the NMR experiments. In addition, residues Gly⁶⁵, Gly²⁰¹, and Met²⁰⁵, all very close to the affected region shown in the simulation, were found to have relaxation-dispersions. The resonances for the directly affected residue Phe²⁰³ were not assigned in the NMR spectrum, probably because they are broadened due to conformational exchange accompanied with the Tyr³⁵-FAD distance fluctuation.

Conclusion

In the present analysis of the response of the NADH:Flavin oxidoreductase (Fre)/FAD complex to the change of an electron transfer distance, large effects far from the perturbation are observed. The structural changes originate primarily from the displacements of the FAD-isoalloxazine cofactor and to a lesser extent from Tyr³⁵. They are propagated through the protein by the coupling between its different parts along a relatively well-defined pathway. The results show that of the three regions that have correlated motions in an NMR study, two appear to be related to the distance fluctuation, while one does not have a significant response to the distance variation. Thus, the molecular dynamics umbrella sampling simulations provide the missing link between the single molecule and NMR experiments.

We note that such remote effects in a protein from a local perturbation are closely related to protein allostery, which concerns the effect on the function of a protein in one region^{12,13} due to binding of a ligand in another¹⁴. The present approach, which uses umbrella potential simulations of the free energy change along a given coordinate to study the response in distant parts of the protein, is expected to be of general interest for understanding allosteric effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Movements of residues under umbrella potentials. (a) Displacements of residues in the presence of the umbrella potentials; for clarity, results of only four umbrella potentials are shown. The Tyr³⁵-isoalloxazine ring distance is 7.8 Å in the average structure. The dotted vertical lines indicate residue Tyr³⁵, to which the umbrella potential is applied and the other point to which the potential is applied is to isoalloxazine ring of FAD, which is nearest to residue Ser¹¹⁵. (b) RMS fluctuations of residues in the unperturbed simulation. (c) Difference between the displacements of residues in the average structures with the umbrella potentials applied at 6.6 Å and at 9.2 Å.

Figure 2. Representative structures obtained with the umbrella potential applied at 6.6 and 9.2 Å, indicating with a color code the residue displacements from the average structure without the umbrella potential. On the top is the free Fre/FAD structure, with positions of every 10th residues labeled in light blue.

Figure 3.

Residues moving in groups and comparison to the NMR results. a) Three groups of residues identified in the NMR experiment reported by Vallurupalli and Kay that move together as a result of spontaneous conformational fluctuations of the protein. b) Groups of residues moving together in the protein (indicated in the same color) are identified from the cross correlation matrix obtained from the unperturbed simulation. In accord with the analysis given in the Supplementary Material, there are twelve such groups. c) Residues in stick model (green, 62–72; blue, 136–150; dark blue, 168–179) that have movements under the applied umbrella potentials with amplitudes significantly larger than the protein RMS fluctuations (see text).

Figure 4. Average positions of the FAD isoalloxazine and Tyr³⁵ (at the top) with no umbrella potential (red) and with the umbrella potential applied for distances of 6.6 Å (green) and 9.2 Å (purple), respectively.

Figure 5.

Propagation of the motions from Tyr³⁵-FAD to remote regions within the protein. Two average structures from simulations with umbrella potentials applied at the shortest (6.6 Å, light gray) and longest (9.2 Å, blue) distances are shown together. Residues with close spatial contact, which are mainly responsible for the propagation of conformational, are shown as sticks in different colors (in red for potential applied at 6.6 Å distance; in blue for potential applied at 9.2 Å distance). The arrows are a schematic indication of the pathways of conformational change.