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Redox Characterization of the FeS Protein MitoNEET and Impact of Thiazolidinedione Drug Binding[†]

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ABSTRACT: MitoNEET is a small mitochondrial protein that has been identified recently as a target for the thiazolidinedione (TZD) class of diabetes drugs. MitoNEET also binds a unique three-His- and one-Cys-ligated [2Fe-2S] cluster. Here we use protein film voltammetry (PFV) as a means to probe the redox properties of mitoNEET and demonstrate the direct impact of TZD drug binding upon the redox chemistry of the FeS cluster. When TZDs bind, the midpoint potential at pH 7 is lowered by more than 100 mV, shifting from ~0 to −100 mV. In contrast, a His87Cys mutant negates the ability of TZDs to affect the midpoint potential, suggesting a model of drug binding in which His87 is critical to communication with the FeS center of mitoNEET.

While the thiazolidinedione (TZD)[1] drugs have been useful in the treatment of type 2 diabetes, their primary mode of action has been attributed to activation of peroxisome proliferator-activated receptor γ (PPARγ) (*1−3*). Recent appreciation of PPARγ-independent modes of TZD action (*4*) spurred the discovery of mitoNEET a mitochondrial protein, through a cross-linking study with a photoactive form of the TZD drug pioglitazone (Figure 1) (*5*). Intriguingly, mitoNEET bears a [2Fe-2S] cluster (*6, 7*), and the cardiac mitochondria of mice lacking mitoNEET display dramatic decreases in respiratory function (*6*). The typical association of FeS proteins with redox events in biology, the growing appreciation of the interplay between mitochondrial dysfunction and type 2 diabetes (*8*), and the observation of oxidative stress in diabetic patients (*9*) led us to posit that mitoNEET may possess redox chemistry that is an important component of mitochondrial function, and that TZD drug binding may stabilize normal function of mitoNEET in diabetes. Here we describe the redox chemistry of the mitoNEET [2Fe-2S] cluster for the first time, demonstrate that TZD binding directly impacts the mitoNEET reduction potential, and show that TZD binding stabilizes the oxidized state of the wild-type protein.

MitoNEET is a small ~17 kDa protein localized to the cytosolic face of the outer mitochondrial membrane by a single transmembrane helix (*6*). X-ray crystallographic analyses of the soluble portion of the protein (Figure 1A) have demonstrated that mitoNEET forms dimers with one [2Fe-2S] cluster per monomer, and a cluster−cluster distance of ~14 Å (*10−12*). The cluster is uniquely ligated by three Cys residues and one His, a novel coordination environment for [2Fe-2S] clusters (Figure 1B), differing from the all-Cys ligation of ferredoxins and the two-Cys, two-His ligation seen in Rieske centers (*13*).

We have used protein film voltammetry (PFV) to interrogate the [2Fe-2S] cluster TZD-free and -bound states of the crystallographically characterized, soluble form of mitoNEET (Figure 1A). Baseline-subtracted oxidative and reductive voltammograms (Figure 2B) were averaged to yield midpoint potentials (E_m) over a pH range from 6 to 12 (Figure 2A). A midpoint potential of approximately 0 mV versus the standard hydrogen electrode (SHE) was observed at pH 7.0 ($E_{m,7}$) for the wild-type protein. The pH dependence of the mitoNEET potential is modeled well assuming a one-electron process that is coupled to two distinct protonation events, similar to Rieske FeS proteins. In the case of Rieske proteins, a region of steep pH dependence (−120 mV/pH) is observed due to the relative values of $pK_{ox1,2}$ and $pK_{red1,2}$ (*14*). Here the relative placements of pK_a values ($pK_{ox1} < pK_{red1} < pK_{ox2} < pK_{red2}$) results in a shallower slope, as shown in Table 1. (An alternate 1:1 model gives reasonable agreement as well and is given as Figure S1 in the Supporting Information for comparison; pK_a values for both models are collected in Table S1.) Finally, we note that the mitoNEET direct electrochemistry data are also in good agreement with solution redox titrations (Figure S2 of the Supporting Information).

Using PFV analyses, we have interrogated the connection between redox chemistry of mitoNEET and TZD drug binding. Data from both the pioglitazone-bound (Figure 2B) and rosiglitazone-bound forms of mitoNEET yield pH-dependent E_m values shown in Figure 2A (red and blue). Both TZD-bound forms of mitoNEET display similar changes: in both cases, the reduction potential is lowered at pH values near neutral, and the decrease in potential can be ascribed to an increase in pK_{ox1} that occurs upon drug binding [from 6.5 to ~8.5 (Table 1)]. The shift in pK_{ox1} results in a decrease in $E_{m,7}$ of > 100 mV for the TZD-bound forms of mitoNEET, where other perturbations to the pH dependence curve upon drug binding are minor.

Presently available crystallographic analyses yield promising possibilities for the identities of sites of protonation and drug binding. In analogy to Rieske FeS proteins (*14*), it is most likely that the site of redox-linked protonation is ε-N of the cluster ligand His87. However, the mitoNEET [2Fe-2S] cluster environment

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[1]Abbreviations: TZD, thiazolidinedione; PFV, protein film voltammetry; mN, mitoNEET; pio, pioglitazone; rosi, rosiglitazone; RsRp, *Rhodobacter sphaeroides* Rieske protein; TtRp, *Thermus thermophilus* Rieske protein; BphF, *Burkholderia* sp. strain LB400 Rieske ferredoxin; PDB, Protein Data Bank.

does display other differences with respect to Rieske proteins, including a lysine residue positioned within ~4 Å of His87 ε-N. A conserved bridging water molecule could theoretically create a hydrogen bond network between His87 and Lys55 (Figure 3), providing an alternative arrangement that leads to proton coupling at His87.

While articulating the details of the mechanism of proton coupling will remain a future challenge, it is clear that His87 is critically important. Replacement of His87 with Cys results in a dramatic decrease in the midpoint potential by more than 300 mV (Figure 2C) to a value of −320 mV at pH 7, suggestive of two-Fe ferredoxins (*16*). The overall pH dependence becomes much more
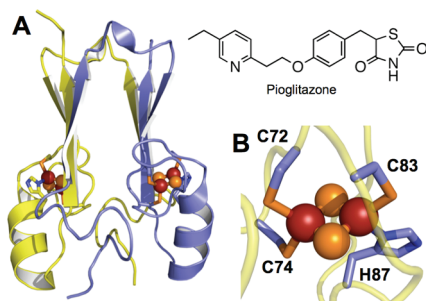

FIGURE 1: (A) Overall fold of the mitoNEET soluble domain (PDB entry 2QH7), which interacts with TZD drugs like pioglitazone. (B) Cluster organization, with the inner iron ligated by Cys72 and Cys74 and the outer iron by Cys83 and His87.

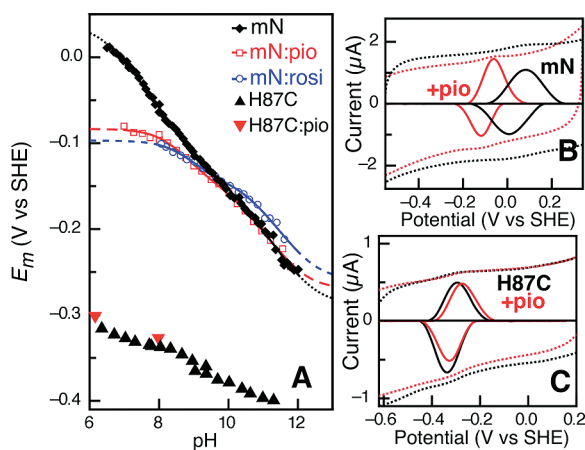nondescript with an apparent linear slope of −15 to −20 mV/pH

FIGURE 2: (A) Comparison of wild-type mitoNEET (mN) pH dependence of midpoint potentials in the TZD-free (black diamonds) and pioglitazone- and rosiglitazone-bound states (red squares and blue circles, respectively). Additional data for the H87C mutant (black triangles) and pioglitazone-treated H87C (red triangles). (B) Raw and baseline-subtracted data for mN alone (black dotted and solid lines, respectively) and in the presence of pioglitazone (red). (C) Analogous data for the H87C mutant in the absence and presence of the TZD drug.

unit [also mimicked by solution redox titrations (Figure S1)]. Most importantly, upon treatment of the H87C mutant with pioglitazone, a nominal change in potential is observed, a positive shift of 5 mV. Taken together, the H87C mutant data suggest that a significant component of the interaction of the TZD drug with mitoNEET occurs via His87, resulting in the modulation of the redox-linked pK_{ox1} and, therefore, the midpoint potential. Notably, this interaction may be through direct hydrogen bonding or van der Waals interactions or might be through a more indirect, allosteric mechanism of binding to another site on mitoNEET.

A clear challenge in this field is to understand TZD drug binding at a higher level of detail. Measuring association constants for TZD binding has not yet been accomplished because of the high insolubility of the drugs in aqueous buffers, and drug solubilization requires high concentrations of DMSO, which denature mitoNEET upon prolonged exposure, as required in equilibrium titrations. Here PFV has the benefit of probing relatively low concentrations of mitoNEET at an electrode (subpicomolar), such that the insolubility of the drug is not an issue. From the overall shift of the E_m values, one can determine the ratio of relative affinities for the drug to the oxidized and reduced states of the protein based on the thermodynamic square shown in Figure 4. Using the Nernst equation,
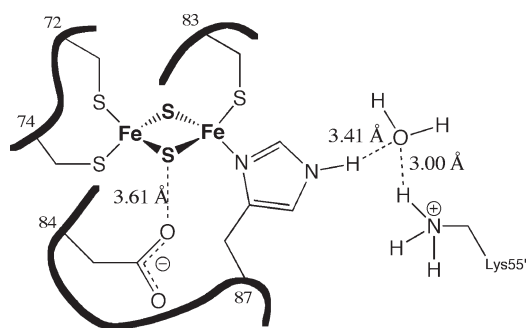

FIGURE 3: Schematic of the [2Fe-2S] cluster of mitoNEET surrounded by ligating residues Cys72, Cys74, Cys83, and His87 and important ionizable residues Asp84 and Lys55′. Potential hydrogen bonds are represented by dashed lines and distances by dotted lines. All distances are averages between non-hydrogen atoms from mitoNEET PDB entries 2QH7, 2R13, and 2QD0.
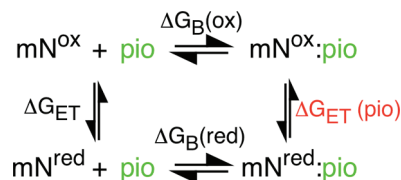

FIGURE 4: Thermodynamic square relating free energies of binding of pioglitazone to mitoNEET in the oxidized and reduced states. $\Delta G_{ET} = -nFE_m$, where n is the number of electrons, F is Faraday's constant, and E_m is the redox potential.

Table 1: Comparison of the Reduction Potentials and pK_a Values of MitoNEET (with TZDs) and Three Rieske Proteins Characterized by Zu and Co-Workers (*11*)

protein	pK_{ox1}	pK_{ox2}	pK_{red1}	pK_{red2}	E_{acid} (mV)	ref
mN	6.5 ± 0.1	10.1 ± 0.1	9.5 ± 0.1	> 12.5	40 ± 4	this work
mN−pio	8.2 ± 0.1	10.0 ± 0.2	9.3 ± 0.2	> 12	−80 ± 3	this work
mN−rosi	8.7 ± 0.1	10.6 ± 0.2	9.3 ± 0.2	> 12	−100 ± 3	this work
RsRp	7.6 ± 0.1	9.6 ± 0.1	12.4 ± 0.4	12.4 ± 0.4	308 ± 3	*11*
TtRp	7.85 ± 0.15	9.65 ± 0.12	12.5 ± 0.5	12.5 ± 0.5	161 ± 4	*11*
BphF	9.8 ± 0.2	11.5 ± 0.4	13.3 ± 0.8	13.3 ± 0.8	−135 ± 5	*11*

we can find a ratio for the ΔG values for pioglitazone binding to the oxidized and reduced states as illustrated in Figure 4 and, thereby, the ratio of affinities (directly related to the free energies of binding). This estimate shows that the drug binds the oxidized state with a roughly 50-fold higher affinity than it does the reduced state.

Collectively, our data may explain the role of the drug in alleviating oxidative stress. MitoNEET is most likely to be found in its reduced state under physiological conditions; on the basis of the reducing environment of the cytosol (*15*) and the potential of $\sim$0 mV at pH 7, the overall lowering of E_m due to drug binding will make mitoNEET a better reducing agent, which may be beneficial under oxidative stress conditions. At the same time, the enhanced TZD affinity for the oxidized form of mitoNEET reflects thermodynamic stabilization of the oxidized [2Fe-2S] cluster, when the drug is bound. It has been appreciated that at pH < 7 the [2Fe-2S] cluster of mitoNEET is labile, and that pioglitazone binding counteracts lability, as does a His87Cys mutation (*9*). Under oxidative stress conditions, cluster oxidation and subsequent lability may be a significant issue that TZD binding counteracts.

Here we have observed for the first time that two members of the TZD drug family similarly affect mitoNEET redox chemistry: pK_{ox1} increases by $\sim$2 units, resulting in an $E_{m,7}$ reduced by > 100 mV. Thus, drug binding has a fundamental impact on the redox activity of mitoNEET. Previous spectroscopic evidence has demonstrated that mitoNEET can exist in both reduced and oxidized forms (*7*), and our data indicate facile electron transfer at an electrode can be achieved [k_0 of 10^2 s^{-1} (data not shown)] and that TZD drug binding impacts the redox properties of the [2Fe-2S] cluster of mitoNEET, likely via His87. While questions remain regarding mitoNEET function and the interplay between cluster lability and redox potential, it is clear that PFV can now serve as an exploitable tool in further assessing the impact of TZD drug binding in this emerging class of FeS proteins related to oxidative stress, mitochondrial function, and type 2 diabetes.

SUPPORTING INFORMATION AVAILABLE

Materials and Methods and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkinson, W. O., Willson, T. M., and Kliewer, S. A. (1995) *J. Biol. Chem. 270*, 12953–12956.
2. Semple, R. K., Chatterjee, V. K. K., and O'Rahilly, S. (2006) *J. Clin. Invest. 116*, 581–589.
3. Tontonoz, P., and Spiegelman, B. M. (2008) *Annu. Rev. Biochem. 77*, 289–312.
4. Feinstein, D. L., Spagnolo, A., Akar, C., Weinberg, G., Murphy, P., Gavrilyuk, V., and Dello Russo, C. (2005) *Biochem. Pharmacol. 70*, 177–188.
5. Colca, J. R., McDonald, W. G., Waldon, D. J., Leone, J. W., Lull, J. M., Bannow, C. A., Lund, E. T., and Mathews, W. R. (2004) *Am. J. Physiol. 286*, E252–E260.
6. Wiley, S. E., Murphy, A. N., Ross, S. A., van der Geer, P., and Dixon, J. E. (2007) *Proc. Natl. Acad. Sci. U.S.A. 104*, 5318–5323.
7. Wiley, S. E., Paddock, M. L., Abresch, E. C., Gross, L., van der Geer, P., Nechushtai, R., Murphy, A. N., Jennings, P. A., and Dixon, J. E. (2007) *J. Biol. Chem. 282*, 23745–23749.
8. Lowell, B. B., and Shulmanz, G. I. (2005) *Science 307*, 384–387.
9. Mehta, J. L., Rasouli, N., Sinha, A. K., and Molavi, B. (2006) *Int. J. Biochem. Cell Biol. 38*, 794–803.
10. Lin, J. Z., Zhou, T., Ye, K. Q., and Wang, J. F. (2007) *Proc. Natl. Acad. Sci. U.S.A. 104*, 14640–14645.
11. Hou, X. W., Liu, R. J., Ross, S., Smart, E. J., Zhu, H. N., and Gong, W. M. (2007) *J. Biol. Chem. 282*, 33242–33246.
12. Paddock, M. L., Wiley, S. E., Axelrod, H. L., Cohen, A. E., Roy, M., Abresch, E. C., Capraro, D., Murphy, A. N., Nechushtai, R., Dixon, J. E., and Jennings, P. A. (2007) *Proc. Natl. Acad. Sci. U.S.A. 104*, 14342–14347.
13. Link, T. A. (2001) in Handbook of Metalloproteins (Messerschmidt, A., Huber, R., Poulos, T., and Wieghardt, K., Eds.) Vol. 1, pp 518−531, Wiley, New York.
14. Zu, Y. B., Couture, M. M. J., Kolling, D. R. J., Crofts, A. R., Eltis, L. D., Fee, J. A., and Hirst, J. (2003) *Biochemistry 42*, 12400–12408.
15. Attene-Ramos, M. S., Kitiphongspattana, K., Ishii-Schrade, K., and Gaskins, H. R. (2005) *Am. J. Physiol. 289*, C1220–C1228.
16. Zanetti, G., Binda, C., and Aliverti, A. (2001) in Handbook of Metalloproteins (Messerschmidt, A., Huber, R., Poulos, T., and Wieghardt, K., Eds.) Vol. 1, pp 532−542, Wiley, New York.