

Electronic states of the O₂-tolerant [NiFe] hydrogenase proximal cluster

Recently, Pandelia et al. (1) used density functional theory (DFT) calculations to interpret their Mössbauer experiments on the superoxidized state of the O₂-tolerant hydrogenase [4Fe3S] proximal cluster. Experimentally (table 2 in ref. 1), the subspectrum (S) exhibits the largest quadrupole splitting ($\Delta E_Q = 2.45$ mm/s) and a positive hyperfine value *A*, indicating that the corresponding iron ion belongs to the minority spin sites (i.e., the projection coefficient *K* of its local spin onto the cluster spin is negative). Among the possible DFT broken symmetry (BS) states Ox_n_ij computed to identify this (S) signal [ij: mixed-valence pair; *n* = 1: nearby protonated glutamate, *n* = 2: deprotonated Glu], the authors have retained Ox2_14 and Ox2_24, as we did previously [BS13 and BS34, respectively (2), considering local iron spin alignments only]. Note that changing Fe1→1, Fe2→4 (unique site), Fe3→2 and Fe4→3 converts Pandelia et al.'s (1) notation into ours (2).

Ox2_14, the spin alignment of which we favored, has been rejected by Pandelia et al. because “this solution does not properly reproduce the experimental δ values or the sign of the *A* values, of which the latter reveals a basically wrong spin-coupling scheme” (1). This statement is incorrect and contradictory to the legend of table S7 in ref. 1. The spin-coupling procedure we proposed for BS13 yields *K* < 0 and *A* > 0, with the largest ΔE_Q (table S5 in ref. 2) for Fe1, in agreement with experiments. Pandelia et al. (1) stated that a ferrous Fe1 is deemed unlikely because of its local quasitrahedral symmetry. However, in this globally asymmetric cluster, in their own Ox2_13 and Ox2_14 states, a ferrous Fe1 site is energetically favored over Fe3 and Fe4, respectively [table S9 in ref. 1, computed with Perdew-Burke-Ernzerhof (PBE) potential]. Moreover, Ox2_14 can hardly be rejected based on

predicted δ values, given the PBE data dispersion in the [0–0.5] millimeter per second range of the calibration curve (figure S11A in ref. 1).

Having ruled out Ox2_14 (hence BS13), Pandelia et al. (1) favored Ox2_24. However, the initially set ferric Fe1-Fe3 pair is changed during calculation into a mixed-valence pair, with the conundrum that “this makes it more difficult to explain why the remaining ferric sites do not determine the majority spin” (1), in which the authors referred to the now ferric Fe2-Fe4 pair of minority spin, required to have *A* > 0, but postulated without explanation. Having reanalyzed their (1) and our (2) data, we realized that this pair becomes ferric because of charge migration from Fe2 to Fe1-Fe3, resulting by BS construction into a local spin state 3/2, instead of 5/2, for Fe2 (spin population in table S9 of ref. 1). This spin lowering not discussed by both teams, rather than “chemical intuition,” leads to a large DFT ΔE_Q (2.25 mm/s) for Fe2 and a short Fe-N bond of 1.9 Å. However, the BS state Ox2_13 with the same Fe1-Fe3 mixed-valence pair [note that Ox2_13 leads to wrong predicted Mössbauer parameters and is therefore rejected on this ground (1, 2)] is energetically favored over Ox2_24 and restores both a local spin 5/2 at Fe2 and a longer Fe-N distance closer to experiment (2.1 Å) (2, 3). Thus, Ox2_24 is an artificially trapped electronic state.

Focusing on structural features, in Ox2_14 the unique Fe2 is coordinated to a deprotonated glutamate carboxylate group not favored by our calculations (2). The protonated Ox1_14 model has been implicitly discarded based on calculated Mössbauer parameters (table S9 in ref. 1), but our calculations on the same state agree with the experiment (table 3 in ref. 2). This discrepancy most probably arises from the authors' use of

small quantum models with spurious constraints (compare with glutamate Cy: figure S8 in ref. 1) instead of the unconstrained models that include the protein matrix we used.

To conclude, we think that the only plausible state is BS13 with a nonbonded nearby glutamate. This state explains why and how the superoxidized state is reached (4).

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The authors declare no conflict of interest.

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