Conformational Properties of Oxidation-Reduction Cofactors

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Abstract: The geometry-energy relationships of three biological redox cofactor models have been examined by using ab initio (Hartree-Fock) calculations with a 3-21G basis set. The results indicate that the aromatic forms of these cofactors are rigidly planar, and a distortion to a nonplanar geometry results in a significant increase in energy. In contrast, the nonaromatic forms are quite flexible and are easily distorted from their own optimum geometries. The implications for biological redox reactions are discussed.

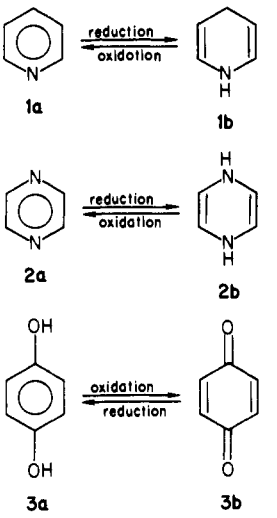
The mechanisms of biological reactions and the details of how enzymes can function as highly effective catalysts with extremely high turnover rates are of great interest to a broad range of chemists.[1] We report here a computational study that may provide fundamental insight into the nature of biological oxidations and reductions. Our results demonstrate a pattern of conformational behavior for cofactors in biological redox reactions that could help to explain both rate and selectivity phenomena for these systems.

Nearly all biological oxidations and reductions of organic substrates involve cofactors that belong to one of three classes: the dihydropyridines[2] (e.g., NAD/NADH, NADP/NADPH), the flavins[2] (e.g., FMN/$FMNH_2$, FAD/$FADH_2$), and the quinones[3] (e.g., coenzyme Q, vitamin K). For each of these redox pairs conformational behavior of the oxidized and reduced forms may differ substantially, and this in turn may be responsible for some of the unique behavior of the enzymatic systems. It has been previously suggested[4] that the preferred planar and nonplanar geometries for oxidized and reduced flavins, respectively, could result in quite different binding ability for the two forms. Alternatively, enzyme-imposed distortions from the optimum flavin geometry could produce variation in its redox potential,[5] and this could in turn affect the enzyme's selectivity toward different substrates.

Each of the three biological redox systems has one member that should show a marked preference for planarity as a result of aromatic character; the other member of the pair has the possibility of being far more flexible. Consequently, we have undertaken a molecular orbital study of the model systems **1-3** to learn if such differences in conformational mobility should indeed be expected. The computational approach permits evaluation of a wide range of molecular geometries in addition to the few minimum-energy structures that might also be observed experimentally.

Results

Computations were carried out at the ab initio level with use of the 3-21G split-valence basis set of the GAUSSIAN 80 system of programs.[6] Complete geometry optimizations were carried out for various distortions from planarity, and the results are summarized in Table I. The planar structures were maintained at C_{2v} symmetry except for **2a** (D_{2h}), **3a** (C_{2h}), and **3b** (D_{2h}). The nonplanar geometries of **1** were restricted to C_s symmetry. The nonplanar structures of **2a**, **2b**, and **3b** were kept at C_{2v} symmetry while those of **3a** were maintained at C_2 symmetry. The folding angle along the axis defined by atoms 1 and 4 was held at the values specified in the table; otherwise all geometric parameters were fully optimized.



Discussion

As anticipated, the aromatic compounds **1a-3a** all exhibit a distinct preference for planarity and can be distorted to a nonplanar geometry only at the expense of a significant increase in energy ranging from 7.5 to 8.5 kcal/mol for folding to 160° (Table II). In contrast the nonaromatic compounds **1b-3b** are much more flexible. The behavior of dihydropyridine (**1b**) is very similar to that which we[7] and Boggs[8] found previously for the hydrocarbon analogue. Folding from planar to 160° requires 1.4 kcal/mol for **1b**, and the value found for 1,4-dihydrobenzene[7] is 1.5 kcal/mol. Dihydropyrazine (**2b**) has a preferred nonplanar geometry similar to that of the reduced flavin analogues,[4] but the energy surface is extremely flat.[9] The array of structures from planar to 160° folded are all within 0.65 kcal/mol of each other. Benzoquinone, although planar (as expected from crystal-structure data for a variety of quinones),[10] is nevertheless quite easily distorted to a nonplanar geometry, and folding by 20° costs only 3.0 kcal/mol.

Since our calculations were carried out with a minimal basis set and neglecting correlation, the reported energies should only be considered as qualitatively correct. More accurate evaluations of the energy would require the use of larger basis sets and the inclusion of polarization functions.[11] For example, the calculated inversion barriers of amines were shown to be in error by several kcal/mol when only a minimal basis set was employed.[12] On the other hand, the deviations from equilibrium geometries were all

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Table I. Ab Initio (3-21G) Calculated Energies for **1–3** as a Function of Distortion from Planarity

	total energy, hartrees					
	aromatic forms			nonaromatic forms		
folding angle[a]	**1a**	**2a**	**3a**	**1b**	**2b**	**3b**
180°	−245.312 01	−261.197 51	−378.297 89	−246.447 27	−262.327 05[b]	−377.100 67
170°	−245.309 03	−261.194 12	−378.294 65	−246.446 74	−262.327 33	−377.099 47
160°	−245.300 04	−261.183 89	−378.284 82	−246.445 07	−262.326 02	−377.095 84
optimum[c]	−245.312 01	−261.197 51	−378.297 89	−246.447 27	−262.327 38[c]	−377.100 67

[a] The dihedral angle, (2)–(1)–(4)–(6), resulting from folding of the molecule along the axis defined by atoms 1 and 4 (cf. footnote of ref 7). [b] If the hydrogens bound to N-1 and N-4 are also restricted to the molecular plane (D_{2h}), the energy increases to −262.32307. [c] The optimum geometry is planar for each compound except **2b**, for which a folding angle of 173° is calculated.

Table II. 3-21G Calculated Energies of Distortion from Planar Geometry for **1–3**

	distortion energy, kcal/mol					
	aromatic forms			nonaromatic forms		
folding angle	**1a**	**2a**	**3a**	**1b**	**2b**	**3b**
170°	1.87	2.13	2.03	0.33	−0.18	0.75
160°	7.51	8.55	8.20	1.38	0.65	3.03

fairly small for the structures considered in this work, and the relative energies of each compound as a function of nonplanar distortion should be qualitatively correct. While the distortion energies reported in Table II might be in error by a factor of 2, this would not alter the conformational preferences and trends that we have calculated.

The previously suggested[4] effect of changes in preferred geometry for the flavins upon oxidation and reduction can now be extended to the pyridine–dihydropyridine[13] and hydroquinone–quinone systems. In addition we can propose yet another way in which these conformational properties may regulate enzymatic reactions. Consider a nonaromatic cofactor bound to the enzyme in a geometry that is distorted from planarity. Oxidation of the cofactor (or reduction in the case of quinones) to the aromatic form could generate substantial strain, and this could have two possible effects, both of which could influence the selectivity and the turnover rate of the enzyme: (1) It might result in release of the aromatic cofactor from the enzyme and thereby allow binding of another nonaromatic cofactor molecule to the enzyme. This would permit a new substrate molecule to undergo reaction. (2) Relaxation of the distorted aromatic cofactor to its preferred planar geometry could induce a conformational change in the enzyme. This in turn could influence binding of substrate at the active site and result in substrate turnover.

The maximum nonplanar distortion of 20° was arbitrarily chosen, although it compares favorably with folding angles of 9–36° for reduced flavins.[5] This geometry is illustrated in Figure 1 for dihydropyrazine (**2b**). While the overall distortion is quite small, the vertical displacement of the hydrogens to one side of the molecule is 0.81 Å from the horizontal plane defined by the other side of the structure. The actual cofactors have substituents on the central ring, and the corresponding linear displacements of various atoms would be much larger. Steric and other intermolecular interactions resulting from such distortions could easily account for energy changes of the magnitude reported in Table II.

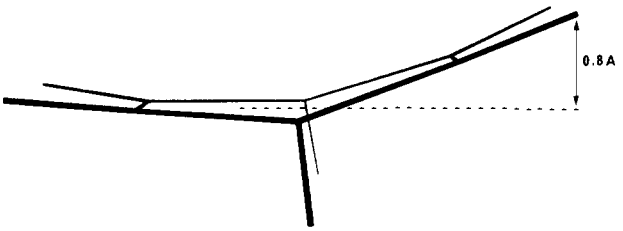

Figure 1. 3-21G optimized structure of dihydropyrazine with a folding angle of 160°.

Still another way in which the conformational behavior of the cofactors may affect biological oxidation–reduction reactions lies in the variation of the oxidation (or reduction) potential with distortion of the cofactor. This need not be restricted to the flavins[4] nor even to the more flexible nonaromatic forms. As long as the inherent binding energy of the enzyme to the (distorted) cofactor is greater than the energy required for distortion of the cofactor from its optimum structure, there will be a favorable net binding energy. Our data show that the distortion energies are particularly small for the nonaromatic forms of the cofactors, so an array of enzyme–cofactor complexes with different distortions and different redox potentials is therefore entirely reasonable. Such behavior may provide part of the basis for high substrate selectivity in enzyme systems.

In summary, we believe that we have identified a pattern of conformational behavior in the cofactors of biological oxidation–reduction reactions which may serve as a common thread in both their catalytic activity and selectivity toward organic substrates. Further work will be required to determine the importance and scope of cofactor conformation with respect to the regulation of enzymatic redox reactions.

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