# Transition Structure for Hydride Transfer to Pyridinium Cation from Methanolate. Modeling of LADH Catalyzed Reaction

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Hydride transfer plays a central role in redox biochemical reactions.[1] The pyridinium cation ($Py^+$)–methanolate ($CH_3O^-$) system represents a simplified, yet realistic, model of electronic aspects of the hydride transfer step in liver alcohol dehydrogenase (LADH)[2] and related enzymes.[3] The study of the hypersurface began with the reactants in a geometric arrangement similar to that found by X-ray crystallography.[4] The transition-state structure (TS) **1** was obtained from a limiting procedure[5] based on constrained energy profiles (CME).[5] In this paper, we report some salient aspects of **1**. For the CME the reactant and product are well defined; they correspond to the di-ionic species $Py^+\cdots CH_3O^-$ and 1,4-dihydropyridine (PyH)···formaldehyde (HCHO) that appear in the proposed mechanism of action in LADH.[2] In order to get an insight into the nature of the reactants and products associated with **1**, descents along the positive and negative directions of the reaction vector (RV) were initiated by using a conjugated gradient method. Points **2** and **3** were reached in relatively flat regions of the global hypersurface (cf. Figure 1). **1** was optimized with gradient methods, and the stationary point was characterized by diagonalizing the force constant matrix in a space of 11 internal geometric parameters,[6,7] using the program MONSTERGAUSS[8] at the 4-31G basis set level.[9]

**Table I.** Energy, Average Gradient Length (mdyn), Geometric Parameters, and Net Atomic Charges for the Stationary Points Depicted in Figure 1[a]

| | **2** | **1** | **3** |
|---|---|---|---|
| total energy (au) | −361.096234 | −361.074626 | −361.156265 |
| rel energy (kcal/mol) | 0.0 | 13.6 | −37.7 |
| gradient length (av) | $1.4 \times 10^{-3}$ | $1.0 \times 10^{-4}$ | $1.6 \times 10^{-3}$ |
| distances (Å) | | | |
| $\rho_{C4-Ht}$ | 2.622 | 1.615 | 1.091 |
| $\rho_{C-Ht}$ | 1.116 | 1.260 | 2.543 |
| $\rho_{C-C4}$ | 3.333 | 2.707 | 3.586 |
| $\rho_{C-O}$ | 1.376 | 1.300 | 1.206 |
| angles (deg) | | | |
| ∠C4HtC | 120.7 | 140.6 | 159.6 |
| ∠OCHt | 113.7 | 110.3 | 113.6 |
| net atomic charges (au) | | | |
| Ht | −0.01 | −0.02 | 0.14 |
| C4 | −0.07 | −0.13 | −0.32 |
| N1 | −0.81 | −0.90 | −0.97 |
| C | 0.00 | 0.06 | 0.18 |
| O | −0.95 | −0.78 | −0.49 |
| ($CH_2O$) | −0.85 | −0.53 | 0.00 |
| charge transfer | 0.14 | 0.45 | 1.00 |

[a] Charge transfer is defined with reference to the pyridynium ring; in the product structure **3** the ring has gained one unit of negative charge.

**Table II.** Gradients (mdyn), Force Constants (mdyn/Å), and Eigenvectors for the Unique Negative Eigenvalue and the Lowest Positive One in the Control Subspace[a]

| | | | | $\epsilon_1 = -1.181$ | $\epsilon_2 = 0.225$ |
|---|---|---|---|---|---|
| | bond angle | grad | *K* | $C_1$ | $C_2$ |
| 1 | $\rho_{C2-C3} + \rho_{C2-C6}$ | 0.0006 | 19.007 | 0.041 | 0 |
| 2 | $\rho_{C-C4}$ | 0.0001 | 0.788 | 0.346 | 0 |
| 3 | $\rho_{O-C}$ | 0.0007 | 7.626 | 0.204 | 0 |
| 4 | $\rho_{H_t-C}$ | 0.0008 | 0.763 | −0.609 | 0 |
| 5 | ∠H–C4–D | 0.0000 | 0.761 | 0.336 | 0 |
| 6 | ∠C–C4–D | 0.0002 | 1.913 | −0.220 | 0 |
| 7 | ∠O–C–C4 | 0.0003 | 1.984 | −0.235 | 0 |
| 8 | ∠H,H(C–O) | 0.0000 | 3.325 | −0.121 | 0 |
| 9 | ∠$H_t$–C–C4 | 0.0004 | 1.475 | 0.369 | 0 |
| 10 | ∠O–C–C4–D | 0.0002 | 0.225 | −0.001 | 0.999 |
| 11 | ∠H,H–(C–O–D) | 0.0001 | 2.032 | 0.314 | 0 |

[a] The method has been described in ref 6 and 7 and the actual procedure is summarized in ref 5. The first variable is the symmetric combination of bond distances $\rho_{C2-C3}$ and $\rho_{C5-C6}$; *D* is a dummy atom located between $C_3$ and $C_5$. Grad is the gradient in mydn, *K* is the force constant, and $C_1$ and $C_2$ are the eigenvectors associated to the eigenvalues $\epsilon_1$ and $\epsilon_2$ of the force constant matrix.

Several features of the saddle point are revealed by the calculations (cf. Table 1 and 2): 1) the syn structure is bent, with a $C_4H_tC$ angle of 140.3°; 2) the forming $C_4$-$H_t$ bond is considerably longer than the breaking C-Ht bond; 3) the ring is slightly bent into a chair form, the nitrogen and $C_4$ are about 5.8° and −8.1° out of the plane f ormed by $C_2$-$C_3$-$C_5$-$C_6$, respectively; as $C_4$ moves towards the alcoholate; the hydrogen bound to $N_1$ becomes pseudo axial as it bends to maximize H-bonding to the alcoholate oxygen (in a N-methylated molecule this effect is likely to be absent); 4) the oxygen atom has transferred ca. 50% of the charge; 5) the charge transfer[10] to the ring is fairly large (ca. 0.5 au), and there is a slight excess of charge on $H_t$; 6) there is a strong coupling between the transferred hydrogen position in the bridge with both the intermolecular distance and orientation (cf. variable 6 in Table 2); 7) all calculated diagonal force constants are positive[11,12] (cf. Table 2); 8) a negative eigenvalue results from the cross-terms in the force constant matrix.

Transition-state structures (TS) for model hydride reactions have been studied by several groups;[13–15] most of them have predicted a linear TS for hydride transfer.[13a–d] The TS relevant

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(1) (a) Klinman, J. P. *Biochemistry* **1976**, *15*, 2018. (b) Limpscomb, W. N. *Annu. Rev. Biochem.* **1983**, *52*, 17.

(2) (a) Branden, C.-I.; Eklund, H. In *Dehydrogenases Requiring Nicotinamide Coenzymes*; Jeffery, J., Ed.; Birkhauser: Basel, 1980; pp 41–84. (b) Eklund, H.; Branden, C.-I. In *Biological Macromolecules and Assemblies. Volume 3. Active Sites of Enzymes*; Jurnak, F. A., McPerson, A., Eds.; Wiley: New York, 1987; pp 74–142. (c) Tapia, O.; Eklund, H.; Branden, C.-I. In *Steric Aspects of Biomolecular Interactions*; Naray-Szabo, G., Simon, K., Eds.; CRC Press: West Palm Beach, 1987; Chapter 8.

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(4) Plapp, B. V.; Eklund, H.; Bränden, C.-I. *J. Mol. Biol.* **1978**, *122*, 23.

(5) The coordinates[4] for the ternary complex benzyl alcohol–$NAD^+$–LADH were used to posit model reactants. As the active site imposes strong constraints on the relative orientation (syn) and distance of the hydride donor–acceptor centers, the reaction was first investigated with a series of energy profiles for hydride transfer by keeping the intermolecular distance, *R*, and syn conformation frozen; *R* was parametrically changed from 3.2 Å down to 2.7 Å, while the orientation was kept fixed; a set of saddle points was obtained that had forces only for the constraints. As $R \rightarrow 2.7$ Å the forces on the constraints tended also to zero and were able to characterize an actual saddle point, **1**, on the global energy hypersurface.

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(9) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724.

(10) (a) Mayer, I. *Chem. Phys. Lett.* **1983**, *97*, 270. (b) Otto, P.; Ladik, J. *Int. J. Quantum Chem.* **1980**, *18*, 1143. These authors have shown that this method gives a qualitatively correct description of the charge-transfer process.

(11) In the initial geometry (distance C–$C_4$ = 3.5 Å) the constrained hypersurface has a saddle point where the C–H bond breaking/forming coordinate has a negative curvature. As we displace downwards along the ridge toward the actual saddle point this curvature is inverted. The VAO5 optimization method[12] with second derivatives is used throughout.

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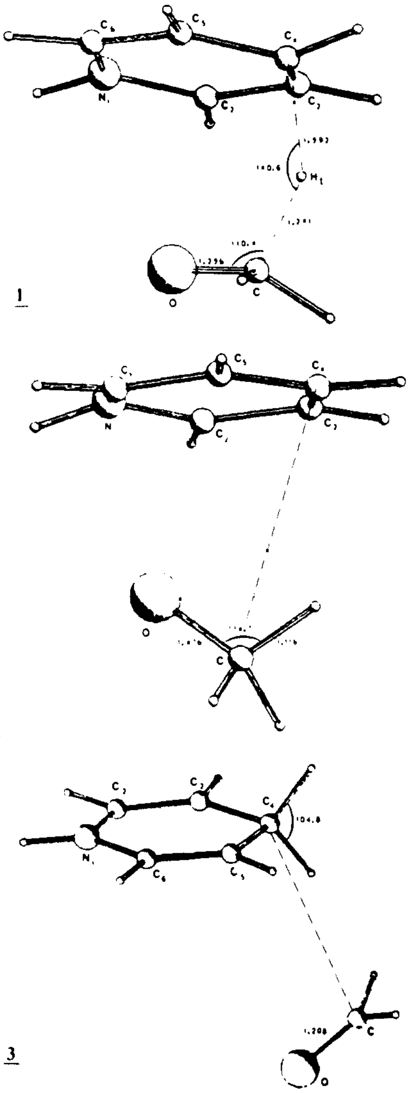

1

2

3

Figure 1. Schematic representation of the TS 1 which is a stationary point of the global energy hypersurface. Descending along the reaction vector with the conjugated gradient method the points 2 and 3 have been reached; 3 represents formic aldehyde interacting with 1,4-dihydropyridine; 2 is located on a flat section of the hypersurface corresponding to the interaction between methanolate and pyridinium cation. However, not all gradients of 2 and 3 are below the threshold of $10^{-3}$ mdyn to qualify as stationary points. Still, they are relevant to the enzyme reaction mechanism as they describe structures therein involved.

to LADH mechanism must be in the syn conformation. Wu and Houk have found a syn TS for systems like $CH_3O^-/HCHO$[13e] and hydride transfer from methylamine and PyH to methyleniminium cation.[15] The latter TS[15] has features resembling **1** and some differences. The differences in structure stem from the ionic nature of the partners in **1**. However, no TS was found with minimum energy profiles for hydride transfer from PyH to HCHO at 3-21G basis set level,[15,16] while we succeeded with a limiting procedure.[5] This situation signals one of the limitations ME profiles may have in finding all relevant stationary points on this particular hypersurface.

Information on the reaction vector for hydride transfer in LADH catalyzed reactions has been obtained experimentally by primary and secondary deuterium isotope effects with NAD-*4-d* and benzaldehyde-*1-d* by Cleland and co-workers.[17] Their results suggest that the motion of the primary hydrogen being transferred as a hydride ion and the $\alpha$-secondary hydrogens is tightly coupled so that motion along the RV is contributing to the observed secondary effect. Huskey and Schowen[18] have carried out model vibrational analysis to explain such anomalies. These authors plotted RV frequencies $\nu^*$ vs the ratio $\lambda$ of the relative amplitude of atoms $H(C_4)$ and $H_t$ (in our nomenclature). The model agreeing with experiments is that one having $\nu^* = 1036i\ cm^{-1}$ and $\lambda = 0.6$.

The eigenvectors corresponding to the negative and smaller positive eigenvalues of the force constant matrix calculated in an 11-parameter subspace are presented in Table II. Using data obtained here, a normal mode calculation[19] without scaling the force constants yield $\nu^* = 1172i\ cm^{-1}$ and $\lambda = 0.7$ in good agreement with the study by Huskey and Schowen. From the RV of **1** it follows that there will be changes in the secondary effect when the primary hydrogen will be changed from H to D, and vice versa, in agreement with experimental information.[17,18,20] Thus, the saddle point **1** has those features required to give an adequate description of kinetic isotope effect observations as they were discussed by Huskey and Schowen.[18]

In the LADH mechanism, the alcohol binds to the catalytic zinc and deprotonates to form an alcoholate bound to Zn. Eklund et al. have studied the ternary complex enzyme–NAD-*p*-bromobenzyl alcohol and proposed a productive structure,[21] while Horjales and Brändén[22] have docked cyclohexanol derivatives into the active site of LADH and determined a productive substrate binding mode. The interesting point is that the coordinates of **1** can be superimposed to those of the productive forms obtained from the experimental and graphics works. The root-mean-square deviation between the coordinates of the productive binding of cyclohexanol[22] and those of **1** for all non-hydrogen atoms but oxygen is 0.1 Å. In the enzyme the C–O axis is nearly parallel to the $C_4$–$C_5$ bond axis, while in our model it is parallel to the $C_4$–N axis to obtain $C_s$ symmetry. Such rotation should not affect the very nature of the RV. If we look at Table II, the amplitude on this variable controlling the syn–anti change (variable 10) in the RV is very small, while the following eigenvector is dominated by this variable.[23] We notice that among the three structures in Figure 1, only the TS fit in the active site of LADH. This result illustrates Pauling's conjecture.[24]

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[16] One of the referees has pointed out that for the reaction starting from the ions $CH_3O^-$ and $Py^+$, a minimum energy profile does not detect a TS because the reaction to form free ions is much too exothermic and methanolate should add to $C_4$ of $Py^+$ without barriers.

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[18] Huskey, W. P.; Schowen, R. L. *J. Am. Chem. Soc.* **1983**, *105*, 5704.

[19] A Normal mode analysis was carried out by using the force constants calculated and supplemented with standard force constants for those degrees of freedom not included in the control space; for a definition of control space see ref 6 and 7.

[20] The chair-like conformation of **1** suggests a secondary isotope effect upon N substitution. This point has been raised by Cleland and co-workers. In a preliminary calculation with an extended active space one gets amplitudes for the $C_4$ and N out-of-plane motion that are of the same order of magnitude between them but they are one order of magnitude smaller than those affecting hydrogen transfer. This result is compatible with experimental evidence on secondary $^{15}N$ isotope effects.[17] In the calculation by Wu and Houk for the dihydropyridine–methyliminium cation reaction, the pyridinyl ring is slightly boat-like.[15] The difference stems in the nature of the partners to Py, but the important point seems to be that the force constant system for ring deformation is smaller in the TS than for reactants and products.

[21] Eklund, H.; Plapp, B. V.; Samama, J.-P.; Brändén, C.-I. *J. Biol. Chem.* **1982**, *257*, 14349.

[22] Horjales, E.; Brändén, C.-I. *J. Biol. Chem.* **1985**, *260*, 15445.

[23] Rotation around coordinate 10 leads to the anti conformation. For the dihydropyridine–methyliminium cation reaction, studied by Wu and Houk,[15] the form syn is 1.4 kcal/mol more stable than the anti structure. In our case, a similar rotation leads to a saddle point which has an energy above the syn of at least 10 kcal/mol (the structure has not yet been fully optimized). This result is understandable since the strong electrostatic interactions in the syn form are lessened as the alcoholate atoms in the anti structure are now at larger distances than in the syn structure.

[24] Pauling, L. *Nature* (*London*) **1948**, *161*, 707. In this seminal paper it was pointed out: "enzymes are molecules that are complementary in structure to the activated complexes of the reaction they catalise".