Hybrid Quantum and Molecular Mechanical (QM/MM) Studies on the Pyruvate to L-Lactate Interconversion in L-Lactate Dehydrogenase

Shoba Ranganathan and Jill E. Gready*

Division of Biochemistry and Molecular Biology, John Curtin School of Medical Research, Australian National University, PO Box 334, Canberra ACT 2601, Australia

Received: March 24, 1997[⊗]

Hybrid quantum mechanical (QM) and molecular mechanical (MM) calculations were undertaken to study the catalytic mechanism for the interconversion of pyruvate to L-lactate by the enzyme L-lactate dehydrogenase, in the presence of the cofactor nicotinamide adenine dinucleotide (NAD). The QM system comprised molecular species or fragments involved in the chemical bond-making and -breaking events: the substrate pyruvate, *trans*-1-methyldihydronicotinamide (a fragment of the cofactor), and His-195. The remainder of the enzyme, cofactor, and water molecules made up the MM system. The QM/MM potential energy surface was calculated as a grid of points for two reaction coordinates representing the transfers of a proton and of a hydride ion. From this surface, two transition states for the two transfers were identified, with the hydride-ion transfer step indicated as being rate-limiting and preceding the proton transfer. The intermediate is deprotonated L-lactate. This result disagrees with our earlier all-QM results for a "supermolecule" system consisting of substrate, cofactor and key active-site residue fragments (Ranganathan, S.; Gready, J. E. *Faraday Trans.* **1994**, *90* 2047), which indicated protonation preceded hydride-ion transfer. Structures, energies, and atomic charges for reactant and product complexes and for the two transition states are reported.

Introduction

L-Lactate dehydrogenase (LDH) performs a central function in anaerobic glycolysis in both higher and lower organisms. It catalyses the reversible interconversion of pyruvate and lactate in the presence of cofactor nicotinamide adenine dinucleotide (NAD), with the transfer of both a proton and hydride ion:

$$CH_3(CO)COO^- + NADH + H^+ \leftrightarrow CH_3CH(OH)COO^- + NAD^+$$

As shown in Figure 1, three possible mechanisms can be proposed: hydride-ion transfer following or preceding protonation, or concerted transfers. On the basis of the structure of the active site, Holbrook and co-workers have suggested a concerted mechanism for the reaction with the transition state (TS) more like pyruvate than lactate.[1] Also, on the basis of the X-ray structure[2] of the ternary complex of dogfish LDH with NADH and the pyruvate-analogue inhibitor oxamate (amino group replacing methyl group) a number of roles have been proposed for key active-site residues (see Figure 2): His-195 as the proton donor, Arg-171 to bind and orient substrate, Arg-191 to activate His-195 and stabilize the TS, Asp-169 to modulate the pK_a of His-195, Val-138 and Ser-163 and Gly-164 with a water molecule, to orient the carboxamide side chain of NAD.

The current hybrid quantum (QM) and molecular (MM) mechanical studies (QM/MM) are the third computational approach we have applied to the LDH mechanism. In initial studies using ab initio QM calculations we found that a proposed reactive intermediate, protonated pyruvate (protonated on carbonyl oxygen), was not a stable species but was in fact a complex of methylhydroxycarbene and carbon dioxide.[3,4] This suggested that if such a species was an intermediate in the enzymic reaction, then the carboxyl group must be stabilized by an interaction in the active site, probably with an arginine side chain as indicated by the X-ray structure. In the next stage a "supermolecule" QM approach was used to study the reaction.[5] Here computations were performed on a complex of substrate, cofactor, and fragments of key active site groups (e.g., His, Arg, and Val (backbone)). This indicated a mechanism of proton transfer to pyruvate preceding hydride-ion transfer. However, extension of this study to include further fragments of interest was unsuccessful as these fragments were so weakly "tethered" that geometry optimization failed. Hence, we have proceeded to the full combined QM/MM approach in this study in order to evaluate the complete effect of the enzyme environment and solvent molecules on the reaction mechanism for interconversion of pyruvate to L-lactate in the active site of LDH.

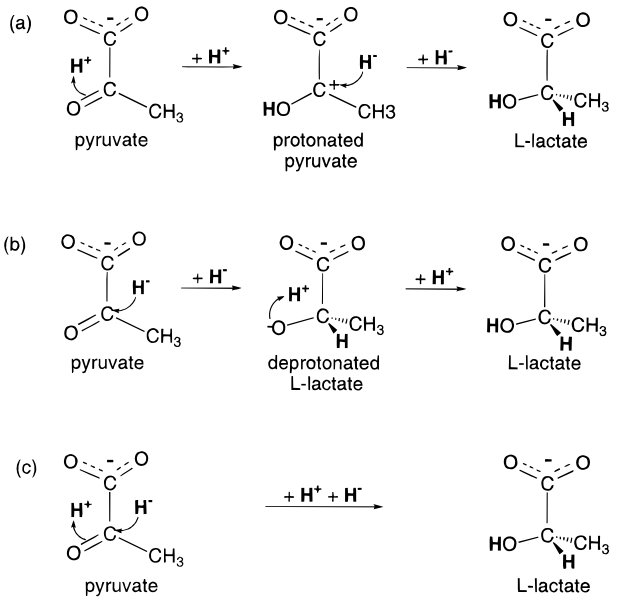

Figure 1. Possible two-step and concerted mechanisms for the L-lactate dehydrogenase reaction. (a) Protonation followed by hydride-ion transfer. (b) Hydride-ion transfer followed by protonation. (c) Concerted transfers of proton and hydride ion.

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1997.

S1089-5647(97)01051-1 CCC: $14.00

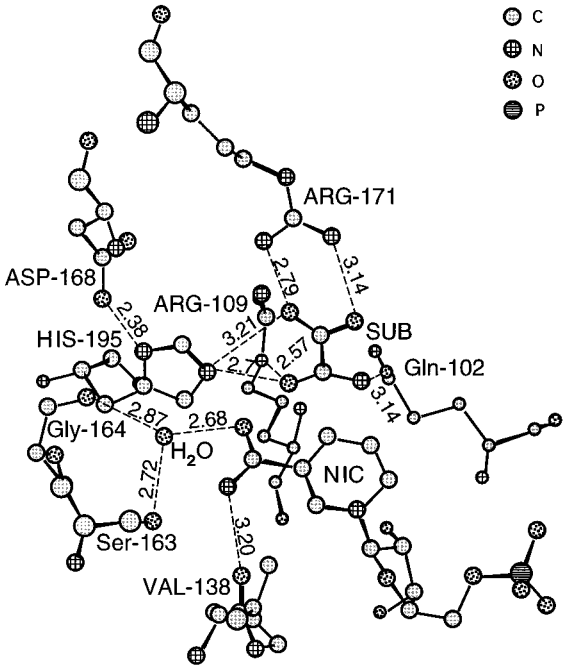

Figure 2. View of the active site of the LDH·NADH·oxamate ternary complex from the X-ray structure 1LDM,[2] showing relative positions of reactants and key residues (see text). Heavy atoms only are shown, i.e., no hydrogens.

The idea of combining QM and MM methods to describe large molecular systems is not new, but its application to systems of real interest has been limited up to now by inadequate computing power. Warshel and Levitt[6] presented the first hybrid study of the enzymic reaction of lysozyme, based on empirical valence-bond and molecular mechanics methods. Singh and Kollman[7] discussed another early implementation and created the program QUEST[7] which is an interface of an ab initio molecular orbital program with the AMBER molecular mechanics/dynamics simulation programs. Recently, Stanton et al.[8,9] have experimented with both semiempirical and density functional QM interfaces to the AMBER simulation programs, while Field et al.[10] have also incorporated semiempirical QM approaches into the CHARMM molecular mechanics/dynamics simulation program. Thompson and co-workers have recently coupled polarizable MM electrostatics with QM in their QM/MM studies.[11,12] Two reviews of QM/MM developments have recently been published.[13,14] Semiempirical QM methods have a 2−3 orders of magnitude speed advantage over ab initio or density functional methods, and consequently only the results of coupled semiempirical/MM potential calculations of enzyme-catalyzed reaction pathways, including transition-state structures, have been reported in the literature.[15−17] A QM/MM free energy perturbation study of proton and hydride transfers in solution has been reported recently[18] for simple substrates mimicking the enzyme reaction of malate dehydrogenase.

The LDH enzyme reaction was studied earlier by Yadav et al.[19] using an empirical valence-bond/molecular dynamics approach and assuming a stepwise reaction, with hydride transfer preceding proton transfer. The work was concerned with investigating one possible contribution to the catalysis, the solvent reorganization energy, and was not a systematic study of the effect of the enzyme environment on the chemical reaction mechanism, i.e., an exploration of the energy surface for the reaction.

The availability of different QM methods and QM/MM potentials raises many important methodological issues, such as treatment of the electrostatics[10,20,21] and choice of van der Waals parameters used to describe the QM/MM interactions. The usefulness of the QM/MM approach will, therefore, depend on careful parametrization of the QM/MM coupled potential. The QM/MM calculations reported in this paper used the general simulation program MOPS,[22] incorporating a number of coupled semiempirical/MM potentials.[20,21] Our approach has been to explore the complete potential energy surface for the enzyme reaction. In contrast to the approach of Yadav and co-workers,[19] our study is without any in-built bias toward a concerted or a stepwise mechanism and uses an explicit particle representation for all atoms of the QM and MM regions.

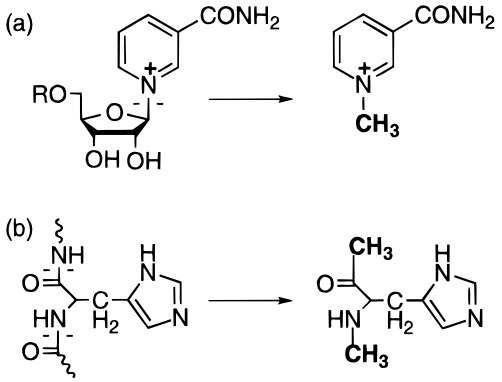

Figure 3. Examples of bond partitioning between QM and MM parts and capping of the QM fragment with a methyl group. Shown for (a) NAD to produce 1-methylnicotinamide ion and (b) His with partitioning at the backbone to produce the capped His residue.

Computational Procedures

Methodology. The system for calculation comprises the entire enzyme, cofactor, substrate, and crystallographic water molecules. This is divided into QM and MM components. The QM component consists of substrate, the reactive part of the cofactor and the enzyme residue His-195 (see Figure 3), i.e., the species involved directly in the chemical reaction. The MM residues are the rest of the system (other enzyme residues, water molecules, and the molecular fragment of cofactor not involved directly in the reaction). When a QM residue is linked covalently to an MM residue, the covalent bond is "capped" by a dummy group. These capping groups appear only in the QM part of the calculation and are not "visible" to the MM atoms. This bond partitioning is a particular problem for macromolecular computations, as otherwise the QM and MM boundary can usually be defined between molecules. Capping can never be ideal as some perturbation is introduced, which will be worse if the division is across a polar bond.[13] In the present work a methyl capping group has been used as it better represents the MM atom across the boundary than the commonly used hydrogen atom.[13] It was also used as the capping group in our earlier QM studies.[5] In this study His has been partitioned at the backbone (see Figure 3).

The total energy of the partitioned system is the sum of the QM, MM, and QM/MM terms,

$$E = E_{\mathrm{QM}} + E_{\mathrm{MM}} + E_{\mathrm{QM/MM}} \qquad (1)$$

E_{QM} is the SCF energy of the quantum system (including capping groups), as polarized by the electrostatic potential produced by the atom-centered charges q_i of the MM system. Thus, the SCF is performed using perturbed Fock matrix elements, in this work at the semiempirical AM1 level:

$$F_{\mu\nu} = F^{\circ}_{\mu\nu} + \sum_i q_i V_{\mu\nu} \qquad (2)$$

where $F^{\circ}_{\mu\nu}$ is the matrix element for the unperturbed (gas phase) system, $V_{\mu\nu}$ is the contribution to the one-electron integrals,[21] and μ, ν are indexes spanning the atomic orbitals. AM1 was chosen as the QM method as it provides a good description of intermolecular interactions.[23] AM1 proton affinities are better[24−26] than those computed by other semiempirical methods such as MNDO and PM3, a factor that is important to the accuracy of the present study as the LDH reaction involves a proton transfer.

E_{MM} is the total energy of the MM part of the system, in this work calculated with the AMBER all-atom force field for enzyme residues,[27] the TIP3P parameters for water molecules,[28] and cofactor residue charges calculated from the AM1 electrostatic potential.[29]

The $E_{QM/MM}$ interaction energy term is the sum of polar (electrostatic) and nonpolar (van der Waals) terms, the latter similar to that in the MM term:

$$E_{QM/MM} = E_{ele} + E_{vdW} \tag{3}$$

In the current treatment the electrostatic term is calculated as the sum of pairwise potential energies in the form

$$E_{ele} = \sum_{ij} q_i V_j(R_i) \tag{4}$$

where $V_j(R_i)$ is the electrostatic potential at the MM atom i produced by atom j of the QM system.[21] Note that this treatment does not allow for polarization of the MM part by the QM part.

The choice of the standard AM1 and AMBER methods provides a general QM/MM methodology which is applicable to any reaction and permits comparison of results. In recent work, Bash et al.[30] reported an extensive reparametrization of the AM1 Hamiltonian to reproduce high-level ab initio QM calculations for some specific solute−solvent interactions. This may improve the accuracy for relevant reactions, including enzyme reactions, but no studies have been reported. We have recently reported a different approach to the parametrization of the QM/MM solute−solvent interactions focusing on the QM−MM interaction terms for the AM1, MNDO, and PM3 methods for a range of neutral organic molecules, including those representative of amino acid side chains.[31] Further work is required on generally applicable QM/MM force fields for use in studying enzymic reactions.

Computational Protocols. A cutoff for nonbonded MM interactions was set at 10.0 Å. A cutoff for the QM/MM interactions was set at 99.0 Å, i.e., the whole enzyme subunit of 329 residues, as the interaction energy leveled off only between 60 and 70 Å. Starting coordinates were taken from the 1.9 Å X-ray structure 1LDM for dogfish LDH ternary complex with NADH and the inhibitor oxamate.[2] After modification of oxamate to pyruvate (NH_2 to CH_3) [and to L-lactate, see below], the whole system was subjected to 100 cycles of MM minimization. There was little change in the structure, including that of the active-site residues. The system was subdivided into QM and MM regions, as described below, and coordinates of all QM and key MM residues were permitted to optimize during minimization. These residues are pyruvate, nicotinamide, His-195, Arg-171, Arg-109, Val-138, Asp-168, and H_2O-351.

In contrast to the earlier supermolecule study,[5] attempts to use the hill-climbing algorithm[32] to locate the geometries of the TS from reactant and product complexes were unsuccessful. Consequently, a grid of energy values was constructed for two reaction coordinates corresponding to proton and hydride-ion transfers. Depending on the position on the surface, each point required 60−600 cycles of optimization to converge to 0.001 kcal/mol. To conserve the stereospecificity of the enzyme reaction, the reaction was followed from the product state, i.e., L-lactate, His-195, and NAD^+ to the reactant state, i.e., pyruvate, protonated His-195, and NADH.

TABLE 1: AM1 Heats of Formation ΔH_f Calculated for the QM System at Points on the Minimum Reaction Pathway (See Figure 4a) Together with Structural Parameters[a]

species	ΔH_f	$r1$ (Å)	$r2$ (Å)	θ (deg)	ϕ (deg)	$r3$ (Å)	$r4$ (Å)
R	−500.5	2.30	1.01	128	199	2.30	1.13
TSH	−481.1	2.10	1.01	135	192	1.30	1.28
DPL	−487.4	1.90	1.03	142	198	1.10	2.13
TSP	−485.9	1.40	1.21	156	196	1.10	2.17
P	−513.0	1.00	2.15	141	195	1.10	2.23

[a] R, Reactants; TSH, TS for hydride-ion transfer; DPL, intermediate state (deprotonated L-lactate); TSP, TS for proton transfer; and P, products. The reaction coordinates are $r1$ and $r2$, and $r3$ and $r4$ for proton and hydride-ion transfers, respectively, and the two angle coordinates are θ and ϕ: see Figure 4.

Calculations were performed on the system with the following partitioning: QM residues: pyruvate, dihydronicotinamide, His-195 (see Figure 3). MM residues: LDH less His-195, NADH less dihydronicotinamide, crystallographic water molecules. All calculations were performed with the locally developed MOPS program[22] running on IBM RS6000 and SGI PowerIndigo2 workstations.

Results

Potential Energy Surface for the Enzymic Reaction. Results for the QM/MM calculations are shown in Figure 4 and Table 1. The QM system and definition of the reaction coordinates, $r1$ and $r3$ for proton and hydride-ion transfers, respectively, and the two angle coordinates θ and ϕ are shown in Figure 4. Molecular definitions of the reactants (R), the TS for hydride-ion transfer (TSH), intermediate state (deprotonated lactate DPL), the TS for proton transfer (TSP), and products (P) are also shown in Figure 4a. Heats of formation for R, TSH, DPL, TSP, and P on the reaction pathway are shown in Table 1, while the position of the pathway on the potential energy grid surface is shown in Figure 4. Finally, the variation of the distance and angle variables along the pathway is shown in Table 1. The θ values indicate that the TS for proton transfer is not linear. The carboxamide side chain remains close to the *trans* conformation in which $\phi = 180°$.

Enzyme Reaction Mechanism. The main feature of the mechanism indicated by the energetics and structural parameters is that hydride-ion transfer precedes proton transfer, with deprotonated lactate (i.e., ionized hydroxyl group)[3] as the reaction intermediate. This result contrasts with the usual chemical and enzymic arguments for hydride-ion transfer reactions which propose preprotonation and is also in disagreement with our results from the supermolecule calculations.[5]

Energetics of the Reaction. Hydride-ion transfer is still the rate-limiting step, in agreement with the supermolecule results. The barrier is only ∼15 kcal/mol in the forward direction. The barrier for the second TS for proton transfer is minimal (2.5 kcal/mol above the intermediate). The energies of the reactant and product complexes differ by 12.5 kcal/mol which agrees broadly with the results from the supermolecule calculations (∼17 and ∼4 kcal/mol for TS2 and TS3, respectively) and is much lower than that for isolated products and reactants (∼40 kcal/mol).[5] This result is consistent with the observed reversibility of the enzymic reaction. We note that the potential energy (PE) surface shown in Figure 4a is "lumpy". This arises from an apparent dependence of the grid-point energies on the order (and, hence, starting structure) in which points were calculated.

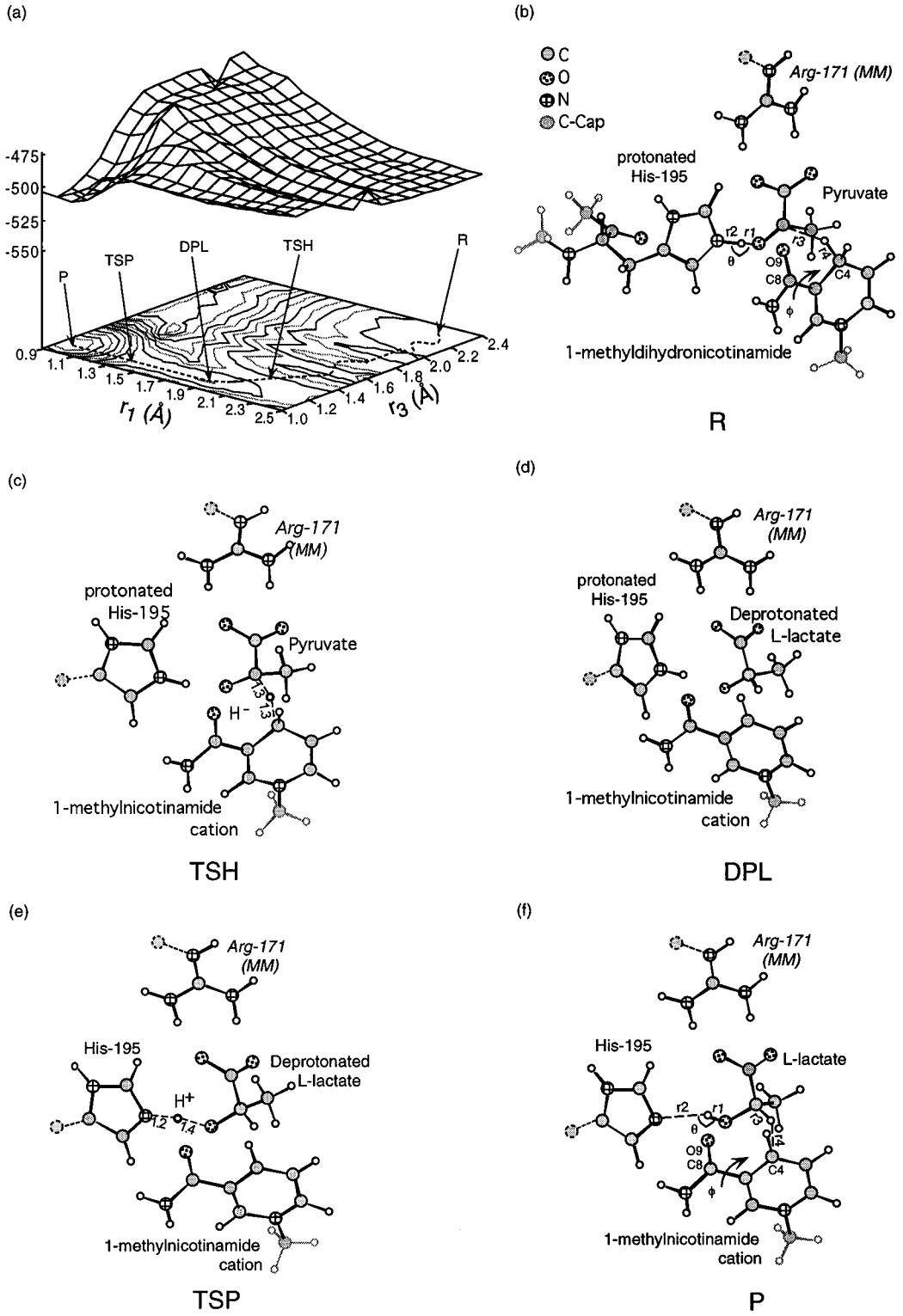

Figure 4. (a) Potential energy grid surface (in kcal/mol) calculated from the QM points, showing the minimum pathway (see also Table 1): R, reactants; TSH, TS for hydride-ion transfer; DPL, intermediate state (deprotonated lactate); TSP, TS for proton transfer; and P, products. (b−f) Definitions of the QM systems for critical points on the minimum reaction pathway of (a). The structures have been drawn using the QM/M optimized coordinates for each critical point and show the subtle reorientations of the component fragments and groups during the reaction. Capping groups are shown as shaded methyl groups. The reaction coordinates, *r*1 and *r*2, and *r*3 and *r*4 for proton and hydride-ion transfers, respectively, and the two angle coordinates θ and ϕ are shown. (b) R, the reactants pyruvate, *trans*-1-methyldihydronicotinamide and protonated His-195, together with one of the MM residues, Arg-171; (c) TSH, the TS for hydride-ion transfer with *r*3 and *r*4 distances, His-195 is protonated; (d) DPL, the intermediate state consisting of deprotonated L-lactate, *trans*-1-methylnicotinamide, and protonated His-195; (e) TSP, the TS for proton transfer with *r*1 and *r*2 distances; (f) P, the products L-lactate, *trans*-1-methylnicotinamide ion and His-195.

Charge Transfer during the Reaction. The AM1 charges for the molecular species of the QM system at the stationary points and the transition states were computed from a Löwdin population analysis[33] (see Table 2). The "hydride ion" is almost uncharged in the TS for hydride-ion transfer, with a charge of +0.14, comparable with the values obtained from the earlier supermolecule calculations (0.00 and 0.01 for TS2 and TS3, respectively).[5] The proton carries a charge of +0.42 in the TS for proton transfer, indicating extensive charge delocalization in the TS.

TABLE 2: Total Net AM1 Charges for Substrate (SUB), Hydride Ion (H^-), Proton (H^+), Cofactor Analogue (NIC), and His-195 (HIS) Species at the Transition States for Hydride-Ion and Proton-Transfer Steps (TSH and TSP), the Reactant and Product Complexes (R and P), and the Intermediate State, Deprotonated L-Lactate (DPL)[a]

species	SUB	H^-	NIC	H^+	HIS	C4	C8	O9	μ
R	−0.99[a]		0.00[b]		0.99[c]	−0.09	0.37	−0.45	2.48
TSH	−1.08[a]	0.14	−0.05[d]		0.98[c]	−0.09	0.37	−0.44	2.45
DPL	−1.48[e]		0.53[d]		0.95[c]	−0.03	0.36	−0.37	2.22
TSP	−1.57[e]		0.84[d]	0.42	0.31[f]	0.05	0.35	−0.33	2.06
P	−0.96[g]		0.95[d]		0.01[f]	0.06	0.34	−0.31	1.95[†]

[a] Partial charges for atoms C4, C8 and O9 (see Figure 4) of the cofactor analogue and the carbonyl bond dipole (μ in Debye) are also given. Molecular species for charge calculation correspond to: [a]Pyruvate; [b]1-methyldihydronicotinamide; [c]Protonated His-195, with 2 capping methyl groups; [d]1-methylnicotinamide ion; [e]Deprotonated L-lactate; [f]His-195, with two capping methyl groups; [g]L-lactate.

La Reau and Andersen[34] have suggested that the stereospecificity of the LDH reaction arises from enhanced polarization of the carbonyl group of the cofactor by charged active-site residues and reduction of electron density at C4 of the nicotinamide ring (see Figure 4b), by the dipole of the α2F helix, leading to stabilization of the TS. Our calculations indicate that there is no charge depletion at C4, compared with the values for isolated 1-methyldihydronicotinamide (−0.05) and 1-methylnicotinamide ion (0.04),[5] similarly to our earlier results.[5] The C8−O9 carbonyl bond polarization has been estimated in terms of the bond dipole moment.[35] The μ value for the reactant complex (2.48 D) is greater than that of 2.26 D for 1-methyldihydronicotinamide,[5] indicating that there is increased carbonyl bond polarization in the active site. From the TS for hydride-ion transfer to the product complex, the μ values decrease gradually from 2.45 to 1.95 D, compared with the value of 1.97 D for 1-methylnicotinamide ion.[5] This trend in the μ values, decreasing from an initial high value, is consistent with the suggestion of La Reau and Andersen[34] and provides a mechanism for the activation of the cofactor in the reactant state.

Discussion

The main conclusion from the QM/MM study is that incorporation of the effects of the complete enzyme environment has a profound effect on the apparent course of the LDH reaction course, compared with the all-QM results from the supermolecule model. Although the rate-limiting step is still the hydride-ion transfer step, this transfer is predicted to occur first by the QM/MM calculations and not following the proton transfer as predicted by the supermolecule calculations. This QM/MM result is at variance with usual solution-chemical mechanisms for hydride-ion reductions, which argue for activation of the hydride-ion acceptor by prior protonation. However, the NADH cofactor is not an indiscriminate reductant, as for inorganic hydrides, but a selective one evolved to react when specifically bound in enzyme-active sites. For reactions in such unusual chemical environments, reaction might be promoted by activation of the cofactor itself, increasing its tendency to donate a hydride ion. The current QM/MM results support this interesting mechanism. We are undertaking further work aimed at testing the stability of the result with respect to the size of the QM system, improving sampling of the critical points on the PE surface, and developing methods for calculating dynamic and entropic contributions[36] to the reaction energetics as a means of establishing how the enzyme environment effects this unusual chemistry.

Acknowledgment. We thank Dr Peter Cummins for advice on use of the MOPS program and the ANU Computing Equipment Committee for a grant to purchase PowerIndigo workstations.

References and Notes

(1) Clarke, A. R.; Atkinson, T.; Holbrook, J. J. *Trends Biochem. Sci.* **1989**, *14*, 101, 145.
(2) White, J. L.; Hackert, M. L.; Buehner, M.; Adams, M. J.; Ford, G. C.; Lentz, P. J. J.; Smiley, I. E.; Steindel, S. J.; Rossmann, M. G. *J. Mol. Biol.* **1976**, *102*, 759.
(3) Norris, K. E.; Gready, J. E. *J. Mol. Struct. (THEOCHEM)* **1993**, *279*, 99.
(4) Norris, K. E.; Bacskay, G. B.; Gready, J. E. *J. Comput. Chem.* **1993**, *14*, 699.
(5) Ranganathan, S.; Gready, J. E. *Faraday Trans.* **1994**, *90*, 2047.
(6) Warshel, A.; Levitt, M. *J. Mol. Biol.* **1976**, *103*, 227.
(7) Singh, U. C.; Kollman, P. A. *J. Comput. Chem.* **1986**, *7*, 718.
(8) Stanton, R. V.; Hartsough, D. S.; Merz, K. M. *J. Phys. Chem.* **1993**, *97*, 11868.
(9) Stanton, R. V.; Little, L. R.; Merz, K. M. *J. Phys. Chem.* **1995**, *99*, 17344.
(10) Field, M. J.; Bash, P. A.; Karplus, M. *J. Comput. Chem.* **1990**, *11*, 700.
(11) Thompson, M. A.; Schenter, G. K. *J. Phys. Chem.* **1995**, *99*, 6374.
(12) Thompson, M. A. *J. Phys. Chem.* **1996**, *100*, 14492.
(13) Gao, J. *Rev. Comput. Chem.* **1996**, *7*, 119.
(14) Bakowies, D.; Thiel, W. *J. Phys. Chem.* **1996**, *100*, 10580.
(15) Bash, P. A.; Field, M. J.; Davenport, R. C.; Petsko, G. A.; Ringe, D.; Karplus, M. *Biochemistry* **1991**, *30*, 5826.
(16) Vasilyev, V. V. *J. Mol. Struct. (THEOCHEM)* **1994**, *304*, 129.
(17) Lyne, P. D.; Mulholland, A. J.; Richards, W. G. *J. Am. Chem. Soc.* **1995**, *117*, 11345.
(18) Ho, L. L.; Mackerell, A. D.; Bash, P. A. *J. Phys. Chem.* **1996**, *100*, 4466.
(19) Yadav, A.; Jackson, R. M.; Holbrook, J. J.; Warshel, A. *J. Am. Chem. Soc.* **1991**, *113*, 4800.
(20) Vasilyev, V. V.; Bliznyuk, A. A.; Voityuk, A. A. *Int. J. Quantum Chem.* **1992**, *44*, 897.
(21) Cummins, P. L.; Gready, J. E. *Chem. Phys. Lett.* **1994**, *225*, 11.
(22) Cummins, P. L.; Gready, J. E. *J. Comput. Chem.* **1994**, *15*, 704.
(23) Stewart, J. J. P. *J. Comput. Aided Mol. Des.* **1990**, *4*, 1.
(24) Dewar, M. J. S.; Dieter, K. M. *J. Am. Chem. Soc.* **1986**, *108*, 8075.
(25) Ozment, J. L.; Schmiedekamp, A. M. *Int. J. Quantum Chem.* **1992**, *43*, 783.
(26) Bliznyuk, A. A.; Schaefer, H. F.; Amster, J. *J. Am. Chem. Soc.* **1993**, *115*, 5149.
(27) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. T. *J. Comput. Chem.* **1986**, *7*, 230.
(28) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D. *J. Chem. Phys.* **1983**, *79*, 926.
(29) SPARTAN 4.0, Wavefunction Inc., 18401 Von Karman, Suite 370, Irvine, CA 92715.
(30) Bash, P. A.; Ho, L. L.; Mackerell, A. D.; Levine, D.; Hallstrom, P. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3698.
(31) Cummins, P. L.; Gready, J. E. *J. Comput. Chem.*, in press.
(32) Dewar, M. J. S.; Healy, E. F.; Stewart, J. J. P. *J. Chem. Soc., Faraday Trans. 2* **1984**, *80*, 227.
(33) Natiello, M. A.; Medrano, J. A. *Chem. Phys. Lett.* **1984**, *105*, 180.
(34) La Reau, R. D.; Andersen, V. E. *Biochemistry* **1992**, *31*, 4174.
(35) Burkert, U.; Allinger, N. L. In *Molecular Mechanics*; American Chemical Society: Washington DC, 1982; p 196.
(36) Cummins, P. L.; Gready, J. E., unpublished work.