Transition-state structural refinement with GRACE and CHARMM: realistic modelling of lactate dehydrogenase using a combined quantum/classical method

Vicente Moliner,† Alexander J. Turner and Ian H. Williams*

School of Chemistry, University of Bath, Bath, UK BA2 7AY

A transition structure for reduction of pyruvate catalysed by lactate dehydrogenase is refined and characterised in hybrid quantum mechanical/molecular mechanical (AM1/CHARMM) calculations involving a fully flexible active-site region by means of a novel procedure (GRACE), and is remarkably similar to that calculated *in vacuo*.

The key to understanding of the fundamental processes of enzyme catalysis, or to designing transition-state analogues (as potential drugs) or modified enzymes (with new specificities), is knowledge of transition-state structure and properties. Computational simulation of enzymic reactions[1] provides a valuable tool for investigation of these issues. We report the results of a combined classical/quantum modelling study of a transition structure (TS) for reduction of pyruvate catalysed by lactate dehydrogenase (LDH). A novel computational procedure is employed, which allows the TS to be located with full gradient relaxation of the positions of 1900 atoms of a solvated enzyme–substrate complex utilising a hessian involving displacements of 147 atoms in the active site. The resulting TS shows remarkable structural similarity to that determined in earlier molecular orbital (MO) calculations[2–4] for the reaction *in vacuo*.

LDH is an NAD-dependent enzyme catalysing the interconversion of pyruvate and lactate. The X-ray crystal structure of its inhibitor-bound ternary complex has been determined,[5] and its mechanism has been the subject of many experimental[6] and several theoretical studies.[2–4,7–9] Although the reaction rate of the wild-type enzyme with pyruvate at room temperature is actually limited by a conformational transition, the chemical step involves hydride transfer (HT) from the nicotinamide ring of NADH to the carbonyl C atom of the substrate. The Empirical Valence-Bond theoretical study by Warshel[7] presupposed that HT to pyruvate occurs first, followed by protonation on the carbonyl O atom of the substrate from His-195. However, all previous MO studies,[2–4,8,9] performed using a variety of *in vacuo* models, concur that proton transfer (PT) precedes HT. These contradictory conclusions beg the question: what influence does the protein environment have upon the reaction mechanism?

We have employed a hybrid theoretical technique in which a quantum mechanical (QM) description of the chemical bond making and breaking events is combined with a molecular mechanics (MM) treatment of the surrounding protein and solvent. The first phase of this work used the CHARMM24 program[10] as described previously for reactions in aqueous solution[11] and in a solvated enzyme active site.[12] Computational limitations made it impractical to include the entire LDH protein in the calculations, so we selected a pseudo-sphere of 1900 atoms of enzyme, co-factor, substrate and solvent centred around the substrate, hydride donor and proton donor groups. This system was divided into a QM region of 39 atoms (the shaded portion of Fig. 1 comprising pyruvate, the nicotinamide ring of NADH and part of His-195), treated by the AM1 semiempirical MO method,[13] and a MM region, comprising the rest of the protein (CHARMM24 potentials) and the solvent water (TIP3P).[14] Two link atoms were inserted where the QM/MM boundary intersected covalent bonds (indicated as ● in Fig. 1). The QM/MM total energy surface shown in Fig. 2 was

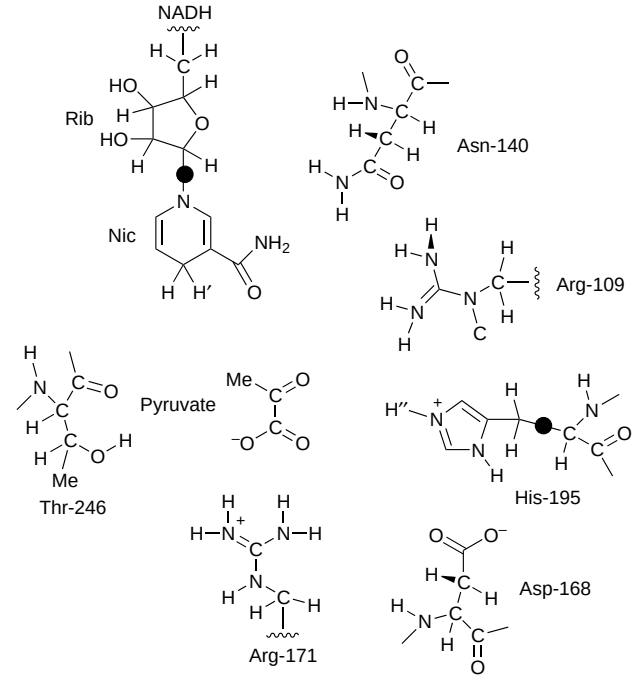

Fig. 1 LDH active site. The shaded region corresponds to the QM atoms; the two link atoms are indicated as ●.

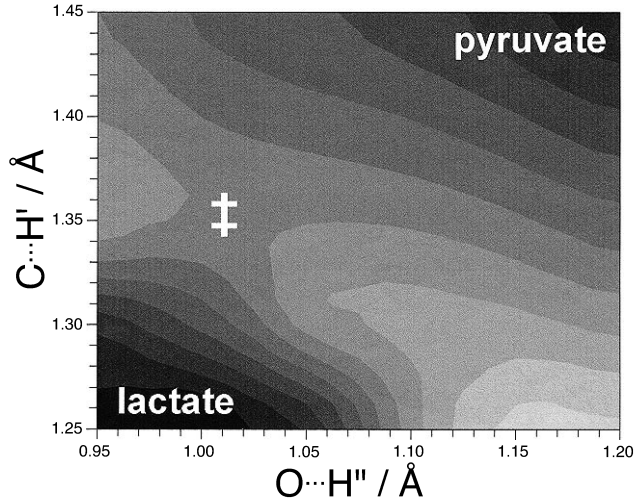

Fig. 2 QM/MM total energy contour map (dark = low energy; light = high energy) for LDH-catalysed pyruvate reduction by NADH obtained as a function of hydride transfer and proton transfer coordinates by means of grid searches using CHARMM

constructed from a grid formed by pairs of rigid internal-constraint values for the making bond distances C··H′ and O··H″, from the carbonyl C atom of pyruvate to the H′ atom of the nicotinamide ring (hydride donor), and from the carbonyl O atom of pyruvate to the H″ atom of His-195 (proton donor), respectively. At each grid point relaxation of the entire hybrid QM/MM potential was performed to the constrained local minimum. At the surface of the pseudo-sphere, a solvent boundary potential was employed to prevent evaporation of water molecules, and harmonic restraints were applied to protein atoms disconnected from the backbone, owing to truncation, in order to preserve the enzyme structure. All non-bonded interactions were included.

The approximate TS, corresponding to the saddle point on Fig. 2, was then refined, using our newly developed GRACE‡ software. A partial-rational-function-operator/adopted-basis-Newton-Raphson method was employed, utilising a hessian matrix of order 441 × 441, describing the curvature of the QM/MM energy hypersurface for a sub-set of the system (larger than that shown in Fig. 1), together with a diagonal hessian plus updates for the rest of the system. A detail of the refined TS is shown in Fig. 3. The r.m.s. residual gradient on the 147 atoms in the sub-set is less than 0.1 kcal mol^{-1} $Å^{-1}$ in the optimized structure, while on the remaining 1753 atoms it is less than 0.005 kcal mol^{-1} $Å^{-1}$; these residual gradients are lower than the commonly accepted convergence criterion for optimized geometries of small molecules in quantum chemistry.[15] Finally, the intrinsic reaction coordinate[15] path was determined from the TS in each direction, leading to pyruvate and to lactate, in order to demonstrate conclusively that the reported structure is indeed a TS for the correct reaction. It is this capability to refine and characterise TSs precisely within a large flexible domain of the reacting system which distinguishes the present work from other recently published QM/MM applications to enzymic reactions.[16]

The transition vector ($\nu^{\ddagger}$ = 568*i* cm^{-1}) for the TS of the LDH-catalysed reaction involves motion of both the transferring hydride H′ and proton H″, indicating a concerted mechanism. However, PT is much further advanced (Pauling bond order $n_{O\cdots H''}$ = 0.81) than HT ($n_{C\cdots H'}$ = 0.33). This is remarkably similar to the TS found in a gas-phase AM1 supermolecule calculation for a *N*-methyl-1,4-dihydronicotinamide–pyruvate–(methylguanidinium)–methylimidazolium complex.[3] The PT and HT components occur in perpendicular planes, the degree of HT is essentially the same (*cf.* 0.31),[3] but PT is slightly earlier (*cf.* 0.93 for specific acid catalysis).[3] The TS for this reaction appears to be a robust entity, the essential features of whose structure are invariant to the nature of its environment.

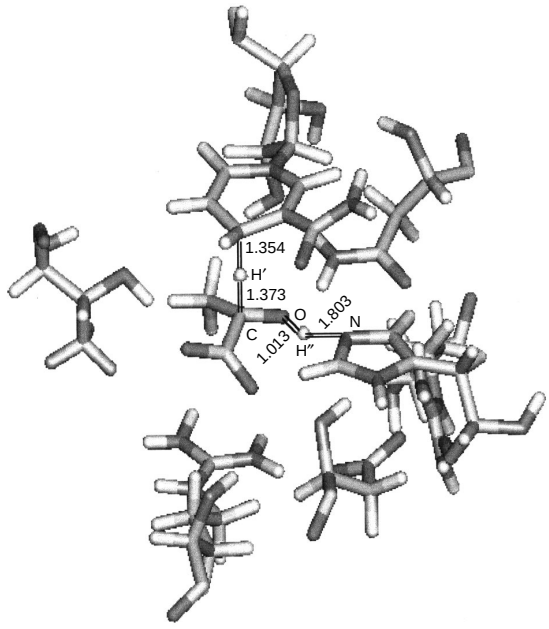

Fig. 3 QM/MM transition structure refined using GRACE for LDH-catalysed pyruvate reduction by NADH (distances in Å)

Finally, several active site residues, notably Arg-109 and Asn-140, display considerable mobility over the range of structures spanned by the energy surface in Fig. 2. We have evidence that the TS reported here is only one of a family of structures of similar energy which differ in regard to conformations of amino acid sidechains; the transition state for the enzymic reaction represents an average of the properties of these many, nearly degenerate TSs. This insight emerges only as a consequence of the flexible model of the active site employed in this study.

We are grateful to the Leverhulme Trust for a Visiting Fellowship (V. M.), the BBSRC for a Studentship (A. J. T.), SmithKline Beecham for an equipment donation, and Glaxo-Wellcome and the EPSRC for financial support. We thank Dr Tim Dafforn for supplying us with initial coordinates for the LDH–NADH–pyruvate ternary complex.

Footnotes

* E-mail: i.h.williams@bath.ac.uk
† On leave from the Departament de Ciencies Experimentals, Universitat Jaume I, Castelló, Spain.
‡ GRACE is a powerful extension to Tool Control Language (TCL), which uses free format data structures and cache technology to interface QM packages (like CADPAC, GAMESS or GAUSSIAN) with CHARMM (or CHARMm) or with its own routines for investigation of QM/MM energy surfaces. The structured programming advantages of TCL are augmented by many novel features including a range of powerful optimisers for minima and saddle points, IRC paths and vibrational frequencies.

References

1 A. J. Mulholland, G. H. Grant and W. G. Richards, *Protein Eng.*, 1993, **6**, 133; A. J. Mulholland and M. Karplus, *Biochem. Soc. Trans*., 1996, **24**, 254.
2 J. Wilkie and I. H. Williams, *J. Am. Chem. Soc.*, 1992, **114**, 5243.
3 J. Andrés, V. Moliner, J. Krechl and E. Silla, *J. Chem. Soc., Perkin Trans. 2*, 1995, 1551.
4 J. Wilkie and I. H. Williams, *J. Chem. Soc., Perkin. Trans. 2*, 1995, 1559.
5 D. B. Wigley, S. J. Gamblin, S. P. Turkenburg, E. J. Dodson, K. Piontek, H. Muirhead and J. J. Holbrook, *J. Mol. Biol.*, 1992, **223**, 317.
6 A. R. Clarke, T. Atkinson and J. J. Holbrook, *Trends Biochem. Sci.*, 1989, **14**, 101 and 145.
7 A. Yadav, R. M. Jackson, J. J. Holbrook and A. Warshel, *J. Am. Chem. Soc.*, 1991, **113**, 4800.
8 J. Andrés, V. Moliner, J. Krechl and E. Silla, *Bioorg. Chem.*, 1993, **21**, 260; J. Andrés, V. Moliner and V. S. Safont, *J. Chem. Soc., Faraday Trans.*, 1994, **90**, 1703.
9 S. Ranganathan and J. E. Gready, *J. Chem. Soc., Faraday Trans.*, 1994, **90**, 2047.
10 B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.*, 1983, **4**, 187; CHARMM24b2, M. Karplus, Harvard University, 1996.
11 J. A. Barnes and I. H. Williams, *Chem. Commun.*, 1996, 193.
12 J. A. Barnes and I. H. Williams, *Biochem. Soc. Trans*., 1996, **24**, 263.
13 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 390.
14 W. L. Jorgensen, J. Chandrasekhar, J. Madura, R. W. Impey and M. L. Klein, *J. Chem. Phys.*, 1983, **79**, 926.
15 J. B. Foresman and Æ. Frisch, *Exploring Chemistry with Electronic Structure Methods*, Gaussian, Inc., Pittsburgh, PA, 1993.
16 *E.g.* M. J. Harrison, N. A. Burton, I. H. Hillier and I. R. Gould, *Chem. Commun.*, 1996, 2769; M. Peräkylä and P. A. Kollman, *J. Am. Chem. Soc.*, 1997, **119**, 1189.

Received in Exeter, UK, 16th April 1997; Com. 7/02600E