Regular article

Quantum mechanical/molecular mechanical methods and the study of kinetic isotope effects: modelling the covalent junction region and application to the enzyme xylose isomerase

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Abstract. Hybrid quantum mechanical and molecular mechanical (QM/MM) methods are used to estimate the kinetic isotope effects (KIEs) for the 1,2 hydride shift reaction of xylose isomerase. Semiclassical transition-state theory with multidimensional tunnelling corrections are evaluated with both semiempirical and ab initio density functional hybrid methods for the study of enzyme catalysis. As part of the study, a link atom and a modified localised self-consistent-field method are assessed to represent the QM and MM covalent interface region. We have shown for some model systems that although the bond orbital method can be adjusted to perform better than the link atom method by using auxiliary density basis functions or by charge scaling, in general the link atom method performs as well. Several QM/MM models are used to calculate the xylose isomerase potential-energy surface and the importance of tunnelling for the KIEs beyond the Wigner correction is demonstrated.

Key words: Xylose isomerase – Quantum mechanical/molecular mechanical methods – Localised self-consistent field – Enzyme catalysis – Kinetic isotope effect

1 Introduction

Xylose isomerase (EC 5.3.1.5) is a much-studied enzyme which catalyses the interconversion of the isomeric forms of a range of sugars [1]. Found in a number of microorganisms, xylose isomerase is particularly important industrially in the production of high-fructose corn syrup, which can be used as a sweetener in preference to sucrose. In this study we have investigated the mechanism of the reversible conversion of the aldose D-xylose isomer (I) into the ketose D-xylulose isomer (II) using hybrid quantum mechanical and molecular mechanical (QM/MM) computational techniques.

The mechanism of xylose isomerase has been previously investigated both experimentally [2–7] and computationally [8–11] and the rate-limiting step is generally agreed to involve a 1,2 hydride shift. There is a great deal of discussion at present regarding enzymatic hydrogen-shift mechanisms and the interpretation of the associated experimental kinetic isotope effects (KIEs), in particular the role of quantum mechanical tunnelling. In this article we discuss the use of QM/MM methods to study the KIEs of the xylose isomerase hydride shift step.

Hybrid QM/MM methods [12–14] are becoming particularly useful for the study of enzymatic mechanisms since they allow the important aspects of reactivity involving electronic rearrangements to be studied quantum mechanically, whilst the remainder of the enzyme system is approximated at a lower theoretical level, here using molecular mechanics. These methods have been reviewed recently [15].

In this study we discuss an ab initio QM/MM implementation which utilises the Hartree–Fock (HF) and density functional theory methods, which should be more reliable than the more widely used semiempirical methods. One of the major problems faced in the development of new techniques for the study of condensed-phase systems such as enzymes is to gauge their applicability and accuracy. High-level ab initio methods have now been shown to be extremely reliable for gas-phase studies of small molecules and it is now possible to favourably compare computationally determined kinetic parameters, such as KIEs or rate constants, with those from experiment [16, 17]. Here, we extend these

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techniques, involving semiclassical variational transition-state theory (VTST), towards the study of the kinetics of enzyme catalysis using QM/MM methods.

In these QM/MM methods the molecular system will be divided into two distinct regions, one of which will be treated quantum mechanically, the other will be treated classically (MM). The MM region is allowed to interact with the QM region as a perturbation to the QM Hamiltonian with the electrostatic and steric effects readily introduced through the interaction with a standard MM force field. In this work we include the electrostatic potential of the partial charges as a one-electron perturbation and the other effects, van der Waals, bond, angle and dihedral interactions between QM and MM regions, are introduced additively [18–20] using the AMBER force field [21].

An important aspect in the development of accurate techniques for the study of condensed-phase reactivity is this interaction between the QM and MM regions. For many enzyme systems it is inappropriate to separate the system into a QM and a MM region without partitioning across a covalent bond. There are two main approaches for approximating this junction between the two regions: the link atom method, whereby covalent bonds are approximated by real bonds to hydrogen atoms (Fig. 1a), and (localised) bond orbital methods [22–27], where an approximate localised orbital is added to the quantum region (Fig. 1b). In both of these approaches it is necessary that the valency in the QM region is fully satisfied. The link atom method [14] is probably the most commonly applied scheme although the bond orbital methods may now be more appropriate to improve the accuracy offered by ab initio QM/MM methods. Here, we compare two schemes, a link atom method and the localised self-consistent-field (LSCF) method [22] and discuss how they may be adjusted for increased applicability. The schemes are applied to study the energetics of the xylose isomerase reaction.

2 Representation of the QM/MM interface region

In this section we discuss two approaches for the description of the QM/MM interface when the QM and MM regions are partitioned across a covalent bond. In the discussion to follow we refer to the QM and MM atoms which form part of this covalent bond as the (QM-) bond atom and the (MM-) junction atom, respectively (Fig. 1).

The first and most common approach is to use a link atom, introduced into the QM region to terminate any dangling bonds at the bond atoms. In our link atom scheme we introduce a hydrogen (link) atom, 1.0 Å from the quantum bond atom. The junction atom now forms part of the MM region and should, therefore, have a partial charge associated with it; however, since this charge would be too close to the new QM hydrogen link atom, it is usually set to zero and to maintain the original (neutral or integral anionic/cationic) charge of the overall system, any excess charge is divided equally amongst the MM atoms adjacent to the junction atom. This method has been applied quite successfully to proteins [18–20] by partitioning between Cα and Cβ atoms since the Cx charge is often near zero and, thus, there is minimal perturbation. Furthermore, the size of the quantum region is generally chosen to be large to avoid adverse termination effects.

An alternative scheme to describe the interface region in ab initio QM/MM methods is the LSCF method originally proposed by Assfeld and Rivail [22]. In this method the dangling bonds are retained in the QM calculation as localised bond orbitals (LOs). Central to this scheme is the reorthogonalisation of the atomic orbital basis to the localised orbitals by way of a transformation matrix, B. This ensures that the final molecular orbitals from the QM calculation are orthogonal to the junction bonds and that the overall valency of the system is maintained.

Following the nomenclature of Assfeld and Rivail, the SCF procedure of an ab initio HF or Kohn–Sham (KS) density functional program can be modified through an initial transformation of the Fock (or KS matrix).

\[ \mathbf{F}' = \mathbf{B}^\top \cdot \mathbf{F}^{AO} \cdot \mathbf{B}, \]

where \( \mathbf{B} = \mathbf{M}^+ \cdot \mathbf{X} \) and

\[ \mathbf{M}_{\mu \nu} = \left( 1 - \sum_i^L S^2_{\mu i} \right)^{-\frac{1}{2}} \delta_{\mu \nu} - \sum_i^L \sum_{\eta} a_{\mu i} \alpha_{\eta i} S_{\eta \nu}. \]

The coefficients, \( a_{\mu i} \), are those of the \( L \) orthogonalised localised orbitals and \( S \) is the usual overlap matrix over \( N \) basis functions.

There are a number of issues associated with the use of the LSCF method. The main issue is that the localised orbital can be associated with two electrons, and thus an additional negative charge is effectively included in the quantum calculation for each LO present. To balance this electron from the junction atom, a charge (\( q_{\text{link}} \)) of 1.0e is added to the original junction atom partial charge. We have found that the value of this charge can become quite problematic, especially for more ionic systems, and we propose a modified scheme later to improve the performance. We have also found that convergence of the SCF procedure required a redefinition

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1 We note that we use a transformation matrix \( \mathbf{B} = \mathbf{M}^+ \cdot \mathbf{X} \) here and not \( \mathbf{B} = \mathbf{M} \cdot \mathbf{X} \) as stated in Ref. [22].