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Combined quantum and molecular mechanics calculations on metalloproteins

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Summary

The combination of quantum mechanics and molecular mechanics (QM/MM) methods is one of the most promising approaches to study the structure, function, and properties of proteins. The number of QM/MM applications on metalloproteins is steadily increasing, especially studies with density functional methods on redox-active metal centres. Recent developments include new parameterised methods to treat covalent bonds between the quantum and classical systems, methods to obtain free energy from QM/MM results, and the combination of quantum chemistry and protein crystallography.

Abbreviations

MM – molecular mechanics

QM – quantum mechanics

QM/MM – combined quantum mechanics and molecular mechanics

Keywords

QM/MM, free energy perturbations, crystallographic refinement, quantum refinement, metalloproteins, reaction mechanisms, protein strain.

Teaser

Recent developments and applications of QM/MM methods on metalloproteins are reviewed, showing that QM/MM is one of the most promising approaches to study the structure, function, and properties of proteins.

Running head: QM/MM calculations on metalloproteins

Introduction

During the last decade, quantum chemical methods have become an important complement to experiments for the study of structure and function of metalloproteins, mainly owing to the increase in computer power and the introduction of accurate density functional methods [1,2]. However, an entire protein is still too large to study by such methods. Therefore, quantum chemical studies have traditionally isolated the interesting part of the protein (e.g. the active site), including only a restricted number of atoms (30–200) in the calculations, whereas the surrounding protein has been ignored or is represented as a homogenous continuum solvent [1,2].

Naturally, such a treatment is not fully satisfactorily, even if it often works surprisingly well [1-3]. A natural improvement is to include the surrounding protein by molecular mechanics, adding the quantum chemical and molecular mechanics energies and forces. This gives the combined quantum mechanics and molecular mechanics methods (QM/MM), pioneered by Warshel, Levitt, and Kollman [4,5]. Many variants of QM/MM methods and programs have been proposed and QM/MM codes are now available in many quantum chemistry and molecular dynamics software [6-11]. This has led to a steadily increasing number of applications of QM/MM methods to proteins, even if there still are almost as many QM/MM method development articles.

Here, I review recent (January 2000 to September 2002) QM/MM applications on proteins with metal ions in the quantum system. QM/MM applications on other proteins have recently been reviewed [6-12]. Model complexes have been excluded, but they are the subjects of a recent review [13]. Likewise, I avoid discussing technical aspects and method developments, unless they are of major interest for metalloprotein applications. I use a strict definition of QM/MM methods; for example, applications of the empirical valence bond method [14,15] are excluded.

Reaction mechanisms

QM/MM methods are often used to study the reaction mechanism of an enzyme, optimising all reactants and transition structures along one or several suggested reaction pathways. The philosophy behind such a treatment is to test if the mechanism is energetically feasible, i.e. if the highest activation energy is in accordance with observed reaction rates [1,2]. Other properties, such as structures, spectra, or isotope effects, are often also studied.

Several groups have studied metalloenzymes in this way. Most of them have dealt with redox-inactive metals, e.g. mandelate racemase (Mg^{2}) [16], cAMP kinase (2 Mg^{2+}) [17], phospholipase A_2 (Ca^{2+}) [18], thermolysin (a Zn^{2+}-containing peptidase) [19], and leucine aminopeptidase (2 Zn^{2+}) [20]. All these studies have been performed with semiempirical methods and they typically give too high activation energies (90–160 kJ/mole), even when recalculated with density functional methods. Only a purely density functional study of stromelysin (another Zn^{2+} peptidase) gave a more reasonable barrier (55 kJ/mole) [21].

Fewer studies have been directed towards proteins containing transition metals with an unfilled *d* shell. Rothlisberger et al. have studied the reaction mechanism of galactose oxidase and compared it with a model complex [22]. This enzyme catalyses the two-electron oxidation of primary alcohols to the corresponding aldehydes, coupled with the reduction of dioxygen to hydrogen peroxide. The rate limiting step is a hydrogen-atom abstraction from the deprotonated alcohol to a tyrosyl radical, which is a ligand of the catalytic copper ion. This residue is covalently attached to a cysteine residue by a thioether link at the *ortho* position. The results indicate that the enzyme obtains a lower activation energy (67 kJ/mole) than the model (88 kJ/mole) by delocalisation of the unpaired spin density over the modified tyrosine ligand. However, this conclusion is

opposed by another QM/MM study, in which the activation energy changes by only 4 kJ/mole when the modification of the tyrosine ligand (modelled by a SH substituent) is removed [23].

Recently, Friesner et al. (personal communication) have studied the first step of the hydroxylation of camphor by cytochrome P450, i.e. the abstraction of a hydrogen atom from the substrate by compound I , a Fe^{IV}=O – haem radical complex. They find that the protein enhances this reaction by over ten orders of magnitude compared to vacuum. The major source of this enhancement is improved electrostatic interactions with the surrounding protein in the transition state, in particular more favourable hydrogen bonds between the propionate side-chains of the haem group and arginine and histidine residues.

Finally, five articles have been devoted to the calculation of hydrogen tunnelling and kinetic isotope effects in alcohol dehydrogenase (hydride transfer in a Zn^{2+} enzyme) [24-27], xylose isomerase (hydride transfer in a di-Mg^{2+} enzyme) [28], and lipoxygenase (hydrogen-atom transfer in a Fe^{3+} enzyme) [26] using variational transition-state theory.

Structures and properties

One important application of QM/MM methods is to predict protein structures, e.g. the effect of mutants on the chlorophyll special pair in the photosynthetic reactions centre [29], the structure of mutants and metal substitutions in blue copper proteins (electron carriers) [30], or the structure and distortion of the porphyrin substrate in ferrochelatase (the enzyme that inserts the iron ion into haem, probably by deforming the porphyrin ring) [31].

Other investigations have studied how much the protein distorts the structure of the

active site, both in geometry and energy terms. Blue copper proteins have been suggested to distort the copper geometry and a QM/MM study of amicyanin and rusticyanin estimates the strain energy (i.e. the QM energy difference of the active site optimised in vacuum and with QM/MM methods) to 74–82 kJ/mole [32]. However, another study gives a much smaller effect, 25 kJ/mole [33], which still is said to be an overestimate [34]. On the other hand, the strain energies for four models of methane monooxygenase and ribonucleotide reductase are 50–200 kJ/mole [35] and similar energies have been observed in several other QM/MM calculations (6–200 kJ/mole) [33,36-38]. Thus, strain is a problematic concept, which depends strongly on the size and charge of the system, the presence of polar groups, what interactions are included, and the reference state [34]. Studies of haemerythrin, cytochrome P450, and blue copper proteins indicate that the functional effect of strain is small [33,39].

Several other properties have been studied, e.g. the binding free energy of O_2 to haemerythrin [39], vibration frequencies and hydrogen bond-strengths in myoglobin [40,41], reorganisation energies of blue copper proteins and iron–sulphur clusters [33, 36], and the relative stability of the Zn–OH and Zn–OH_2 forms in metallo-β-lactamase [42]. Two groups have studied the electronic structure of various intermediates of cytochrome P450, which is extremely sensitive to the surroundings and the theoretical treatment [43,44]. Finally, QM/MM methods have also been used to identify Cu^{2+} binding sites in the prion protein [45] and to improve the results of molecular dynamics simulations of metallothioneins with Zn^{2+} and Cd^{2+} ions [46].

Free energies from QM/MM calculations

The previous studies have obtained only pure (internal) energies or have estimated free energies from a harmonic vibrational analysis of the quantum system [1-3,21,23,

39]. Combinations of QM methods and free energy techniques have recently begun to be developed [11,47,48], but only a few studies of metalloenzymes combine QM/MM techniques and methods to obtain free energies. This can be predicted to be an important area of developments in the future.

Yang et al. have studied two consecutive reactions catalysed by enolase (2 Mg^{2+}) [49]. They calculate QM/MM structures along a postulated reaction coordinate and perform free energy perturbations between these structures, keeping the whole quantum system fixed. Four studies of alcohol dehydrogenase (Zn^{2+}) [24,25,27] and acetohydroxy acid isomeroreductase (2 Mg^{2+}) [50] use umbrella sampling along a simple reaction coordinate to obtain the potential of mean force for the reaction, keeping only this coordinate fixed. Thus, they do not use the QM/MM structures in the free energy calculations.

We have tested alternative methods to get free energies from QM/MM calculations for the enzyme catechol O-methyltransferase (Mg^{2+}), viz. Langevin-dipole models or Poison– Boltzmann solvation methods on the QM/MM geometries, combined with entropy obtained from a vibrational analysis of the whole enzyme (T Rasmussen, K Nilsson & U Ryde, unpublished data), i.e. methods similar to the MM/PBSA method [51]. The results indicate that stable and accurate results can be obtained in a relatively short time compared to free energy perturbations.

Effect of the protein environment

Is a QM/MM treatment of a protein really necessary? Of course, this depends on the protein and properties of interest. Siegbahn et al. has argued that it is possible to discriminate between various reaction mechanism of metalloenzymes by performing QM calculations on small models of the active site, once a few key residues are

included [1,2]. Such a small environmental effect has been demonstrated in myoglobin: The difference in hydrogen-bond energy between O_2 or CO and the distal histidine residue is 21, 24, and 21–22 kJ/mole when calculated in vacuum without or with a few constraints to mimic steric effects of the protein, and with QM/MM methods, respectively [3,41]. Likewise, no important effects of the protein were found for the reaction energies of galactose oxidase [22].

However, for other systems, the protein environment has an appreciable effect. For example, it reduces inner-sphere reorganisation energies of iron–sulphur clusters and blue copper proteins by a factor of two or more [33,36] and reduces the activation energy by 20 kJ/mole in phospholipase A_2 [18].

An important goal of QM/MM calculations is to identify the mechanisms and interactions by which enzymes accomplish their function. This has been done in several studies. For example, Friesner and coworkers show that haemerythrin stabilises bound O_2 by 57 kJ/mole, compared to aqueous solution, using van der Waals interactions in a pre-formed cavity and electrostatic stabilisation by a second-sphere glutamate residue [39]. Similarly, Yang et al. have studied two consecutive reactions catalysed by enolase and show that the first step is favoured by the two Mg^{2+} ions in the active site, whereas the second step is disfavoured by these ions, but this is more than compensated by other residues in the protein [49].

Method development

A well-know problem of QM/MM methods is how to treat covalent bonds between the QM and MM systems. The QM system can either be terminated by a dummy atom or by a special localised orbital [52]. Many variants have been suggested. Both methods have their shortcomings [52] and a direct comparison of the two methods indicated that

the simple hydrogen link-atom method gave the best results [28]. Recently, Friesner and coworkers have developed a strongly parameterised localised frozen-orbital approach [43,53]. They have performed a detailed analysis of the accuracy of the junctions and the effect of increasing the quantum system [54]. Interestingly, out of 35 recent QM/MM investigations on metalloenzymes, only five [19,25,39,43,49] do not use hydrogen link atoms.

Electrostatic interactions can be treated in several different ways in QM/MM calculations [10]. Although methods to include polarisation of both the QM and MM systems self-consistently have been presented [55] and the first polarisable force fields for biological systems have appeared [56], most QM/MM implementations only include electrostatic interactions in the QM calculations. Moreover, some QM/MM applications still treat electrostatics only in the MM calculations [21,23,30,32], thus excluding effects of hydrogen bonds and solvation. Interestingly, such an approach [30] actually gave improved geometries of blue copper proteins, indicating problems in the point-charge model.

The selection of the QM system is of course crucial. For metal-containing systems, an important rule is that all metal ligands should be included in the QM system; otherwise huge errors may arise, e.g. ~70 kJ/mole in the activation barriers for xylose isomerase [28] and cAMP kinase [17].

As to the level of the QM calculations, more than half of the applications employ density functional methods (typically B3LYP), whereas almost all the others use semiempirical methods (AM1 or PM3). Most of the latter calculations are calibrated to or use single point density functional calculations to improve the accuracy. Therefore, the recent development of a semiempirical density functional method, with an improved performance over standard semiempirical methods, is of interest [57]. An alternative to

QM/MM calculations could be to use frozen or constrained density functional [58] or linear-scaling methods [9] for parts of the protein.

Many QM/MM projects start by reoptimising a crystal structure to get structures and energies that are comparable with QM results. Unfortunately, there is no guarantee that the reoptimised structure stays close to the crystal structure; inaccuracies in the MM force field may distort the structure if the surrounding protein is allowed to move, as two recent investigations show [38,59]. On the other hand, crystal structures involve significant errors, which together with systematic errors in the theoretical method will lead to nonsense energies if the crystal structure is used directly.

A natural solution to this dilemma is to include the crystallographic raw data (the structure factors) in the QM/MM calculations by replacing the MM potential with a crystallographic refinement penalty function. We have implemented such an approach [38] and obtain good results in terms of the crystallographic R_{free} factor and electron-density maps. For example, the method brings a low-resolution structure closer to an atomic-resolution structure of the same protein, as can be seen in Figure 1 [60]. Thus, the structure is *improved* locally.

This method can also be used to interpret crystal structures (what oxidation states and atoms are present). For example, the protonation states of metal ligands can be determined by comparing which of the possible candidates fit the raw data best, as is shown in Figure 2 [60]. The method has been applied for superoxide dismutase, compound II in myoglobin, and hydrogenase [60, K Nilsson, L Rulisek & U Ryde, unpublished results].

Conclusions

QM/MM is a valuable method to study the structure and function of metalloproteins,

including the effect of the surroundings in an unbiased way. The applications range from studies of structures and spectroscopic or functional properties to investigations of the full reaction mechanisms of enzymes. A clear trend is seen towards the study of redox-active metal centres using density functional methods and the combination of QM/MM methods with free-energy techniques.

It interesting to note that all except two of the QM/MM applications on metalloproteins have been done with one of the developers as a coauthor. This shows that QM/MM methods are still not widely used. It will be interesting to see if the latest developments of general-purpose QM/MM methods, e.g. ONIOM [35] and QSite [53, 54], may change this.

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- It is shown that quantum refinement can be used to deduce the protonation state of metal-bound solvent molecules from crystal structures combined with QM calculations. The method is calibrated on alcohol dehydrogenase and applied on

iron superoxide dismutase and ferrochelatase.

Figure 1. The low- (magenta) and high-resolution (orange) crystal structure of haem cytochrome c_{553} compared to the ComQum-X structure and the electron density ($2f_o - f_c$ omit map at the 2.5 σ level) from the high-resolution data [60]. Note the differences in the position of the iron ion, the histidine ring, and the ethyl side chain on the left.

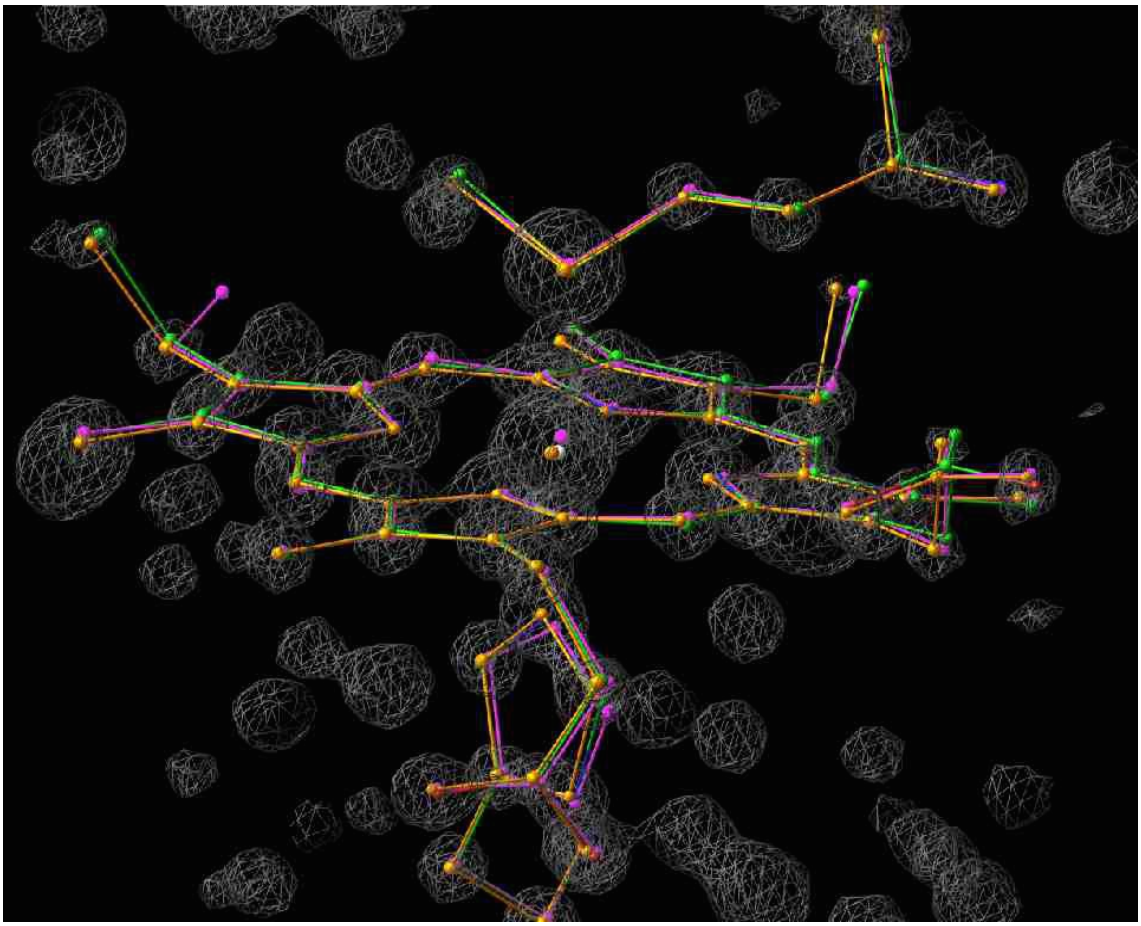

Figure 2. The structure of the catalytic zinc ion in alcohol dehydrogenase in complex with NAD^+ and trifluoroethanol (1axe, 2.0 Å resolution). Structures with a protonated (magenta) and a deprotonated alcohol are compared, together with the corresponding electron density $f_o - f_c$ difference maps at the ±2.8 σ level (white and green for the protonated structure, blue and red for the deprotonated structure) [60]. The picture shows the zinc ion (cross), the alcohol (left), the histidine ligand (bottom), and the two cysteine ligands (right). The improvement is seen in the smaller red than green volumes in the centre and the smaller blue than white volume on the right. The alkoxide also gives a lower R_{free} factor and strain energies, and better Zn–ligand distances than the protonated alcohol [60]. Kinetic data confirm that the alcohol should be deprotonated at the pH (8.4) at which the crystals were grown [60].