

**QUANTUM AND QUANTUM-CLASSICAL STUDIES OF
PHOSPHORYL-TRANSFER MECHANISMS IN MODEL SYSTEMS**

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Phosphoryl-transfer processes are involved in many aspects of gene regulation, metabolism and signal transduction. A number of enzyme families which take part in such processes, like kinases, phosphatases or GTPases, catalyze nucleophilic substitution of a hydroxyl group by the gamma phosphate of ATP or GTP. Typically, this substitution is correlated with an in-line phosphoryl-transfer mechanism, through a pentacoordinate transition state, with an inversion of configuration at phosphorus, (see e.g. [1]). To ensure an optimal stereochemistry and facilitate the transfer process, negative charges at oxygen atoms of the phosphate are neutralized by Mg^{2+} ions and Lys/Arg residues.

When studying mechanisms of the phosphoryl-transfer reactions, using quantum-mechanical methods, one requires detailed knowledge of the protonation states of all molecular fragments/groups which take part in the reactions. The total number of protons and their proper assignments in the substrate, transition-state and product domains are of key importance for computing the energy and structural changes during the phosphoryl-transfer reaction. Depending on the number of protons, a free phosphate group has a total charge of -1 or -2, and the energy barrier for phosphate transfer strongly depends on this charge.

One should also note that the phosphorylated serine, threonine or tyrosine influence long-range conformational changes in proteins. In particular, the "closed" and "open" conformational states of PKA are correlated with the degree of ionization of phosphorylated threonine 197 [2]. A virtual titration procedure for phosphorylated proteins has been elaborated [3].

The main purpose of this presentation is analysis of the energy profiles for the phosphoryl-transfer reaction in a model phosphodiester system, depending on the ionization state of the phosphate group. This is followed by analysis of the energy profiles in the active site of PKA. The following quantum mechanical methods are applied in our studies:

- Density Functional Theory (DFT) calculations at the B3LYP/6-31D+(d,p) level,
- Approximate Valence Bond (AVB) method, very fast quantum generator of the potential energy function, developed by us during the past several years [4],
- Self-Consistent Charge Density-Functional based Tight-Binding Method (SCC-DFTB) [5].

The B3LYP calculations for the model system in the "vapour" state reveal a high energy for dissociation of the phosphoester, and also a large activation energy for formation of the phosphodiester, suggesting that attaining reliable results in the real molecular environment is a difficult task. The potential energy landscape for these processes can be quite well reproduced with the AVB and SCC-DFTB methods. With the AVB method it is difficult, however, to find a universal set of parameters capable of describing both dissociative and associative reaction mechanisms at different protonation states.

The SCC-DFTB method was applied for the reaction site comprising about 390 atoms, with a view to selection between associative and dissociative mechanisms and accompanying proton transfer processes [6]. These problems, and our current attempts at simulation of phosphoryl-transfer reactions, using our Quantum-Classical Molecular Dynamics (QCMD) method [7], will be discussed in more detail.

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