Conformational Dependence of Enzyme Function

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Proteins are dynamic molecules that must move to function. For proteins that are catalytic (i.e., enzymes) some of these motions regulate their catalytic function. Although plausible models of the allosteric regulation of enzymatic reactions by protein conformation were first proposed 40 years ago to great acclaim [1], the elucidation of the physical basis for this control has taken a steady, decades-long course and is still incomplete. Much has been learned in the intervening decades about the physical basis of allostery, for instance the relationship between conformational heterogeneity and ligand binding [2], and the stereochemical basis of allostery in a prototypical protein [3]. However, answers to a number of questions, such as the quantitative dependence of an enzymatic reaction barrier on structurally characterized protein conformations, have been elusive.

Protein kinases compose an important family of enzymes that site-specifically catalyze the transfer of the terminal phosphoryl group from adenosine triphosphate (ATP) to a protein or peptide substrate. This site-specific catalytic addition of a bulky, charged phosphoryl group, in the form of a phospho-ester, to the side chain of the aminoacids serine, threonine, or tyrosine plays an essential biological function by changing the conformation of the substrate protein and thus allosterically regulating protein function. This regulatory mechanism is widespread in biology, playing both regulatory and signaling roles in all phases of cell growth and regulation. The ability to manipulate this mechanism has become medically useful, the most striking example being the recent cure of many cases of adult mylogenous leukemia. This cure is effected by a single 590 Dalton tyrosine kinase-inhibiting compound, imatinib.

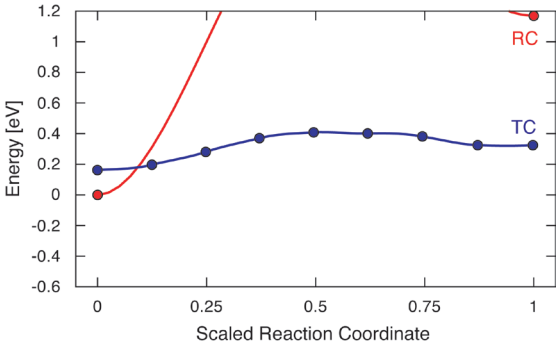

Fig. 1.
PKA phosphoryl transfer energetics depend on protein conformation. (a) The energy along the reaction pathway for 244 atoms involved in the reaction. The reactant conformation (inactive, RC) and the transition conformation (active, TC) are markedly different. If the protein conformation is RC (inactive), there is a large barrier and more than 1eV of energy must be absorbed to reach the product state.

The activity of the enzymatic region of protein kinases is related to the conformation of the protein, and is thus also an example of allostery. The structure of many protein kinases is known, many with a peptide substrate and the product ligand adenosine diphosphate (ADP) bound to the enzyme. While this combination of enzyme, ligand-product and an appropriate substrate often allows the crystal structure of a kinase to be determined, the protein conformation of these kinases is uniformly enzymatically inactive. In the last few years the structure of a transition-state analog for a phophoryl transfer reaction was published, revealing an enzymatically active conformation of the kinase PKA. These structural data allowed us to compute the reaction barrier energy for this phosphoryl transfer reaction in the enzyme [4]. Our technique for computing the energetics along the path of the chemical reaction uses density functional theory to calculate electron densities and system energies at a series of points along the reaction path. The reaction path is identified using a nudged-elastic band method.

Using the inactive conformation of the protein (PKA) and the active conformation, we modeled in atoms

missing from the experimentally determined structures. Primarily the atoms modeled in were those in the missing transferred phosphoryl group, either attached to the ATP (to make the reactants state) or to the substrate serine residue (to make the products state). A few missing protein atoms were also added to complete the picture. Having pictures of the protein with reactants or products bound in two different conformations (one active and one inactive) we were able to compute the energy barrier and change in equilibrium energy during transfer of the phosphoryl group in both conformations of the protein. The details of this density functional calculation reveal much of interest about the coupling between (slow) protein conformational motions and (fast) chemical reaction dynamics.

Calculation of the energetics during phosphoryl transfer is computationally intensive; no more than 250 atoms from the protein are included in the density functional calculation of the chemical reaction. By varying the size of the model system included in the density functional calculation, it becomes eminently clear that if too little of the protein is included in the calculation (less than about 200 atoms) two problems arise. First, the electronic state of the computational model system does not accurately mimic the electronic state of the real system. Second, the active and inactive conformations of the protein merge into a single, physically irrelevant conformation during the geometry optimization steps of the calculation.

Of fundamental interest are the results in larger systems (greater than 200 atoms): the chemical reaction in the inactive conformation requires the absorption of 1.2 eV to proceed (thus the barrier must be at least this large), while the reaction in the active protein conformation is nearly isoenergetic and

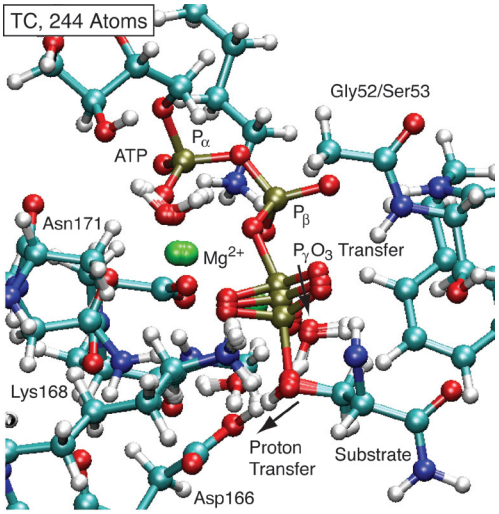

Fig. 2.
A picture of the reaction center in the protein kinase. The gold atom is the phosphorus atom in the transferred phosphoryl group. ATP and the substrate are the reactants, ADP and a phosphorylated substrate the products of the reaction.

the barrier is 0.2 eV. This demonstrates that, at least in some proteins, the conformational, allosteric motions of the protein are a slow modulation of the reaction barrier and that the reaction proceeds not by the vibrational crossing of a comparatively high barrier, but instead by the protein slowly lining up requisite atoms for the chemical reaction to proceed in a semireversible manner. The theoretical underpinnings for this kind of reaction were published by Agmon and Hopfield in 1983 [5].

This work points toward a scheme for analyzing protein catalysis that decouples the explicit protein conformational dynamics responsible for allostery from the chemical reaction, and then screens protein conformations for those that are reactive. Calculations of the barrier between chemical reactants and products can then be done using large-scale, but standard, density functional calculations.

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