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PREDICTION OF THE GTPASE ACTIVITIES BY USING THE SEMIEMPIRICAL MOLECULAR ORBITAL THEORY

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Abstact: Assuming that substrate-assisted catalysis is the mechanism of GTP hydrolysis for *ras* p21 and other GTP-binding proteins,[1] we used the PM3 semiempirical molecular orbital method to predict from the calculated reaction profiles of GTP hydrolysis reactions the changes in GTPase activities caused by mutations. We succeeded in making qualitative predictions for mutants.

Introduction: GTP hydolysis reactions play central roles in the functions of proteins belonging to the GTPase superfamily,[2] and neither why these proteins have low GTPase activities nor why the rate of GTP hydrolysis is increased by their activator proteins has not been clear. GTP hydrolysis by both *ras* p21 and EF-Tu causes inversion of the configuration at the γ–phosphate as a result of a direct attack of a water molecule on the phosphorus atom. A catalytic water molecule was observed to be located at a position 3.4–3.8 Å from the P_γ atom in the crystal structures of the GppNHp complexes of *ras* p21[3] and EF–Tu[4] as well as those of the GTPγS complexes of $G_t\alpha$[5,6] and $G_i\alpha_1$.[7] It has been suggested on the basis of an EVB (Empirical Valence Bond) study[8] and an experimental study[1] that the base which abstracts a proton from the water molecule might be the substrate itself (i.e. the γ–phosphate of GTP). This hypothesis is called the substrate-assisted catalysis, and in the work descrived in this paper we used it to generate GTP hydrolysis reaction models consisting of an amino acid residue, a diphosphate, and a water molecule.

Methods: Semiempirical molecular orbital calculation methods such as MNDO,[9] AM1,[10] and PM3[11] have recently been widely used to investigate the properties of medium-sized and large systems. They have also been applied to the analysis of enzymatic reactions. The PM3 method is the only one thought to be able to reproduce hypervalent molecules,[12] so in the present work we evaluated the GTP hydrolysis reaction by using the semiempirical PM3 method contained in the MOPAC ver.6.0 package.[11,12] Each model in our calculation was composed of an amino acid residue, a diphosphate, and a water molecule (**Figure 1**).

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To investigate the effects of specific amino acid residues on the GTPase reaction, we constructed two kinds of reaction models: the wild-type model containing the wild-type residue, and the mutant model containing a mutant residue.

To search for new mutant structures that have novel activities, we constructed several mutant model structures using natural residues as well as unnatural residues that had not been found in x-ray crystal structure analysis, and we analyzed the GTPase reaction by using their reaction models. The amino acid structure of the wild-type model was derived from the X-ray structure of *ras* p21[3] (PDB code:121P), and the N-terminus and C-terminus of the amino acid structure were protonated. The amino acid structure of the mutant model was constructed from the structure of the wild-type model by substitutiing an atom and using the PM3 method for energy optimization. The structures and relative coordinates of the diphosphate and the reactive water were extracted from the x-ray structure of GTP and the crystal water.

The initial structure of our calculation was subjected to energy miminization by the PM3 method. Considering the mechanism of substrate-assisted catalysis, we classified the GTPase hydrolysis reaction into the following three elementary reactions (**Figure 1**): (a) the reaction generating OH^- from a water molecule (the reaction coordinate (RC) is the bond length of H–O (RC=H–O)), (b) the in-line nucleophilic reaction in which OH^- is added to the γ phosphorus (RC=P–O), and (c) the reaction to disconnect γ phosphate (RC=O–P).

We obtained the reaction profiles from the observation of the change in the heat of formation by the structural optimization of PM3 method for the degrees of freedom without the reaction coordinate.

In this reaction profile calculation, we introduce the minimum constraints of the distance between the amino acid residue and the β-phosphate in order to keep the active site structures. The value of $\Delta\Delta H_f^{\ddagger}$ is defined as the enthalpy difference between the peaks of the wild-type's and mutant's reaction profiles at their rate limitting steps (**Figure 2**). Using the transition state theory, we predicted an activity

index k_{rel}[calculation] based on $\Delta\Delta H_f^{\ddagger}$:

$$k_{rel}[calculation] = exp(\frac{\Delta\Delta H_f^{\ddagger}}{RT}). \quad (1)$$

We also obtained corresponding values of k_{rel}[experiment] from the experimental k_{cat} data:

$$k_{rel}[experiment] = \frac{k_{cat}[mutant]}{k_{cat}[wild\text{-}type]}, \quad (2)$$

where $k_{cat}[mutant]$ is the k_{cat} data for the mutant, and $k_{cat}[wild-type]$ is the k_{cat} data for the wild-type.

Result and Conclusion: The energy minimization of the initial structure results in the catalytic water molecule being stabilized at a position 3.6 Å from the oxygen atom of the γ-phosphate. This position agrees with that determined from x-ray crystal data.

The relationship between k_{rel}[experiment] and k_{rel}[calculation] is shown in **Figure 3**, where each point shows the ratio change of k_{cat} caused by the mutation of an amino acid residue. The line shows the ideal prediction of k_{rel}, so the points near the line are ones that are predicted accurately. The symbols used for each point (**Figure 3**) represent the types of structure used in the reaction models. Open circles and closed circles represent values for which both the wild-type and the mutant crystal structures are known, so the accuracy with which those values are predicted shows the validity of our method using k_{rel}[calculation] to predict k_{rel}[experiment]. Open squares represent values for which the mutant crystal structures are unknown, so the accuracy of their prediction shows the validity of our method of modeling the side-chain structures of amino acid residues. The data around zero are those for which the mutations extinguish the activities, and the data around the intersection of k_{rel}[calculation]=1 and k_{rel}[experiment]=1 are those for which the changes in activity are very small.

Both wild-type and mutant structures for G12V, G12P, and D38E have already been known from x-ray crystal analysis. (The name of each mutant model is a concatenation of the name of the wild-type residue, the residue number and the name of the mutant residue. For example, G12V designates the mutant with valine substituted for the glycine at position 12 of the wild-type protein sequence.) The errors for G12V and D38E are only −4.5% and −3.0%, so the activities for these mutant are predicted quantatively. The activity calculated for Q61E is 4.5 times larger than that obtained experimentally, but the two values are in good agreement in that both are of in the order of 10^1. Similarly, the calculated activity for K16Hec is 4.2 times larger than the activity obtained from the experimental data but both

are of the order of 10^{-3}. We have succeeded in making qualitative predictions for other residues of *ras* p21 and the G_α subunit (**Figure 3**),and we have also used this method to predict activities for Ile36 mutants whose GTPase activities have not been measured previously (**Table 1**). These mutants include the unnatural amino-acid residues shown in **Figure 4**. These results show that the GTPase activities tend to be increased when Ile36 is replaced by residues that have polar groups (–OH) in their side chains, but they are decreased by the replacement Ile36 with residues that have negatively charged groups in their side chains. This method enables us to predict the rate of the GTP hydrolysis in GTP binding proteins, such as *ras* p21, EF-Tu, and the G_α subunit.

Reference and Notes:

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Table 1. Values of k_{rel}[calculation] for Ile36 mutants of *ras* p21.

Amino acid	k_{rel}[*calculation*]	k_{rel}[*experiment*]
Homoserine (Hse)	1.90	
Serine (Ser)	1.53	
α-Amino-β-methyl-aminopropionic acid (Ama)	1.17	1.00
Alanine (Ala)	1.15	1.04
Norvaline (nVal)	1.09	0.98
Methylthreonine (mThr)	1.09	
Threo-α-amino-β,γ-hydroxybutylic acid (Tah)	1.08	
Valine (Val)	1.06	1.02
α-Aminobutylic acid (Aba)	1.06	
β-Hydroxynorvaline (Hnv)	0.963	
Glutamic acid (Glu)	0.537	
β-Methylaspartic acid (Mtd)	0.182	
Aspartic acid (Asp)	0.170	

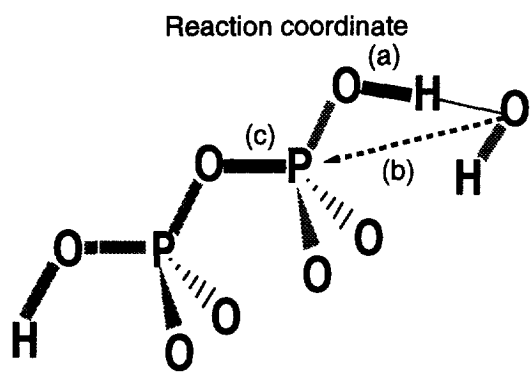

Figure 1. Elementary reactions (a),(b), and (c)

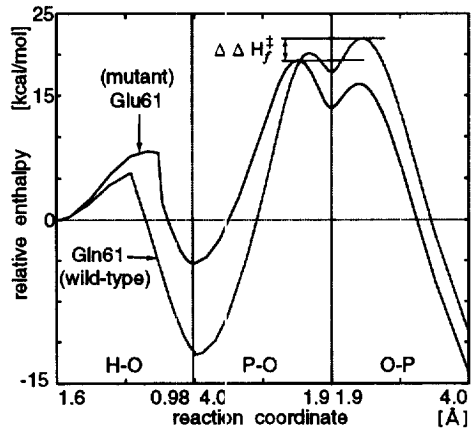

Figure 2. Reaction profiles for Gln61 and Glu61

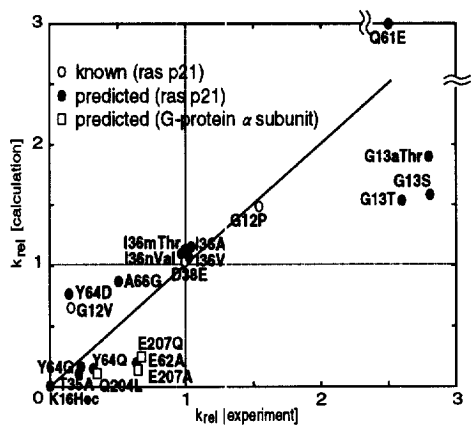

Figure 3. Calculated activity vs. experimental activity)

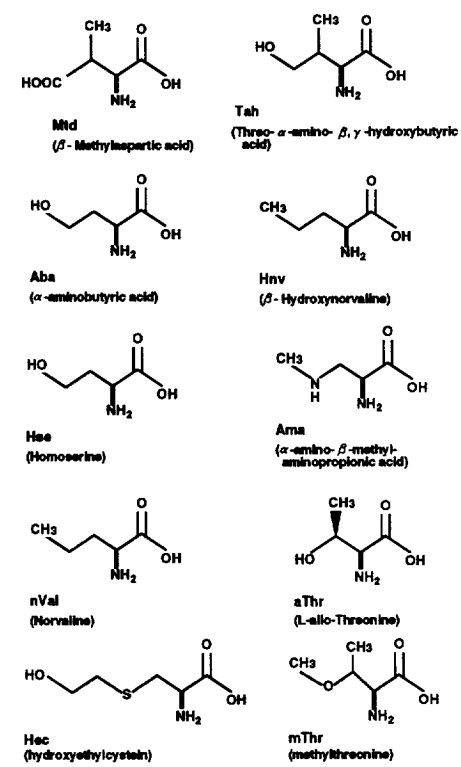

Figure 4. Side-chain structures of unnatural amino acids.