# Atomic-Scale Mechanism of the GTP → GDP Hydrolysis Reaction by the Giα1 Protein

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Quantum chemical calculations were carried out to investigate the hydrolysis reaction of GTP → GDP by Giα1. Giα1 is one kind of G protein and contains a GTPase-activating protein (GAP) inside it. The reason for the increase in GTPase activity in the presence of GAP was examined in detail. Geometry optimization was performed at the Hartree−Fock level using the 3-21G** basis functional set. The energy levels required for all optimized structures of stable and transition states were re-estimated by the density functional theory (B3LYP) method using the 6-31G** basis set. It was revealed that the GTP → GDP hydrolysis reaction proceeds through a proton relay process assisted by a Lys residue. This reaction is a one-step reaction and gives an activation-energy barrier of 35 kcal/mol. A Gln residue, a part of GAP, plays a role in maintaining the position of an $H_2O$ molecule near the $\gamma$-phosphate of GTP, and the $H_2O$ molecule is therefore kept in an appropriate position for initiating the hydrolysis.

## 1. Introduction

G proteins, which consist of α, $\beta$, and $\gamma$ subunits, function as signal transmitters in the living cell machinery.[1] A GTP-bound α subunit is in the active state, and a GDP-bound α-$\beta$-$\gamma$ complex is in the inactive state.[2] A hydrolysis reaction of GTP → GDP plays an important role in controlling the activity of an adenylate cyclase (AC), which produces cAMP as a second messenger of hormones.

G proteins are separated into two groups, Gs and Gi proteins. Gs proteins activate AC in the active state, whereas Gi proteins inactivate AC in the active state. Giα1, one kind of Gi protein, possesses an intrinsic GTPase activating protein (GAP) inside it. GAP was discovered by McCormick et al. in 1987 and was revealed to play an important role in increasing GTPase activity.[3]

Ras protein p21 is a G protein and belongs to the same superfamily as Giα1. Since the variant Ras protein p21 plays a role in carcinogenicity, many experimental and theoretical studies on this protein have been carried out. It has been reported that the structure of the active site of Ras protein p21 combined with GAP is similar to that of the active site of Giα1 possessing intrinsic GAP.[4] It has also been shown that the GTPase activity levels of Ras protein p21 increase greatly in the presence of GAP.[5−8] It would therefore be interesting to analyze the reaction of Giα1 in comparison with that of Ras protein p21, two contrasting G proteins with and without GAP, respectively. It has also been demonstrated that a mutation of Ras protein p21 causes a significant decrease in the level of GTPase activity and that this decrease is closely associated with the development of human endocrine tumors.[9,10] Hence, an elucidation of the hydrolysis mechanism of GTP → GDP and the role of GAP would be very useful in developing effective tumor treatments.

The mechanism of the hydrolysis reaction of GTP → GDP and the role of GAP have still not been elucidated despite the availability of some crystallographic and mutagenic data.[2] Therefore, studying the hydrolysis reaction of GTP → GDP at the atomic level is of great importance. In this study, the reaction process of Giα1 was examined by performing quantum chemical computations, and the function of GAP was examined in detail. The results of this study provide atomic-level insight into the process of information transmission for maintaining homeostasis in a living cell.

## 2. Methods

**2.1. Construction of a Model Reaction System by Molecular Mechanics (MM) Calculations.** To clarify the mechanism of Giα1 in terms of the hydrolysis reaction of GTP → GDP, a quantum chemical molecular orbital (MO) calculation using an appropriate model reaction system must be performed. For this purpose, a molecular mechanics (MM) calculation was first carried out. In the MM calculation, the structure of the ES complex, consisting of Giα1 and $Mg^{2+}$−GTP, was determined as follows. An X-ray crystallographic structure of Giα1[11] (pdb code: 1gia) in the active form (GTP-$\gamma$-S) was placed in a large box filled with TIP3P water molecules generated by the Monte Carlo method.[12] Energy minimization using the molecular mechanics (MM) method was executed under the periodic boundary condition. In the computation of energy minimization, the steepest descent method was used for the first 1000 cycles, and the conjugate gradient method was used for next 1000 cycles. An all-atom force field[13] was applied to every amino acid. The SHAKE method[14] was used to simplify the calculation in which all bond distances in the molecule were maintained at the equilibrium distance. The Amber, version 4.1[15] program package was used.

Because of the limitation of the size of the model for MO calculations, the active site must be extracted from the above energy-minimized structure and represented by a model reaction system. To reproduce the atomic geometry involved in the active reaction site, the triphosphate of GTP, $Mg^{2+}$, three water molecules, Ser44, Lys46, Arg178, Thr181, and Gln204 were extracted from the ES complex obtained by the MM calculation.

**2.2. Molecular Orbital (MO) Calculation.** The Schrödinger equation for the model reaction system was solved at the

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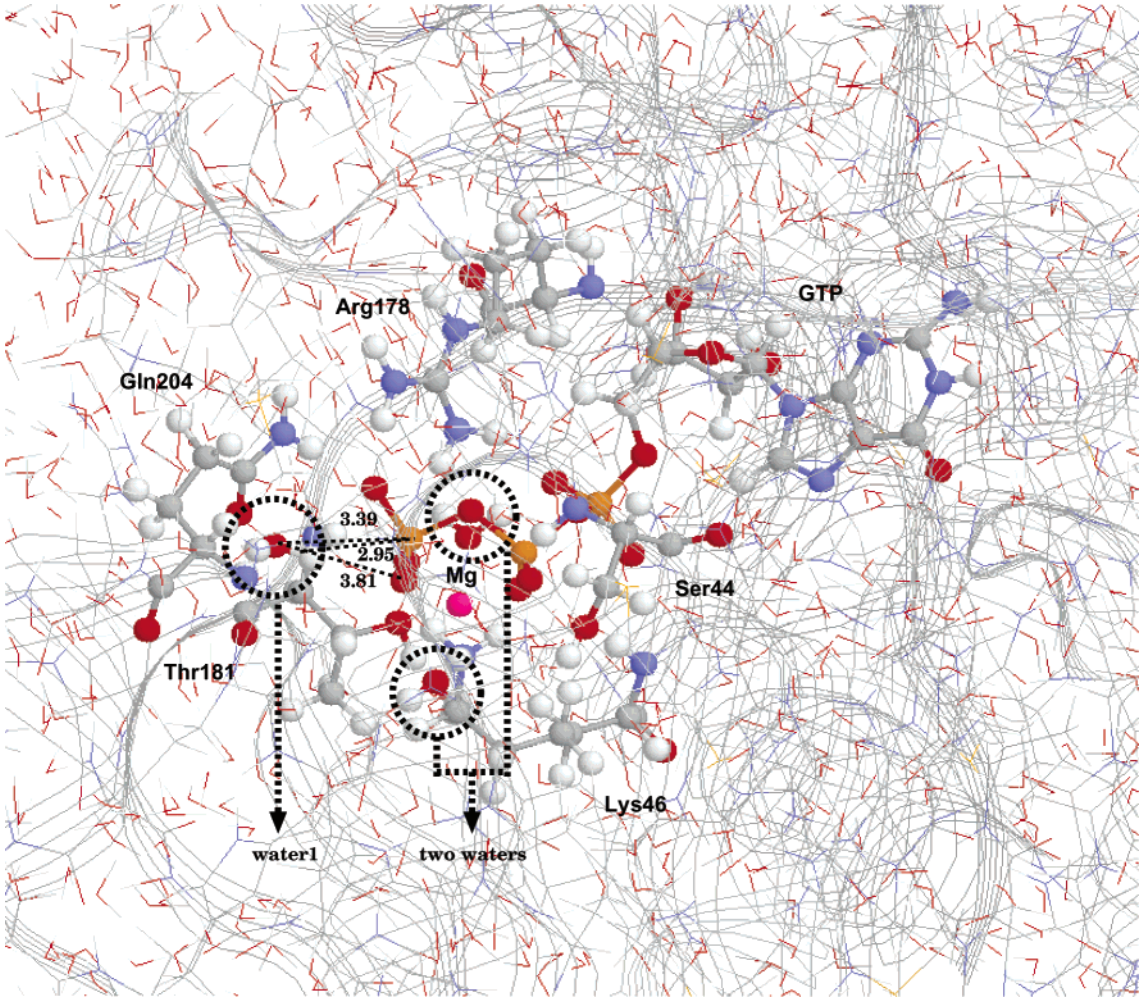


**Figure 1.** Energy-minimized structure of the Giα1 protein incorporating GTP and $Mg^{2+}$, which was obtained by molecular mechanics calculations. Ser44, Lys46, Arg178, Thr181, Gln204, water 1, two water molecules coordinated to $Mg^{2+}$, $Mg^{2+}$, and GTP are emphasized.

Hartree−Fock (HF) level using the 3-21G** basis functional set,. The minimum state and the transition state (TS) on the potential energy hypersurface were obtained by geometry optimization using the energy gradient method. Frequency analysis was carried out for the optimized structure of TS because the vibration of the molecular system in the TS must contain only one imaginary frequency. The steepest descent path from the TS was calculated for both the forward and reverse directions of the normal vibrational mode of the imaginary frequency, and the minimum points on both sides of the TS were determined. The above procedure provided the lowest-energy reaction path connecting a reactant and a product via the TS. Energy levels that were required for all optimized structures for stable and transition states were re-estimated by the density functional theory (DFT) method, which was executed to include the effect of electron correlation using the 6-31G** basis functional set. Becke's three-parameter functional[16] incorporating the LYP correlation term[17,18] was used in the DFT calculation. The Gaussian 98[19] program package used was

## 3. Results

**3.1. Structure of the ES Complex.** The energy-minimized structure obtained by the MM calculation is shown in Figure 1. The triphosphate part of GTP is surrounded by several amino acid residues: Ser44, Lys46, Arg178, Thr181, and Gln204. Gln204 is a part of the GAP protein. A $Mg^{2+}$ ion is located near the $\beta$- and $\gamma$-phosphate and is coordinated with two O atoms bonded to $\beta$- or $\gamma$-phosphate, the two O atoms of two water molecules, the O atom of Ser44, and the O atom of Thr181. Furthermore, a water is held by Gln204. The distances between the oxygen atom of the water molecule (water 1) and the phosphorus atom ($P_\gamma$) of $\gamma$-phosphoric acid, between the hydrogen atom of water 1 and the oxygen atom ($O_{\gamma 1}$) bonded to $P_\gamma$ and between the oxygen atom of water 1 and $O_{\gamma 1}$, are 3.39, 2.95, and 3.81 Å, respectively. The oxygen atom of water 1 forms a hydrogen bond with the oxygen atom of the main chain of Gln204, and the nitrogen atom of the main chain of Gln204 forms a hydrogen bond with the oxygen atom ($O_{\gamma 2}$) of $\gamma$-phosphoric acid. The distances between these atoms are 2.76 and 3.11, respectively. These values suggest that there are interactions between these atoms.

Geometry optimization of the model reaction system via the MO calculation gave the initial structure (A) as shown in Figure 2. In A, the distances between the oxygen atom of water 1 and $P_\gamma$, between the hydrogen atom of water 1 and $O_{\gamma 1}$, and between the oxygen atom of water 1 and $O_{\gamma 1}$ are 3.20, 1.95, and 2.82 Å, respectively. The oxygen atom of water 1 forms a hydrogen bond with the oxygen atom of the main chain of Gln204, and the nitrogen atom of the main chain of Gln204 also forms a hydrogen bond with $O_{\gamma 2}$ of $\gamma$-phosphoric acid. The distances between these atoms are 3.00 and 2.91, respectively. These values are compatible with the results obtained by the MM calculation, and the other interatomic distances also show a high degree of compatibility.

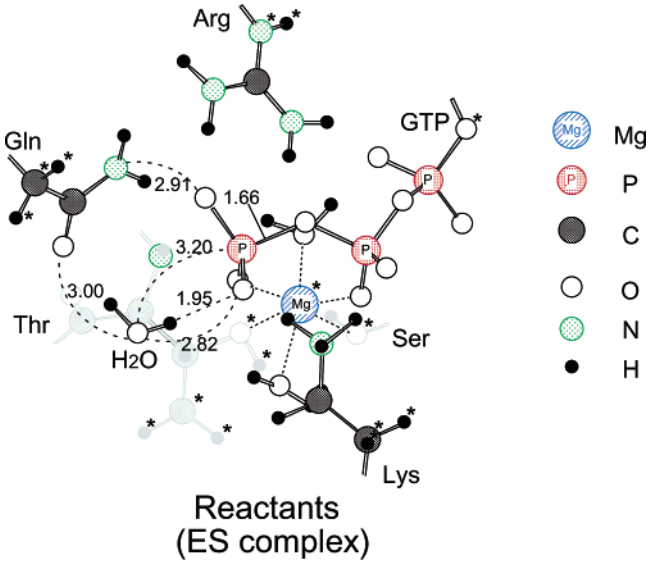


**Figure 2.** Structure of the ES complex obtained by ab initio MO calculations starting from the atomic configuration shown in Figure 1. GTP, $Mg^{2+}$, three water molecules, and amino acid residues (Ser44, Lys46, Arg178, Thr181, and Gln204) were extracted from the energy-minimized structure of Figure 1. To make a model cluster for MO calculations, these amino residues were truncated and capped with H atoms. The capping H atoms were fixed at the initial position during the geometry optimization. They are not shown for the sake of visual clarity. Atoms with asterisks are fixed through the calculation.

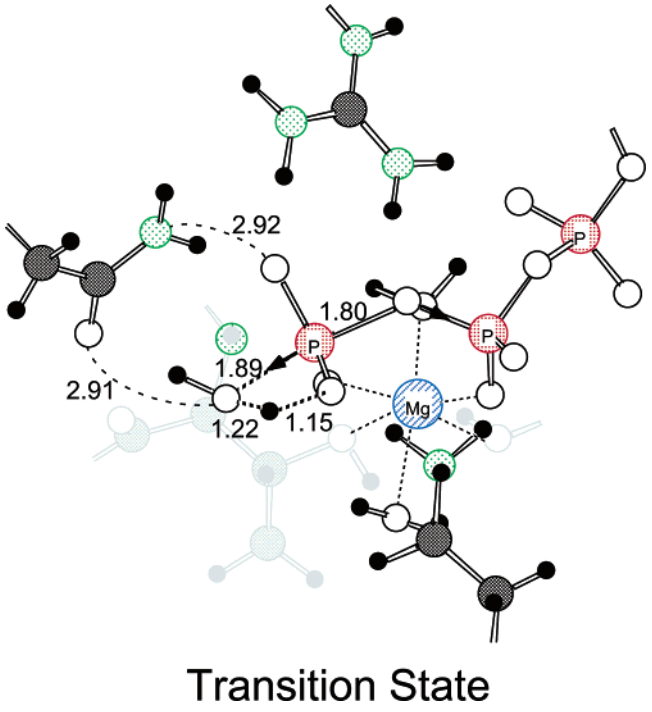


**Figure 3.** Optimized structure for the transition state (TS) of the GTP hydrolysis reaction. See also the legend of Figure 2. The arrows indicate the direction of the vibrational mode with an imaginary frequency.

**3.2. Structure of the Transition State.** The final product of hydrolysis should have a covalent bond between the P atom of phosphoric acid from GTP and the O atom of a water molecule. The transition state (TS) was determined by the water molecule approaching the P atom of $\gamma$-phosphoric acid. The TS determined in this study is shown in Figure 3. This TS structure has only one vibration mode with an imaginary frequency. It should be emphasized that this TS structure was obtained through geometry optimization under the condition that the fixed atoms

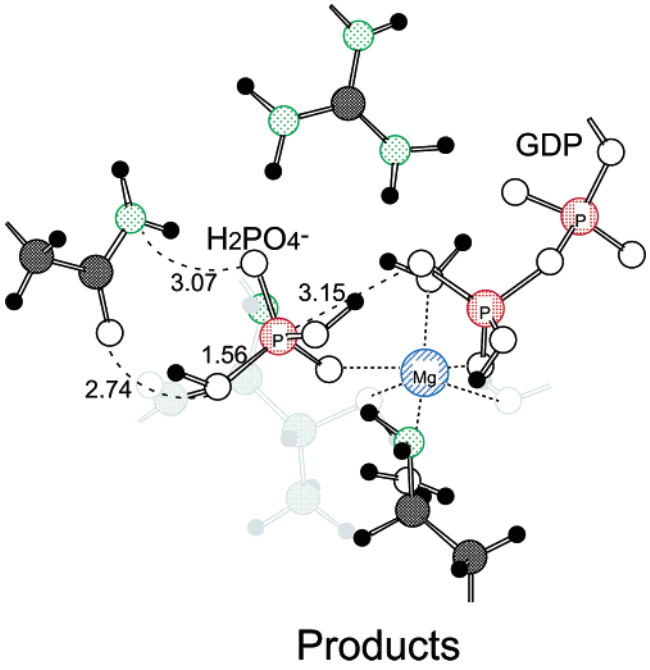


**Figure 4.** Stable structure (final product) obtained at the last point of the steepest descent path for the forward direction from the TS.

were the same as those in the model system shown in Figure 2. The distances between the oxygen atom of water 1 and $P_\gamma$, between a hydrogen atom of water 1 and $O_{\gamma 1}$, and between the oxygen atom of water 1 and the hydrogen atom of water 1 are 1.89, 1.15, and 1.22 Å, respectively. These values suggest that the water molecule ($H_2O$) has broken into OH and H and that the dissociated hydrogen atom is transferring from the water molecule to $O_{\gamma 1}$ of $\gamma$-phosphoric acid. The distance between the oxygen atom ($O_{\beta\gamma}$) that is bonded to $\beta$- and $\gamma$-phosphate and $P_\gamma$ is 1.80 Å, which is slightly longer than the value in the ES complex, 1.66 Å. $Mg^{2+}$ is 6-fold coordinated by two oxygen atoms of $\beta$- and $\gamma$-phosphoric acid ($O_{\beta 1}$ and $O_{\gamma 3}$), two oxygen atoms of water, and two oxygen atoms of the OH groups of Ser44 and Thr181.

**3.3. IRC Calculations.** Next, the steepest descent paths were calculated for the forward and reverse directions of the vibrational mode, and this calculation eventually provided the lowest potential energy path connecting the reactant and the product. As a result of the computation for the reverse direction, a stable structure, which is the same structure as that shown in Figure 2, was obtained. Therefore, this structure was confirmed to be identical to structure A in Figure 2. This means that the optimized structure shown in Figure 3 is an appropriate TS leading from the initial reactant. The potential energy difference between the ES complex and the TS is 40.1 kcal/mol.

Finally, an intrinsic reaction coordinate (IRC) calculation was performed for the forward direction following an imaginary vibrational mode of the TS. The potential energy decreased spontaneously, and the structure shown in Figure 4 (structure B) was obtained. In this structure, the distance between $O_{\beta\gamma}$ and $P_\gamma$ is 3.15 Å, indicating that one phosphoric acid has separated from GTP and that GDP has then been generated. A new chemical bond is produced between the oxygen atom ($O_\beta$) bound to $P_\beta$ and the hydrogen atom detached from the nitrogen atom of Lys46. This structure is a stable structure after the hydrolysis of GTP; therefore, this structure is the final product of hydrolysis. The final product is more stable than the ES complex by 19.3 kcal/mol.

**TABLE 1: Comparison of Activation Energies**

| method | activation energy (kcal/mol) |
|---|---|
| HF/3-21G** | 40.1 |
| DFT/6-31G** | 35.5 |

**3.4. Single-Point Energy Calculations.** The above geometry optimizations were performed at the computational level of HF/3-21G**. Hence, additional calculations were carried out for a better estimation of the potential energy change during the hydrolysis reaction. Since the Hartree−Fock method does not take the effect of electronic correlation into consideration, it is not sufficient for a precise evaluation of the energy changes of reactions.[19] Hence, HF/3-21G** was used for the determination of the optimized structure because of the reduction in calculation cost. DFT/6-31G** was used for the evaluation of the energy.

In the three structures of the ES complex (A), the transition state (TS), and the products (B), single-point energy calculations were executed using the DFT/6-31G** method. (See Table 1.) Assuming that the DFT/6-31G** method provides the most reliable computational results for this reaction, the activation energy is estimated to be 35.5 kcal/mol. The computation by the DFT/6-31G** method shows that the final product is more stable than the ES complex by 13.6 kcal/mol.

## 4. Discussion

**4.1. Proton Relay Mechanism and the Role of GTP.** The distance between $O_\beta$ and the hydrogen atom bound to the $N_\zeta$ atom of Lys46 in the ES complex was 1.65 Å. The distances in the TS and in the final product were 1.57 and 0.97 Å, respectively. Thus, a hydrogen atom is detached from Lys46 and makes a covalent bond with $O_\beta$ as the hydrolysis reaction proceeds. The water molecule also approached GTP at the same time. This means that the transfer of a hydrogen atom of Lys46 to $O_\beta$ and the transfer of a hydrogen atom of a water molecule to $O_{\gamma 1}$ occur concertedly. Therefore, it is thought that the GTP → GDP hydrolysis reaction proceeds through a proton relay mechanism assisted by the Lys residue. Schematic drawings of the catalytic reaction mechanism are presented in Figure 5.

The IRC calculation showed that the hydrolysis reaction of GTP is a one-step reaction. According to the estimation of the potential energy change using the DFT/6-31G** method, the final product is more stable than the ES complex by 13.6 kcal/mol. That is, the hydrolysis of GTP is an exothermic reaction, releasing of 13.6 kcal/mol of energy. The reaction requires a large activation energy (35.5 kcal/mol), and the energy necessary for this reaction should thus be supplied from other parts of a living cell. Since enzymes usually function at a temperature of around 310 K, a living cell would provide the energy required for this reaction; that is, the cell would act as a heat source for the energy. These results indicate that the main role of GTP is not to supply the energy necessary for the hydrolysis, which is conventionally called "an energy current",[20] but to determine the direction of the information transmission by acting as a trigger through the hydrolysis. A similar role has been reported for ATP in its phosphorylation caused by the action of c-AMP-dependent protein kinase (cAPK).[21]

**4.2. Role of GAP.** Figure 2 shows the ES complex structure for the hydrolysis of GTP by Giα1. Gln204 is a part of GAP. There are two hydrogen bonds in Gln204—one between the nitrogen atom of the main chain and $O_{\gamma 2}$ and one between the oxygen atom of the main chain and the oxygen atom of water 1. These two hydrogen bonds function to maintain an adequate distance between the water molecule and $P_\gamma$, enabling the reaction to be initiated. This means that GAP assists the beginning of the hydrolysis reaction in the ES complex.

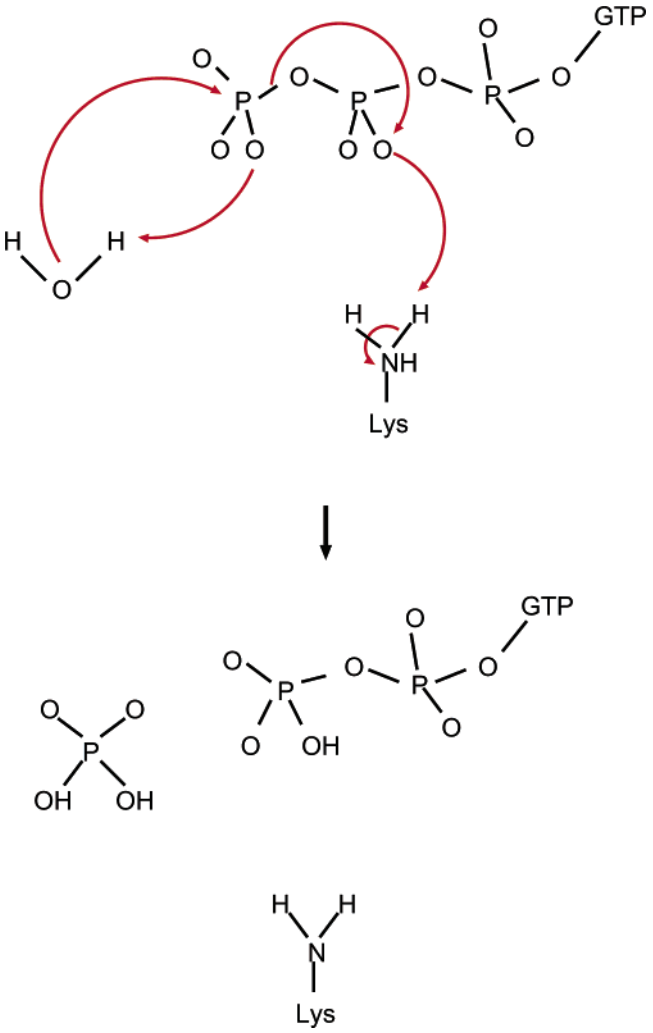


**Figure 5.** Schematic drawings of the catalytic reaction mechanism.

Figure 3 shows the TS structure of GTP hydrolysis. Gln204 also formed two hydrogen bonds with the nitrogen and oxygen atoms of the main chain. The hydrogen bond between the oxygen atom of the main chain and the oxygen atom of water 1 restricts the movement of the water molecule and assists the smooth dissociation of the catalytic water, keeping out unfavorable fluctuations of reacting atoms. Therefore, GAP contributes to the progress of the hydrolysis reaction in the TS.

Figure 4 shows the final product structure of the GTP hydrolysis. Gln204 also formed two hydrogen bonds—one between the nitrogen atom of the main chain and $O_{\gamma 2}$ and one between the oxygen atom of the main chain and the oxygen atom of the inorganic phosphoric acid. These two hydrogen bonds give strength to the inorganic phosphoric acid and keep inorganic phosphoric acid away from GDP. That is, GAP plays a role in the separation of the inorganic phosphoric acid to generate GDP and assists the progress of the hydrolysis reaction in the final product.

The above results indicate that the role of GAP is to keep the water molecule in a favorable position when it is supplied to the catalytic site and to keep inorganic phosphoric acid away from GDP after the hydrolysis. These two important roles produce a high level of GTPase activity. This mechanism underlying this high level of GTPase activity of GAP has also been reported in Ras protein p21.[22,23]

In contrast to the above-described theoretical findings, the role of Gln204 as a GAP seems to be underestimated in experiments. David et al. suggested that Gln204 did not substantially contribute to the binding of GTP or to the attack of the water molecule in the ES complex.[2] The results of their experiments showed that the replacement of Gln204 by Leu in Giα1 did not perturb the configuration of the active site or the binding mode of the nucleotide and that the nucleophilic water molecule remained bound. Their results also suggested that the Gln204 side chain has no hydrogen bonds or any close contact with the solvent molecules. Similar results were obtained by Prive et al.[24] from their crystallographic analysis of Gln61 → Leu Ras protein p21. These experimental results indicate that the Gln204 side chain forms no hydrogen bonds with GTP or with a water molecule; however, it should be emphasized that the hydrogen bonds with GTP and a water molecule are formed by the main chain of Gln204. Accordingly, we propose that the side chain of Gln204 (Gln61 in Ras protein p21) is not important but that the main chain is essential to the function of GAP.

The hydrogen bond system can be observed by molecular dynamics (MD) calculations. However, it is difficult to observe bond dissociation and reconnection with the progress of the reaction (ES complex → TS → final product) by using the molecular dynamics (MD) method. Furthermore, MD calculations do not clearly reveal the structure of the TS; only the ES complex and final product can be discussed on the basis of the results of MD calculations. In this study, the role of Gln204 was discussed in terms of the exchange of a bond network. Thus, each stationary structure was determined by using molecular orbital (MO) calculations. For the purpose of determing the stability of the catalytic water, it would also be interesting to perform MD computations on an ES complex.

**4.3. Comparison with Other Proteins with Regard to the Hydrolysis of Phosphoric Acid.** Both Ser44 and Thr181 were coordinated to $Mg^{2+}$ throughout the GTP hydrolysis reaction of Giα1. The coordination of these residues effectively stabilized the $Mg^{2+}$ to large fluctuations that were present and assisted the GTP hydrolysis. Lys46 operated as a proton donor in the hydrolysis. It is notable that these three kinds of residues (Ser, Thr, Lys) commonly exist at the active sites of several proteins as well as in the hydrolysis of GTP or ATP. As examples, Giα1, Ras protein p21, and myosin are shown in Figure 6, and the atomic geometries of these proteins are compared. In the GTP hydrolysis of Ras protein p21, Ser, Thr, and Lys have been suggested to operate in the same manner as in the case of Giα1.[23] The positions of these three residues in Ras p21 are very similar to those in Giα1, as shown in Figure 6. ATP hydrolysis by myosin has also been demonstrated to be caused by similar actions of the same three residues.[25] Accordingly, it is concluded that the three residues Ser, Thr, and Lys are fundamental parts of those enzymes and play an essential role in GTP or ATP hydrolysis.

Ras p21 does not have a GTPase activating protein (GAP) inside it; that is, the reaction active site is usually open to the outside. A GAP is thought to be attached to the reaction site and to stabilize a catalytic water molecule to settle down at its adequate location. Hence, the role of GAP in Giα1 can also be adapted to the Ras protein p21 combined with GAP (i.e., Gln61 of Ras protein p21 would form a hydrogen bond with a water molecule and assist the GTP hydrolysis). There is no obviously defined GAP region for myosin; however, similar amino residues Arg and Glu have been shown to exist at the position at which GAP is located in G proteins. Figure 6 shows that

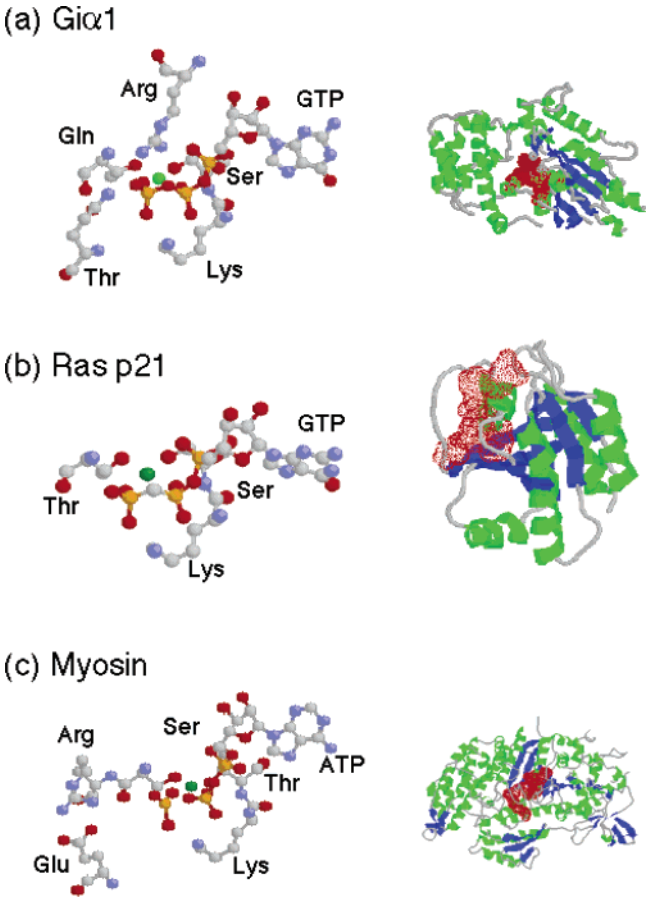


**Figure 6.** Structural comparison of Giα1, Ras p21, and myosin. Atomic geometry at the reaction active site, which is located in the red dotted area of the ribbon-and-tube representation of each protein, is depicted on the left.

Arg and Glu residues in myosin correspond to Arg and Gln residues in Giα1. Arg238 and Glu459 of myosin have also been suggested to stabilize a water molecule at its adequate location and to assist in the ATP hydrolysis. Consequently, the role of Arg238 and Glu459 of myosin is equivalent to the role of GAP. It will also be informative to compare the activation energies for the hydrolysis caused by these proteins. The activation energy required for the GTP → GDP reaction by Giα1 was calculated in the present study to be 35.5 kcal/mol. The energy barrier for the GTP → GDP reaction by Ras p21 is thought to be about 42 kcal/mol. This difference may be due to the absence of an intrinsic GAP in Ras p21. The ATP → ADP reaction induced by myosin was also found to impose a similar value of the energy barrier, about 42 kcal/mol. Consequently, it is concluded that Ser, Thr, and Lys residues are fundamental building blocks of the reaction active site and that Arg and Gln residues are important assisting components of the hydrolysis of phosphoric acids.

## 5. Conclusions

Quantum chemical calculations were performed to clarify the hydrolysis mechanism of the Giα1 protein, and the following conclusions were drawn:

(1) The GTP → GDP hydrolysis reaction is a one-step reaction.

(2) The GTP → GDP hydrolysis reaction proceeds through a proton relay mechanism assisted by a Lys residue.

(3) The activation energy estimated by the DFT/6-31G** 

computation is 35.5 kcal/mol. This hydrolysis reaction releases 13.6 kcal/mol of energy.

(4) The role of GAP is to keep the water molecule in a favorable position before the hydrolysis and to keep the inorganic phosphoric acid away from GDP after the hydrolysis reaction.

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