A cavity with an appropriate size is the basis of the PPIlase activity

Teikichi Ikura1,4, Kengo Kinoshita2,3 and Nobutoshi Ito1

1Laboratory of Structural Biology, School of Biomedical Science, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, 2Human Genome Institute, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639 and 3Structure and Function of Biomolecules, SORST, Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

To whom correspondence should be addressed.
E-mail: ikura.str@tmd.ac.jp

Peptidyl-prolyl isomerases (PPIases) are biologically very important enzymes but their catalytic mechanism is not fully understood. Recently, our comprehensive mutational study on a PPIase, human FK506-binding protein 12 (FKBP12), suggested that only presence of a cavity was required for the catalysis. This study, however, could not determine what properties of the cavity were essential for the catalysis. In the present study, we focused on the size of the cavity and examined if an artificial PPIase activity could be achieved by a protein with a cavity of a size similar to that of FKBP12. We designed such a cavity on barnase, a bacterial nuclease without the PPIlase-like activity, by a quadruple mutation F56G/R59G/H102Y/ Y103G. The mutant barnase successfully exhibited weak yet significant PPIase activity. Furthermore, we searched the Protein Data Bank for proteins natively possessing such a cavity. Two of the identified non-PPIase proteins, α-amylase and prolyl endopeptidase, were tested for the PPIase activity and indeed catalyzed the isomerization of peptide bonds. These results suggest that a cavity with an appropriate size is the basis of the PPIase activity.

Keywords: barnase/cavity-creating mutation/design of protein function/peptidyl-prolyl isomerase activity/stochastic binding and releasing model

Introduction

Peptidyl-prolyl isomerase (PPIase) catalysis is a well-known reaction that accelerates the conformational change between the cis and trans forms of the Xaa-Pro peptide bond. This catalysis is performed by a large number of proteins belonging to the PPIase family, which is classified into three subfamilies, namely, cyclophilin, FK506-binding protein (FKBP), and parvulin (Fischer and Schmid, 1999). The amino acid sequences of these proteins are highly conserved within each member of the subfamilies, but share limited similarity among each other (Wiederrecht et al., 1991). The similarity in sequence and structure across the subfamilies is, however, slightly higher for the substrate-binding cavities than for the entire molecule, thereby suggesting a convergent evolution of the enzyme catalytic site (Denesyu et al., 1993). Therefore, the catalytic mechanism of PPIase is expected to be common to all the members of the PPIase family.

To date, several mechanisms have been proposed in order to explain PPIase catalysis (Fischer et al., 1989a, 1989b; Harrison and Stein, 1990; Kofron et al., 1991; Ke et al., 1993); however, none of these mechanisms could describe the catalytic process satisfactorily.

In a recent study, six highly conserved residues in a typical PPIase, human FK506-binding protein 12 (FKBP12), were selected for the comprehensive mutation analysis to examine the importance of each residue (Ikura and Ito, 2007). As a result, no single substitution of the six residues (Asp37, Arg42, Phe46, Val55, Trp59 and Tyr82) could completely abolish the PPIase activity (Fig. 1A). Furthermore, a sextuple mutant protein, namely, D37G/R42G/F46G/V55G/ W59G/Y82G, where all the side chains of the six residues were removed, retained 90% of the activity of wild-type FKBP12, which showed these side chains were unessential for the activity. Thus, we concluded that the site-specific interactions of the main-chain atoms of the substrate-binding cavity and/or the hydrophobic nature of the cavity were adequate for PPIase catalysis. These results lead to possibility of designing an artificial PPIase by creating a cavity with appropriate properties to unrelated proteins.

In the present study, we focused on the size of the cavity and introduced a set of cavity-creating mutations in proteins without PPIase-like activity to confirm the idea that a cavity with an appropriate size is enough to achieve PPIase activity. A set of a quadruple mutation was introduced to barnase, an extracellular ribonuclease from Bacillus amyloliquefaciens (Matouschek et al., 1989; Serrano et al., 1992; Schreiber and Fersht, 1993; Buckle and Fersht, 1994; Schreiber and Fersht, 1995; Ikura et al., 2004), which has no PPIase-like activity. The mutant barnase indeed exhibited a PPIase activity that was 4% of that of the wild-type FKBP12 to support our notion.

This result prompted us to search the Protein Data Bank (PDB) for proteins possessing a cavity similar in size to the substrate-binding cavity of FKBP12 (Kinoshita et al., 1999; Berman et al., 2000; Wang and Dunbrack, 2003), in hope that they may also exhibit PPIase activity, since such simple cavities are likely to exist in non-PPIase proteins. We found various non-PPIases possessed a comparable cavity and two of them, α-amylase and prolyl endopeptidase, were tested for the PPIase activity. Both of them clearly performed PPIase catalysis: α-amylase and prolyl endopeptidase exhibited 0.5 and 5% of the activity of wild-type FKBP12, respectively.

Taken together, these results strongly indicate that a cavity with an appropriate size is enough to perform the PPIase activity at least to some extent and suggest discussions on the catalytic mechanism of the PPIase should not be limited to site-specific interactions conferred by the residues specific to PPIases. We accordingly propose a catalytic mechanism based on the ‘stochastic binding and releasing’ model, which developed from the ‘catalysis by distortion’ model (Harrison and Stein, 1990).
albumin (BSA) was purchased from Takara Bio Inc. and Wako Pure Chemical Industries, Ltd., and bovine serum from Seikagaku Corporation. Lysozyme was purchased from Amylase and prolyl endopeptidase were purchased from Professor Sir A.R. Fersht, University of Cambridge.

Materials and methods

Materials

The expression plasmid of wild-type barnase was a gift from Professor Sir A.R. Fersht, University of Cambridge. α-Amylase and prolyl endopeptidase were purchased from the Seikagaku Corporation, lysozyme was purchased from the Wako Pure Chemical Industries, Ltd., and bovine serum albumin (BSA) was purchased from the Takara Bio Inc.

Mutagenesis and preparation of wild-type barnase and its mutant

For the cavity creation, we selected one of the positions that were the most dissimilar to the substrate-binding cavity of FKBP12 (Fig. 1A) in a similarity search (Kinosita et al., 1999; Wang and Dunbrack, 2003). As a result, the position comprising Phe56, Ser57, Asn58, Arg59, Glu60, Lys62, Leu63, Glu73, Arg87, Leu89, His102, Tyr103 and Phe106 was determined (Fig. 1B). A set of mutations – F56G/R59G/H102Y/Y103G – was introduced to create a cavity with a diameter and depth of ~10 and 6 Å, respectively, because most PP1ases commonly had such dimensions in their substrate-binding cavities (Ke et al., 1993; Wilson et al., 1995; Konno et al., 1996); an additional mutation, i.e. H102Y, was introduced to compensate for the loss of hydrophobicity caused by the mutation Y103G (Fig. 1C). The quadruple mutant barnase F56G/R59G/H102Y/Y103G was constructed using the QuikChange site-directed mutagenesis kit (Stratagene Co.) containing specific mutagenic primers. The expression and purification of wild-type barnase and its quadruple mutant have been described elsewhere (Serrano et al., 1990).

The concentration of wild-type barnase and its mutant was determined by UV absorption at 280 nm using the molar extinction coefficients determined previously (Loewenthal et al., 1992).

Circular dichroism spectroscopy

Circular dichroism (CD) spectra were taken on a Jasco J-720 spectropolarimeter using an optical cuvette with a pathlength of 1.00 mm for measurements in the peptide region and a path-length of 10.0 mm for measurements in the aromatic region. All the measurements were carried out at 25 ± 0.5°C. Protein concentration was adjusted to 0.1 mg/ml in 50 mM sodium acetate buffer, pH 5.0.

Assay of the isomerase activity

The cis–trans isomerization of the Ala-Pro peptide bond in the synthetic peptide N-succinyl-Ala-Ala-Pro-Phe-4-methylcoumaryl-7-amide (AAPF-MCA) was measured in a coupled assay with α-chymotrypsin; this reaction was based on the ability of this protease to cleave the synthetic peptide only when the Ala-Pro bond, which is in equilibrium between cis and trans, is in the trans configuration (Hayano et al., 1991). Twenty microliters of a 1.68 mM solution of the peptide was preincubated with appropriate concentrations of the target protein in 35 mM HEPES-Na buffer (pH 7.8) containing 5 mM ß-mercaptoethanol. We initiated the assay at 30°C by manually mixing 20 μl of 0.76 mM α-chymotrypsin in the above solution in a spectrophotometer cell mounted in a Hitachi U-1800 spectrophotometer connected to a NesLab temperature-controlled liquid system. Hydrolysis of the MCA in the cis peptide was monitored by the increase in absorbance at 354 nm, whereas the trans peptide was cleaved within the dead time of the experiment. The kinetic curves were analyzed using KaleidaGraph software (Hulinks).

Similarity search for the substrate-binding cavity through the protein data bank

We searched for structures analogous to the substrate-binding cavity of FKBP12 among 5588 non-redundant proteins in the PDB (Kinosita et al., 1999; Berman et al., 2000; Wang and Dunbrack, 2003). The configurations of five residues – Asp37, Glu54, Val55, Ile56 and Tyr82 – located in the cavity of FKBP12 were selected as the search query because these residues well determined the size of cavity (Ikura and Ito, 2007). For the three residues, Glu54, Val55 and Ile56, only the main-chain atoms were applied rather than the whole residues, because any correlation between the type of side chain of this 55th residue and PP1ase activity could not be observed in the comprehensive mutational study on Val55, and the main-chain atoms of Glu54 and Ile56 were...
included in the query in order to weight the configuration of the main-chain atoms at the 55th residue. On the contrary, the side-chain atoms of Asp37 and Tyr82 were also included because side-chain specificity on the PPIase activity was rather observed at these positions. Finally, the configuration of 32 atoms was applied as a query: 12 atoms of the main chain from Glu54 to Ile56, 12 atoms of Tyr82, and eight atoms of Asp37 (Fig. 1A). In order to evaluate the degree of satisfaction to the above query, the score (Z) for each protein in the database was defined as follows (Kinoshita et al., 1999): 

\[ Z = MNR - RMSD \]

where MNR represents the maximum number of residual atoms involved in the final query when the atoms involved in the query were decreased from the original query so that the root-mean square deviation between the query and the target protein was not more than 1.0Å, and RMSD represents the root-mean square deviation between the final query and the target protein. A maximum score (32.0) was obtained only for wild-type FKBP12 from which the atoms were extracted as the query (PDB ID: 1FKJ).

Results

The PPIase activity of the quadruple mutant barnase

We selected, an extracellular ribonuclease from \textit{B. amyloquefaciens}, as a model protein to create a cavity with artificial PPIase activity because it was without PPIase-like activity and exhibited a robust structure against mutations, i.e. the overall protein structure changed little after any of more than 130 mutations (Matouschek et al., 1989; Serrano et al., 1992). We tested structural integrity of the quadruple mutant barnase with CD spectroscopy, which is regarded as one of the most sensitive indexes of the structural assessment for barnase for the following three reasons (Vuilleumier et al., 1993). (i) The far-UV CD spectrum of barnase is well characterized by the minimum around 230 nm (Fig. 2), which is closely associated with the presence of the major hydrophobic core consisting of His18, Tyr90 and Trp94. (ii) The intensity in the far-UV CD spectrum is extremely low because of extensive canceling out of positive and negative CD bands caused by interaction between aromatic residues and neighboring residues (Fig. 2); the prediction of secondary structure content by the far-UV CD spectrum indicates only 5% \( \alpha \)-helix content for the wild-type barnase, although the X-ray structure gives an \( \alpha \)-helix content of 22% for wild-type protein (Kabsch and Sander, 1983; Vuilleumier et al., 1993). (iii) In the near-UV, the complex spectrum reflects the high proportion of aromatic residues in barnase (Vuilleumier et al., 1993).

In the CD spectrum of our quadruple mutant barnase F56G/R59G/H102Y/Y103G, the far-UV CD spectrum had low overall spectral intensities and a minimum around 230 nm, while in the near-UV, it had the positive complex CD spectrum at 270–320 nm (Fig. 2). Comparing the CD spectra between the wild-type barnase and the mutant, the feature of these spectra was totally similar to that of wild-type barnase, although the intensities in the CD spectra of the mutant was much lower at 205–222 and 270–288 nm than those of the wild-type protein. These discrepancies in far- and near-UV CD spectra may be caused by the three mutations of aromatic residues, F56G, H102Y and Y103G, because the CD spectra of barnase was highly correlated to amount of aromatic residues in general. In addition, it was known that the mutation on Phe56 decreased the intensity of the far-UV CD spectrum at 205–222 nm as reported by Vuilleumier et al. (1993). Judging from the research on the CD spectra of wild-type barnase and its various mutants (Vuilleumier et al., 1993), the far-UV and near-UV CD spectra of the quadruple mutant strongly suggested that the mutant protein retained the native structure of wild-type barnase.

When its PPIase activity was measured using the synthetic peptide AAPF-MCA, the mutant barnase clearly exhibited PPIase activity whose catalytic efficiency \( k_{cat}/K_m \) at 30°C was 92 ± 3.6 mM\(^{-1}\) s\(^{-1}\), while the PPIase activity of the wild-type protein was negligible (Table I and Fig. 3). Although this value represents only 4.6% of the activity of wild-type FKBP12 — the \( k_{cat}/K_m \) value of which was 1980 ± 250 mM\(^{-1}\) s\(^{-1}\) at 30°C (Ikura and Ito, 2007), it is much larger than experimental errors and thus is significant. Therefore, this result indicated that barnase acquired the PPIase activity as a result of the mutations.

The PPIase activity of non-PPIases with appropriate sizes of cavities

In the mutational study on barnase described above, only the size of the substrate-binding cavities of PPIases was...
considered for the cavity creation. The activity of the mutant barnase, therefore, implies that any protein might perform PPIase catalysis if they simply possessed cavities with the appropriate size. Accordingly, we searched proteins in the PDB for those with a cavity similar in size to the substrate-binding cavity of FKBP12. Five thousand five hundred and eighty-eight non-redundant proteins were screened in the similarity search (Kinoshita et al., 1999; Wang and Dunbrack, 2003) (Fig. 4 and Table II). The average and standard deviation of the total scores were 17.2 and 1.7, respectively, suggesting that most of the proteins satisfied half of the search query as a perfect match would give a score of 32. The atoms corresponding to the main-chain conformation from Glu54 to Ile56 were frequently found since this conformation is ubiquitous. In contrast, the simultaneous occurrence of atoms corresponding to both Asp37 and Tyr82 and the atoms corresponding to the main-chain conformation from Glu54 to Ile56 was infrequent. Moreover, the atoms did not always form a cavity even if they adequately satisfied the conditions of the query; this failure was often due to steric hindrance in which other amino acid residues filled the cavity. Thus, we checked if a cavity really existed on the protein surface by manual inspection. As PPIases are also included in the database, the scores of the PPIases were utilized as a guide to evaluate the scores of other proteins. All the proteins in the FKBP family achieved high scores (32.0–22.4), while the score of hen egg white lysozyme without PPIase activity was 20.0 (Tables I and II). We used this value as a threshold and carefully examined the structures of 245 non-PPIases with a score higher than 20. Although many of these could not form the cavity because of the steric hindrance mentioned above, some of the proteins, e.g. human Chk1 checkpoint kinase (score = 22.3) and the Spred1 EVH-1 domain from Xenopus tropicalis (score = 21.0), formed cavities fully exposed to solvent. Unfortunately, many of the proteins with such cavities are not commercially available and thus would be difficult to obtain. Eventually, we identified two commercially available proteins with high scores, α-amylase (score = 21.2) and prolyl endopeptidase (score = 20.1), and measured the PPIase activities of these two proteins.

α-Amylase is an enzyme that breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylase (Junge et al., 1959). This enzyme can be considered as an example of a typical non-PPIase. The predicted

### Table I. Catalytic activities of FKBP12 and non-PPIases on prolyl cis→trans isomerization of a tetrapeptide AAPF-MCA at pH 7.8 and 30°C

<table>
<thead>
<tr>
<th>Protein</th>
<th>$k_{cat}/K_m$ (mM$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FKBP12</td>
<td>1980 ± 250</td>
</tr>
<tr>
<td>Wild-type barnase</td>
<td>1.6 ± 1.7</td>
</tr>
<tr>
<td>Barnase F56G/R59G/H102Y/Y103G</td>
<td>92 ± 3.6</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>9.4 ± 3.0</td>
</tr>
<tr>
<td>Prolyl endopeptidase</td>
<td>94 ± 7.1</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.36 ± 1.1</td>
</tr>
<tr>
<td>BSA</td>
<td>0.040 ± 1.5</td>
</tr>
</tbody>
</table>

Fig. 3. Peptidyl-prolyl isomerizations catalyzed by wild-type barnase and its quadruple mutant. The apparent rates ($k_{obs}$) of cis-to-trans isomerization of a tetrapeptide (AAPF-MCA) were plotted for different concentrations of wild-type barnase (filled circles) and the quadruple mutant (open circles). The solid and dashed lines represent the linear least-square fittings of the data of wild-type barnase and its quadruple mutant, respectively. The $k_{cat}/K_m$ values for the PPIase activities of wild-type barnase and its quadruple mutant were calculated at 30°C as $1.6 ± 1.7$ mM$^{-1}$ s$^{-1}$ and $92 ± 3.6$ mM$^{-1}$ s$^{-1}$, respectively.

Fig. 4. Summary of the similarity search. Five thousand five hundred and eighty-eight non-redundant proteins were investigated in the similarity search. The average and standard deviation of the total scores were 17.2 and 1.7, respectively.
The predicted substrate-binding cavity comprises Tyr495, Tyr98, Phe228 and Phe257, residing in the bottom of the cavity (Fig. 5A). The protein clearly exhibited PPIase activity, with a catalytic efficiency ($k_{\text{cat}}/K_\text{m}$) of $9.4 \pm 3.0 \text{ mM}^{-1} \text{s}^{-1}$ at $30^\circ\text{C}$ (Table I). Although this value represents only 0.5% of the activity of wild-type FKBP12, it was clearly greater than the values obtained for the two control proteins, hen egg white lysozyme and BSA, whose PPIase activities were negligible (Table I).

Prolyl endopeptidase can also be regarded as a typical non-PPIase. It is a serine protease that captures peptides containing at least one Pro residue and then cleaves the peptide bond immediately following the Pro residue (Yoshimoto et al., 1980). Although this protein can bind a prolyl imide bond, its PPIase activity has not been reported previously. The predicted substrate-binding cavity comprises Tyr495, Gly496, Gly497, Tyr620 and Val661, and two aromatic amino acid residues, Phe498 and Trp616, which reside in the bottom of the cavity (Fig. 5B). The protease also exhibited PPIase activity, with a $k_{\text{cat}}/K_\text{m}$ value of $94 \pm 7.1 \text{ mM}^{-1} \text{s}^{-1}$ at $30^\circ\text{C}$ (Table I), corresponding to 5% of the activity of wild-type FKBP12. The peptidase activity of prolyl endopeptidase was considered negligible because its catalytic rate was considerably slower than both the rate of peptidyl-prolyl isomerization of the substrate AAPF-MCA and the rate of peptidase activity of $\alpha$-chymotrypsin under the same reaction conditions (Yoshimoto et al., 1980).

Discussions

Our previous work showed side-chains of the six highly conserved residues in FKBP12 were not essential to its PPIase activity (Ikura and Ito, 2007). The new results presented in this paper extended the idea and strongly indicate that a simple cavity with an appropriate size on a protein surface is enough to generate such activity at least to some extent, suggesting that the fundamental PPIase activity is achieved by a considerably simple mechanism.

### Table II. Summary of the similarity search for a substrate-binding cavitya

<table>
<thead>
<tr>
<th>Rank</th>
<th>Score</th>
<th>Protein (FKBP/Cyclophilin/Parvulin)</th>
<th>PDB ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.0</td>
<td>Human FKBP12 (FKBP)</td>
<td>1FKJ</td>
</tr>
<tr>
<td>2</td>
<td>31.5</td>
<td>Human FKBP52 (FKBP)</td>
<td>1Q1C</td>
</tr>
<tr>
<td>3</td>
<td>31.4</td>
<td>Arabidopsis thaliana AtFKBP13 (FKBP)</td>
<td>1L79</td>
</tr>
<tr>
<td>4</td>
<td>26.5</td>
<td>Caenorhabditis elegans FKBP-type PPIase (FKBP)</td>
<td>1R9H</td>
</tr>
<tr>
<td>5</td>
<td>24.5</td>
<td>Trypansoma cruzi TCMIP (FKBP)</td>
<td>1JVW</td>
</tr>
<tr>
<td>6</td>
<td>23.3</td>
<td>Human UDP-glucuronate decarboxylase 1</td>
<td>2B69</td>
</tr>
<tr>
<td>7</td>
<td>23.2</td>
<td>Human dual-specificity protein kinase CLK1</td>
<td>1Z57</td>
</tr>
<tr>
<td>8</td>
<td>22.4</td>
<td>Human FKBP8 (FKBP)</td>
<td>2AWG</td>
</tr>
<tr>
<td>9</td>
<td>22.3</td>
<td>Human Chk1 checkpoint kinase</td>
<td>1IA8</td>
</tr>
<tr>
<td>10</td>
<td>21.2</td>
<td>Bacillus licheniformis $\alpha$-amylase</td>
<td>1VJS</td>
</tr>
<tr>
<td>11</td>
<td>21.0</td>
<td>X. tropicalis spred1 EVH-1 domain</td>
<td>1XOD</td>
</tr>
<tr>
<td>141</td>
<td>20.1</td>
<td>Novosphingobium capsulatum prolyl oligopeptidase</td>
<td>1YR2</td>
</tr>
<tr>
<td>252</td>
<td>20.1</td>
<td>E. coli catalase HPII</td>
<td>1P80</td>
</tr>
<tr>
<td>262</td>
<td>20.0</td>
<td>Hen egg white lysozyme</td>
<td>3LZT</td>
</tr>
<tr>
<td>5000</td>
<td>15.2</td>
<td>Human SRP19</td>
<td>1JID</td>
</tr>
<tr>
<td>5204</td>
<td>15.0</td>
<td>Crambe abyssinica crambin</td>
<td>1EJG</td>
</tr>
</tbody>
</table>

The proteins were ranked according to their scores. The type of peptidyl-prolyl isomerase (PPIase) is indicated in parenthesis if a protein belongs to the PPIase family.

On the other hand, the activity of quadruple mutant barnase as well as the two non-PPIase proteins were rather weak: 4.6% (barnase), 5% (prolyl endopeptidase) and 0.5% ($\alpha$-amylase) of the wild-type FKBP12 (Table I). Such low activities of these proteins appear to be partly due to the inexact size of their cavities. Although the dimensions of the cavity we created were set at a diameter and depth of $\sim 10$ and 6Å, respectively, similar to those of the substrate-binding cavities of PPLases, the actual dimensions of the cavity achieved should be confirmed in further structural study.

We should also take into account the difference in the substrate specificity as another possible reason for the weak activity of three proteins. While a tetrapeptide (AAPF-MCA) was used as the substrate in the present study, the amplitude of PPIase activity often highly depends on the amino acid residue preceding the Pro residue of the peptide bond which gets rotated. The wild-type FKBP12 strongly prefers Leu to Ala at this position, whereas human cyclophilin A (hCyPA) strongly prefers Ala to Trp (Harrison and Stein, 1990). In the case of wild-type FKBP12, the PPIase activities for the Glu-Pro and Gly-Pro peptide bonds corresponded to only 1.1 and 2.3%, respectively, of the activity for the Ala-Pro peptide bond, suggesting that the PPIase activity of the mutant barnase might show higher activity to other substrates; that is, the optimal Xaa component of the substrate Xaa-Pro dipeptides might be different from Ala. In the present study, however, we did not perform further mutational analysis improving the PPIase activity of the mutant.
barnase or search the specific substrates of the protein, because our aim was to elucidate the importance of the cavity itself for the PPIase activity.

Another possible reason we should consider is contributions of site-specific interactions by the side chains of the amino acid residues constituting the cavities. In a complex between hCyPA and a dipeptide (Ala-Pro), the guanidinium group of Arg55 and the side chain carboxyl group of Gln63 were considered to facilitate the isomerization of the peptide (Ke et al., 1993). This suggests that the side chains of the residues constituting the substrate-binding cavity of PPIase were tuned up for the PPIase activity and their contributions are needed to achieve high activity.

Nonetheless, the present results clearly indicate that fundamental PPIase activity can be achieved by the presence of a simple cavity with an appropriate size. In the case of the mutant barnase, three of the four mutations introduced were only to delete the bulky side chains of the residues of Phe, Arg and Tyr, while the fourth mutation merely transposed the aromatic side chain from Tyr103 to the preceding residue (His102). Thus, except for influence of such deletion and transposition, these mutations did not add any new attributes common to ordinary PPIases to barnase, but only created a simple cavity with an appropriate size. In a complex of two possible conformations, cis or trans, by chance upon the reduction of prolyl imide bond distortion. As a result, the PPIase functions as an enzyme that accelerates the rate of isomerization. This is because the abovementioned process involves the frequent exchange of the conformation of the prolyl imide bond, but does not involve a change in the ratio of the two conformations in the equilibrium state. Although this model is almost equivalent to the ‘catalysis by distortion’ model (Harrison and Stein, 1990), it differs in that it emphasizes the ‘quick release’ of the substrate, since frequent exchange of the prolyl imide bond conformation is necessary in order to accelerate the rate of isomerization.

Acknowledgements

We thank Professor Sir A.R. Fersht for providing the expression plasmids of barnase. We thank Professor K. Akiyoshi and S. Sawada for allowing us use the CD spectropolarimeter.

Funding

This work was supported by Grants-in-Aid for Scientific Research (Nos 16041210 and 18031009) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. K.K. was supported by a Grant-in-Aid for Scientific Research (Nos 16041210 and 18003109) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References


Received September 19, 2007; revised December 3, 2007; accepted December 10, 2007

Edited by Haruki Nakamura