The crystal structure of the Pyrus pyrifolia pistil ribonuclease (S₃-RNase) responsible for gametophytic self-incompatibility was determined at 1.5-Å resolution. It consists of eight helices and seven β-strands, and its folding topology is typical of RNase T₂ family enzymes. Based on a structural comparison of S₃-RNase with RNase Rh, a fungal RNase T₂ family enzyme, the active site residues of S₃-RNase assigned were His³³ and His⁸⁸ as catalysts and Glu⁴⁴ and Lys⁸⁷ as stabilizers of an intermediate in the transition state. Moreover, amino acid residues that constitute substrate binding sites of the two RNases could be superimposed geometrically. A hypervariable (HV) region that has an S-allele-specific sequence comprises a long loop and short α-helix. This region is far from the active site cleft, exposed on the molecule’s surface, and positively charged. Four positively selected (PS) regions, in which the number of nonsynonymous substitutions exceeds that of synonymous ones, are located on either side of the active site cleft, and accessible to solvent. These structural features suggest that the HV or PS regions may interact with a pollen S-gene product(s) to recognize self and non-self pollen.

Many flowering plants have a self-incompatibility system that recognizes the self or non-self between the pistil and pollen (tube) after pollination and suppresses growth of the self-pollen tube to prevent self-fertilization (1, 2). Gametophytic self-incompatibility (GSI) is controlled genetically by a single locus (S-locus) with multiple alleles (1, 2). When a pollen grain lands on a stigma of the pistil, a process that discriminates as to whether an S-allele of the pollen matches one of the two S-alleles of the pistil takes place. The pollen grain germinates on the stigma and grows into the style toward the embryo. If its S-allele matches one of the two S-alleles of the pistil, pollen tube growth is arrested in the style, and no fertilization takes place.

In solanaceous, scrophulariaceous, and rosaceous plants that have GSI, the pistil glycoproteins that cosegregate with the S-alleles have been identified as ribonucleases of the RNase T₂ family (S-RNase) (3). McClure et al. (4) reported that pollen rRNA is degraded after self-pollination but not after cross-pollination and suggested that GSI expression is mediated by degradation of the pollen rRNA of self-pollen tubes by S-RNase, leading to depletion of protein biosynthesis and the eventual arrest of tube growth. S-RNase has been confirmed necessary for GSI from results of gain-of-function and loss-of-function experiments on transgenic plants of solanaceous species (5, 6). Transgenic experiments also have shown that the RNase activity of S-RNase is necessary for GSI (7), which in petunia the carbohydrate moiety is not responsible for GSI (8), and that mutant S-RNase, which has lost RNase activity, acts as a dominant negative for GSI (9).

Based on these findings, two models have been proposed to explain S-allele-specific inhibition of pollen tube growth; the receptor and inhibitor models (10). In the receptor model, the pollen S-gene product(s) is(are) the receptor that incorporates the S-RNase that matches the pollen S-allele. In the inhibitor model, S-RNase enters the pollen tube nonspecifically and is inhibited, except for the enzyme that has the same pollen S-allele. Recently, immunocytological research has shown that S-RNase enters and is accumulated in the cytoplasm of all pollen tube haplotypes without S-allele specificity, thus experimentally supporting the inhibitor model (11). The function of the GSI mechanism, however, is still not clear, because no pollen S-gene products that interact with S-RNase and cause self and non-self recognition between the pollen and pistil have yet been found.

Amino acid sequence analyses of solanaceous S-RNases have identified two hypervariable regions, HVa and HVb, with extremely high levels of divergence between allelic sequences; candidates for sequences that would be recognized as S-allele-specific by pollen (12). In fact, the S-allele specificity of the Solanum chacoense S₁₁-RNase was changed to the S₁₃-allele by swapping the HVa and HVb domains (13), and mutation in the HVa and HVb regions of S₁₁-RNase produced a new S-RNase with dual specificity, S₁₁- and S₁₃-alleles (14). In contrast, only one hypervariable region (HV), corresponding to the HVa of the solanaceous S-RNase, has been detected in sequences of rosaceous S-RNases (15, 16), the HVb region apparently being deleted (16, 17). This structural feature suggests that the recognition mechanism of S-allele specificity may differ for solanaceous and rosaceous S-RNases.
phases at 2.5-Å resolution by DM with solvent flattening and histogram matching. The phases were improved by the DM (23) and wARP (26) programs. First, the phases were located by the program SHARP (25) (Table I). The experimental MIR data set was collected at the Photon Factory with a charge-coupled device detector Quantum 4R (ADSC) at the beam line of BL6A and processed by program O (27). X-ray crystallographic refinement was done by the CPS program (28). One cycle of simulated annealing refinement, followed by positional and temperature factor refinements, was repeated several times. Throughout the refinements bulk solvent correction was applied, and the resolution range was gradually extended from 2.3 to 1.5 Å. At each stage of crystallographic convergence, the model was carefully checked and rebuilt into the simulated annealed omit map calculated by CNS. Solvent water molecules were picked up from a difference Fourier map by use of the automated scripts implemented in CNS. The lower cut-off level for picking up the solvent water molecules was set at 3σ. High resolution model coordinates of sugar chains were obtained from the Uppsala web server, HIC-Up (xray.hmc.uu.se/hicup/) (29). The crystal structure was refined to the respective R- and free R-factors of 17.2 and 20.2% in the resolution range of 500–1.5 Å. The final model has 1643 protein atoms, 59 sugar atoms, and 266 water atoms. A Ramachandran plot from the program PROCHECK (30) shows that 91.5% of the residues are in the most favorable region, 8.5% in the additional allowed region, and none in the generally allowed and disallowed regions. The coordinates have been deposited in the Protein Data Bank with the accession code 1IQQ.

**EXPERIMENTAL PROCEDURES**

Crystallization and Data Collection—P. pyrifolia S3-RNase was purified and crystallized as described previously (20). The crystals belong to the P21 space group with unit cell dimensions of a = 45.65, b = 52.59, c = 47.57 Å, and β = 106.45° (20). Diffraction data were collected with an image plate detector Raxis IV (Rigaku) at the beam line of BL40B2 at SPring-8 to 1.5 Å resolution. Image data were processed by the programs DENZO and SCALEPACK (20) (native) or MOSFLM (21) and SCALA (22) (derivative).  

- Structural analysis of P. pyrifolia S3-RNase was performed by the program SOLVE (24) to locate the heavy atom structure factor amplitudes.
- **Table I** summarizes the data collected and gives the final refinement statistics. The geometry of the current model is such that the root mean square deviations from the ideal values are 0.022 Å for bond length and 2.02° for bond angles.

Knowledge of the three-dimensional structure is required for further investigation of the recognition mechanism of rosaceous S-RNases at the molecular level. We therefore made a three-dimensional structural analysis of the S-RNases from *Pyrus pyrifolia* (Japanese pear), a member of the Rosaceae family. Seven S-RNases (S1- through S7-RNase) have been identified in and purified from the fruits of *Pyrus pyrifolia* (15, 18). From these we chose S3-RNase for this x-ray crystallographic analysis, because it has only two N-glycosylation sites (19), and its amino acid sequence is highly homologous to that of *P. pyrifolia* S2-RNase (95.5% identity) (15). Identification of the three-dimensional structures of the S1- and S3-RNases will pave the way for a structural comparison that provides high resolution information on molecular recognition between these S-RNases and the pollen S-gene product(s). We report the crystal structure of *Pyrus pyrifolia* S3-RNase at 1.5 Å resolution and discuss the recognition site(s) present on S3-RNase and the structural basis of its enzymatic activity. This is the first report on the three-dimensional structure of a rosaceous S-RNase.

**RESULTS**

Overall Structure—The crystal structure of *P. pyrifolia* S3-RNase was determined by the multiple isomorphous replacement (MIR) and refined to an R-factor of 17.2% at 1.5 Å resolution. Table I summarizes the data collected and gives the final refinement statistics. The geometry of the current model is such that the root mean square deviations from the ideal values are 0.022 Å for bond length and 2.02° for bond angles.

A stereo view of the three-dimensional structure of S3-RNase is shown in Fig. 1. The molecule’s size is ~40 × 50 × 30 Å. This protein has an α + β type structure consisting of eight helices (six α-helices (α1, α2, α4, and α6-α8)), two 3_10-helices (α3 and α5), and seven β-strands (β1-β7) (Fig. 1a). The folding topology of its main chain is very similar to the topologies of the RNase T$_2$ family enzymes: the RNase Rh from *Rhizopus niveus* (31, 32), RNase LE from cultured tomato cells (33), and RNase MC1 from *Macrocystis pyrifera* (34). The molecule’s size is ~40 × 50 × 30 Å. This protein has an α + β type structure consisting of eight helices (six α-helices (α1, α2, α4, and α6-α8)), two 3_10-helices (α3 and α5), and seven β-strands (β1-β7) (Fig. 1a). The folding topology of its main chain is very similar to the topologies of the RNase T$_2$ family enzymes: the RNase Rh from *Rhizopus niveus* (31, 32), RNase LE from cultured tomato cells (33), and RNase MC1 from *Macrocystis pyrifera* (34).
from bitter gourd seeds (34), all of whose tertiary structures have been determined (Fig. 1). Two secondary structural element characteristics are present in S₃-RNase; the length of the α₂-helix is shorter than the lengths of the other RNases, and a very short 310-helix (α₃), Arg⁷⁴-Lys⁷⁶, not seen in the RNase T₂ family, is present (Fig. 2). The electron density map for S₃-

\[ \text{FIG. 1. Stereo diagram showing the overall structure of } P. \text{ pyrifolia } S₃-\text{RNase. } \]

\[ \text{a, stereo ribbon diagram of } S₃-\text{RNase. } N-\text{Glycosylated asparagines, N18 and N116, are shown as } \text{ball-and-stick models. } \]

\[ \text{b, stereo diagram showing the superposition of RNase T₂ family enzymes. } S₃-\text{RNase is in red, RNase Rh in cyan (32), RNase LE in yellow (33), and RNase MC1 in green (34). } \]

\[ \text{c, stereo drawing of the space filling model of } S₃-\text{RNase. Amino acid residues that constitute the P₁, } B₁, \text{ and } B₂ \text{ sites (46), respectively, are colored red, blue, and green. Orientations of the } S₃-\text{RNase molecules in } a, b, \text{ and } c \text{ are identical. } a \text{ and } b \text{ were prepared by the programs MOLSCRIPT (54) and RASTER3D (55); } c \text{ was prepared by the program GRASP (56).} \]
RNase clearly shows four disulfide bridges: Cys$^{35}$-Cys$^{36}$, Cys$^{45}$-Cys$^{46}$, Cys$^{55}$-Cys$^{56}$, and Cys$^{75}$-Cys$^{76}$. The topologies of these bridges are the same as those of the $P_{y}$ pyrifolia $S_{r}$-RNase and Nicotiana alata $S_{r}$-RNase determined by mass spectrometry of fragmented peptides (35, 36).

Catalytic Site—The structure of $S_{r}$-RNase was compared in detail with that of RNase Rh to search for catalytic site residues, because many RNase Rh studies on the structure-function relationship of this RNase $T_{2}$ family enzyme have been done by chemical modification (37–39), site-directed mutagenesis (40–45), and x-ray crystallographic analysis (31, 32). The main-chain frameworks, including the catalytic $P_{1}$ site residues of RNase Rh ($\beta_{2}$ and $\alpha_{4}$), were well superimposable on those of $S_{r}$-RNase (Fig. 1b), indicating that their $P_{1}$ site topologies are similar. The $P_{1}$ site is here defined according to the nomenclature of the subclasses of RNase A given by Richards and Wyckoff (46).

As shown in Fig. 3a, the His$^{33}$ and His$^{88}$ of $S_{r}$-RNase were superimposable geometrically on the general acid and base functionalities at the $B_{1}$ and $B_{2}$ sites of RNase Rh (data not shown). This extended base specificity may be due to substitution of Asp(51) with Ser$^{38}$, to single-sided stacking interaction together with Tyr$^{57}$ (called double-sided stacking) (32, 33). Trp$^{36}$ may bind to the base of the substrate in the same manner as Trp$^{49}$, but the position corresponding to Tyr$^{57}$ is occupied by Asp$^{38}$, consequently double-sided stacking recognition is impossible in $S_{r}$-RNase (Fig. 3b). The base therefore probably is recognized at the $B_{1}$ site of $S_{r}$-RNase by a single-sided stacking interaction with Trp$^{36}$, unlike RNase Rh and RNase LE (32, 33). X-ray crystallographic data on the RNase Rh/2'-AMP complex shows that Asp$^{51}$ contributes to the adenine base preference at the $B_{1}$ site of RNase Rh by hydrogen bonding to the adenine base of 2'-AMP (32, 33). The position corresponding to Asp$^{51}$, however, is occupied by Ser$^{38}$ in $S_{r}$-RNase (Fig. 3b).

The $B_{2}$ site of $S_{r}$-RNase seems to be comprised of Gln$^{9}$, Pro$^{10}$, Asn$^{70}$, Val$^{71}$, Phe$^{72}$, and Phe$^{80}$ (Fig. 3c), which geometrically correspond to the Glu$^{92}$, Pro$^{92}$, Ser$^{93}$, Asn$^{94}$, Gln$^{95}$, and Phe$^{101}$ of RNase Rh that have been identified as components of the $B_{2}$ site based on x-ray crystallographic data for the
complex with d(ApC) (33). In RNase Rh, the base is thought to be recognized at the B₂ site by an aromatic stacking interaction with Phe^{(101)} and van der Waals contact with the side chains of Asn^{(94)} and Gln^{(95)} (33). Similar recognition should occur at the B₂ site of S₃-RNase, because Val^{71}, Phe^{72}, and Phe^{80}, respectively, can be superimposed geometrically on Asn^{94}, Gln^{95}, and Phe^{101} (Fig. 3c).

**Fig. 3. Superposition of the active sites of P. pyrifolia S₃-RNase and RNase Rh.** a, catalytic P₁ site; b, base-binding B₁ site; c, base-binding B₂ site. Names of the P₁, B₁, and B₂ sites are based on the nomenclature of the subsites of RNase A (46). The amino acid residues of S₃-RNase and RNase Rh, respectively, are shown as ball-and-stick and wireframe constructions. The graphics were prepared by using the program MOLSCRIPT (54).

**Hypervariable Region**—One hypervariable (HV) region with an allele-specific sequence is present in the rosaceous S-RNases (Fig. 4a) and is a candidate for the recognition site of...
**FIG. 4.** Location of the HV and PS regions on the primary and tertiary structures of *P. pyrifolia* S₃-RNase.  

**a**, sequence alignment of Rosaceae S-RNases. *P. pyrifolia*, Japanese pear; *Malus domestica*, apple. HV and PS regions are represented as aligned colored bars: HV (blue), HVb (black), PS1 (red), PS2 (yellow), PS3 (green), and PS4 (purple). Amino acid residues that are the same as those of S₃-RNase are shaded, and positions of the amino acid substitutions between S₃- and S₅-RNases are indicated by the symbol # over the alignment. Numbering is the same that for S₅-RNase. Potential N-glycosylation sites are in red with white letters.  

**b**, location of HV and PS regions in the tertiary structure of S₃-RNase. These regions are in the same colors as in a; HV (blue), PS1 (red), PS2 (yellow), PS3 (green), and PS4 (purple). The substituted amino acids in the S₃- and S₅-RNases are labeled and shown as ball-and-stick constructions. The S₃-RNase molecule orientation is the same as in Fig. 1. This figure was prepared by using the program GRASP (56).
pollen S-gene product(s) (15). The HV region of S3-RNase (Pro19-Gln63) is made up of a loop (Pro19-Glu57) and half of an α2-helix (Lys58-Gln63) and is exposed on the molecule’s surface (Figs. 1a and 4b). Although the exposed loop appears to be somewhat flexible, three of its nine amino acid residues (Asn52, Arg54, and Arg56) interact with the amino acid residues inside the molecule by hydrogen bonding (Fig. 5). Asn52N is hydrogen-bonded to the carbonyl group of Asp97 directly, and Asn52O is to the amide of Glu99 via a water molecule. The δ-guanidino group of Arg54 forms water-mediated hydrogen bonds individually with Asn100N and Asp87O. The δ-guanidino group of Arg56 directly forms three hydrogen bonds with Asp80O, Glu101O, and Glu101O’ (Fig. 5).

In contrast, the half of the α2-helix (Lys58-Gln63) located within the HV region has six amino acid residues. Of these, the side chains of Leu60 and Glu61 interact with other amino acids (Fig. 5). The side chain of Leu60 is packed into a hydrophobic space composed of the side chains of Leu64, Asn69, Phe103, and Ile104, and the main chain of Arg56-Glu57. The O5’ and O2’ of Glu61, respectively, are hydrogen-bonded to Arg54N and Asn72N (Fig. 5). These described interactions may fix and stabilize the conformations of the loop and helix; i.e. the HV region.

This HV region is comprised of 15 amino acid residues, ten of which, Pro19, Ile50, Lys55, Lys58, Glu57, Lys58, Leu59, His62, and Glu61, are widely exposed to solvent. The side chains of Ile53, Lys55, Glu57, Lys58, Leu59, and His62, in particular, prominently extend into the solvent (Fig. 5). Given that the HV region is in contact with the pollen factor(s), these residues may have importance in pollen factor binding.

**Positively Selected Regions**—Recognition sites in some proteins (e.g. the major histocompatibility complex (47–49), antigenic surface proteins of parasites and viruses (49), and acrosomal proteins of the abalone (50, 51)) are reported to be regions in which the number of nonsynonymous nucleotide substitutions (dN) exceeds that of synonymous substitutions (dS), and positive selection probably takes place in these regions. Window analysis of the dS and dN in rosaceous S-RNases detected four regions with an excess of dN over dS, in which positive selection may operate (PS1–PS4) (Fig. 4) (52).

PS1 nearly duplicates the HV region. It is interesting that the four positively selected (PS) regions were detected by window analysis of the rosaceous S-RNase genes but that there is only a single region with an allele-specific amino acid sequence. PS1 (HV) and PS2, as well as PS3 and PS4, respectively, are close in the three-dimensional structure of S3-RNase, on either side of the active site cleft (including the P1, B1, and B2 sites) and accessible to solvent (Figs. 1c and 4b).

**Sugar Chains**—P. pyrifolia S3-RNase has two potential N-glycosylation sites, Asn18 and Asn116 (Fig. 1a). The Asn18 site is specific to the S3- and S5-RNases, whereas the Asn116 site is conserved among all the rosaceous S-RNases (Fig. 4a). A two-dimensional sugar map and mass spectrometry of fragmented peptides showed that both sites have heterogeneous N-glycans, Asn18 mainly glycosylated by a chitobiose (GlcNAcβ1-4GlcNAc) and Asn116 primarily occupied by a xylomannose type sugar chain (19). The core structure, Manβ1-4GlcNAcβ1-4GlcNAc, of the xylomannose type sugar chain at Asn116, clearly visible in the difference Fourier map, was modeled to fit the densities (data not shown). No clear electron densities corresponding to the chitobiose at Asn18 and the mannose and xylose moieties of the sugar chain at Asn116 were observed, indicating that their conformations are highly disordered. Because Asn18 and Asn116, respectively, are located at the end of and on the opposite side of the active site cleft (Fig. 1, a and c), the S3-RNase sugar chains probably are not involved in its enzymatic properties and the recognition of the self and non-self.

**DISCUSSION**

The structure of the active site (the catalytic P1 site and substrate binding B1 and B2 sites) of S3-RNase is typical of the structures of the RNase T2 family enzymes. Probably, His83 and His86 function as general acid and base catalysts, and Glu84 and Lys87 stabilize the pentavalent intermediate in the transition state. One marked difference between the P1 sites of S3-RNase and RNase Rh is that the imidazole ring of His83 is rotated about 90° from that of His33 (32) (Fig. 3a), even though the distance between His83N and His86N (7.15 Å) is nearly equal to that between His33N and His36N (6.71 Å) (32). Because S3-RNase has ribonuclease activity (20), rotation of the ring of His83 is not crucial for its catalytic activity. Another difference is that the His104 of RNase Rh, which is expected to bind to the phosphate group of the substrate RNA (32, 41), is replaced by Lys83 in S3-RNase (Fig. 3a). Because the ε-amino group of Lys83 extends outside the P1 site and is about 8 Å from the imidazole ring of His104, it is unlikely that it interacts with the phosphate group of the substrate unless a large induced fit occurs on the side chain of Lys83 during catalysis. A more detailed x-ray crystallographic study of the S3-RNase complex...
with nucleotides is required to clarify how the rotation of the imidazole ring of His$^{55}$ and substitution of His$^{(104)}$ for Lys$^{33}$ affect the ribonuclease activity of $S_3$-RNase.

Although the in vivo substrate of $S$-RNase during the GSI reaction has not been identified experimentally, it must be a pollen (tube) rRNA according to the receptor and inhibitor models (10). Because the overall shape of the active site of $S_3$-RNase is very similar to the active sites of the other RNase T$_2$ family enzymes (except for a few amino acid substitutions), $S_3$-RNase is not likely to have a strict substrate specificity corresponding to the S-alleles. Actually, $S_3$-RNase can hydrolyze dinucleotide monophosphate nonspecifically (data not shown); therefore, it is reasonable that S-allele specificity in the inhibition of pollen tube growth is not expressed due to the enzyme’s restricted substrate specificity but to its interaction with one or more pollen S-gene products, consistent with either the receptor or inhibitor model.

Domain swapping experiments proved that, in the solanaceous S-RNases, two hypervariable regions, HVa and HVb, essentially are responsible for S-allele-specific pollen recognition (13, 14). Only one hypervariable (HV) region appears to be present in the rosaceous $S$-RNases, two hypervariable regions, HVa and HVb, with the sequences of the solanaceous S-RNases (16, 17), the potential displayed represents a range of $-10$ to $+10$ $k_B T$, red being negative and blue positive. This graphic was prepared by using the program GRASP (56).

Fig. 6. Electrostatic surface potential of the HV and PS regions of $P$. pyrifolia $S_3$-RNase. a, surfaces of the HV (PS1) and PS2 regions; b, surfaces of the PS3 and PS4 regions. The potential displayed represents a range of $-10$ to $+10$ $k_B T$, red being negative and blue positive.

Five basic amino acids, Lys$^{31}$, Arg$^{54}$, Lys$^{55}$, Arg$^{56}$, and Lys$^{58}$ and two acidic amino acids, Glu$^{57}$ and Glu$^{61}$, are present in the HV region of $S_2$-RNase (Fig. 4a). Although they seem to form a positively or negatively charged cluster on the molecule’s surface, no such cluster was clearly detected, because the side chains of the basic or acidic amino acids are oriented in different directions (Figs. 5 and 6). Because the δ-guanidino groups of Arg$^{54}$ and Arg$^{56}$ are neutralized by the formation of hydrogen bonds, the surface of HV carries a weaker rather than strong positive charge (Figs. 5 and 6). If the other six $P$. pyrifolia $S$-RNases ($S_1$, $S_3$, and $S_4$ through $S_7$) have the same main-chain framework as $S_2$-RNase, their HV regions also would similarly have a weak positive charge, which might be important for binding to one or more pollen factors (Figs. 4 and 5).

PS regions with an excess of dN over dS, like the HV region, which is a candidate for the binding site to one or more pollen factors, are located on either side of the active site cleft (Fig. 4b). Although there is no marked charge cluster in any of these regions, PS1 (HV), PS2, and PS3 are hydrophilic and weakly basic (Fig. 6). In contrast, PS4 is neutral and hydrophobic despite its exposure to solvent. Why the rosaceous S-RNase has been positively selected on such a wide area of its molecular surface is not clear, but location topology of the PS regions suggests that $S_2$-RNase simultaneously interacts with multiple pollen factors and the substrate RNA.

The amino acid sequence identity between the $P$. pyrifolia $S_3$- and $S_2$-RNases is 95.5%, and there are only nine substitutions in their 200 amino acid residues, all concentrated on the N-terminal half of $S_3$-RNase (Fig. 4a). Two of the nine substitutions, Lys/Arg$^{94}$ and His/Pro$^{95}$, are the most likely to contribute to recognition between the two alleles, because they are located on the molecular surface of the HV region (amino acids in parentheses are those of $S_2$-RNase) (Fig. 4b). The side chain of Lys/Arg$^{94}$ is fully exposed to solvent and, therefore, has no interaction with the other residues (Fig. 5). In contrast, His/Pro$^{95}$ is close to Glu/Ala$^{95}$ in the three-dimensional structure, although at separate locations in the primary structure. His$^{95}$N$^{3+}$ forms a water-mediated hydrogen bond with Glu$^{65}$O$^{4-}$, and both are appreciably exposed to solvent (Fig. 5).

Two possibilities are suggested as to how one or more pollen factors discriminate $S_2$-RNase from $S_3$-RNase. The pollen factor(s) must come in contact with Lys$^{51}$, the site consisting of His$^{62}$ and Glu$^{65}$, or both to recognize the difference between the
S₃- and S₅-RNases. Or, substitution of Hia to Pro produces a large conformational change in the α2-helix and the pollen factor(s) recognize such a conformational change rather than the amino acid substitutions. In general, substitution for proline tends to bend the helix structure (53). An x-ray crystallographic study of *P. pyrifolia* S₅-RNase is in progress in our laboratory. If the three-dimensional structure of S₃-RNase can be determined, a detailed structural comparison can be made of the two S-RNases, which should shed light on the recognition mechanism that operates between S₃- and S₅-alleles.

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