INTRODUCTION

Ribosome-inactivating proteins (RIPs) (also known as polynucleotide:adenosine glycosidases; PAGs) are a family of plant proteins with strong inhibitory activity of protein synthesis [1]. Based on their molecular structure, RIPs are divided into three families [2]. Type-1 RIPs, which consist of a single polypeptide chain with a molecular mass of approx. 30,000 Da, show a wide spectrum of activities toward several nucleic acid substrates, such as ribosomal RNA from different sources, DNA and poly(A) [3–5]. They can remove one or more adenines from the nucleic acid substrates, thus damaging the nucleic acids and affecting their biological activities [6]. Type-2 RIPs are built up of one, two or four protomers, each consisting of two functional peptides [7]. The A chain is similar to the type-1 RIPs, whereas the B chain possesses carbohydrate-binding activity. Though not required for enzymic activity, the additional B chain is helpful for type-2 RIPs to recognize and bind specific receptors on the cell membrane and subsequently to enter the cell [8]. Hitherto, only one type-3 RIP has been identified. The so-called JIP60 [9] from barley leaves is a single-chain protein of 60 kDa, consisting of an N-terminal domain similar to type-1 RIPs linked to an unrelated C-terminal domain with unknown function.

The apparent widespread occurrence of RIPs in flowering plants raises the question of their biological role(s) in plants. Though it has been demonstrated that most probably all RIPs can act on conspecific ribosomes in vitro [10,11], it is still unknown whether RIPs damage ribosomes in planta. If so, the questions have to be addressed as to what the purpose of the in planta inactivation of ribosomes is, and how plants can protect themselves against undesired damage of ribosomes by these toxic proteins [12]. One possible mechanism to prevent RIPs killing the plant cell is to separate the RIPs and ribosomes into different cell compartments. Alternatively, plants can synthesize enzymically inactive forms of RIPs that are activated only under certain conditions [13]. There is also a possibility that some unknown proteins or nucleic acids can bind and regulate the activity of RIPs in vitro. A complex form of pokeweed antiviral protein with an unknown protein factor was identified in the leaves of pokeweed, and this complex shows reduced activity as compared with the native pokeweed antiviral protein [14]. Recently developed in vitro genetics and binding site selection techniques allow the isolation from a nucleic acid library of those nucleic acid fragments that can bind with specific protein ligands (for review see [15] and references therein). RNA molecules that can specifically bind and inhibit a type-1 RIP from Cucurbita pepo and the A chain of a type-2 RIP from Ricinus communis have been selected from a pool of random RNA sequences [16,17]. These RNA sequences, which were shown to inhibit the RNA N-glycosidase activity of the respective RIPs, were quite different from each other and showed no sequence homology with the sequence of the toxin-substrate domain (i.e. the so-called sarcin/ricin loop) in rat 28S rRNA. In contrast with RNA sequences, no evidence has been reported yet that conspecific plant DNA sequences can bind and affect RIP activity.

A few years ago [18] one of the predominant proteins from bulbs of Iris hollandica was identified as a highly active type-1 RIP (called IRIP). This abundant, well-characterized type-1 RIP was used as a model to study the in vitro interactions between a RIP and DNA fragments from the same plant species. Several DNA fragments that specifically bind to IRIP were selected from a pool of conspecific genomic DNA fragments and sequenced. Some, but not all, selected fragments inhibited the RNA N-glycosidase activity of IRIP. None of the bound DNA fragments...
can be used as a substrate by IRIP. The selective binding of IRIP to conspecific non-substrate DNA sequences containing stable hairpin structures suggests that RIPs may act as DNA-binding proteins with a regulatory activity on gene expression.

EXPERIMENTAL

Proteins, antibodies and enzymes

IRIP was isolated as described previously [18] and checked for purity by SDS/PAGE. Primary antibodies against IRIP were raised in male New Zealand White rabbits. Rabbits were injected subcutaneously with 1 mg of purified protein dissolved in PBS buffer and emulsified in 1 ml of Freund’s complete adjuvant. Four booster injections (with 1 mg of purified protein dissolved in PBS) were given with 10-day intervals. Ten days after the final injection, blood was collected from an ear marginal vein. Serum was collected by centrifugation and the antibodies purified by affinity chromatography on a Sepharose 4B column with immobilized IRIP. The monospecificity of the antiserum was checked by Western-blot analysis as described in [19].

The restriction enzymes, kinase and ligase were obtained either from Roche Diagnostics (Mannheim, Germany) or MBI Fermentas GmbH (St Leon-Rot, Germany).

Isolation of genomic DNA

Total genomic DNA of Iris was prepared from young shoots buried within resting Iris bulbs. The plant tissue was ground in liquid N\textsubscript{2} with mortar and pestle, and incubated with prewarmed (at 65 °C) DNA isolation buffer [1.0 %, sarcosyl, 0.8 M NaCl, 0.022 M EDTA, pH 8.0, 0.22 M Tris/HCl, pH 7.8, 0.8 % cetlytrimethylammonium bromide (‘CTAB’), 0.14 M mannitol and 0.014 % 2-mercaptoethanol]. An equal volume of chloroform/isopropylalcohol was added, and the tube was inverted gently to form an emulsion. After incubation for 10 min with occasional gentle inversion, the mixture was centrifuged at 4000 °C for 5 min to separate the phases. The aqueous layer was transferred to a new centrifuge tube and an equal volume of isopropanol was added. After standing at room temperature for 5 min, the DNA was precipitated by centrifugation at 4000 °C for 5 min and dissolved in double-distilled water. The DNA preparation was then treated with RNase to remove contaminating RNA and extracted with phenol/chloroform and precipitated overnight with ethanol and glycogen at −20 °C. The DNA was amplified by PCR and used as the starting material for the next round of binding to IRIP. Four cycles of binding, isolation and amplification were performed. Finally, the selected DNA molecules were either analysed by Southern blotting or cloned into a vector.

PCR, cloning and sequencing of the amplified DNA molecules

PCR reactions were typically performed in a PerkinElmer DNA Thermal Cycler Model 480 in the step-cycle mode. The PCR program consisted of an initial denaturation step at 94 °C for 3 min, followed by 25 cycles of 94 °C for 1 min, 50 °C for 2 min, and 72 °C for 2 min. The final step was an incubation at 72 °C for at least 10 min in order to fulfill the requirement of the TOPO\textsuperscript{®} PCR cloning kit (Invitrogen, Carlsbad, CA, U.S.A.). The amplified DNA from the final PCR reactions was purified by High Pure PCR Product Purification kit (Roche Diagnostics) and cloned into Escherichia coli cells using the TOPO\textsuperscript{®} PCR cloning kit according to the manufacturer’s instructions. The plasmids were isolated from purified single clones on a miniprep scale using the alkaline lysis method described by Mierendorf and Pfeffer [21]. They were then cleaved with EcoRI to check the presence and length of the insertion fragments. Manual sequencing of both ends of the DNA clones was performed by the dyeoxy method [22].

Purification of DNA fragments

The plasmids containing the binding sequences were cleaved with EcoRI to release the binding sequence, and run on a 1.5 % agarose gel. The gel was stained in 0.5 mg/ml ethidium bromide, and the DNA visualized on an UV transilluminator. DNA fragments were excised from the gel and purified using the Mermaid\textsuperscript{®} kit (for purification of low-molecular-mass DNA fragments) (Bio 101 Inc., La Jolla, CA, U.S.A.).

Sequence analysis

Analysis of sequence similarity between different sequences was performed using the program GenePro (Riverside Scientific, Seattle, WA, U.S.A.). A search for hairpin structures and calculation of the free energy was performed using the HAIRPIN package from the programme PC/GENE (IntelliGenetics, Inc., Mountain View, CA, U.S.A.). The sequences (without catch-linkers) were also compared with entries in GenBank\textsuperscript{®} using a Basic BLAST search (BLAST 2.0 version) [23].
The Endo’s fragment was detected by running the RNA in a reaction mixture, in which no DNA fragment was added to the samples. The RNA N-glycosidase activity of IRIP was determined by the method of Endo et al. [3] with minor modifications. Ribosomes from rabbit reticulocyte lysate and 60 μl of RIP buffer (25 mM Tris/HCl, pH 7.4, 5 mM KCl, 5 mM MgCl₂, 2 ng/ml BSA and 1 mM dithiothreitol) were added to form IRIP–DNA complexes. After standing at room temperature for 30 min, 65 μl of the rabbit reticulocyte lysate and 60 μl of RIP buffer (25 mM Tris/HCl, pH 7.4, 5 mM KCl and 5 mM MgCl₂) were added, and the mixture was incubated at 37°C for 30 min. SDS (final concentration 0.5%) was added to stop the reaction. RNA was isolated by phenol/chloroform extraction and precipitated by adding 2.5 vol. of ethanol. The recovered RNA was divided into two parts: one part was used as control, and the second part treated with acidic aniline to cleave the so-called Endo’s fragment. The Endo’s fragment was detected by running the RNA in a 1.2% agarose-formamide gel and visualized by UV detection. To quantify the extent of depurination of the rRNA, the ratio of the 28S and 5.8S rRNA was determined as described in [24]. Agarose gels were scanned with an AlphaImager™ 2200 Documentation & Analysis system (Alpha Innotech Corporation, San Leandro, CA, U.S.A.), which allows the total absorbance for each band to be calculated and corrected for the background. This assumes that the 28S and 5.8S rRNA are present in equimolar amounts in the ribosomes. A sample from a reaction mixture, in which no DNA fragment was added to IRIP, was used as a control. The depurination activity of this control sample was defined as 100%. The results presented here are the average of two measurements.

**Assay for PAG activity**

The possible PAG activity of RIPs on DNA fragments was quantified using the method developed by Zamboni et al. [25], as described by Wang and Tumer [26]. Purified DNA fragments (approx. 2 ng) were incubated with 6 μg of RIP in a 50 μl volume containing RIP buffer at 37°C for 1 h. After incubation, DNA fragments were precipitated with 2.5 vol. of ethanol. The ethanol-soluble fractions (containing the released adenine residues) were diluted to 1 ml with water, and 0.4 ml of 0.14 M chloroacetaldehyde containing 0.1 M sodium acetate (pH 5) was added to each sample. The samples were incubated at 80°C for 1 h. After cooling to room temperature, fluorescence was measured in a Spex-25 spectrophotofluorometer at an excitation wavelength of 310 nm and an emission wavelength of 410 nm. Adenine (Sigma) at concentrations ranging from 1–1200 pmol was used as a standard for the calculation of the adenine concentrations in the samples.

**RESULTS**

**Selection of genomic DNA fragments that bind to IRIP**

The strategy used to select DNA fragments that bind to IRIP was essentially the same as that described by Kinzler and Vogelstein [20] for RNA fragments. Genomic DNA from *Iris* was first extensively cleaved with *MboI* (recognition sequence GATC) to produce a library of DNA fragments of 50–2000 bp (average length 200 bp). The genomic DNA fragments were then ligated with two linkers containing a half *XhoI* site and one *EcoRI* site to facilitate further amplification and subcloning. Catch-linked DNA fragments were incubated with RIP to generate the RIP–DNA complexes. After incubation, the IRIP–DNA complexes were precipitated by adding specific antibodies against the IRIP to separate the bound DNA fragments from the unbound DNA fragments. The precipitates were washed, resuspended in water, and digested with *EcoRI* to release binding fragments. After the restriction enzyme was inactivated by heating at 75°C for 5 min, 150 pg of RIP in 15 μl of water and 10 μl of binding buffer (25 mM Tris/HCl, pH 7.4, 25 mM KCl, 5 mM MgCl₂, 2 mg/ml BSA and 1 mM dithiothreitol) were added to form IRIP–DNA complexes. After standing at room temperature for 30 min. SDS (final concentration 0.5%) was added to stop the reaction. RNA was isolated by phenol/chloroform extraction and precipitated by adding 2.5 vol. of ethanol. The recovered RNA was divided into two parts: one part was used as control, and the second part treated with acidic aniline to cleave the so-called Endo’s fragment. The Endo’s fragment was detected by running the RNA in a 1.2% agarose-formamide gel and visualized by UV detection. To quantify the extent of depurination of the rRNA, the ratio of the 28S and 5.8S rRNA was determined as described in [24]. Agarose gels were scanned with an AlphaImager™ 2200 Documentation & Analysis system (Alpha Innotech Corporation, San Leandro, CA, U.S.A.), which allows the total absorbance for each band to be calculated and corrected for the background. This assumes that the 28S and 5.8S rRNA are present in equimolar amounts in the ribosomes. A sample from a reaction mixture, in which no DNA fragment was added to IRIP, was used as a control. The depurination activity of this control sample was defined as 100%. The results presented here are the average of two measurements.

**Table 1 Characteristics of the IRIP-binding sequences**

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<tr>
<th>DNA-binding sequence</th>
<th>Accession number in GenBank®</th>
<th>Number of bp</th>
<th>Number of possible hairpin structures</th>
<th>Total number of BLAST hits in database</th>
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**Southern-blot analysis**

PCR products of the selected DNA fragments from the first to the fourth selection round were separated by electrophoresis on a 1% agarose gel, transferred to Immobilon N membranes (Millipore, Bedford, MI, U.S.A.) and hybridized at 65°C. The IRIP-binding DNA sequences isolated from agarose gels were radiolabelled by the random primer method and used as hybridization probes.

**Assay for RNA N-glycosidase activity**

The RNA N-glycosidase activity of IRIP was determined by the method of Endo et al. [3] with minor modifications. Ribosomes from rabbit reticulocyte lysate were used as a substrate. The purified plasmids containing binding sequences were first cleaved with EcoRI to release binding fragments. After the restriction enzyme was inactivated by heating at 75°C for 5 min, 150 pg of IRIP in 15 μl of water and 10 μl of binding buffer (25 mM Tris/HCl, pH 7.4, 25 mM KCl, 5 mM MgCl₂, 2 mg/ml BSA and 1 mM dithiothreitol) were added to form IRIP–DNA complexes. After standing at room temperature for 30 min, 65 μl of the rabbit reticulocyte lysate and 60 μl of RIP buffer (25 mM Tris/HCl, pH 7.4, 25 mM KCl and 5 mM MgCl₂) were added, and the mixture was incubated at 37°C for 30 min. SDS (final concentration 0.5%) was added to stop the reaction. RNA was isolated by phenol/chloroform extraction and precipitated by adding 2.5 vol. of ethanol. The recovered RNA was divided into two parts: one part was used as control, and the second part treated with acidic aniline to cleave the so-called Endo’s fragment. The Endo’s fragment was detected by running the RNA in a 1.2% agarose-formamide gel and visualized by UV detection. To quantify the extent of depurination of the rRNA, the ratio of the Endo’s fragment/5.8S rRNA was determined as described in [24]. Agarose gels were scanned with an AlphaImager™ 2200 Documentation & Analysis system (Alpha Innotech Corporation, San Leandro, CA, U.S.A.), which allows the total absorbance for each band to be calculated and corrected for the background. This assumes that the 28S and 5.8S rRNA are present in equimolar amounts in the ribosomes. A sample from a reaction mixture, in which no DNA fragment was added to IRIP, was used as a control. The depurination activity of this control sample was defined as 100%. The results presented here are the average of two measurements.

**Assay for PAG activity**

The possible PAG activity of RIPs on DNA fragments was quantified using the method developed by Zamboni et al. [25], as described by Wang and Tumer [26]. Purified DNA fragments (approx. 2 ng) were incubated with 6 μg of RIP in a 50 μl volume containing RIP buffer at 37°C for 1 h. After incubation, DNA fragments were precipitated with 2.5 vol. of ethanol. The ethanol-soluble fractions (containing the released adenine residues) were diluted to 1 ml with water, and 0.4 ml of 0.14 M chloroacetaldehyde containing 0.1 M sodium acetate (pH 5) was added to each sample. The samples were incubated at 80°C for 1 h. After cooling to room temperature, fluorescence was measured in a Spex-25 spectrophotofluorometer at an excitation wavelength of 310 nm and an emission wavelength of 410 nm. Adenine (Sigma) at concentrations ranging from 1–1200 pmol was used as a standard for the calculation of the adenine concentrations in the samples.
DNA fragments that bind to IRIP are not sequence-specific

Most of the plasmids contained inserts ranging in length between 50 and 2000 bp, which is in good agreement with the length of the catch-linked genomic DNA fragments. To compare the sequence similarity among these DNA fragments, 14 clones of IRIP-binding DNA fragments were sequenced (Table 1). It is noteworthy that only clones with an insert length smaller than 400 bp were selected for sequencing. All the DNA fragments contained the linker sequence at each end, ruling out the possibility that some fragments were derived from contaminating DNA. Most DNA fragments also contained the GATC sequence at one end or at both ends, which is a characteristic of MboI cleavage. The length of the sequenced IRIP-binding DNA fragments ranged from 89–380 bp, and only a single copy of each fragment was identified. Attempts to trace conserved sequences in all the DNA fragments were not successful because the sequences were highly diverse, suggesting that IRIP does not recognize genomic DNA fragments in a strictly sequence-specific manner.

A BLAST search was performed to trace possible similarities between the binding sequences predicted for IRIP, and sequences deposited in GenBank®. No complete matches were found between the identified sequences and the sequences in the database; however, in many cases partial matches were observed. For example, the DNA fragment I7 shows homology with the Arabidopsis thaliana BAC clone F9G14 (chromosome 5, accession number emb}AL162973.1}ATF9G14). In this sequence a stretch of 40 nt matches exactly part of the sequence of I7 (error value 1.1). Fragment I17 shows homology with clones T16B24 and F12L6 (accession numbers gb}AC004697.2}AC004697) of A. thaliana located on chromosome II, section 211 of 255. A stretch of 40 nt of these sequences matches exactly a part of the sequence of I17 (error value 0.37). DNA fragment I12 shares the highest sequence similarity with plant genomic sequences. In total, 97 matches with an error value < 0.002 were identified for I12, 53 matches being plant genome sequences. Among these plant genome sequences nine matches have an error value < 0.002.

Most IRIP-binding DNA fragments contain possible energy-stable hairpin structures

RIPs are known as RNA N-glycosidases which recognize a conserved GAGA hairpin structure (Figure 1A). Therefore the possible hairpin structures in the DNA-binding sequences were determined, and the free energy (dG) required to maintain these hairpin structures calculated (Table 1). A total of 12 out of the 14 IRIP-binding DNA fragments contain hairpin structures (except I1 and I5). The number of hairpin structures for a given fragment ranges from 1–8. Most of the presumed hairpin structures are at the low-energy level. A selection of the possible hairpin structures found in the IRIP-binding DNA fragments with a dG < −7 kcal are shown in Figure 1(B).

Southern-blot analysis

To confirm the occurrence of the IRIP-binding sequences throughout the selection cycles, the clones encoding I7 and I18 were selected for Southern-blot analysis. The insert encoding I7 total mixture of the genomic DNA fragments. The IRIP–DNA–antibody complexes were then dissociated with phenol/chloroform extraction, and the DNA fragments recovered by ethanol precipitation and amplified using the two catch-linkers as primers. The amplified DNA fragments were used as the substrate for the second round of selection. In total, four rounds of selection were carried out. After the final amplification round, the DNA fragments were cloned into the TOPO pCR® 2.1-TOPO® vector. Plasmids from single bacterial colonies were purified and analysed.

Several control experiments were designed to assure the success of this strategy. When catch-linked DNA was incubated with either IRIP or anti-IRIP antibody, no pellet was formed after centrifugation; when anti-IRIP antibody was incubated with IRIP without catch-linked DNA, pellets were observed, but no DNA fragments were amplified after PCR reaction.
The possible PAG-activity can also be detected by treatment of RIP-modified nucleic acid substrates with acidic aniline. When adenine(s) is removed from nucleic acids, the modified site(s) become sensitive to β-elimination, and as a result the nucleic acids are cleaved at the abasic site(s) in the presence of acidic aniline. Several DNA fragments (I7, I12, I18 and I23) were incubated with IRIP at a 100:1 protein/DNA molar ratio for 1 day at 37°C, and subsequently treated with acidic aniline. No cleavage products could be detected (results not shown), which confirms that IRIP does not act as a PAG on the IRIP-binding DNA fragments.

**DISCUSSION**

During the last few years several reports appeared on the DNA-binding activities of RIPS from different plant species. However, all the identified RIP-binding DNA fragments were either derived from plasmid DNA or were oligonucleotides [27–29]. To check whether RIPS have the ability to bind to specific DNA fragments from the plant genome, and, if so, whether these fragments possibly affect the activity of the RIPS, we used a combination of immunoprecipitation and a PCR-associated method to select DNA fragments from the *Iris* genome that have the ability to bind IRIP.

The results of the selection experiments indicate that IRIP selectively binds to some conspecific genomic DNA fragments. Analysis of the RIP-binding DNA fragments revealed little or no conserved DNA sequences, indicating that IRIP does not bind genomic DNA in a strictly sequence-specific manner. Most of the IRIP-binding DNA fragments have little, if any, effect on the RNA N-glycosidase activity of the enzyme. Only three fragments (I17, I18 and I26) are capable of inhibiting the depurination activity of IRIP on rabbit reticulocyte ribosomes by 30–40%. Interestingly, none of the IRIP-binding DNA fragments serves as a substrate for the PAG activity of IRIP (which has been shown to deadenylate a wide range of nucleic acids) [18]. This implies that the weak inhibition of the RNA N-glycosidase activity of IRIP by some of the DNA fragments is most probably not due to an interference with the catalytic activity of IRIP, but rather due to a binding mechanism, which may cause a steric hindrance or keep the RIP molecule in a low-active or inactive conformation.

The identification of conspecific RIP-binding DNA fragments in the genome of *Iris* has important consequences. First, our results demonstrate for the first time that conspecific genomic DNA fragments are capable of binding and affecting the activity of RIPS. Though no highly conserved motifs could be identified in these DNA fragments, there is no doubt that RIPS bind very selectively to certain fragments, presumably through the recognition of stable hairpin structures in the DNA sequences. Secondly, the binding of IRIP to specific DNA sequences without subsequent deoxyadenylation of the bound sequence may have profound effects on the replication and/or transcription of the target DNA. For example, one can imagine that binding of RIP to specific sequences may activate or inactivate specific genes. Such a RIP-mediated regulation of gene expression may explain why some RIPS, such as JIP60 [30] and IRIP (W. J. Peumans, B. Hause and E. J. M. Van Damme, unpublished work), are (partly) located in the nucleus. Moreover, gene inactivation by RIPS may also explain why in many cases the synthesis of RIPS is closely associated with senescence [9,31]. Though these presumed regulatory activities are still speculative, they can explain some biological activities of RIPS that definitely do not rely on their RNA N-glycosidase or PAG activity. For example, the selective toxicity of type-1 RIPS, such as momordica anti-HIV protein-30 (‘MAP30’) from *Momordica charantia* and gelonin from *Gelonium multiflorum*, towards human/animal cancer cells and cells infected with viruses [32,33], which cannot be explained in terms of a selective enzymic activity [28,34], may rely on the inactivation of crucial genes in these cells. These considerations on a possible novel activity of RIPS justify detailed...
studies of RIP–nucleic acid interaction in vitro, not only because these studies may give valuable clues to the understanding of the role and mode of action of RIPS in planta, but also because they may contribute to an improvement of medical and therapeutical uses of RIPS.

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REFERENCES


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