Theor Appl Genet (1989) 78:581–588

Characterization of proteins associated with self-incompatibility in *Solanum tuberosum*

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Received April 20, 1989; Accepted May 9, 1989
Communicated by H.F. Linskens

Summary. The gametophytic self-incompatibility system of *Solanum tuberosum* is controlled by a single locus, designated as the *S*-locus. Protein extracts from potato styles of defined *S*-genotypes have been analysed by two-dimensional gel electrophoresis, and found to contain a group of basic glycoproteins. Each genetically determined allele S_1 to S_4 was associated with the presence of one of a number of these polypeptides differing slightly in isoelectric points (in the range 8.3–>9.1) and/or apparent molecular weight (ranging from 23,000 to 29,000). Two abundant basic polypeptides, one of which is apparently not glycosylated, were present in all genotypes examined. Amino-terminal protein sequence determinations revealed homologies of the *S. tuberosum* stylar proteins S_2, S_3 and S_4 with SI-associated polypeptides from *Nicotiana alata* and *Lycopersicon peruvianum*. With an oligonucleotide generated to the potato-S_2 N-terminal protein sequence, it was possible to detect a style-specific RNA species of ~920 nucleotides. The oligonucleotide also behaved as an allele-specific probe when hybridized to total RNA of different *S*-genotypes.

Key words: *Solanum tuberosum* – Self-incompatibility – *S*-alleles – Glycoprotein – Style

Introduction

The cultivated potato (*S. tuberosum* ssp. *tuberosum*) exhibits gametophytic self-incompatibility (SI) in the diploid state (2n = 2x = 24) (Pandey 1962a, b; Cipar et al. 1964). As in many other species of the Solanaceae, SI is governed by a series of codominant alleles at a single genetic locus, *S*. Tetraploid potato lines (2n = 4x = 48), for example cultivars, are expected to be self-compatible, a situation attributed to the action of two different *S*-alleles within one pollen grain (Crane and Lawrence 1929). The genetical principles underlying the action of the *S*-locus in plants have been extensively studied (reviewed in De Nettancourt 1977).

One approach to elucidate the molecular basis of self-incompatibility has been to look for polypeptides associated with and possibly encoded by the *S*-locus. Such SI-associated polypeptides have been identified in both sporophytic (Hinata and Nishio 1978; Nasrallah and Nasrallah 1984) and gametophytic (Bredemeijer and Blaas 1981; Kamboj and Jackson 1986; Mau et al. 1986) systems. In *Nicotiana alata,* the best-characterized gametophytic SI-system to date, SI-associated polypeptides are abundant, basic glycoproteins of M_rs (molecular weight) between 28,000 and 32,000. Each *S*-allele examined is linked to a polypeptide differing in charge and/or molecular weight.

Both for *N. alata* and *L. peruvianum,* SI-associated proteins have been located in sectioned styles. The highest concentrations are found just below the stigma, which is the normal zone of inhibition of pollen tube growth observed in incompatible interactions. Immature styles, which are phenotypically self-compatible, contain only low amounts of SI-associated proteins, confirming their spatial and temporal correlation with the expression of SI (Anderson et al. 1986; Mau et al. 1986). The major SI-associated proteins of *N. alata* have been further characterized by N-terminal sequencing, followed by isolation of corresponding cDNA clones (Anderson et al. 1986).

We have examined style extracts from diploid potato clones of defined SI-genotype for the presence of SI-associated polypeptides, and report here evidence for a family of proteins showing both similarities and differences to SI-associated polypeptides characterized in other members of the Solanaceae.

Materials and methods

Plant material

Plant material used in the present study was as follows: (1) Commercial tetraploid *S. tuberosum* clones cv 'Hansa' and 'Roxy' with unknown SI-genotypes; (2) a dihaploid (2n = 2x = 24) *S. tuberosum* clone (1506/60) with unknown SI-genotype, which was originally isolated from a tetraploid clone; (3) self-incompatible diploid *S. tuberosum* clones of genetically determined SI-genotypes S_1S_2 (191/2), S_1S_3 (192/2), S_1S_2 (193/2), S_3S_4 (194/1), S_1S_4 (195/5) and S_1S_3 (196/1). Classified diploid genotypes were kindly made available by Prof. J.G.Th. Hermsen (Wageningen, The Netherlands).

Potato styles for analysis of proteins and RNA were collected with forceps and immediately frozen in liquid nitrogen. (The term "style" is used for that segment of the pistil separated from the ovary, and thus includes the stigma.) Styles from completely closed flowers were considered to be immature, while those from open flowers having anthers with still enclosed pollen sacs were regarded as mature. All plant tissues were stored at −70 °C until use.

Extraction of stylar proteins and analysis by two-dimensional gel electrophoresis

Extraction of proteins was as described by Hurkman and Tanaka (1986), except that the extraction buffer was supplemented with 2 m*M* PMSF (Phenylmethylsulfonyl fluoride) and 10 µg/ml Leupeptin. Proteins were extracted from about 0.15 g frozen style tissue. The protein-pellet was resuspended in 100 µl 50 m*M* TRIS (2-amino-2[hydroxymethyl]-1,3-propandiol)-HCl pH 6.8, 0.5 m*M* $MgCl_2$, 1% (w/v) SDS (Ames and Nikaido 1976) for 30 min at room temperature, boiled for 3 min and the remaining insoluble material was removed by centrifugation. Protein content was measured according to Bradford (1976) using a standardized Bio-Rad protein assay. Protein samples could be stored at −70 °C for several months.

Proteins were resolved by IEF (isoelectric focussing) or NEPHGE (non-equilibrated pH-gradient electrophoresis) in the first dimension followed by SDS-PAGE in the second dimension, as described by O'Farrell (1975) and O'Farrell et al. (1977), respectively, with slight modifications. Protein solution of desired concentration (60 µg) was mixed with 2 vol of 2-D sample buffer [9.5 *M* urea, 2% LKB ampholines pH 3.5–10, 0.5% dithiothreitol (DTT), 6% Nonidet P-40] (Ames and Nikaido 1976, modified) and urea to obtain a saturated solution. After removing undissolved urea by centrifugation, the samples were applied onto cylindrical electrofocussing gels (15 cm length, 1.5 mm internal diameter) containing 4% (w/v) acrylamide, 0.21% (w/v) N,N'-methylene bis-acrylamide, 9.15 *M* urea, 2% (v/v) NP-40 and 1.4% (w/v) ampholines pH 3.5–10, 0.6% (w/v) ampholines pH 9–11 for IEF or 1% (w/v) ampholines pH 7–9, 0.8% (w/v) ampholines pH 8–9.5, 0.5% (w/v) ampholines pH 9–11 for NEPHGE. Both for IEF and NEPHGE, samples were loaded on the acid end of the gel (O'Farrell et al. 1977; Sanchez-Martinez et al. 1986), covered completely with overlay solution (8.0 *M* urea, 2% NP-40, 1% ampholines pH 3.5–10) and electrofocussed for 20 min at 200, 300, 400 and 500 V, 15 h at 800 V and 1 h at 1,000 V or 4 h at 300 V, respectively.

The rod gels were equilibrated in 10% (w/v) glycerol, 2.3% (w/v) SDS, 5% (w/v) DTT and 62.5 m*M* TRIS-HCl pH 6.8 for 30 min with gentle agitation, and placed individually onto 12% SDS-polyacrylamide gels overlaid with a 4% polyacrylamide stacking gel. Electrophoresis was carried out according to Laemmli (1970).

Protein detection was either by silver staining (Morrissey 1981) or, for glycoproteins, by Concanavalin A-Peroxidase staining, using 4-chloro-1-naphthol as substrate (Hawkes 1982). For the latter, style proteins, separated by 2-D gel electrophoresis, were electrotransferred to nitrocellulose (Schleicher-Schüll, Dassel, FRG) according to Towbin et al. (1979). As a control for the protein-transfer, the filter was first stained for 10 min with 0.2% (w/v) Ponceau S in 3% (w/v) TCA (trichloroacetic acid).

Purification and amino-terminal sequencing of SI-associated proteins

A method for isolating SI-associated components from styles is based on the fact that most of the examined SI-proteins of gametophytic systems have a high pI. As a result, SI-associated polypeptides can be purified by anion exchange chromatography at a pH just below their pI. The purification was accomplished essentially as described by Anderson et al. (1986), with diethylaminoethyl (DEAE)-cellulose (DE-52, Whatman Maidstone) as exchanger material. Frozen styles (0.4 g) were ground to a fine powder in liquid nitrogen using a mortar and pestle, transferred to an Eppendorf-tube to which was added 2 ml ice-cold extraction buffer (50 m*M* TRIS-HCl pH 8.5, 10 m*M* EDTA (ethylene-diaminetetraacetic acid), 1 m*M* $CaCl_2$, 1 m*M* DTT, 1 m*M* PMSF, 10 µg/ml Leupeptin). The homogenate was mixed for 1 min, centrifuged twice (8,500 × g, 5 min, 4 °C) to get rid of any insoluble material and the supernatant was equilibrated with 5 m*M* NH_4HCO_3 pH 8.6, 1 m*M* NaCl, 1 m*M* $CaCl_2$, 1 m*M* EDTA by passage through a Sephadex G-25 column (bed volume 2 ml). Protein-containing fractions were pooled and applied in 500 µl aliquots to a DEAE-cellulose column (bed volume 2 ml) equilibrated with the same ammonium bicarbonate buffer. Under these conditions SI-associated proteins do not bind. The unbound fractions were collected on ice and the elution was continued with ammonium bicarbonate buffer for another 2–3 ml. The purified protein-containing samples were combined, concentrated by lyophilization and subsequently used for amino-terminal sequencing.

Purified protein samples for N-terminal sequencing were separated by SDS-polyacrylamide gel electrophoresis (Laemmli 1970), the proteins were transferred to siliconized glass-fiber sheets (GF/C, Whatman) (Eckerskorn et al. 1988) and stained with 0.1% (w/v) Coomassie blue R 250 in 40% (v/v) methanol/10% (v/v) acetic acid. Protein bands were cut out and the filter strips put into the cartridge of a gas-phase sequencer (470A, Applied Biosystems). The polypeptides were degraded to phenylthiohydantoin derivatives and analysed by an on-line isocratic high-performance liquid chromatography system (Lottspeich 1985).

Isolation of RNA and Northern blot analysis

Isolation of total RNA from potato style tissue was as described by Logemann et al. (1987) with the following modifications. Frozen style tissue (0.3 g) was crushed to a fine powder in liquid nitrogen and transferred to a 2.2-ml Eppendorf-tube. After addition of sand and 400 µl guanidine buffer (8 *M* guanidine-hydrochloride, 20 m*M* MES (4-morpholineethanesulfonic acid), 20 m*M* EDTA, 50 m*M* β-mercaptoethanol at pH 7.0), the tissue was homogenized with a fast rotating pin fitting into the tube, frozen in liquid nitrogen and homogenized again until the extract was completely thawed. The homogenate then was mixed with another 200 µl guanidine buffer and centrifuged for 10 min (3,000 × g, 4 °C). The supernatant was collected, the pellet resuspended in 200 µl guanidine buffer and centrifuged again. Both supernatants were combined, extracted twice with 1 vol phenol/chloroform (1:1) and centrifuged for 10 min at 14,000 × g. The aqueous phase was mixed with 0.7 vol ethanol and 0.05 vol 1 *M* acetic acid and stored overnight for precipitation of the RNA.

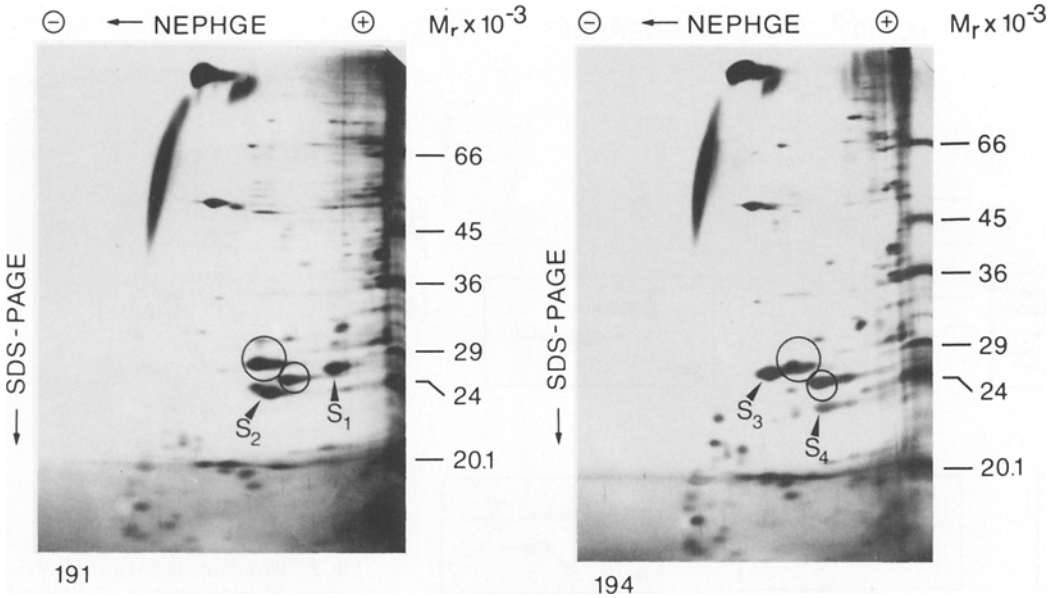

Fig. 1. Two-dimensional gel electrophoresis of style extracts of potato clones 191 (S_1S_2) and 194 (S_3S_4). Stylar proteins were separated by NEPHGE/SDS-PAGE. Polypeptides were detected by silver stain. Each extract shows in the high pI-range two specific *S*-allele-associated proteins indicated by *arrows*, as well as two constant polypeptides (*circled*, ○ = S_{k1}, o = S_{k2})

The precipitated RNA was pelleted (10 min, 14,000 × g, 4 °C) and washed twice with 3 *M* sodium acetate pH 5.0. The salt was removed by a final wash with 70% ethanol and the RNA was subsequently dissolved in sterile water.

RNA samples (90 µg) were denatured with formaldehyde and electrophoresed in formaldehyde/1.5% agarose gels (Maniatis et al. 1982) using a running buffer containing 20 m*M* morpholinopropanesulphonic acid pH 7.0, 5 m*M* sodium acetate and 1 m*M* EDTA. The size-fractionated RNAs were transferred to nylon filters (Hybond N, Amersham Buchler Braunschweig) and hybridized with two different oligodeoxyribonucleotide probes. A 17-mer oligonucleotide (with 16-fold degeneracy), based on the amino-terminal sequence of the potato S_2-protein, and a 47-mer oligonucleotide, based on the S_2-cDNA sequence from *N. alata* (Anderson et al. 1986) were synthesized by the solid-phase phosphoramidite method (Beaucage and Caruthers 1981) using an Applied Biosystems model 380B DNA synthesizer. Both oligonucleotides were labelled at the 5′ end using γ-[^{32}P]-ATP (5,000 Ci/mmol) and T_4 polynucleotide kinase (BRL) ($6 \times 10^6 - 7 \times 10^7$ cpm/0.2 µg DNA), RNA-blot filters were prehybridized for 2 h at 37 °C in 1 × Denhardt's, 6 × SSC, 0.5% SDS, 100 µg/ml sonicated, denatured herring-sperm DNA and 0.05% sodium pyrophosphate. Hybridization (20 h at 37 °C) was carried out in 1 × Denhardt's, 6 × SSC, 20 µg/ml tRNA, 0.05% sodium pyrophosphate. Filters were subsequently washed several times in 6 × SSC, 0.05% sodium pyrophosphate at 37 °C (Woods 1984). Hybridization signals were visualized by autoradiography.

Results

Identification of S-allele associated proteins in styles of S. tuberosum

Styles for the protein analysis were collected from flowers at a stage when self-incompatibility is expressed, but prior to anther dehiscence, to avoid contamination with pollen.

Preliminary analysis of the proteins on 2-D IEF/SDS-PAGE gels revealed the presence of abundant basic polypeptides, as seen for other SI-associated polypeptides (Anderson et al. 1986; Mau et al. 1986; Kamboj and Jackson 1986). The potato stylar proteins were, therefore, optimally resolved using the non-equilibrated pH-gradient electrophoresis (NEPHGE) technique.

Extracts of the clones 191/2 (S_1S_2) and 194/1 (S_3S_4) were examined by two-dimensional gel electrophoresis (Fig. 1). Both extracts showed a relatively similar two-dimensional distribution of protein spots. Within the quantitatively prevalent group of polypeptides with M_r 23,000–29,000, there were protein spots for both genotypes which corresponded to certain *S*-alleles. Certain proteins outside this group differed somewhat in intensity among the genotypes, but none of these spots could be associated with the *S*-genotype. Based on these separations and analogous separations for the other genotypes, for clone 194/1 (S_3S_4) a 23-kilodalton protein was linked to S_4 and a 27-kilodalton protein was linked to S_3. Protein extract of clone 191/2 (S_1S_2) contained a 24-kilodalton polypeptide, correlating with S_2 and another 27-kilodalton protein, which was assigned to S_1. As well as the putative *S*-proteins indicated (Fig. 1), there were two proteins of M_r 26,000 and 28,000 (circled in Fig. 1) with a high pI common not only to both *S*-genotypes shown, but also to all other genotypes tested. Since both polypeptides seemed to be related to the "*S*-protein" group by virtue of their M_r, charge and abundance, they

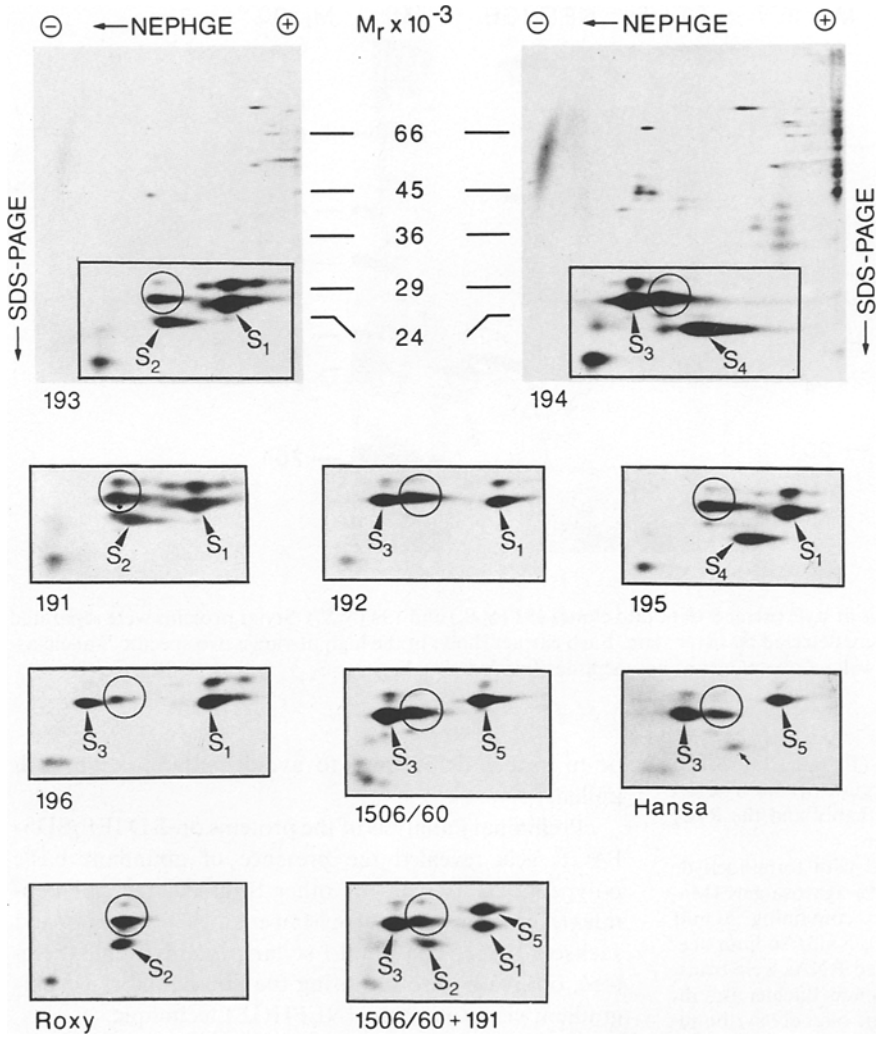

Fig. 2. Two-dimensional glycoprotein pattern of style extracts of different potato clones (genetically defined genotypes given in *brackets*): 191 (S_1S_2), 192 (S_1S_3), 193 (S_1S_2), 194 (S_3S_4), 195 (S_1S_4), 196 (S_1S_3). Polypeptides were separated by NEPHGE/SDS-PAGE, transferred to nitrocellulose and glycoproteins were subsequently detected enzymatically with Con A-Peroxidase. The complete 2-D pattern is only shown for two extracts. For all other style extracts, only the framed area where the *S*-polypeptide group migrates is shown. *S*-proteins are marked with *arrowheads*. The S_{k1}-polypeptide, which is common to all extracts, is *circled*

were designated as S_{k1} and S_{k2}, respectively. Similar constant, basic proteins have been noted in stylar extracts of *L. peruvianum* (Mau et al. 1986) and *Prunus avium* (Rosaceae) (Raff et al. 1981).

To further characterize the *S. tuberosum* SI-associated polypeptides, style extracts of all available *S*-genotypes and three unknown potato lines were separated by NEPHGE/SDS-PAGE and examined by "Con A-blotting", in order to reveal glycoproteins (Fig. 2). All polypeptides of the so called "S-protein" group, except for S_{k2}, were glycosylated and could be detected by Con A/Peroxidase-staining. Proteins labelled S_1-S_4 were only found in stylar extracts of clones possessing the corresponding *S*-alleles. In contrast to the *S*-specific polypeptides, the relative amount of the S_{k1}-protein (circled in Fig. 2) varied in style extracts of different genotypes, although it was present in all extracts tested. Some glycoproteins outside the framed region showed qualitative as well as quantitative differences, but nevertheless were not connected with specific *S*-alleles. As is shown in Fig. 2, it was possible to assign two glycoproteins, in correlation to the corresponding *S*-alleles, in every SI-genotype. In style extracts of the self-compatible clone 1506/60 and the tetraploid cv "Hansa", a glycoprotein of about 29–30 kilodaltons could be detected, which possibly corresponds to a new *S*-allele and was, therefore, designated S_5. Moreover, in Hansa-extracts, a weakly stained protein spot of 23 kilodaltons was found, which corresponded with S_4 in its migration (Fig. 2/Hansa). Due to its low concentration in comparison to S_3 or S_5, it has not been assigned as S_4 in the Figure. A third *S*-allele in a tetraploid potato clone nevertheless would not be unexpected.

An unusual complement of SI-associated proteins was found in style extracts from the variety "Roxy" (4×): Only a single putative *S*-protein with the M_r and pI characteristic of S_2 could be detected. Perhaps other *S*-alleles have been inactivated by mutation or deletion.

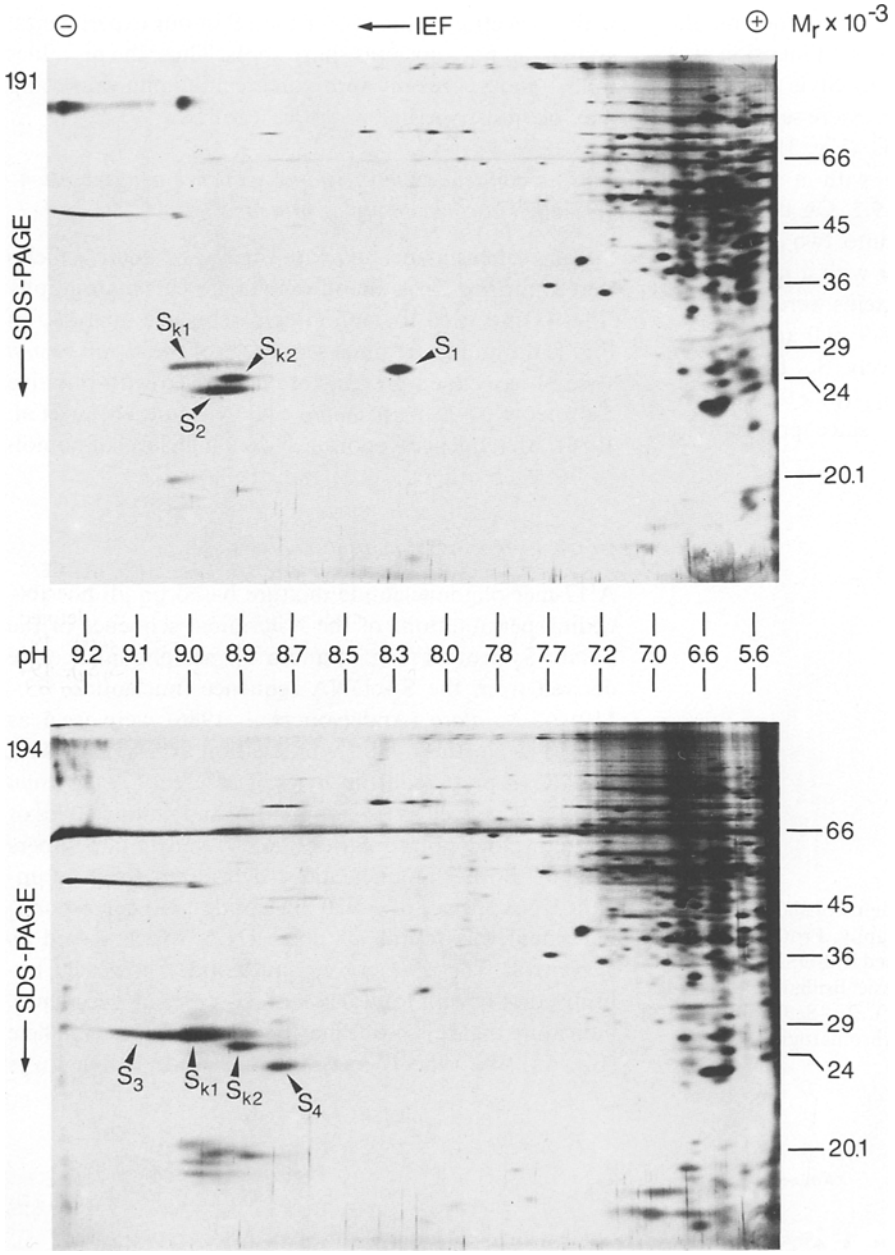

Fig. 3. pI-determination of SI-associated proteins from style extracts of the potato clones 191 (S_1S_2) and 194 (S_3S_4). Polypeptides were separated by two-dimensional gel electrophoresis and detected by silver staining. The values of the apparent pH-gradient are indicated. SI-associated proteins are marked by *arrowheads*

Another possibility would be a mutation at the glycosylation-sites or of associated glycosyl transferases. No extra non-glycosylated polypeptides were, however, observed on silver-stained 2-D gels of Roxy style extracts (data not shown).

In addition to the SI-associated proteins in all stylar extracts, some glycoproteins with a slightly higher M_r (≤ 2 kilodaltons) could be identified (Fig. 2). These polypeptides had similar pIs to S_1, S_3 and S_{k1}, however, their relative concentrations varied greatly among extracts. They may represent higher glycosylation products of the corresponding *S*-proteins. The differing proportions indicate a possible precursor relationship to S_1, S_3 and S_{k1}. In this respect, the protein pattern of the mixed style extract (1506/60 + 191) seemed interesting, because S_5 co-migrated exactly with the putative higher glycosylated form of S_1.

Determination of isoelectric points of SI-associated proteins

Isoelectric points of different SI-associated proteins of *S. tuberosum* have been determined in an IEF/SDS-PAGE system. Unfortunately, basic proteins are not well-separated in a normal IEF system, because in general they

enter the IEF gel poorly. To avoid this problem, the direction of electrophoresis in the first dimension was reversed (Sanchez-Martinez et al. 1986). Style protein extracts of the *S*-clones, 191/2 and 194/1, were separated by two-dimensional gel electrophoresis (Fig. 3). The SI-associated proteins were focussed with a few other polypeptides in a range of pH 8.3–9.2. On the basis of their pI, they could be subdivided into two groups. S_1 had by far the most acidic character with a pI of about 8.3. All other *S*-allele-associated proteins were separated in a range of pH 8.7–9.1. A pI of 8.9–9.0 and 8.7–8.8 was assigned to S_2 and S_4, respectively. S_{k2} focussed at about pH 8.9. The pI values for S_{k1} ($pI \geq 9.0$) and S_3 ($pI > 9.1$) could only be estimated, since polypeptides with isoelectric points higher than 9 in our experimental system did not appear as sharp spots. Thus, the pI-values for S_{k1} and S_3 are only approximate minimum values and may obviously be higher, at least for S_3.

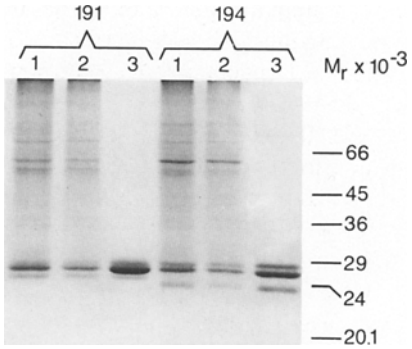

Fig. 4. Purification of SI-associated proteins of the clones 191 and 194 by anion-exchange chromatography. Protein solution of different purification steps was separated by SDS-PAGE. The polypeptides were stained with Coomassie Brilliant Blue. *1* – Total style extracts (100 µg protein/lane), *2* – Sephadex G-25 eluate (100 µg protein/lane), *3* – DE-52-chromatography eluate (50 µg protein/lane)

Purification and amino-terminal sequence determination of S-allele-associated stylar proteins

Stylar proteins associated with the S_2, S_3 and S_4 alleles were purified by anion-exchange chromatography (Fig. 4) and used for amino acid sequence analysis. In Fig. 5, the amino-terminal sequences of the *S. tuberosum* style SI-associated proteins are compared with putative *S*-proteins of *L. peruvianum* and *N. alata* (Mau et al. 1986). All sequences reported show a high level of homology to each other.

Northern-blot hybridization analysis

A 17-mer oligonucleotide mixture based on all possible coding permutations of the N-terminal sequence of the potato S_2-protein (Fig. 5) and a 47-mer oligonucleotide derived from the S_2-cDNA sequence (nucleotides 73–119) of *N. alata* (Anderson et al. 1986) were used as probes in Northern-blot hybridization experiments with total RNA prepared from styles of different *S. tuberosum* genotypes, as well as from immature and mature styles of the tetraploid potato variety, Roxy, and potato tubers (Fig. 6). Both oligonucleotides hybridized to an abundant RNA species of ~920 nucleotides. No corresponding signal was found in tuber RNA, which served as a control. The 17-mer oligonucleotide, however, hybridized only with total RNA of S_2- carrying genotypes, indicating that this probe may be specific for the S_2-allele (Fig. 6a). With the 47-mer oligonucleotide, a signal was

	pI	$M_r \times 10^{-3}$	Amino-terminal sequence (1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21)
L. peruvianum			
S_1	7.5	28	Y F E Y L Q L V L Q X P T T F
S_3	>9.5	28	D F D Y L Q L V L Q X P R S F
N. alata			
S_2	>9.5	32	A F E Y M Q L V L T W P I T F
S_6	>9.5	31	A F E Y M Q L V L Q W P T A F
S_z	9.0	30	D F D Y M Q L V L T X P A S F
S_{f11}	9.5	27	D F E Y L Q L V L T W P A S F
S. tuberosum			
S_2	8.9–9.0	24	(D) F D Y M Q L V L T W P R S F X Y P (R) G F
S_3	>9.1	27	X F E L L E L V (S)(T)(W) P X X F
S_4	8.7–8.8	23	(D) F D S L Q L V L T W P A S F X Y L N N

Fig. 5. pI, M_r and amino-terminal sequences of potato SI-associated proteins in comparison to *S*-proteins of *L. peruvianum* and *N. alata*. Amino acids are identified by their single-letter code, with X and ( ) indicating residues which could not be assigned with certainty. The data for *L. peruvianum* and *N. alata* *S*-proteins are from Mau et al. (1986). The *underlined* amino acids are those residues from which a 17-mer oligonucleotide of 16-fold degeneracy was prepared

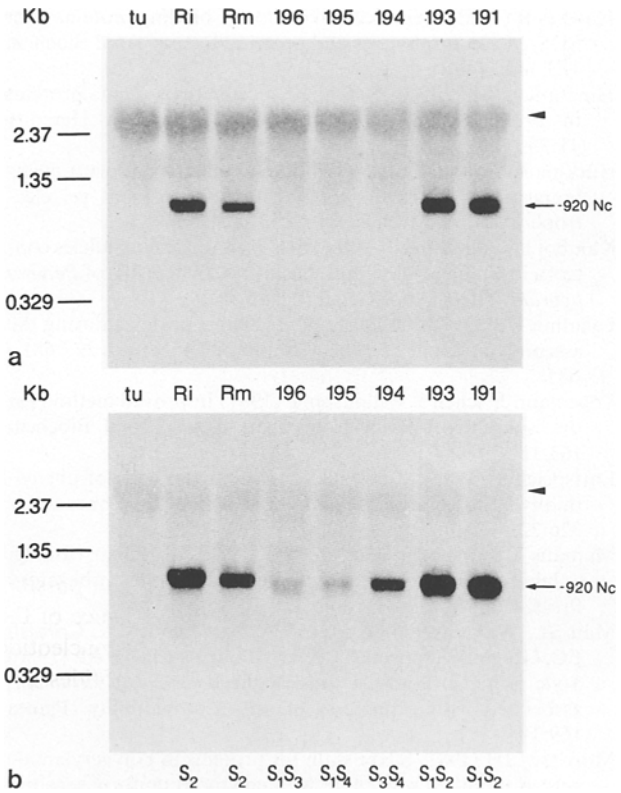

Fig. 6 a and b. Northern-blot analysis of total RNA from styles of different potato *S*-genotypes. Ninety micrograms of RNA/lane was electrophoresed in formaldehyde/1.5% agarose gels, transferred to a nylon filter and probed with ^{32}P-labelled oligonucleotides. **a** 17-mer oligonucleotide hybridization, **b** 47-mer oligonucleotide hybridization. 191 = S_1S_2, 193 = S_1S_2, 194 = S_3S_4, 195 = S_1S_4, 196 = S_1S_3, Rm = mature Roxy styles, Ri = immature Roxy styles, tu = potato tuber-RNA. The SI-specific RNA is marked by an *arrow*, the position of a potato-rRNA band, which served as a control for equivalent RNA-quantities, is indicated by *arrowheads*. Positions of size markers are drawn on the left

detected in all style extracts (Fig. 6b). The extent of hybridization was again strongest in S_2-genotypes. A somewhat weaker signal was found in genotype 194 (S_3S_4). RNA extracts of the clones 195 (S_1S_4) and 196 (S_1S_3), however, hybridized to a very low extent to the oligonucleotide probe. Thus, both oligonucleotides can be used to distinguish allele-specific differences, suggesting that the *S*-locus-associated gene involved shows substantial allelic variation in its coding sequence.

Discussion

The two-dimensional electrophoretic separation of proteins from potato styles shows the presence of abundant basic glycoproteins associated with genetically determined SI-alleles. The proteins characterized here, corresponding to alleles S_1–S_4, are best resolved by NEPHGE. The SI-related polypeptides have pIs estimated from IEF separations to be in the range 8.3–>9.1 and M_rs of 23–29 kilodaltons. These properties closely resemble those reported for SI-associated proteins in other members of the Solanaceae, *Nicotiana alata*, *Lycopersicon peruvianum* and *Petunia hybrida* (Anderson et al. 1986; Mau et al. 1986; Kamboj and Jackson 1986). Amino-terminal protein sequences obtained for S_2, S_3 and S_4 show a clear homology to sequences of SI-associated proteins from *N. alata* and *L. peruvianum* (Mau et al. 1986). This provides strong evidence that the proteins characterized here are products of different alleles of a highly conserved gene, involved in the mechanism of self-incompatibility. This proposal is further supported by Northern-blot experiments, where a 17-mer oligonucleotide based on the N-terminal sequence of the potato S_2-protein and a 47-mer oligonucleotide derived from the *N. alata* S_2-cDNA sequence (Anderson et al. 1986) were used as hybridization probes. Both oligonucleotides hybridized to a mRNA of 920 nucleotides present in total RNA purified from potato styles of different S-genotypes. The size of this transcript is almost identical to that of the *N. alata* S_2-mRNA of 940 nucleotides found by Anderson et al. (1986).

S_{k2}, one of the most prominent members of the abundant basic protein group in potato styles, is not a glycoprotein in contrast to the other SI-related polypeptides, and does not display allelic variation among the genotypes studied. We have shown (H. H. Kirch and R. D. Thompson, in preparation) that the S_{k2}-protein is style-specific and shows temporal and spatial correlation with self-incompatibility in potato. This may indicate that, whilst not directly determining SI-specificity, S_{k2} plays a role in the mechanism of self-incompatibility as an associated component. A further invariant protein, which is glycosylated (S_{k1}) has not yet been characterized to the same scale.

Differences in the extent of glycosylation of the S_1–S_4 proteins appear to account for most of the molecular weight differences observed. Enzymatic deglycosylation of S_2 and S_4 gave rise to polypeptides of nearly identical molecular weight (H. H. Kirch and R. D. Thompson, unpublished observations). The sizes of the deglycosylated products are consistent with the proteins being allelic variants of a single locus.

The diploid line 1506/60 is self-compatible (H. Uhrig, personal communication) and the tetraploids examined here would also be expected to be self-compatible. The protein patterns observed in these clones indicate this is not merely due to the loss of major style-specific polypeptides, although cv "Roxy" appears to possess only one polypeptide co-migrating with an SI-associated spot (S_2). Possibly this spot is not homogeneous, i.e. comprises more than one protein species. An alternative would be

an inactivation, deletion or homogenization in cv "Roxy" of some of the *S*-alleles, as the existence of a tetraploid clone possessing four identical *S*-alleles seems unlikely.

Proof that the proteins characterized in this work are involved in or responsible for the self-incompatibility character would be provided by isolation and transformation of the corresponding genes into an appropriate potato tester line in order to modify its SI-phenotype. The studies presented here have provided us with probes for this objective.

Acknowledgements. The authors thank Prof. J. G. Th. Hermsen for his generous gift of potato lines of defined *S*-genotype. We are indebted to B. Nolden for skilled technical assistance. The work was supported by the Deutsche Forschungsgemeinschaft-Schwerpunktprogramm "Mechanisms of Hybrid Breeding".

References

Ames G, Nikaido K (1976) Two-dimensional gel electrophoresis of membrane proteins. Biochemistry 15:616–623

Anderson MA, Cornish EC, Mau SL, Williams EG, Hoggart R, Atkinson A, Bonig I, Grego B, Simpson R, Roche PJ, Haley JD, Penschow JD, Niall HD, Tregear GW, Coghlan JP, Crawford RJ, Clarke AE (1986) Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. Nature 321:38–44

Beaucage SL. Caruthers MH (1981) Deoxynucleoside phosphoramidites: a new class of key intermediates for deoxypolynucleotide synthesis. Tetrahedron Lett 22:1859–1862

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bredemeijer GMM, Blaas J (1981) S-specific proteins in styles of self-incompatible *Nicotiana alata*. Theor Appl Genet 59:185–190

Cipar MS, Peloquin SJ, Hougas RW (1964) Inheritance of incompatibility in hybrids between *Solanum tuberosum* haploids and diploid species. Euphytica 13:163–172

Crane MB, Lawrence WJC (1929) Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. J Pomol Hortic Sci 7:276–301

De Nettancourt D (1977) Incompatibility in angiosperms. (Monographs on theoretical and applied genetics, vol 3). Springer, Berlin Heidelberg New York

Eckerskorn C, Mewes W, Goretzki H, Lottspeich F (1988) A new siliconized-glass fiber as support for protein-chemical analysis of electroblotted proteins. Eur J Biochem 176:509–519

Hawkes R (1982) Identification of Con A-binding proteins after SDS-gel electrophoresis and protein blotting. Anal Biochem 123:143–146

Hinata K, Nishio T (1978) S-allele specificity of stigma proteins in *Brassica oleracea* and *Brassica campestris*. Heredity 41:93–100

Hurkman WJ, Tanaka CK (1986) Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. Plant Physiol 81:802–806

Kamboj RK, Jackson JF (1986) Self-incompatibility alleles control a low molecular weight, basic protein in pistils of *Petunia hybrida*. Theor Appl Genet 71:815–819

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:681–685

Logemann J, Schell J, Willmitzer L (1987) Improved method for the isolation of RNA from plant tissues. Anal Biochem 163:16–20

Lottspeich F (1985) Microscale isocratic separation of phenylthiohydantoin amino acid derivatives. J Chromatogr 326:321–324

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbour Laboratory Press, Cold Spring Harbour/NY

Mau SL, Williams EG, Atkinson A, Anderson MA, Cornish EC, Grego B, Simpson RJ, Kheyr-Pour A, Clarke AE (1986) Style proteins of a wild tomato (*Lycopersicon peruvianum*) associated with expression of self-incompatibility. Planta 169:184–191

Morrissey JH (1981) Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity. Anal Biochem 117:307–310

Nasrallah JB, Nasrallah ME (1984) Electrophoretic heterogeneity exhibited by the S-allele specific glycoproteins of *Brassica*. Experientia 40:279–281

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

O'Farrell PZ, Goodman HM, O'Farrell PH (1977) High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133–1142

Pandey KK (1962a) Interspecific incompatibility in *Solanum* species. Am J Bot 49:874–882

Pandey KK (1962b) Incompatibility in some *Solanum* crosses. Genetica 33:24–30

Raff JW, Knox RB, Clarke AE (1981) Style antigens of *Prunus avium* L. Planta 153:125–129

Sanchez-Martinez D, Puigdomenech P, Pages M (1986) Regulation of gene expression in developing *Zea mays* embryos. Plant Physiol 82:543–549

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354

Woods DE (1984) Oligonucleotide screening of cDNA libraries. BRL Focus 6, No 3:1–2