

Update section

Identification of the viroid-induced tomato pathogenesis-related (PR) protein P23 as the thaumatin-like tomato protein NP24 associated with osmotic stress

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Abstract

P23, a 23 kDa pathogenesis-related (PR) protein, was purified from citrus exocortis viroid (CEVd)-infected tomato leaves. Partial amino acid sequencing of this protein including the N-terminal and nine additional tryptic fragments covering about 50% of its primary structure revealed extensive homologies to the members of the family of plant thaumatin-like proteins. Sequence alignment revealed that tomato P23 is the previously described NP24 protein found to be associated to osmotic stress in tomato. In view of this fact the possible role of pathogenesis-related P23 protein as a component of a general mechanism of response of the plant is discussed.

Introduction

Pathogenesis-related (PR) proteins are a family of host-encoded plant proteins induced and accumulated in the plant tissues under a number of stress situations provoked by different biotic or abiotic pathogenic agents [1, 21]. The biological function of these proteins is not clearly understood despite the large number of studies dealing with their cellular and molecular biology. A possible role for these proteins as defensive tools against bacteria and fungi has been proposed on the ground that some PR proteins display hydrolytic activities such as chitinase, β -1,3-

glucanase and protease [9, 12, 14, 22]. However, their putative role in limiting virus multiplication or spread needs to be shown [3, 15].

The systemic infection of tomato plants with citrus exocortis viroid (CEVd) results in the *de novo* synthesis and accumulation of a set of ten major different cationic PR proteins [11]. Among these, P1(p14), shows extensive peptide sequence homologies with tobacco PR 1 proteins [16], P69 has been found to display proteinase activity [22] and P32 and P34 are chitinases [9]. A 23 kDa PR protein which accumulates upon CEVd infection of tomato plants is one of the most evident. This protein (P23) was purified and characterized.

Here we report the partial peptide sequencing of P23 and its identification as the tomato NP24 protein which accumulates in tomato plant roots upon osmotic stress [13]. This finding, together with the possible defensive role of the viroid-induced chitinases [9], reinforces our idea that viroid infection triggers non-specifically a system of coordinated responses against different kinds of pathogens or pathogenic and stress situations.

Materials and methods

Tomato PR P23 was purified from CEVd-infected leaves bearing strong symptoms of viroid disease. Details of the purification procedure and biochemical and serological properties will be given elsewhere. To obtain the N-terminal amino acid sequence, the protein was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate as described [5] and electroblotted onto polyvinylidene difluoride membranes (0.45 μ m pore size Immobilon transfer sheets, Millipore) in accordance with the manufacturer's instructions. Bands corresponding to P23 were cut out and a 20-amino acid N-terminal sequence was determined [17]. For more complete sequence analysis, the protein was digested with trypsin and the tryptic fragments were separated by HPLC. For sequence analysis 100–500 pmol peptide was subjected to Edman degradation in a gas-phase sequencer constructed and operated as described [10]. On-line analysis of the phenylthiohydantoin amino acids was performed on a Hewlett-Packard 1090 liquid chromatograph using a PTH-C18 column (Applied Biosystems).

Results and discussion

Figure 1 shows the N-terminal sequence of PVDF-immobilized P23. A search of this partial sequence (20 amino acids long) indicated striking homologies with those previously published for thaumatin-like proteins, including tobacco, potato and barley PR proteins [2, 6, 18], a maize α -

P23	A	T	F	E	V	R	N	N	C	P	Y	T	V	W	A	A	S	T	P	I
NP24	A	T	I	E	V	R	N	N	C	P	Y	T	V	W	A	A	S	T	P	I
OSM	A	T	I	E	V	R	N	N	C	P	Y	T	V	W	A	A	S	T	P	I
PR 5	A	T	F	D	I	V	N	Q	C	T	Y	T	V	W	A	A	A	S	P	
PR C	A	T	F	D	I	T	N	R	C	T	Y	P	V	W	A	A	A	S	P	
Hv-1	A	T	F	N	I	K	N	N	C	G	S	T	I	W	P	A	G	I	P	V
THA	A	T	F	E	I	V	N	R	C	S	Y	T	V	W	A	A	A	S	K	G
MAI	A	V	F	T	V	V	N	Q	C	P	F	T	V	W	A	A	S	V	P	V

Fig. 1. Alignment of the 20 amino acid N-terminal sequence of tomato PR P23 with the first 20 N-terminal amino acid positions from tomato NP24 [13], tobacco osmotin [20], tobacco PR 5 [6], potato PR C [18], barley Hv-1 pathogenesis-related protein [2], thaumatin II [8] and maize bifunctional α -amylase/trypsin inhibitor [19]. Amino acids are expressed in single-letter code. Positions homologous to P23 are boxed.

amylase/trypsin inhibitor [19], tobacco and tomato osmotins [13, 20] and thaumatin itself [8]. Alignment of the sequenced amino acids revealed homologies greater than 60% in most cases. Among them, a virtual identity was found with both tobacco osmotin and tomato NP24 in this N-terminal sequence, being the third amino acid position (Phe instead of Ile) the only difference found at this level. On the basis of this result we performed a more complete sequencing of P23. Tryptic fragments randomly distributed along the molecule were sequenced, covering about 50% of the total protein sequence. When these partial sequences were aligned to those of the previously mentioned proteins (not shown here; see [20] for a recent alignment) it was observed that the degree of homology was also similar along the different tryptic fragments. The amino acid sequence homology with tobacco PR 5 (Fig. 2) was similar (about 65%) to that already reported for other tomato and tobacco counterparts such as tomato P1(p14) and tobacco PR 1 proteins [16]. The most outstanding fact was, however, the matching of the amino acid sequence with two salt-induced proteins: more than 90% homology with tobacco osmotin [20] and a virtually 100% identity with tomato NP24 [13]. As shown in

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NP24  Nt-ATIEVRNNCPYTVWAASTPIGGRRRLNRGQTVVINAPRGTKMARIWGRTGCNFAAGRGCQTGD
P23   Nt-ATFEVRNNCPYTVWAASTPI-----GQTVVINAPR-----IWGR-----
PR 5  Nt-ATFDIVNQCTYTVWAAASP  GGGRQLNSGQSW SINVNPGTVQARIWGRTNCNFDGSGRGNCETGD

NP24  CGGVLQCTGWGKPPNTLAEYALDQFSNLD FWDISLVDGFNIPMTFAPTKPSGGKCHAIHCTANIN
P23   -----?HAIH?TANIN
PR 5  CNGMLECQGYGKPPNTLAEFALNQ  PNQDFVDISLVDGFNIPMEFSPTNG  G  CRNLRCTAPIN

NP24  GECPRALKVP  GGCNNPCTTFGGQQYCCTQ  GPCGPTELSKFFKKRCPDAYSYPQDDPTSTFTC
P23   GE?PR---VP  GG?NNP?TTFGGQQY??TQ  GP?GP-----FFK--?PDAYSYPQDDPTSTFT?
PR 5  EQCPAQLK  TQGGCNPCTVIKTNEFCCTNGPGSCGPTDLSRFFKARCPDAYSYPQDDPPSLFTC

NP24  PGGSTNYRVVFCPNGVADPNFPLEMPASTDEVAK-Ct
P23   PG-----VVF?PNGVA-----
PR 5  PPG  TNYRVVFCP-Ct

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Fig. 2. Alignment of the amino acid sequences determined from tryptic fragments of tomato P23 protein with the published sequences of tomato NP24 [13] and tobacco PR 5 [6]. The 20 N-terminal amino acid sequence of P23 correspond to that of Fig. 1. Gaps have been inserted in the sequences to achieve best matching. Non determined sequences of P23 have been indicated by hyphens (-). Question marks (?) correspond to amino acid positions that are not positively identified and most probably correspond to cysteine residues [10].

Fig. 2, P23 tryptic fragments match perfectly to the NP24 amino acid sequence with the single exception of the third residue. This punctual conservative change in amino acid sequence can be explained by a single nucleotide change in the genomic sequence (e.g. the third amino acid position in tomato NP24 is Phe (ATT) [13] whereas tobacco PR 5 shows Ile (TTT) in this position [6]). In fact, only Phe or Ile have been found in this position on the published thaumatin-like proteins [2, 6, 8, 13, 18, 19, 20]. The only difference observed between P23 and NP24 is most probably due to cultivar variation. If this is so, our results would indicate that P23 and NP24 are the same functional protein.

The fact that a viroid induces a salt-stress protein with no obvious relationship to viroid replication or pathogenesis poses two main questions: i) How is this accomplished?, ii) What is its biological significance?. In line with our idea that PR proteins are part of a non-specific, coordinately triggered mechanism of response against different kinds of pathogenic situations [4], P23 (NP24) may have been developed as a specific response to a salt stressing environment. This particular response to osmotic stress, together with other responses independently developed against fungi, bacteria or other stress agents, might have been integrated into a general system

of response with the capability of being coordinately triggered by different pathogens. Such a system would prepare the afflicted plant to withstand subsequent stresses more effectively. Thus there would be two different possibilities of activating the synthesis of this tomato osmotin: either specifically in osmotic adaptations or non-specifically, coordinately triggered as part of a general response to pathogen attack. In this respect, it is worth noting that osmotin has recently been shown to be transcriptionally induced in tobacco leaves upon TMV infection and wounding [7] and thus seems to behave as a PR protein. The finding we present in this communication is complementary and symmetrical: a viroid-induced tomato PR protein turns out to be an osmotic stress protein. Which is the specific biological function of this protein and how its induction can be either specifically or coordinately activated as a part of a general mechanism of response remains to be determined.

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