Journal of Experimental Botany, Vol. 52, No. 362, pp. 1947–1948, September 2001

Journal of Experimental Botany

GENE NOTE

Cryptogein affects expression of α3, α6 and β1 20S proteasome subunits encoding genes in tobacco

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Received 26 April 2001; Accepted 4 June 2001

Abstract

Twelve α and β 20S proteasome subunits cDNAs showing 70–82% identity with the corresponding genes in *Arabidopsis* or rice, and features of eukaryotic proteasome subunits were cloned in tobacco. Only β1-tcI 7, α3 and α6, 20S proteasome subunits encoding genes were up-regulated by cryptogein, a proteinaceous elicitor of plant defence reactions. These results led to the hypothesis that the activation of β1-tcI 7, α3 and α6 could induce a specific proteolysis involved in the hypersensitive response and systemic acquired resistance monitored by cryptogein.

Key words: Elicitins, 20S proteasome subunits, plant defence reactions.

In eukaryotes, the 26S proteasome is the central multicatalytic proteinase complex comprising two subcomplexes: the 20S core particle that performs proteolysis and the 19S regulatory particle that recognizes the protein targeted for degradation. The 20S proteasome is a stack of four seven-membered rings, the two outer rings being formed by seven α subunits and the two inner rings by seven β subunits. The central β rings enclose a cavity that houses the active sites allowing the progressive degradation of proteins. In mammals, proteasomes are shown to be involved in the degradation of misfolded proteins and also in biological functions such as cell cycle progression or cell death, and specific proteolysis such as the cleavage of propeptides in order to activate peptides or allowing the antigene presentation (for a review see Coux *et al*., 1996).

In plants, the 20S proteasome was shown to be involved in cell cycle progression (Genschik *et al*., 1994), in senescence (Bahrami and Gray, 1999), in the early stages of seedling development (Ito *et al*., 1999), and in defence responses (Becker *et al*., 2000). Since it was previously shown that *tcI 7*, a gene encoding a β1 proteasome subunit, was up-regulated by cryptogein, a proteinaceous elicitor of the defence reaction (Petitot *et al*., 1997; Etienne *et al*., 2000), it was interesting to investigate how genes encoding other proteasome subunits are regulated during the initiation of defence reactions. The published sequences of *Arabidopsis thaliana* (Parmentier *et al*., 1997) and rice (*Oryza sativa*) (Sassa *et al*., 2000) α and β subunits were used to determine degenerated primers for RT-PCR and RACE. Reverse transcription was performed using the 'First strand cDNA synthesis kit for RT-PCR' (Boehringer) with 3 μg total RNA. RACE amplifications were performed from 1 μg poly(A)$^+$ using the 'Marathon^{TM} cDNA Amplification kit' (Clontech). The cDNAs were cloned in pGEM-Teasy vector (Promega, France) and sequenced (Genome Express, France). Sequences of all obtained cDNAs were submitted to the EMBL database. The corresponding genes were named: *NtPA1* (100% identity with *NtPSA1*, Y16644, previously isolated from tobacco; Bahrami and Gray, 1999), *NtPA3* (AJ291733), *NtPA4* (AJ291734), *NtPA5* (AJ291735), *NtPA6* (AJ291737), and *NtPA7* (AJ291738) for α subunits and *NtPB1* (= *tcI 7*, Y09505, Petitot *et al*., 1997), *NtPB2* (AJ291736), *NtPB3* (AJ291739), *NtPB4* (AJ291740), *NtPB5* (AJ291741), *NtPB6* (AJ291742), and *NtPB7* (AJ291743) for β subunits. The amino acid sequence homologies between the tobacco α and β subunits and the corresponding genes in *Arabidopsis thaliana* and in rice are high (75–98%), confirming the characterization of cloned cDNAs as 20S proteasome α or β subunits.

Figure 1 shows the alignments of the deduced amino acid sequences of the α (Fig. 1A) or β (Fig. 1B) subunit family of tobacco. Four of the six characterized α subunits (*NtPA1*, *NtPA3*, *NtPA6*, *NtPA7*) were found to have the α-type signature at their N-termini and two (*NtPA6* and *NtPA7*) showed a Tyr residue (Y), essential for the assembly of the α ring (Groll *et al*., 1997). Moreover, the amino acid sequences of NtPA1, NtPA3 and NtPA4 show a putative nuclear localization signal (NLS), with a typical KKXXXK sequence (Tanaka *et al*., 1990). The β-type signature was conserved in all of the cloned tobacco β subunits. In animals, β1, β2 and β5 were previously shown to be processed to remove their propeptides (Chen and Hochstrasser, 1996), and their N terminal Thr residues to form the catalytic site of the 20S proteasome (Groll *et al*., 1997). The sequence of tobacco β1-tcI 7 (*NtPB1*) and β2 (*NtPB2*) show such a propeptide and a Thr residue (T) preceded by a Gly (G), the Gly/Thr pair representing the cleavage site (Groll *et al*., 1997). The sequence analysis of tobacco 20S proteasome subunits also shows the presence of conserved glycine residues (Chen and Hochstrasser, 1996). Thus, the sequences obtained in this study showed sufficient characteristics of α or β proteasome 20S subunits to allow the analysis of the expression of the various α and β proteasome subunits during the induction of plant defence mechanisms monitored by cryptogein in tobacco.

Figure 2 shows the kinetics of accumulation of mRNA corresponding to α and β 20S proteasome subunits: for α1, α4, α5, α7, β2, β3, β4, β5, β6, and β7 proteasome subunits, the transcripts remained undetected. A high accumulation of transcripts was observed from 9 h or 12 h only for α3, α6 and β1-*tcI 7* and hypersensitive-like lesions appeared within 16–24 h after the treatment. A treatment of leaves with bovine serum albumin instead of cryptogein confirmed that infiltration of a foreign protein was not sufficient to induce any proteasome subunits (not shown). These data indicate a specific induction of β1, α3 and α6 subunits of the 20S proteasome encoding genes, which begins before the appearance of the hypersensitive response and correlated with the induction of systemic acquired resistance monitored by cryptogein. It could be possible that during the induction of defence reactions, the incorporation of elicitin-stimulated subunits into the proteasome structure leads to the replacement of constitutive corresponding subunits, as previously observed in 'immunoproteasomes' in animals. In mammalian cells, three β subunits X, Y and Z were replaced by other interferon-induced β subunits named LMP7, LMP2 and MECL1 modifying the proteolytic activities of these 'immunoproteasomes' (Eleuteri *et al*., 1997). The results obtained in this work indicate specific activation of α3, α6 and β1 subunits, probably in order to monitor specific proteolysis involved in defence reactions. Only further detailed

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A

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NtPA1   ---------------SPEGRLFQVEYAFKAVKAAGITSIGVRGKDSVCVVTQKKVPDKLL   45
NtPA3   --------DSRTTIFSPEGRLYQVEYAMEAIGNAGS-AIGILAKDGVVLVGEKKVTSKLL   51
NtPA4   ------------------------------------GVRGTVTVVLGVEKKSTPKLQ   21
NtPA5   ------------------------------------TKEGVVLAVEKRVTSPLL   18
NtPA6   ----QKIYDTDVTTWSPAGRLFQVEYAMEAVKQ-GSAAIGLRSKTHVILACVNKANSELS   55
NtPA7   MSSIGTGYDLSVTTFSPDGRVFQIEYAGKAVDNSG-TVVGIKCKDGIVLGVEKLIASKMM   59
                                                 . : :   :    :

NtPA1   DQ-TSVSHLFPITKYLGLLATGMTADARTLVQQARNEAAEFRFKYGYEMPVDVLSKWIAD  104
NtPA3   QTSTSTEKMYKIDDHVACAVAGIMSDANILINTARVQAQRYTFSYQEPMPVEQLVQSLCD  111
NtPA4   DS-RSARKIVNLDDHIALACAGLKADARVLVNRARIECQSHRLTVEDPVTVEYITRYIAG   80
NtPA5   EP-SSVEKIMEIDEHIGCAMSGLIADARTLVEHARVETQNHRFSYGEPMTVESTTQALCD   77
NtPA6   SH---QKKIFKVDDHIGVAIAGLTADGRVLSRYMRNECINYSYAYESPLPAGRLVVQLAD  112
NtPA7   LP-GSNRRIHSVHRHSGMAVAGLAADGRQIVARTKSEATNYESTYGEPILVKELAERVAS  118
              ::  :  :     :*: :*.. :    : :   .       : .      :..

NtPA1   KSQVYTQHAY---MRPLGVVAMILGIDEEKGP-QLFKCDPAGHFFGHKATSAGSKEQEAI  160
NtPA3   TKQGYTQFGG---LRPFGVSFLFAGWDKNYGF-QLYMSDPSGNYGGWKAAAIGANNHAAQ  167
NtPA4   LQQKYTQSGG---VRPFGLSTLIIGFDPYTGVPALYQTDPSGTFSAWKANATGRNSHSLR  137
NtPA5   LALRFGEGDEESMSRPFGVSLLIAGHD----------------------------------  104
NtPA6   KAQVCTQRSWK---RPYGVGLLVGGLDESGAH--LYYNCPSGNYFEYQAFAIGL------  161
NtPA7   YVHLCTLYWWL---RPFGCGVILGGYDRDGPQ--LYMVEPSGVSYRYFGAAIGKGRQAAK  173
                    ** *   :. * *             .         .

NtPA1   NFLEKKMKNDPAFSYEETVQTAISALQSVLQEDFKASEIEVGVVKKEDPIFRV-------  213
NtPA3   ----------------------------------------------------------
NtPA4   EFLEKNYKKT---SGQETVKLAIRALLEVVESGGKNIEVAVMTKEHGLKQLEEAEIDAIV  194
NtPA5   ----------------------------------------------------------
NtPA6   ----------------------------------------------------------
NtPA7   TEIEKLKLSEMTCRQGVIEVAKIIYGVHDEAKDKAFELETSWVCDESNRQHQKVPADLLE  233

NtPA4   AEIEAEKAAAEAAKKAPKET  214
NtPA7   EAKAAAKAALEEMDAD----  249
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B

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                                                  #          #
NtPB1   ---------------------------mentdvdqphsmgTTIIGVTYNGGVVLGADSRT   33
NtPB2   mtakttmdvpqkggfsfdlcrrnemlvnkglrspsflktgTTIVQLIFQDGVILGADTRA   60
NtPB3   ------------------------------MSIFEYNGSALVAMVGKNCFAIASDRRL   28
NtPB4   ------------------------------------MECVFGMVGNGFALVVADSSA   21
NtPB5   ----------------------------------------------------------A    1
NtPB6   ---------------------------------SPYDNNGGTCVAVAGADYCVIAADTRM   27
NtPB7   ----------------------------------------------------------GG   2

                #
NtPB1   STGMYVANRASDKITQLTDNVYVCRSGSAADSQIVSDYVRYFLHQHTIQL-GQPATVKVA   92
NtPB2   TEGPIVADKNCEKIHYMAPNIYCCGAGTAADTEAVTDMVSSQLKLHRFHT-GRESRVVTA  119
NtPB3   GVQLQTIATDFQRIFRIHDKVFIGLSGLGTDVLTLYQRLVFRHKLYQLRE-ERDMKPETF   87
NtPB4   VHSILVHKSNEDKIMILDSHKLMGASGEAGDRAQFTEYVQKNVALYQFRN-GIPLTTAAA   80
NtPB5   SMGGYISSQSVKKIIEINPYMLGTMAGGAADCQFWHRNLGIKCRLHELAN-KRRTSVAGA   60
NtPB6   SSGYNILTRDYSKIIKLADKCVLASSGFQADVKALQKVLAARHLIYQHQH-NKQMSCPAM   86
NtPB7   SYGSTIRYKSVERLKSVGKHSLLGASGEISDFQEISRKLDELILYDNMWDDGNSLGPKEV   62
                .::  :        :*   *       :

                                                          #
NtPB1   ANLTRLLSYNN--KDRLQTGMIIGGWDKYEGGKIYGIPPGGTVLEQPFAIGGSGSSYLY  149
NtPB2   LTLLKTHLFSYQ---GYVSAALVLGGVDVT-GPHLXTIYPHGSTDTLPYATMGSSSLAAI  176
NtPB3   ASLVSALLYEKR--FGPYFCQPVIAGLGDDDKPFICTMDSTGAKELAKDFVVAGTASESL  145
NtPB4   ANFTRGELATALR-KNPYMVNIILAGYDKETGPSLYYVDYIATLHKVDKGAFGY------  133
NtPB5   SKLLANILYSYR--GMGLSVGTMIAGWDEK-GPGLYYVDSEGGRLKGNRFSVGSGSPYAY  117
NtPB6   GQLLSNTLYYKR--FFPYYSFNVLGGLDNEGKGCVFTYDAVGSYGRVGYSSQGSGSTLIM  144
NtPB7   HNYLTRMMYNRRNKFNPLWNSLVLGGVKNGQKYLGTVSMI-GVHYEDDHVATGFGNHLAR  122
                        ::.*            .        .

                                  #  #
NtPB1   GFFDQAWKEGMTQEEAEKLVVTAVSLAIARDGASGGVVRTVTINKDGATRKFYSGDSLQL  209
NtPB2   AIFESKYREGLITPLREVVEVIEGGDAMEK-----------------------------  205
NtPB3   YGACEAMFKPDMGPEELFETISQALIASVDRDC---------------------------  178
NtPB4   ----------------------------------------------------------
NtPB5   GVLDSGYRFDLSVEEAAELARRAIYHATFRDGASGGVASVYHVGPNGWKKLSGDDVG---  174
NtPB6   PFLDYQLKSPSPLLLPAKDAVTPLSESEAIDLVKTCFVSATERDIYT-------------  191
NtPB7   PILRDEWH-----------------------------------------------------  129

NtPB1   WHEELEPVNSLLDVVFASSPVPMVS  234
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Fig. 1. Deduced amino acid sequence alignments of the α-type (A) and β-type (B) proteasome subunits of tobacco (*Nicotiana tabacum* cv. *Xanthi*). The alignments were created using the program 'Clustalw' of the GCG package. Identical and similar residues are indicated by asterisks and dots or colons, respectively. Grey letters indicate conserved glycine residues present in many α and β subunits. (A) The Tyr residue essential for the assembly of the α ring is indicated in reverse type. The proteasomal α-type signature found in subunits (PROSITE database) is in bold. The underlined residues correspond to putative Nuclear Localization Signal. (B) The proteasomal β-type subunit signature (PROSITE database) is in bold. # indicates residues involved in proteolytic activities. Putative propeptide sequences are indicated in lower-case letters.

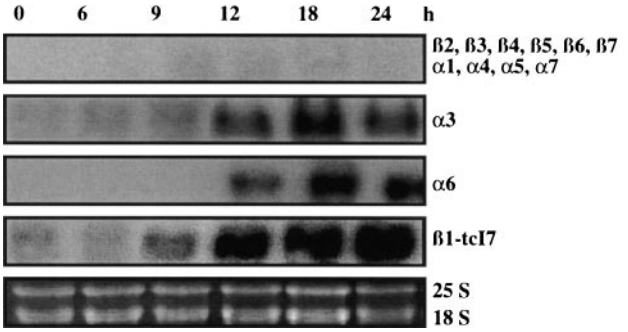

Fig. 2. The effect of cryptogein on the accumulation of transcripts corresponding to genes encoding α and β 20S proteasome subunits in tobacco leaves. Total RNA (15 μg per lane) were extracted from leaves treated by infiltrating cryptogein (100 ng) directly through the abaxial epiderm for various times. The Northern blots were hybridized with each α and β subunit cDNA probe ^{32}P labelled by 'random priming' (*redi* prime, Amersham, France) and analysed with a PhosphorImager: α1 (*NtPA1*), α3 (*NtPA3*), α4 (*NtPA4*), α5 (*NtPA5*), α6 (*NtPA6*), α7 (*NtPA7*), β1-tcI7 (*NtPB1*), β2 (*NtPB2*), β3 (*NtPB3*), β4 (*NtPB4*), β5 (*NtPB5*), β6 (*NtPB6*), and β7 (*NtPB7*). Equal loading is controlled by staining 18S and 25S rRNAs with ethidium bromide.

multiparametric studies will lead to an understanding of their different putative functions.

Acknowledgements

This work is supported by INRA and by the 'Conseil Régional de Bourgogne'. Ph Etienne is supported by a grant from the 'Ministère de l'Education Nationale, de la Recherche et de la Technologie' and Anne-Sophie Petitot by Genoplante.

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