European Journal of Cell Biology 55, 114–121 (1991) © Wissenschaftliche Verlagsgesellschaft mbH · Stuttgart

Prosomes (proteasomes) of higher plants

Martin Schliephacke[2)], Anette Kremp, Hans-Peter Schmid[3)], Kurt Köhler, Ulrich Kull[1)]

Biologisches Institut der Universität, Stuttgart/Bundesrepublik Deutschland

Received November 7, 1990
Accepted February 24, 1991

Prosomes — proteasomes — multicatalytic protease — serological relationship — glycoproteins

From different plant tissues such as tobacco (Nicotiana rustica), potato (Solanum tuberosum), and mung bean (Phaseolus radiatus), ring- or cylinder-shaped particles called prosomes were isolated by either sucrose gradient centrifugation or fast protein liquid chromatography (FPLC). These particles have a diameter of 12 to 14 nm and a length of 16 to 18 nm. They migrate under conditions of nondenaturing gel electrophoresis as one distinct band. Sedimentation coefficient and buoyant density in Cs_2SO_4 of the plant prosomes were determined by analytical ultracentrifugation to be approximately 23S and 1.23 g/cm^3, respectively. The total molecular mass was estimated by gel filtration to be 650 kDa. Plant prosomes are composed of 12 to 15 proteins with molecular masses in the range of 24 to 35 kDa with isoelectric points of *p*H 5 to 7 as revealed by two-dimensional gel electrophoresis. The protein patterns of prosomes from the three different plant species are very similar. Polyclonal antisera against potato prosomes reacted in Western blots with prosomal proteins of all three plant species. They also bind to some prosomal proteins of animal species. Antisera against animal prosomes react with some proteins of plant prosomes. As shown by lectin blotting, plant prosomes are glycosylated carrying glucosyl- or mannosyl, and N-acetylgalactosaminyl residues. Prosomal preparations contain non-stoichiometric amounts of small RNA of about 80 kDa. These results suggest that plant prosomes are structurally and functionally homologous to prosomes of other eukaryotic cells.

Introduction

Prosomes are small particles with sedimentation coefficients of 19 to 22S in the cytoplasm and in nuclei of various animal tissues with very similar morphology and biochemical properties [11, 22, 33, 38, 40]. These ring-shaped or cylindrical particles have a diameter of 10 to 14 nm and a length of 16 to 19 nm. Although various functions have been proposed for these particles, so far only an involvement in the posttranscriptional control of gene expression has been demonstrated [21, 29, 35, 37]. Because of this function the particle was named "prosome" (short version for "programosome", [37]). Independently, an intracellular, non-lysosomal proteinase was described ("multicatalytic proteinase" [8, 46]; "high molecular weight proteinase" [41, 42]), which strikingly resembles the prosome in structure and biochemical properties. Lately it was shown that both types of particles share antigenic determinants [4, 13], and the new name "proteasome" was proposed. Quite likely, proteasome and prosome designate the same particle; we prefer to use the latter name in the following investigation.

Prosomes of animal tissues are resistant to 1% of the detergent sodium-N-lauroylsarcosinate and consist of a set of slightly acidic polypeptides with molecular masses of about 20 to 35 kDa. They are often reported to be associated with small RNAs of about 80 nucleotides [2, 10, 29]. The protein subunit pattern and the number of the associated RNAs show species-specific differences [3, 29], and also developmental changes in the protein subunit pattern are reported [16].

Prosomes were also found in higher plants and in yeast [3, 25], and one may consider them to be ubiquitous in eukaryotic cells. Whereas the isolation of prosomes from Escherichia coli was unsuccessful [3], recently prosome-like particles were detected in archaebacteria [9]. In this paper we present comparative biochemical and immunological data of prosomes isolated from three different species of higher plants (tobacco, potato and mung bean).

Materials and methods

Culture of plants

a) Tobacco (Nicotiana rustica). Plants were grown in pots in a green-house at temperatures of 25 °C (day, 12 h) and 20 °C (night). Leaves of 6 to 8-week-old plants or stems and petals of 10 to 12-week-old plants were cut and frozen at −20 °C.

b) Potato (Solanum tuberosum var. "Aula"). Potato tubers were kept in darkness at 18 °C and at a relative air moisture of about 40%. The germinating sprouts were removed when they reached about 20 cm and then kept frozen at −20 °C.

c) Mung bean (Phaseolus radiatus). Seedlings were grown in the dark at 25 °C for 3 days and then stored at −20 °C.

[1)] Prof. Dr. Ulrich Kull, Biologisches Institut der Universität, Pfaffenwaldring 57, D-7000 Stuttgart 80/Federal Republic of Germany.
[2)] Present address: Institut für Klinische Genetik der Universität, Wilhelmstr. 27, D-7400 Tübingen/Federal Republic of Germany.
[3)] Present address: Biochimie, Université de Clermont-Ferrand III, 24 Avenue de Landais, F-63170 Aubière/France.

Purification by sucrose gradient centrifugation

The homogenization of the plant material, preparation of the post-ribosomal pellet and purification of the particles by sucrose gradient centrifugation was done as described [25].

Purification by fast protein liquid chromatography (FPLC)

The postribosomal pellet was resuspended in TBK 100 (20 mM Tris-HCl, *p*H 7.4, 100 mM KCl, 3 mM $MgCl_2$, 7 mM 2-mercaptoethanol) and aliquots of about 20 A_{280} units applied to an anion exchange column of a FPLC system (Mono Q, Pharmacia, Freiburg/FRG) at a salt concentration of 240 mM KCl. The chromatography was performed at a flow rate of 1 ml/min, and a program comprising a linear salt gradient from 240 mM to 600 mM KCl in FPLC buffer (20 mM Tris-HCl, *p*H 7.4, 2 mM $MgCl_2$, 7 mM 2-mercaptoethanol) was used. The elution of bound material took place within an elution volume of 13 ml and the absorption at 280 nm was measured during the run. The prosomal fractions of several runs were pooled and concentrated by anion exchange chromatography using a salt step from 360 mM to 560 mM KCl in FPLC buffer. Subsequently, aliquots of 200 µl were subjected to a gel filtration column of a FPLC system (Superose 6B; Pharmacia, Freiburg/FRG) equilibrated with 560 mM KCl in FPLC buffer. The chromatography was performed at a flow rate of 0.5 ml/min, and the absorption at 280 nm was measured during the run. Fractions of 1 ml were collected and precipitated with 2.5 vol. ethanol.

Determination of buoyant densities

Buoyant densities of purified prosomes were determined by analytical ultracentrifugation. Prosomal fractions in FPLC buffer were brought to a density of 1.19 to 1.22 g/cm^3 with Cs_2SO_4 and centrifuged in a Centriscan 75 (48 h, 45000 rpm, 20°C; MSE, Crawley/UK). After reaching the equilibrium, the absorbance at 280 nm was measured along the sample cells and recorded. Bouyant densities were calculated as described [19, 27, 45].

Determination of sedimentation coefficients

Sedimentation coefficients of plant prosomes were determined by analytical ultracentrifugation. Prosomes were centrifuged through 5% (w/w) sucrose in FPLC buffer using Vinograd cells (Centriscan 75, six-place analytical rotor, 40000 rpm, 20°C; MSE). During centrifugation the absorbance at 280 nm was measured along the sample cells and recorded. Sedimentation coefficients were calculated as described [32].

Gel electrophoresis of proteins

Denaturing polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was performed in 12.5% polyacrylamide (PA) gels with 5% PA stacking gels [26]. Molecular weight markers (LKB-Pharmacia, Freiburg/FRG) were phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carboanhydrase (30 kDa), trypsin inhibitor (20 kDa) and lactalbumin (14.4 kDa). Non-equilibrium electrofocusing [34] was performed using ampholines (LKB-Pharmacia) in the range of *p*H 3.5 to 10. The gels for the first dimension were run for 4 h at 400 V. For the second dimension 12.5% PA gels (see above) were used.

Non-denaturing gel electrophoresis [7] was carried out using 2% PA/0.5% agarose gels in Tris-borate buffer, *p*H 8.3. Prosomal fractions were concentrated by ultracentrifugation, resuspended in sample buffer (10 mM Tris-HCl, *p*H 7.4, 20 mM EDTA, 10 mM NaCl, 1 mM 2-mercaptoethanol, 20% (w/w) sucrose) and after short centrifugation (12000 rpm, 1 min) to remove insoluble material, used for electrophoresis. After 20 min pre-electrophoresis at 200 V and 4°C, the samples were applied to the gel, and electrophoresis took place for 2 h at 200 V. The gels were stained with Coomassie Brilliant Blue.

Gel electrophoresis of RNA

Ethanol-precipitated material from prosomal fractions was resuspended in digestion buffer (10 mM Tris-HCl, *p*H 7.5, 100 mM NaCl, 2 mM EDTA, 0.5% Na-N-lauroylsarcosinate) and digested for 2 h at 37°C with proteinase K (10 mg/ml, Boehringer, Mannheim/FRG). RNA was extracted with chloroform/phenol [28], precipitated with 2.5 vol. ethanol/0.5 vol. 3 M sodium acetate/acetic acid, *p*H 5.5, and analyzed in 11% PA gels containing 7 M urea, 50 mM Tris-borate buffer, *p*H 8.3, and 1 mM EDTA [14]. Molecular weight markers were phe-tRNA, TMV-RNA, 5S-, 18S-, and 28S-rRNA. The gels were silver-stained [39].

Digestion of RNA

The postpolysomal pellet was resuspended in Tris buffer (10 mM Tris-HCl, *p*H 7.5, 15 mM NaCl) and incubated with 670 µg/ml RNase A in this buffer for 5 min. After addition of detergent buffer (20 mM Tris-HCl, *p*H 7.4, 1% Na-N-lauroylsarcosinate, 7 mM 2-mercaptoethanol), the postpolysomal pellet was centrifuged in sucrose gradients as described [25].

Electron microscopy

Drops of purified prosomal fractions were adsorbed onto freshly glow-discharged and carbon-coated copper grids. The grids were rinsed with water and the particles stained with 2% uranyl acetate. They were then rinsed again and air-dried. The micrographs were taken with a EM 10 electron microscope (Zeiss, Oberkochen/FRG) at 80 kV.

Immunoblotting

An antiserum against potato prosomes was raised in rabbit using prosomal particles isolated and concentrated by FPLC. For immunization, about 1.2 mg of prosomes, mixed with Freund's complete adjuvants (Difco, Detroit, MI/USA), were injected. After two and three weeks, the rabbit was boostered with about 1.0 and 0.7 mg of prosomes, respectively, each mixed with Freund's incomplete adjuvants (Difco).

Prosomal proteins were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose [44]. After transfer, the remaining binding sites on the nitrocellulose sheets were blocked with 2% gelatine/0.5% Tween 20 in TBS (20 mM Tris-HCl, *p*H 7.5, 500 mM NaCl). After washing with TTBS (20 mM Tris-HCl, *p*H 7.5, 500 mM NaCl, 0.05% Tween 20), the nitrocellulose sheets were incubated for 3 h at 37°C with antisera (rabbit anti-potato-prosome, rabbit anti-calf-prosome) diluted 1:100 in TTBS. After repeated washing in TTBS, the nitrocellulose sheets were incubated at 37°C with peroxidase-labeled swine anti-rabbit antibodies (Dakopatts, Hamburg/FRG) diluted 1:200 in TTBS for 1 h. After washing in TTBS and TBS, the sheets were assayed for peroxidase activity by incubation in peroxidase substrate solution I (50 ml 100 mM Tris-HCl, *p*H 7.6, 10 ml 3 mg/ml 4-Cl-1-naphthol, 50 µl 30% H_2O_2).

Lectin blotting

After separation on SDS-PAGE, prosomal proteins were transferred electrophoretically from the gel to nitrocellulose [44]. After transfer, the remaining binding sites were blocked by incubation for 2 min in 2% Tween 20 in TBS. After washing in TBS and lectin buffer (10 mM Tris-HCl, *p*H 7.2, 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 10 mM NaN_3, 0.05% Tween 20), the nitrocellulose sheets were incubated overnight with biotinylated lectins (2 µg/ml; Kem-En-Tec, Hellerup/Denmark) in lectin buffer. The following biotinylated lectins were used: Bandeiraea simplicifolia agglutinins I and II (BS I and BS II), Concanavalin A (Con A) from Canavalia ensiformis, and agglutinins from Dolichos biflorus (DBA), Lens culinaris (LCH), Limulus polyphemus (LPA, Limulin III), Phaseolus vulgaris (PHA), Arachis hypogaea = peanut (PNA), Pisum sativum (PSA), Glycine max = soybean (SBA), Solanum tuberosum (STA), Ulex europaeus (UEA), Vicia faba (VFA), Triticum vulgaris = wheat germ (WGA). After incubation with the lectins, the nitrocellulose sheets were washed in lectin buffer and incubated for 3 h with horseradish peroxidase-conjugated biotinylated streptavidin (Amersham, Braunschweig/FRG) diluted 1:400 to 1:1000 in lectin buffer. After washing in lectin buffer and TBS, the nitrocellulose sheets were assayed for peroxidase activity by incubation in peroxidase substrate solution II (100 ml 50 mM Na-acetate/acetic acid, *p*H 5.5, 4 ml 1% 3-amino-9-ethylcarbazole in acetone, 50 µl 30% H_2O_2). For competition experiments with Con A, VFA, and DBA, the corresponding sugar residues methyl-α-D-mannoside, methyl-α-D-glucoside, and N-acetyl-D-galactosamine (0.5 M; Sigma, Deisenhofen/FRG) were added to the reaction mixtures and treated as described above.

Results and discussion

Prosomes can be prepared from plant tissues by two different methods. Initially, prosomes were purified by sucrose gradient centrifugation made up with 1% sodium-N-lauroylsarcosinate, since prosomes are known to be resistent to this strong detergent [37]. Alternatively, fast protein liquid chromatography (FPLC) was chosen for the isolation of prosomes. Quantities of pure prosomes can be obtained with non-green plant tissues (etiolated mung bean seedlings and potato sprouts or tobacco stems and petals), whereas preparations derived from green leaf tissue tend to be heavily contaminated with ribulose-1,5-bisphosphatecarboxylase/-oxygenase (rubisco, 14 kDa and 55 kDa subunits); this protein is most abundant in green leaves and cannot be easily separated from the prosomal particles (Fig. 1).

Analysis of the protein composition of prosomes of the three species revealed a characteristic set of proteins in the molecular mass range of 25 to 35 kDa (Fig. 1). When prosomes of different tissues of mung bean and tobacco were analyzed, no differences in the protein pattern from the different kinds of tissues of the same species were detected (Fig. 1). Obviously, there is no tissue specificity of the protein pattern.

Electron microscopic examination of the prosomal fractions revealed identical structures for the prosomes of all three plants (tobacco, potato, and mung bean), having the same characteristic feature as prosomes of animal tissues. As shown in Figure 2, two types of structures are visible in

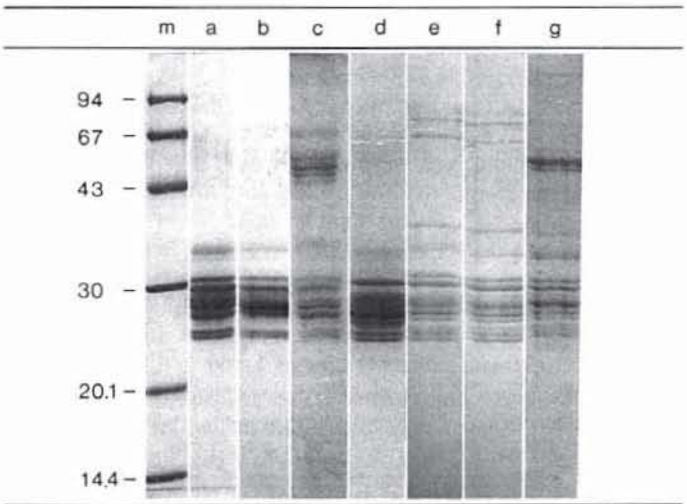

Fig. 1. SDS-polyacrylamide gel electrophoresis of prosomal fractions from different plant tissues. Prosomal fractions of different plant tissues were analyzed by one-dimensional (17.5%) polyacrylamide gel electrophoresis. All tissue fractions revealed a characteristic set of proteins in the range of 25 to 35 kDa. The two fractions from leaf tissue in addition showed a contamination with proteins of about 55 kDa (rubisco). — *Lanes a* to *c*: tobacco: blossoms (*lane a*), stems (*lane b*), leaves (*lane c*); *lane d*: potato sprouts; *lanes e* to *g*: mung-bean seedlings: stems (*lane e*), roots (*lane f*), cotyledons (*lane g*); *lane m*: molecular weight marker proteins. All proteins Coomassie-stained. The prosomal fractions were isolated by FPLC (*lanes a, b, d*) and sucrose gradient centrifugation, respectively.

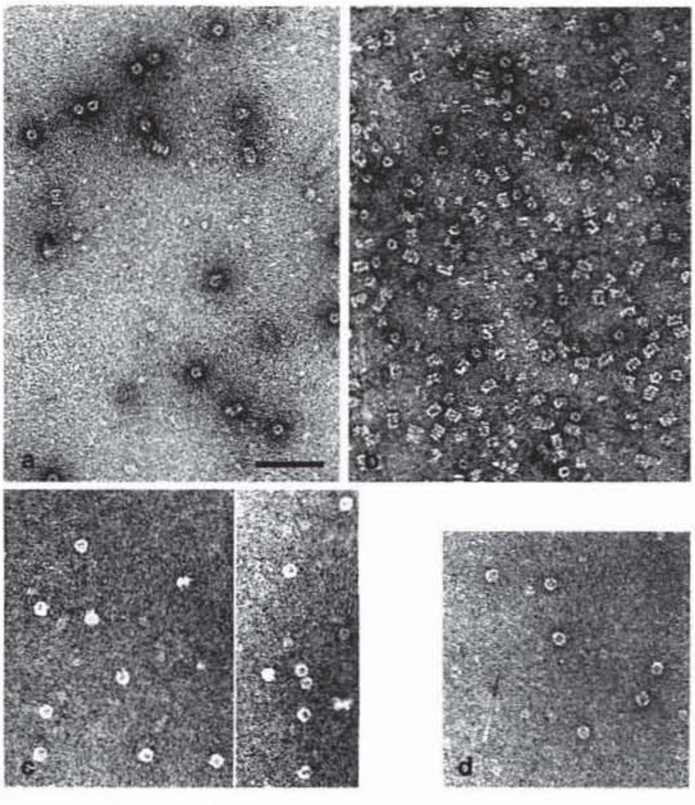

Fig. 2. Electron micrographs of prosomal fractions from different plant species. Prosomal fractions of tobacco (**a**), potato (**b**) and mung bean (**c, d**) were examined by electron microscopy after staining with 2% uranyl acetate. Two types of structures are visible: ring-shaped particles of 12 to 14 nm diameter and cylindrical particles of the same diameter, but they probably represent different perspectives of the same particle. Digestion of mung-bean particles by RNase A prior to examination did not alter the shape of the particles (**d**). — Bar 100 nm.

the various prosomal preparations: One type is ring-shaped with a diameter of 12 to 14 nm, the other one is cylindrical with the same diameter and a length of 16 to 18 nm. These two structures probably represent side views and the end-on views of the same cylindrical particle [5]. To verify the homogenous character of the prosomal preparations, we analyzed them further by electrophoresis under non-denaturing conditions. Since only one distinct band could be detected, we assume that only one type of particle was present. Further, when sedimentation coefficients of purified plant prosomes were determined by analytical ultracentrifugation, again a single peak was visible as shown for tobacco prosomes in Figure 3b.

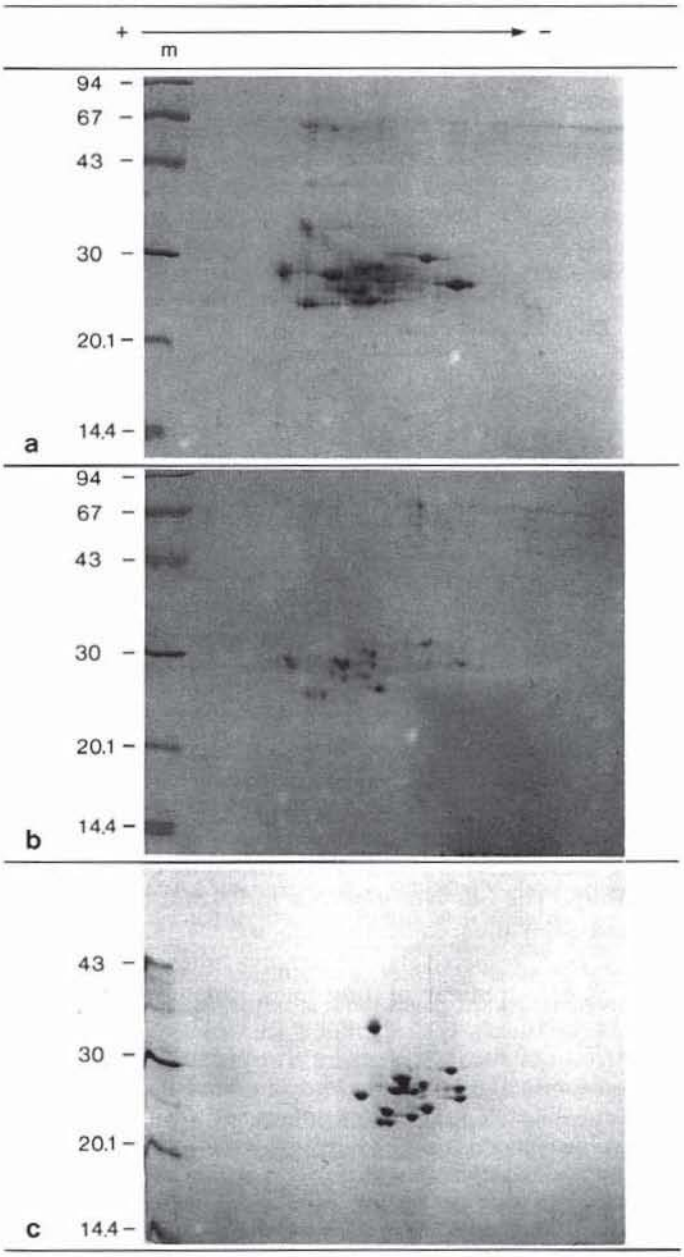

Fig. 3. Analysis of protein and RNA components. Two-dimensional PAGE of prosomal proteins. Prosomal fractions were analyzed by two-dimensional PAGE, revealing similar patterns of 12 to 15 slightly acidic proteins. — **a**. Tobacco. — **b**. Potato — **c**. Mung bean. — m: Molecular weight marker proteins. All proteins Coomassie-stained.

The fact that plant prosomes migrated as one band under conditions of non-denaturing electrophoresis, has also been reported for prosomes/proteasomes of other species [3, 8, 41] and suggests the presence of one distinct particle. This was further supported by analytical ultracentrifugation analysis by us and by others [42], where only one peak of sedimenting particles was detected.

The sedimentation coefficients of prosomes from the three different plant species were between 22S and 23S. This is slightly higher than the coefficients of animal prosomes, reported to be between 19S and 22S [3, 22, 37].

Prosomes/proteasomes are particles of approximately 650 kDa total molecular mass, but the values reported in the literature vary, which is likely to be due to the different evaluation methods used. Our data suggest that prosomes of higher plants seem to be somewhat larger complexes than the prosomes/proteasomes of most animal species. This assumption is consistent with results of a comparative study, in which the sizes of prosomes from several eukaryotic species were determined; the largest dimensions were found for prosomes from wheat germ, the only plant tissue tested [3].

Analysis of the protein constituents of plant prosomes by two-dimensional gel electrophoresis revealed a complex pattern of 12 to 15 slightly acidic proteins with isoelectric points of *p*H 5 to 7 (Fig. 3). This corresponds with the protein pattern of animal prosomes [3, 29]. The prosomal protein patterns of tobacco and potato are nearly identical. Ten out of the twelve proteins of the two Solanaceae have the same electrophoretic mobilities. The protein pattern of prosomes from mung bean is somewhat different but shares several subunits with the other plant prosomes, as far as molecular weight and isoelectric point are concerned. These similarities could be supported by immunological studies.

A rabbit antiserum against prosomal particles of potato sprouts recognized all proteins of the homologous potato prosome in Western blot analysis. When tested on prosomal proteins of the other two species, tobacco and mung bean, the antibody reacted with all proteins with the exception of the smallest ones, p24 and p25 (Fig. 4b). This antiserum cross-reacted also with prosomes from animal tissues. It recognized only the 29 kDa protein of prosomal proteins from calf liver and two proteins (25 kDa and 29 kDa) of prosomes from mouse spleen cells (Fig. 4b). On the other hand, an antiserum against calf liver prosomes, which recognized at least 4 proteins of the homologous calf liver prosome, reacted with only one prosomal protein from mouse, tobacco, potato, and mung bean in the molecular mass range of 25 to 26 kDa (Fig. 4c). Previously, immunological relationship between proteins from prosomes/proteasomes of different animal species was reported in several studies [13, 15], and even immunological relationships between proteins of prosomes/proteasomes from Xenopus and yeast [23], and from rat and archaebacteria [9] are reported. From these data a rather high evolutionary conservation of some components of the prosome may be concluded.

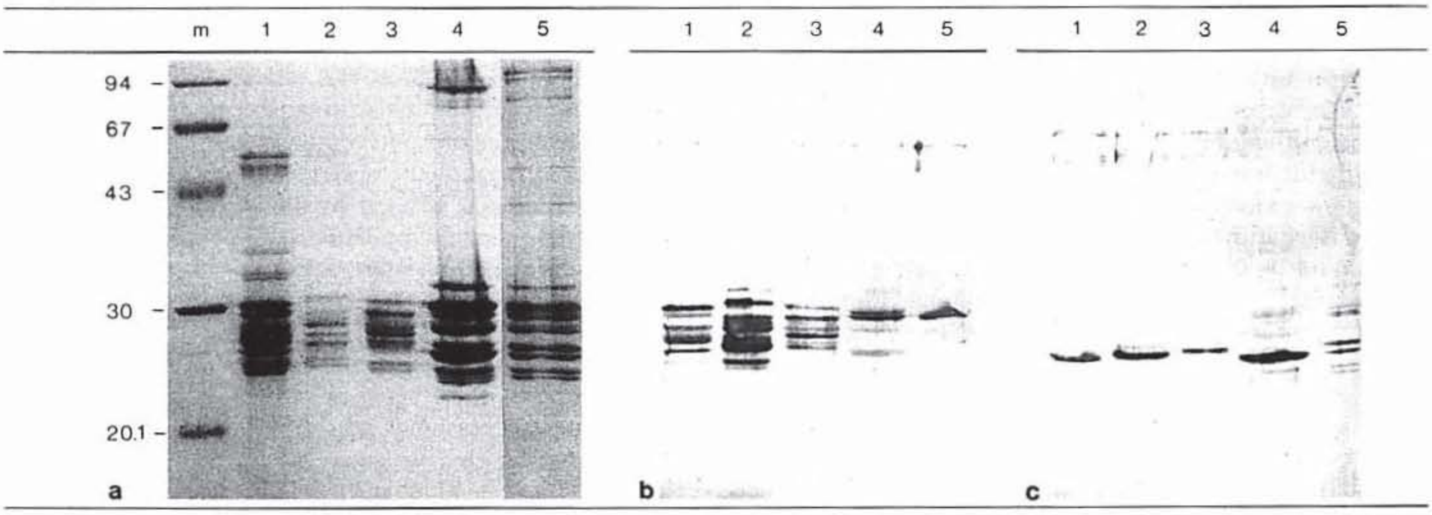

Fig. 4. Cross reactions of antisera against potato and calf liver prosomes with prosomal proteins of different species. Prosomal proteins of different species separated on 12.5% polyacrylamide gels were transferred to nitrocellulose sheets and incubated with rabbit antiserum against potato or calf liver prosomes. Binding reactions were visualized by incubation with peroxidase-labeled swine anti-rabbit antibodies after peroxidase/substrate reaction. — **a**. Polyacrylamide gels (12.5%) of prosomal fractions of mung bean (*lane 1*), potato (*lane 2*), tobacco (*lane 3*), mouse erythroblasts (*lane 4*), calf liver (*lane 5*). — m: Molecular weight marker proteins, Coomassie-stained. — **b**. Incubation after Western blotting with an antiserum raised against prosomal proteins of potato. The other two plant species showed positive signals for all proteins except in the range of 24 to 25 kDa. Cross reaction with calf liver and mouse proteins was obtained with a protein of about 29 kDa and an additional protein of about 25 kDa for mouse. — **c**. Incubation after Western blotting with an antiserum raised against calf liver prosomal proteins. This antiserum recognized proteins in the range of 25 to 26 kDa in all plant species.

However, the question arises whether or not proteins of plant prosomes are glycosylated, as it had been reported for animal prosomes previously [43]. To verify this, prosomal proteins from potato and mung bean were transferred to nitrocellulose and incubated with various biotinylated lectins. As shown in Figures 5a and 5c, the lectins Con A, VFA, and DBA bind to some prosomal proteins, regularly to p27, indicating the presence of glucosyl- or mannosyl-, and N-acetylgalactosaminyl residues. The specificity of these reactions was demonstrated in competition experiments, with the corresponding 1-methylsaccharides. Incubation with these sugars resulted in reduced lectin binding to the respective prosomal proteins (Fig. 5b). These findings were not unexpected since prosomal proteins of animal cells are modified by phosphorylation, ADP-ribosylation, and glycosylation [30]. Glucosyl- or mannosyl residues have also been found in prosomal proteins in mouse [43]. So far N-acetylglucosamine and N-acetylneuraminic acid were identified in mouse prosomes [43], while, according to our investigations, N-acetylgalactosamine is likely to be present in plant prosomes. One may argue that sugar residues could be important for the stability of the particle against self-digestion or proteolysis by other proteases [20]. It was also proposed that glycosylation of cytoplasmic proteins might be essential for the formation and stabilization of multiprotein complexes or could direct particles to specific intracellular targets [17, 18].

It has been reported that prosomes of animal cells or rather the 19S fractions of the postribosomal supernatants contain RNA. These RNA as well as purified prosomes react with viral mRNA and may thus be of functional importance for the cell [21]. From prosomal fractions of all three plant species RNA could be purified either by FPLC or by sucrose gradient centrifugation followed by Cs_2SO_4-gradient centrifugation. When examined by PAGE, a small RNA band of approximately 80 nucleotides was detected (Fig. 6). From FPLC-isolated prosomes, the yield of RNA was lower than from prosomes purified through sucrose gradients. The amount of RNA in prosomal fractions varied somewhat, but stoichiometric amounts of RNA could not be detected. This could be confirmed by density determinations. The buoyant densities in Cs_2SO_4 of prosomes isolated from tobacco and potato were 1.232 g/cm^3 and 1.229 g/cm^3, respectively. Preparative ultracentrifugation in a Cs_2SO_4-gradient of prosomes isolated from mung bean revealed a similar low value of 1.24 g/cm^3. RNAs of about 70 to 80 nucleotides have also been detected in prosomes of animal tissues with buoyant densities of 1.29 and 1.31 g/cm^3 in Cs_2SO_4, which allows to estimate a RNA content of 12 to 15% [2, 37, 38]. But in other studies, no RNA or only non-stoichiometric amounts of RNA could be detected [4, 6, 22, 23, 42]. The buoyant density and absorption spectra (not shown) rather indicate that prosomes in higher plants are comprised exclusively of proteins. After incubation with RNase, no RNA could be detected in purified prosomal fractions from mung bean (Fig. 4b), which is in contrast to findings in animal prosomes [10]. The morphology of the plant particles is not affected by the RNase treatment (Fig. 2d). It seems as if RNA is not a

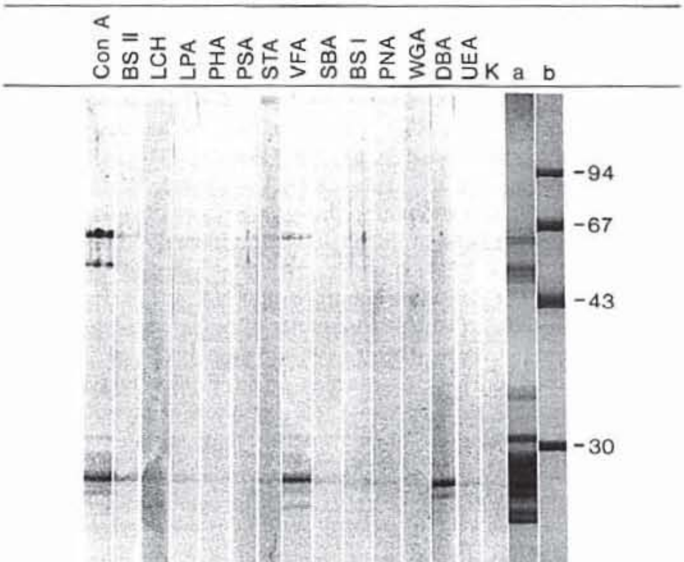

Fig. 5. Analysis of glycoproteins. Glycoproteins were determined after separation of prosomal proteins by 12.5% polyacrylamide gel electrophoresis and Western blotting, followed by incubation with different biotinylated lectins. The lectins were visualized by incubation with streptavidin-conjugated peroxidase and subsequent peroxidase/substrate reaction. — **a**. Prosomal proteins of potato were incubated with different biotinylated lectins (abbreviations see Materials and methods). Positive reactions, mainly with a protein of about 27 kDa, were obtained with Con A and VFA (both specific for α-D-glucosyl and α-D-mannosyl residues) as well as with DBA (specific for N-acetyl-α-D-galactosaminyl-residues). — *Lane a*: PAGE-separated proteins, Coomassie-stained; *lane b*: molecular weight marker proteins; *lane K*: streptavidin control. — **b**. The specificity of the reactions is shown by competition experiments. Western blotted prosomal proteins of potato were incubated with the biotinylated lectins Con A, VFA and DBA and with the corresponding 1-methylglycosides. — *Lane a*: without glycoside; *lane b*: methyl-α-D-glucoside; *lane c*: methyl-α-D-mannoside; *lane d*: N-acetyl-α-D-galactosamine; *lane K*: streptavidin control; *lane m*: molecular weight marker proteins, Amido black-stained. Concentration of haptens: 0.5 M. — **c**. Prosomal proteins of mungbean seedlings revealed a positive reaction with the biotinylated lectins VFA and DBA in the range of 25 to 26 kDa. — *Lane K*: streptavidin control; *lane m*: PAGE-separated proteins, Coomassie-stained.

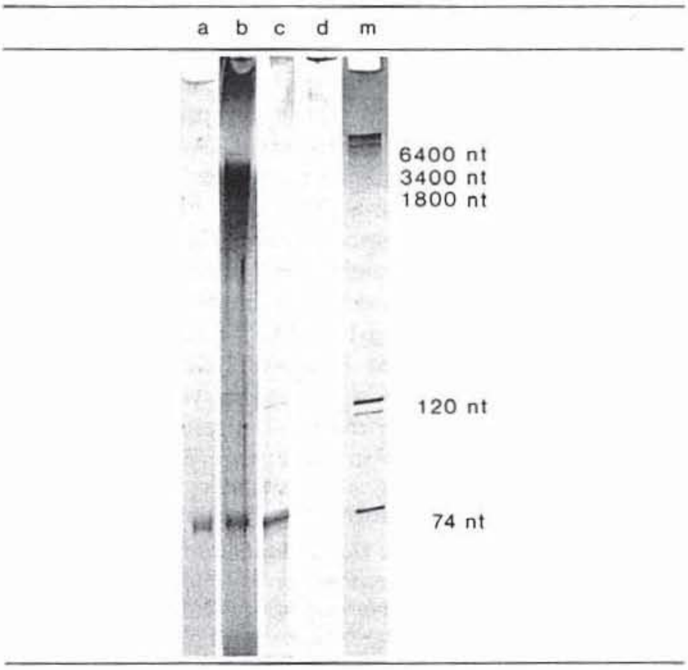

Fig. 6. Gel electrophoresis of RNA. RNA extracted from prosomal fractions was submitted to 11% polyacrylamide urea gels. The preparations of all plants showed an RNA of about 80 nucleotides (nt). When digested by RNase A prior to RNA isolation, the prosomal fraction of mung bean revealed no presence of RNA. — *Lane a*: tobacco; *lane b*: potato; *lane c*: mung bean; *lane d*: mung bean after digestion by RNase A; *lane m*: molecular weight marker RNA. All RNAs silver-stained.

genuine structural component of plant prosomes but rather associates in a nonspecific way.

In summary, our data indicate that plant prosomes are identical with prosomes/proteasomes of animal tissues, supporting the idea of ubiquity of these particles. Thus, a basic cellular function can be envisaged [35]. It has been shown that prosomes play a role in the posttranscriptional regulation of gene expression, since prosomes were initially found to cosediment with repressed messenger ribonucleoprotein particles in extracts of animal tissues [1, 29, 37]. An interesting inhibitory effect of prosomes and/or RNA of prosomal fractions from mouse on the expression of viral mRNA has been reported. This inhibition is thought to be due to an association of prosomal RNA with the 5′-regions of viral mRNA, thus preventing initiation of translation [21]. Surprisingly, in preliminary experiments an inhibitory effect of purified plant prosomes (which do not contain stoichiometric amounts of RNA) on the translation of tobacco mosaic virus RNA was also observed, whereas the translation of globin-mRNA was not affected [24]. Possibly, the inhibition is not likely to be due to the action of RNA. One may rather speculate that it is a result of a proteolytic activity of the prosome, affecting some viral-specific messenger binding proteins, since a multicatalytic proteinase activity is present in prosomes/proteasomes [4, 8, 9, 12, 13, 23, 31, 35, 41]. As was found in our laboratory, purified mung bean prosomes were able to hydrolyze methylcasein [36], a proteolytic activity which is known from animal proteasomes [4, 8, 13].

By the physical, biochemical and immunological data presented in this paper, and by functional aspects [36], the plant prosomes are demonstrated to be particles homologous to the proteasomes (multicatalytic protease) described from animals, yeast and prokaryotes. Therefore, the need of evaluation of the basic physiological role of these enzyme complexes in eukaryotic and prokaryotic cells still increases.

Acknowledgements. We thank Dr. G. Adam for his help in raising antibodies against potato prosomes, Dr. L. Kühn, W. Tomek, S. Großkopf, and R. Vallon for providing antisera and animal prosomes, K. Maurer for the non-denaturing gel electrophoresis, M. Flachmann and Dr. H. Ungibauer-Kremp for help in electron microscopy and analytical ultracentrifugation, Prof. K. Scherrer for discussions, and U. Vallon for help in preparing the manuscript. — This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Ku 310/7).

References

[1] Akhayat, O., M.-F. Grossi de Sa, A. A. Infante: Sea urchin prosome: Characterization and changes during development. Proc. Natl. Acad. Sci. USA **84**, 1595-1599 (1987).

[2] Arrigo, A.-P., J.-L. Darlix, E. W. Khandijan, M. Simon, P.-F. Spahr: Characterization of the prosome from Drosophila and its similarity to the cytoplasmatic structures formed by the low molecular weight heat-shock proteins. EMBO J. **4**, 399-406 (1985).

[3] Arrigo, A.-P., M. Simon, J.-P. Darlix, P.-F. Spahr: A 20S particle ubiquitous from yeast to human. J. Mol. Evol. **25**, 141-150 (1987).

[4] Arrigo, A.-P., K. Tanaka, A. L. Goldberg, W. J. Welch: Identity of the 19S "prosome" particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature **331**, 192-194 (1988).

[5] Baumeister, W., B. Dahlmann, R. Hegerl, F. Kopp, L. Kuehn, G. Pfeifer: Electron microscopy and image analysis of the multicatalytic proteinase. FEBS Lett. **241**, 239-245 (1988).

[6] Castaño, J. G., R. Ornberg, J. G. Koster, J. A. Tobian, M. Zasloff: Eukaryotic pre-tRNA 5'processing nuclease: Copurification with a complex cylindrical particle. Cell **46**, 377-387 (1986).

[7] Dahlberg, A. E., C. W. Dingman, A. C. Peacock: Electrophoretic characterization of bacterial polyribosomes in agarose-acrylamide composite gels. J. Mol. Biol. **41**, 139-147 (1969).

[8] Dahlmann, B., L. Kuehn, M. Rutschmann, H. Reinauer: Purification and characterization of a multicatalytic high-molecular-mass proteinase from rat skeletal muscle. Biochem. J. **228**, 161-170 (1985).

[9] Dahlmann, B., F. Kopp, L. Kuehn, B. Niedel, G. Pfeifer, R. Hegerl, W. Baumeister: The multicatalytic proteinase (prosome) is ubiquitous from eukaryotes to archaebacteria. FEBS Lett. **251**, 125-131 (1989).

[10] Dineva, B., W. Tomek, K. Köhler, H.-P. Schmid: Evidence for a highly nuclease resistant RNA fragment in prosomes. Mol. Biol. Rep. **13**, 207-211 (1989).

[11] Domae, N., F. R. Harmon, R. K. Busch, W. Spohn, C. S. Subrahmanyam, H. Busch: Donut-shaped "miniparticles" in nuclei of human and rat cells. Life Sci. **30**, 469-477 (1982).

[12] Driscoll, J., A. L. Goldberg: Skeletal muscle proteasome can degrade proteins in an ATP dependent process that does not require ubiquitin. Proc. Natl. Acad. Sci. USA **86**, 787-791 (1989).

[13] Falkenburg, P.-E., C. Haass, P.-M. Kloetzel, B. Niedel, F. Kopp, L. Kuehn, B. Dahlmann: Drosophila small cytoplasmic 19S ribonucleoprotein is homologous to the multicatalytic proteinase. Nature **331**, 190-192 (1988).

[14] Grierson, D.: Gel electrophoresis of RNA. In: D. Rickwood, B. D. Hames (eds.): Gel electrophoresis of nucleic acids: a practical approach. IRL Press. Oxford, Washington 1982.

[15] Grossi de Sa, M.-F., C. Martins de Sa, F. Harper, M. Olink-coux, M. Huesca, K. Scherrer: The association of prosomes with some of the intermediate filament networks of the animal cell. J. Cell Biol. **107**, 1517-1530 (1988).

[16] Haass, C., P.-M. Kloetzel: The Drosophila proteasome undergoes changes in its subunit pattern during development. Exp. Cell Res. **180**, 243-252 (1989).

[17] Hart, G. W., G. D. Holt, R. S. Haltiwanger: Nuclear and cytoplasmic glycosylation: novel saccharide linkages in unexpected places. Trends Biochem. Sci. **13**, 380-384 (1988).

[18] Hart, G. W., R. S. Haltiwanger, G. D. Holt, W. G. Kelly: Glycosylation in the nucleus and cytoplasm. Annu. Rev. Biochem. **58**, 841-874 (1989).

[19] Hennig, W.: Ultrazentrifugation und ihre Anwendung in der Untersuchung des Eukaryoten-Genoms. Instrument und Forschung **3/75**, 3-22 (1975).

[20] Holzer, H., P. C. Heinrich: Control of proteolysis. Annu. Rev. Biochem. **49**, 63-91 (1980).

[21] Horsch, A., C. Martins de Sa, B. Dineva, E. Spindler, H.-P. Schmid: Prosomes discriminate between mRNA of Adenovirus-infected and uninfected HeLa cells. FEBS Lett. **246**, 131-136 (1989).

[22] Kleinschmidt, J. A., B. Hügle, C. Grund, W. W. Franke: The 22S cylinder particles of Xenopus laevis. I. Biochemical and electron microscopic characterization. Eur. J. Cell Biol. **32**, 143-156 (1983).

[23] Kleinschmidt, J. A., C. Escher, D. H. Wolf: Proteinase yscE of yeast shows homology with the 20S cylinder particles of Xenopus laevis. FEBS Lett. **239**, 35-40 (1988).

[24] Kremp, A.: Isolierung und Charakterisierung von 23S-Partikeln (Prosomen) aus Nicotiana rustica L. und Solanum tuberosum L. Dissertation, Universität Stuttgart (1989).

[25] Kremp, A., M. Schliephacke, U. Kull, H.-P. Schmid: Prosomes exist in plant cells too. Exp. Cell Res. **166**, 553-557 (1986).

[26] Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680-685 (1970).

[27] Ludlum, D. B., R. C. Warner: Equilibrium centrifugation in cesium sulfate solutions. J. Biol. Chem. **240**, 2961-2965 (1965).

[28] Maniatis, T., E. F. Fritsch, J. Sambrook: Molecular Cloning. A laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY, 1982.

[29] Martins de Sa, C., M.-F. Grossi de Sa, O. Akhayat, F. Broders, K. Scherrer, A. Horsch, H.-P. Schmid: Prosomes: Ubiquity and inter-species structural variation. J. Mol. Biol. **187**, 479-493 (1986).

[30] Martins de Sa, C., E. Rollet, M.-F. Grossi de Sa, R. M. Tanguay, M. Belpomme, K. Scherrer: Prosomes and heat-shock complexes in Drosophila melanogaster cells. Mol. Cell. Biol. **9**, 2672-2681 (1989).

[31] Matthews, W., K. Tanaka, J. Driscoll, A. Ichihara, A. L. Goldberg: Involvement of the proteasome in various degradative processes in mammalian cells. Proc. Natl. Acad. Sci. USA **86**, 2597-2601 (1989).

[32] MSE Technical Publication No. 73 (Supplement): Basic theory and application of analytical ultracentrifugation.

[33] Narayan, K. S., D. E. Rounds: Minute ring-shaped particles in cultured cells of malignant origin. Nature New Biol. **243**, 146-150 (1973).

[34] O'Farrell, P. Z., H. M. Goodman, P. H. O'Farrell: High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12**, 1133-1142 (1977).

[35] Scherrer, K.: Prosomes, subcomplexes of untranslated mRNP. Mol. Biol. Rep. **14**, 1-9 (1990).

[36] Schliephacke, M.: Isolierung und Charakterisierung von 22S-Partikeln ("Prosomen") aus Tabak (Nicotiana rustica) und Mungbohne (Phaseolus radiatus). Dissertation, Universität Stuttgart (1989).

[37] Schmid, H. P., O. Akhayat, C. Martins de Sa, F. Puvion, K. Scherrer: The Prosome: an ubiquitous morphologically distinct RNP particle associated with repressed mRNPs and containing specific scRNA and a characteristic set of proteins. EMBO J. **3**, 29-34 (1984).

[38] Schuldt, C., P.-M. Kloetzel: Analysis of cytoplasmic 19S ring-type particles in Drosophila which contain hsp 23 at normal growth temperature. Dev. Biol. **110**, 65-74 (1985).

[39] Schumacher, J.: Horizontale Elektrophorese in ultradünnen Polyacrylamidgelen zur Auflösung von RNAs und DNA-Fragmenten. LKB Sonderdruck RE 040 (1985).

[40] Shelton, E., E. L. Kuff, E. S. Maxwell, J. T. Harrington: Cytoplasmic particles and aminoacyl transferase I activity. J. Cell Biol. **45**, 1-8 (1970).

[41] Tanaka, K., K. Ii, A. Ichihara, L. Waxman, A. L. Goldberg: A high molecular weight protease in the cytosol of rat liver. I. Purification, enzymological properties, and tissue distribution. J. Biol. Chem. **261**, 15197-15203 (1986).

[42] Tanaka, K., T. Yoshimura, A. Ichihara, K. Kameyama, T. Takagi: A high molecular weight protease in the cytosol of rat liver. II. Properties of the purified enzyme. J. Biol. Chem. **261**, 15204-15207 (1986).

[43] Tomek, W., G. Adam, H.-P. Schmid: Prosomes, small cytoplasmic RNP particles, contain glycoproteins. FEBS Lett. **239**, 155-158 (1988).

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

[45] Vinograd, J.: Sedimentation equilibrium in a buoyant density gradient. Methods Enzymol. **6**, 854-870 (1963).

[46] Wilk, S., M. Orlowski: Evidence that pituitary cation-sensitive neutral endopeptidase is a multicatalytic protease complex. J. Neurochem. **40**, 842-849 (1983).