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Oligomerization of profilins from birch, man and yeast. Profilin, a ligand for itself?

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Abstract Profilins are structurally well conserved low molecular weight (12–15 kDa) eukaryotic proteins which interact with a variety of physiological ligands: (1) cytoskeletal components, e.g., actin; (2) polyphosphoinositides, e.g., phosphatidylinositol-4,5-bisphosphate; (3) proline-rich proteins, e.g., formin homology proteins and vasodilator-stimulated phosphoprotein. Profilins may thus link the microfilament system with signal transduction pathways. Plant profilins have recently been shown to be highly crossreactive allergens which bind to IgE antibodies of allergic patients and thus cause symptoms of type I allergy. We expressed and purified from *Escherichia coli* profilins from birch pollen (*Betula verrucosa*), humans (*Homo sapiens*) and yeast (*Schizosaccharomyces pombe*) and demonstrated that each of these profilins is able to form stable homo- and heteropolymers via disulphide bonds in vitro. Circular dichroism analysis of oxidized (polymeric) and reduced (monomeric) birch pollen profilin indicates that the two states have similar secondary structures. Using ¹²⁵I-labeled birch pollen, yeast and human profilin in overlay experiments, we showed that disulphide bond formation between profilins can be disrupted under reducing conditions, while reduced as well as oxidized profilin states bind to actin and profilin-specific antibodies. Exposure of profilin to

oxidizing conditions, such as when pollen profilins are liberated on the surface of the mucosa of atopic patients, may lead to profilin polymerization and thus contribute to the sensitization capacity of profilin as an allergen.

Key words Profilin · Pollen · Cytoskeleton · Type I allergy · Oligomerization

Introduction

Profilins are ubiquitous eukaryotic proteins that are involved in microfilament dynamics as they bind to monomeric actin (G-actin) with an equilibrium dissociation constant in the micromolar range in vitro (Pollard and Cooper 1986). In vivo, profilin is thought to regulate the pools of G- and F-actin by simple monomer sequestration (Pollard and Cooper 1986), catalytic enhancement of actin-bound adenine nucleotide exchange (Mockrin and Korn 1980; Goldschmidt-Clermont et al. 1992; Carlier et al. 1993) and coupling of monomer addition to the growing filament with ATP hydrolysis (Pantaloni and Carlier 1993). In addition to actin, several other ligands have been identified for profilin. They include proline-rich peptides/proteins (Tanaka and Shibata 1985; Lindberg et al. 1988), e.g., vasodilator-stimulated phosphoprotein (Reinhard et al. 1995), formin homology proteins (Chang et al. 1997; Evangelista et al. 1997; Frazier and Field 1997; Imamura et al. 1997; Watanabe et al. 1997), and phosphatidylinositol-4,5-bisphosphate (PIP₂) (Lassing and Lindberg 1985; Machesky et al. 1990; Goldschmidt-Clermont et al. 1991; Drobak et al. 1994). By interacting with various natural ligands, profilin is thought to represent a link between the microfilament system and signal transduction pathways (Machesky and Pollard 1993; Sohn and Goldschmidt-Clermont 1994).

Plant profilins were discovered to be potent allergens which bind IgE antibodies of almost 20% of plant-allergic patients (Valenta et al. 1991, 1992). Thus IgE antibodies represent unwanted profilin ligands, which according to epitope mapping and structural analysis of birch pollen

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profilin, bind to exposed surface areas that are also involved in the actin and PIP₂ interaction (Valenta et al. 1993; Fedorov et al. 1997; Thorn et al. 1997). From these observations it has been concluded that the ligand-free profilin represents the sensitizing agent which induces IgE antibody responses in atopic individuals.

Likewise, the physiological functions of profilin appear to be controlled by alternating ligand interactions. Poly (L-proline)-bound, but not PIP₂-bound profilin, is able to interact simultaneously with actin. Hydrolysis of PIP₂ by phospholipase C has therefore been proposed as a mechanism to release membrane-bound profilin to allow its interaction with G-actin, thus establishing a link between signal transduction pathways and microfilament assembly (Goldschmidt-Clermont et al. 1990). Interestingly, profilin actions on actin can be divergent. The sequestering model suggests that profilin interaction with G-actin prevents microfilament assembly while under certain conditions, via promotion of exchange of the actin-bound adenine nucleotide, monomeric actin can be converted into a highly polymerizable form (Carlier and Pantaloni 1997).

The findings that profilins possess various functions, can accumulate in areas of rapid actin filament growth (Buss et al. 1992; Rothkegel et al. 1996), and are located in different cell compartments close to the cell membrane (Buss et al. 1992; Rothkegel et al. 1996), in the cytoplasm (Buss et al. 1992; Grote et al. 1995; Mittermann et al. 1995; Rothkegel et al. 1996; Vidali and Hepler 1997) and in the nucleus (Hess and Valenta 1997; Holzinger et al. 1997) suggest a tight control of profilin functions by various ligands, many of which remain to be discovered. Recently it was reported that natural human platelet profilin can form tetramers that bind to G-actin, indicating that profilin may be able to bind to itself, and simultaneously to actin (Babich et al. 1996). In order to investigate whether multimerization may be a feature common to profilins from various species and to study the conditions under which profilin can be a ligand for itself, we expressed and purified from *Escherichia coli* profilins from evolutionarily highly divergent organisms (birch pollen, humans and yeast). Purified recombinant profilins were then allowed to bind to each other in overlay experiments. We investigated the effects of reducing and non-reducing conditions on the homo- and heteropolymerization of profilins from birch, humans and yeast; the interaction of oxidized and reduced birch profilin with actin and antibodies; and the secondary structural characteristics of monomeric and polymeric birch pollen profilin.

Materials and methods

Reagents

Competent *E. coli* BL 21 (DE3) and plasmid pET 17b were purchased from Novagen (Madison, Wis.). The rabbit antisera raised against purified celery profilin (RP1), a C-terminal peptide of birch profilin (RP3) and recombinant tobacco pollen profilin RP5

were described previously (Vallier et al. 1992; Valenta et al. 1993; Mittermann et al. 1995). Purified rabbit skeletal muscle actin and bovine β -lactoglobulin were purchased from Sigma (St. Louis, Mo.).

Expression and purification of recombinant profilins

Recombinant birch profilin was expressed in *E. coli* BL21 (DE3) using plasmid pMW175 which contained the cDNA coding for birch profilin (Susani et al. 1995). Human profilin and *Schizosaccharomyces pombe* profilin were expressed using plasmid pMW172 (Almo et al. 1994; Fedorov et al. 1994). Plasmid pET17b expressing human acrosin, a proline-rich protein, was constructed using human acrosin-encoding cDNA isolated from a human testis cDNA library (Clontech, Palo Alto, Calif.) (Valenta et al., unpublished data). Freshly transformed *E. coli* BL21 (DE3) was inoculated directly into LB medium containing 100 mg/l ampicillin, grown to an optical density (600 nm) of 0.3 at 37°C and then induced by addition of IPTG (final concentration 1 mM) for 4 h. Recombinant profilins were purified by affinity to poly (L-proline) Sepharose as described (Lind et al. 1987; Lindberg et al. 1988) except that dialysis of the profilin-containing samples was carried out in 20 mM TRIS-HCl, pH 7.6, 150 mM NaCl and 0.1% v/v β -mercaptoethanol.

Colony lift, SDS-PAGE, immunoblotting

Protein interactions under non-denaturing conditions (i.e., without SDS or heating) were studied by colony overlays. *E. coli* BL21 (DE3) transformed with empty plasmid pET17b, plasmid pET17b containing the human acrosin-encoding cDNA, plasmid pMW175 expressing birch profilin or plasmids pMW172 expressing human and yeast profilins, respectively, were streaked onto LB plates containing 100 mg/l ampicillin. After *E. coli* colonies were visible, synthesis of proteins was induced at 37°C by a 4-h overlay with nitrocellulose filters (Schleicher and Schuell, Dassel, Germany) soaked in 10 mM IPTG. Proteins were liberated from nitrocellulose-bound *E. coli* cells by freeze thawing.

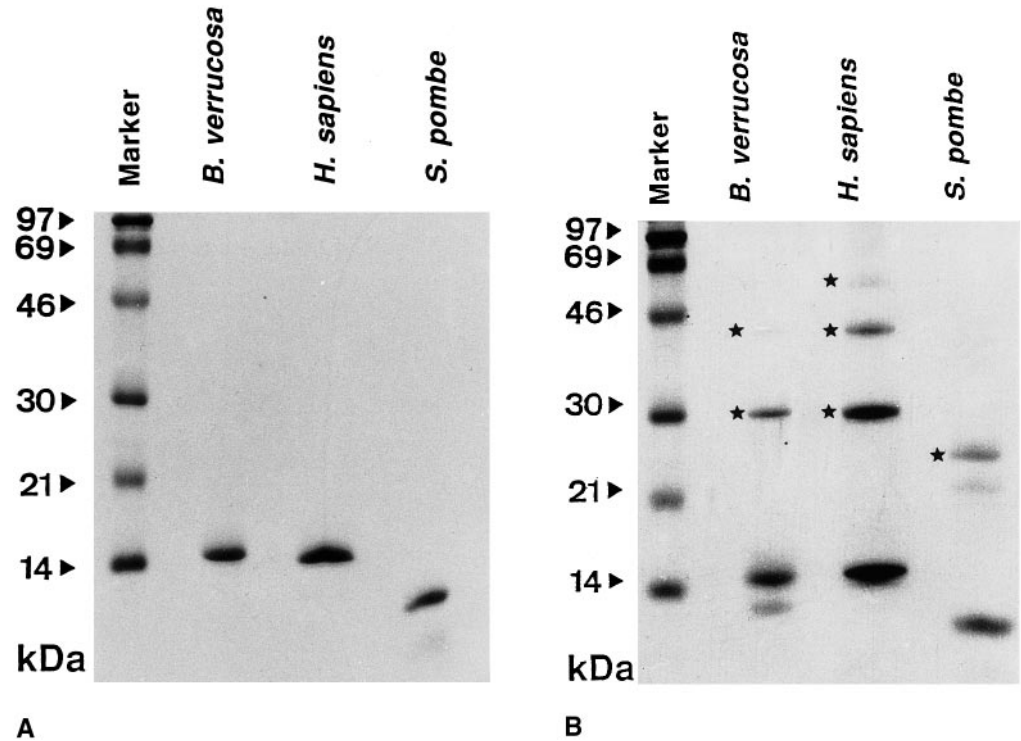
Purified proteins (profilin, actin, BSA) were separated by 12.5% SDS-PAGE (Fling et al. 1986) under reducing (sample buffer containing 5% v/v β -mercaptoethanol) or non-reducing (sample buffer without β -mercaptoethanol) conditions and transferred onto nitrocellulose membranes (Schleicher and Schuell) by electroblotting (Towbin et al. 1979). Nitrocellulose-blotted recombinant birch profilin separated under reducing and non-reducing conditions was exposed to rabbit anti-profilin antisera; bound rabbit Ig was detected with ¹²⁵I-labeled donkey anti-rabbit antiserum (Amersham, Buckinghamshire, UK) and visualized by autoradiography using X-OMAT films (Kodak, Heidelberg, Germany).

Iodine labeling of purified recombinant profilins, colony and blot overlay experiments

Thirty micrograms of purified recombinant birch pollen, human and yeast profilins were ¹²⁵I-labeled using the chloramine T method (Sambrook et al. 1989). ¹²⁵I-labeled proteins were separated from unincorporated ¹²⁵NaI and desalted using Sephadex PD10 columns (Pharmacia, Uppsala, Sweden). The ¹²⁵I-labeled proteins were stored in PBS containing 1% BSA at 4°C until use.

Nitrocellulose filters containing *E. coli* BL21 (DE3) colonies expressing birch, human and yeast profilins, and for control purposes human acrosin as a proline-rich protein or *E. coli* proteins, as well as nitrocellulose-blotted purified recombinant profilins (birch pollen, man, yeast), rabbit skeletal muscle actin and BSA (each 10 μ g/cm gel) were exposed to ¹²⁵I-labeled profilins. Nitrocellulose-blotted proteins were preincubated three times in overlay buffer (10 mM Tris-HCl, pH 7.5, 90 mM NaCl, 0.05% v/v Tween 20, 5% w/v BSA) for 30 min each, exposed to ¹²⁵I-labeled profilins (250000 cpm/ml overlay buffer) for 4 h, washed three times

Fig. 1A, B Purified recombinant profilins exhibit different migration patterns in SDS-PAGE under reducing and non-reducing conditions. Twenty micrograms of recombinant birch pollen (*Betula verrucosa*), human (*Homo sapiens*) and yeast (*Schizosaccharomyces pombe*) profilin were separated by 12.5% SDS-PAGE under reducing (Fig. 1A) and non-reducing (Fig. 1B) conditions. The molecular weight marker is shown on the left side of each gel. Profilin polymers in **B** are indicated by asterisks



for 30 min in overlay buffer, dried and exposed to Kodak X-OMAT films. To perform overlay assays under reducing conditions, overlay buffer containing 5 mM dithiothreitol (DTT) was used. The influence of different concentrations of Tween 20 (0.05%, 0.5%, 1%) in the overlay buffer was tested as well.

Circular dichroism spectroscopy and thermal stability measurements

Circular dichroism (CD) spectroscopy was accomplished on an Aviv 62DS spectropolarimeter essentially as described by Fetrow et al. 1997. Briefly, birch pollen profilin was dissolved in 10 mM phosphate buffer, pH 7.0, to a concentration of about 17.8 mM. Samples analyzed under denaturing conditions contained 6 M guanidine hydrochloride. Reduced samples contained 10 mM DTT. Degassed samples were assayed in 1-mm quartz cuvettes. Spectra were acquired from 200 to 250 nm, with a 1-nm bandwidth. The averaging time per data point was 15 to 30 s, depending on the experiment. To visualize the spectrum of each protein, mean residue ellipticity was calculated (Strickland 1974) and plotted against wavelength.

Thermal stability was assessed by monitoring the CD spectrum at 218 nm from 5–80°C, with a 30–90 s equilibration time, and a 30 s averaging time per data point, essentially as previously described (Fetrow et al. 1997). Mean residue ellipticity was calculated at each temperature and data were processed by a non-linear least squares fitting procedure (Kaleidagraph) to the equations of Elwell and Schellmann (1977), essentially as previously described (Cohen and Pielak 1994; Fetrow et al. 1997). The equation fits the mean residue ellipticity and temperature data to native and denatured baseline points, ΔH_m , and T_m . $\Delta G_{D,298}$ was not calculated because of a lack of an estimate for ΔC_p . Errors in these calculations were estimated using a standard analysis of error. Reported results are the averages of two to three experiments. To apply this type of analysis, the unfolding reaction must be two-state and reversible. To show reversibility, a complete scan from 205–250 nm was collected at 5°C before melting and another complete scan was acquired at 5°C after quickly cooling the sample following denaturation to 80°C. Scans acquired pre- and post-thermal denaturation

were superimposable for all samples (data not shown), demonstrating reversibility of the unfolding reaction.

Results

Self-association of purified recombinant profilins from birch pollen, man and yeast as determined by SDS-PAGE and Westernblot

SDS-PAGE analysis and Coomassie blue-staining under reducing and non-reducing conditions revealed a varying electrophoretic mobility pattern of purified recombinant profilins from birch pollen, man and yeast (Fig. 1). Under reducing conditions, birch pollen and human profilins appeared as single 14-kDa bands, and yeast profilin as a single 12-kDa moiety (Fig. 1A). Under non-reducing conditions (i.e., omission of β -mercaptoethanol from the sample buffer), monomer bands (15 kDa), lower molecular weight species (birch), bands of double molecular weight (birch, man, yeast) as well as tri- (birch, human) and tetramers (human) were observed (Fig. 1B) in Coomassie blue-stained SDS-PAGE containing aliquots of the same profilin preparations analyzed in Fig. 1A. Using three antisera raised against the plant profilins RP1 (celery root profilin, Vallier et al. 1992), RP3 (C-terminus of birch profilin, Valenta et al. 1993), RP5 (tobacco pollen profilin, Mittermann et al. 1995), mono-, di-, tri-, tetra-, and pentamers of birch profilin were detected under non-reducing conditions while under reducing conditions, predominantly the monomeric form was found (Fig. 2). Interestingly, the rabbit antiserum (RP3) raised against the C-terminal 25 amino acids of birch profilin

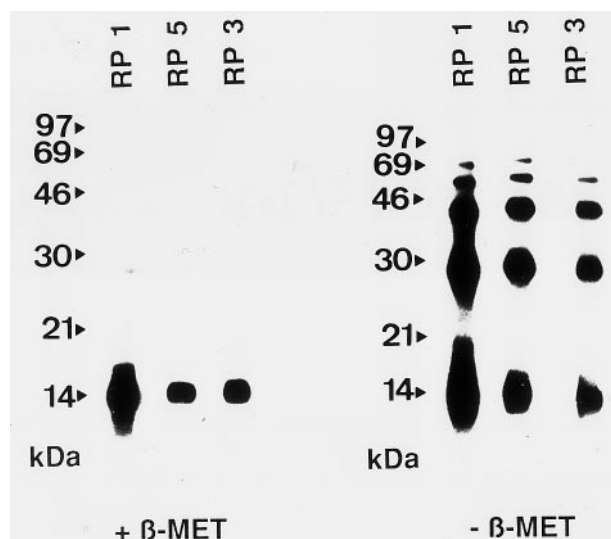


Fig. 2 Detection of nitrocellulose-blotted birch profilin and birch profilin polymers using anti-plant profilin antisera. Purified recombinant birch profilin was separated by SDS-PAGE under reducing (*left panel*, + β -MET) and non-reducing (*right panel*, - β -MET) conditions and blotted onto nitrocellulose. Nitrocellulose strips were incubated with three rabbit anti-plant profilin antisera (*lanes*, RP1, RP3, RP5), bound rabbit antibodies were detected with a ^{125} I-labeled donkey anti-rabbit antiserum and visualized by autoradiography. Molecular weights are shown on the *left side* of each panel

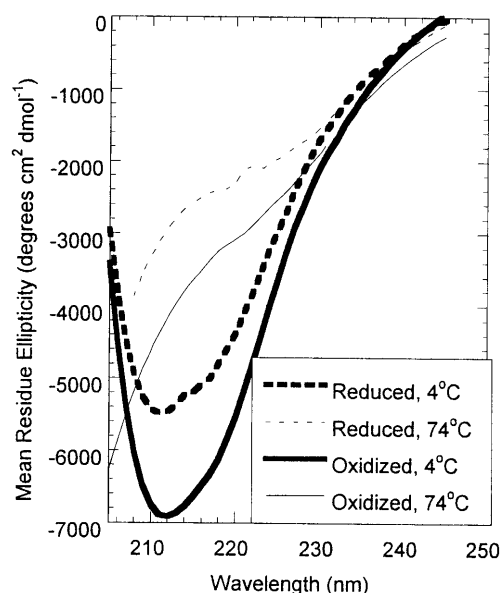


Fig. 3 Circular dichroism (CD) analysis of reduced and oxidized recombinant birch pollen profilin. CD spectra of reduced and oxidized profilin were recorded at 4°C (*thick lines*) and 74°C (*thin lines*). Reduced protein spectra are shown as *dashed lines*; oxidized spectra are shown as *solid lines*. The mean residue ellipticity (*y-axis*) is displayed for a given wavelength (*x-axis*)

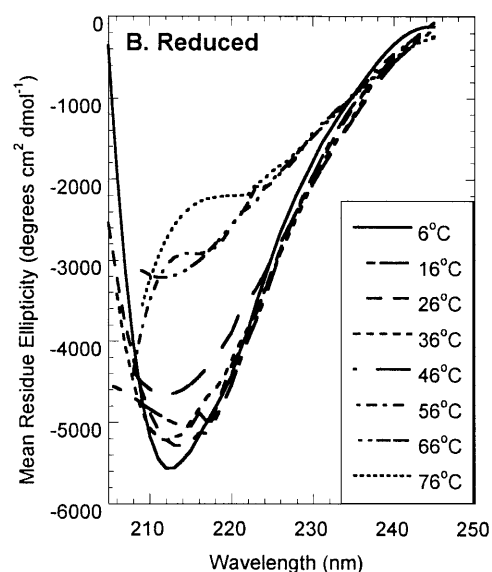
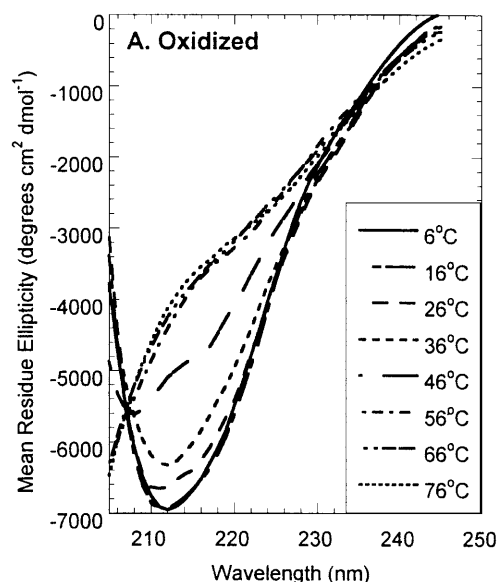


Fig. 4 CD spectra of oxidized (A) and reduced (B) birch pollen profilin recorded at several gradually increasing temperatures. The mean residue ellipticity (*y-axis*) at a given wavelength (*x-axis*) is displayed for the various temperatures

reacted equally well with the polymeric and monomeric profilin states although one of the two cysteines present in the birch profilin sequence is part of the carboxyterminal alpha helix.

Purified recombinant birch pollen profilin exhibits similar secondary structural features under reducing and non-reducing conditions as measured by circular dichroism analysis

Circular dichroism analysis of oxidized and reduced birch pollen profilin was performed at different temperatures: 4°C and 74°C (Fig. 3), and 6°C, 16°C, 26°C,

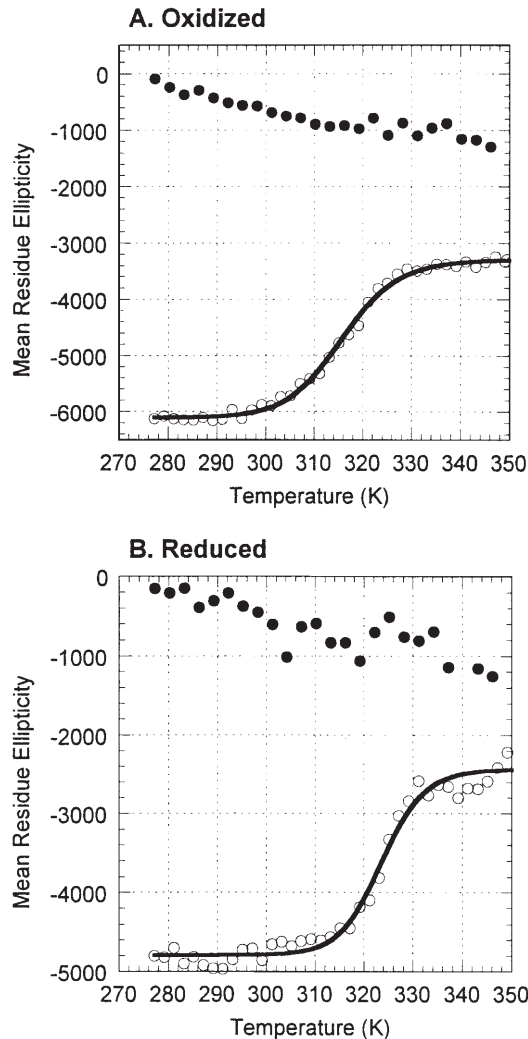


Fig. 5 Thermal denaturation curves show the temperature-dependent unfolding transition of oxidized (**A**) and reduced (**B**) recombinant native (open circles) and denatured (closed circles) birch pollen profilin recorded at 218 nm. Curves represent the best fit of thermodynamic equations to the data, as described in Materials and methods. Both curves fit with an R value greater than 0.99. The mean residue ellipticity (y -axis) is displayed at a given temperature (x -axis)

36°C, 46°C, 56°C, 66°C and 76°C (Fig. 4). The spectra of oxidized and reduced birch profilin qualitatively look very similar. Reduced birch profilin samples exhibited more noisy spectra than oxidized samples. Due to the minimum ellipticity centered between 212 and 214 nm, beta-sheet type structure was prominent, which is in agreement with the data obtained from the X-ray analysis of crystals grown from recombinant birch profilin. According to the X-ray data, birch profilin is composed of three alpha helices, seven beta sheets and ten turns (Fedorov et al. 1997). The unfolding transition of oxidized and reduced birch profilin was recorded at 218 and 222 nm between 4° and 80°C (Fig. 5). A temperature (T_m) of 42.6(±6.3)°C for the unfolding transition of oxidized and 50.5(±0.6)°C for reduced birch profilin was

Fig. 6 SDS-PAGE analysis of purified ^{125}I -labeled recombinant profilins. Approximately 5000 cpm of ^{125}I -labeled recombinant birch pollen (*Betula verrucosa*), and human (*Homo sapiens*) and yeast (*Schizosaccharomyces pombe*) profilin were separated by 12.5% SDS-PAGE and subjected to autoradiography

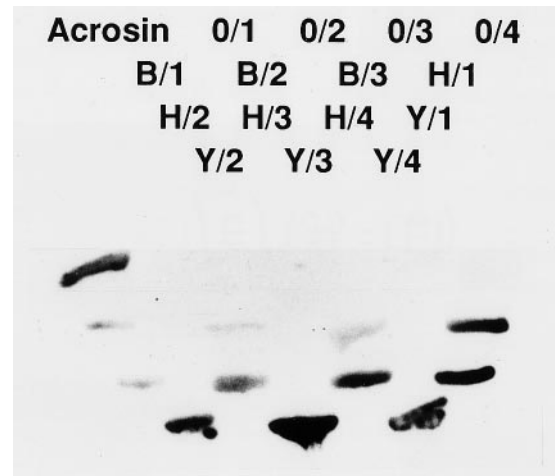
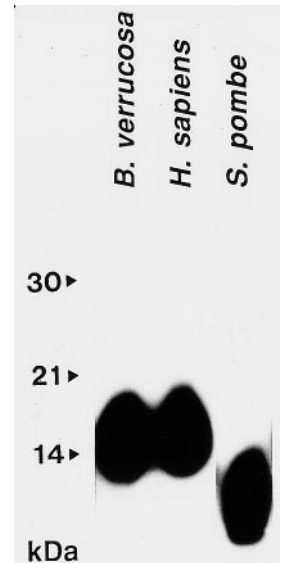


Fig. 7 Binding of ^{125}I -labeled purified recombinant birch pollen profilin to a nitrocellulose filter containing *E. coli* colonies. The order in which the colonies were streaked out and transferred to the filter is indicated on top of the figure. One *E. coli* colony expressed human acrosin (Acrosin), four colonies contained no recombinant protein (negative control: 0/1–0/4), three colonies synthesized birch profilin (B/1–B/3), four colonies produced human profilin (H/1–H/4) and yeast profilin (Y/1–Y/4). The filter was exposed to ^{125}I -labeled birch profilin and colony-bound birch profilin was visualized by autoradiography (bottom)

computed. Thus the reduced protein was slightly more stable to heat than the oxidized protein. The data recorded at 222 nm were essentially identical to those collected at 218 nm (data not shown).

Purified recombinant profilins from birch pollen, man and yeast bind to each other

To investigate the interaction of profilins to each other by overlay experiments, birch pollen, human and yeast profilins were ^{125}I -labeled. Figure 6 shows the autoradiogra-

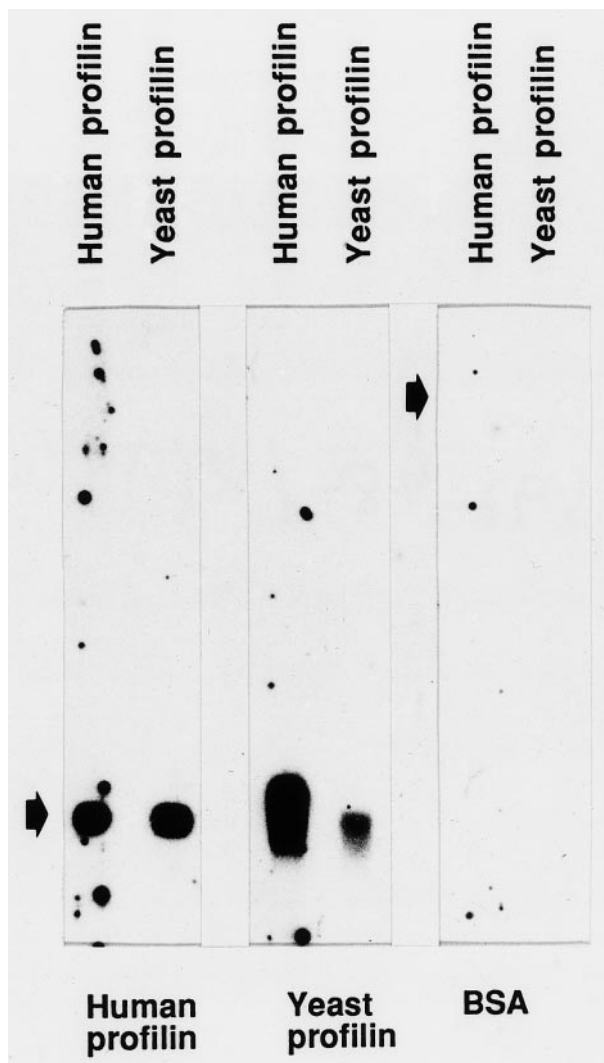


Fig. 8 Specific binding of ^{125}I -labeled profilins from man and yeast to each other. Nitrocellulose-blotted human and yeast profilins and, for control purposes, bovine serum albumin (*BSA*), positions indicated by arrows, were incubated with ^{125}I -labeled profilins as indicated for each lane on the top of the figure. Bound ^{125}I -labeled birch profilin was visualized by autoradiography

phy of an SDS-PAGE performed under reducing conditions containing 5000 cpm of pure ^{125}I -labeled birch, human and yeast profilin, respectively. The interaction of ^{125}I -labeled birch pollen profilin was first studied in a colony lift assay under non-denaturing conditions (i.e., without SDS or heating). Although this assay does not allow a quantitation of binding, ^{125}I -labeled birch pollen profilin clearly bound to nitrocellulose adsorbed *E. coli* colonies expressing profilins from birch, human and yeast, to a recombinant proline-rich protease, i.e., human acrosin (Baba et al. 1989), but not to *E. coli* colonies transformed with the empty pET17b plasmid (Fig. 7). Likewise, ^{125}I -labeled purified human and yeast profilins bound to nitrocellulose-blotted purified human and yeast profilins but not to BSA in a blot overlay experiment (Fig. 8).

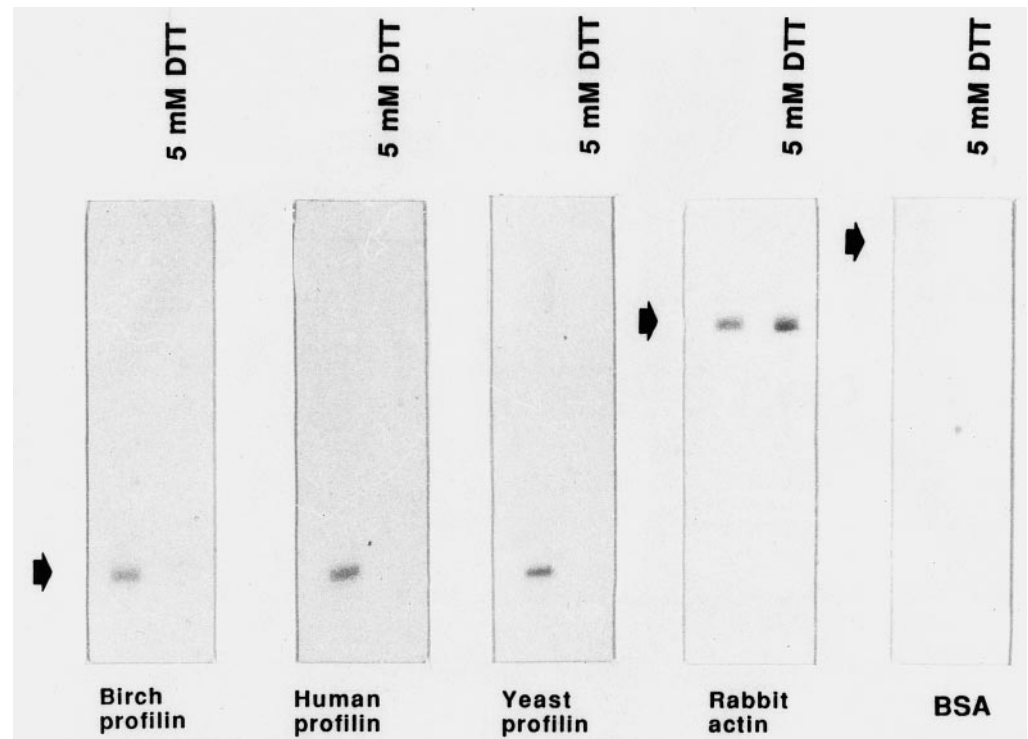
Reducing conditions prevent the binding of profilins from birch pollen, humans and yeast to each other but not to actin

To investigate the mechanisms of profilin homo- and heteropolymerization, overlay experiments were performed under non-reducing and reducing conditions. Under non-reducing conditions, ^{125}I -labeled recombinant birch profilin bound to itself, to human and to yeast profilin (Fig. 9). Addition of DTT (5 mM) to the overlay buffer prevented the interaction of birch profilin with itself, and with human and yeast profilins but it did not prevent binding to rabbit skeletal muscle actin (Fig. 9). Binding of profilin to BSA or to bovine β -lactoglobulin (data not shown) was not observed under either oxidizing or reducing conditions (Figs. 8, 9). Different concentrations of Tween 20 (0.05%, 0.5% and 1%) in the overlay buffer did not influence binding of profilins to each other or to actin (data not shown). These results indicate that birch, human and yeast profilins bind to each other via disulphide bonds. Indeed, birch, human and yeast profilins contain cysteines in their sequence, which are located in the C-terminal alpha helix and in the case of birch and human profilins are also found in the N-terminal helix. Although the C-terminal helix of profilins is thought to be involved in the actin interaction (Schutt et al. 1993; Fedorov et al. 1997), we found that oxidized (polymeric) as well as reduced (monomeric) birch pollen profilin states bound to rabbit skeletal actin. This implies that monomeric as well as polymeric states of profilin are able to bind to actin.

Discussion

Profilins are small (12–15 kDa) proteins regularly found in vertebrates, invertebrates, fungi and plants. The functions of profilin are very diverse and may depend on the interaction of profilin with various ligands (Carrier and Pantaloni 1997). Actin, proline-rich peptides/proteins and PIP_2 represent the best-characterized profilin ligands. It is assumed that alternating binding of profilin to PIP_2 and actin may link the signal transduction and microfilament systems (Machesky and Pollard 1993; Sohn and Goldschmidt-Clermont 1994). While the binding regions of profilin which are recognized by proline-rich peptides have been determined for profilins of *Acanthamoeba* (Archer et al. 1994), mammalian (Metzler et al. 1994; Björkegren et al. 1993) and plant origin (Domke et al. 1997) as not overlapping with the actin-binding site, the functional role of this interaction is not completely clear. Vasodilator-stimulated phosphoprotein is assumed to convey signal transduction to actin filament formation (Reinhard et al. 1995) and formin homology proteins may control actin activities during polarized cell growth and cytokinesis (Frazier and Field 1997). Control of the actin filament assembly is the best-documented biological function of profilin. It has been proposed that by sequestering actin, profilin may control

Fig. 9 Reducing conditions prevent binding of ^{125}I -labeled birch pollen profilin to profilins from birch, human and yeast but not to rabbit skeletal muscle actin. Purified ^{125}I -labeled birch profilin was allowed to bind to nitrocellulose-blotted birch, human and yeast profilins, rabbit actin, and bovine serum albumin (positions are indicated by arrows) under non-reducing (left lanes) or reducing conditions (right lanes indicated by 5 mM DTT). Bound ^{125}I -labeled birch profilin was visualized by autoradiography



focal actin dynamics (Buss et al. 1992), cytoplasmic streaming (Staiger et al. 1994), growth and development (Holzinger et al. 1997), as well as cytokinesis (Valster et al. 1997). Despite their conserved physiological functions, plant profilins represent highly crossreactive allergens, which cause type I allergic symptoms in a substantial proportion of allergic patients (Valenta et al. 1991, 1992).

In the present study we showed that purified recombinant profilins from three evolutionarily highly divergent organisms (birch, humans, yeast) are able to form spontaneously homo- and heteropolymers in vitro. These findings establish extracellularly occurring profilins as ligands for themselves. Although the DNA and amino acid sequences of plant, yeast and mammalian profilins display rather low sequence homology (approximately 25% sequence identity), they adopt the same fold (Fedorov et al. 1997). In blot overlays, birch pollen, human and yeast profilins bound to each other and actin equally well, but not to BSA or to a cysteine-rich control protein, bovine β -lactoglobulin (data not shown). Oligomerization of profilins was observed over a broad range of concentrations (ng/ml in the blot overlay experiments to mg/ml in non-reducing SDS PAGE analyses). While profilin polymerization was sensitive to reducing agents (β -mercaptoethanol, DTT), profilin binding to actin was not affected by reducing conditions. Indeed all profilins tested in this study contained cysteines in their C-terminal and/or N-terminal portion. In birch, human and yeast profilins the C- and N-terminus constitute alpha helices that are brought into close proximity on the molecular surface (Fedorov et al. 1997). Although the crystal structure of human profilin suggests that the sulfur atoms on

cysteines are all buried, it is possible that these sites are exposed in solution. Based on the experiments performed with recombinant profilins in solution we would thus propose that disulphide bond formation represents one of the major mechanisms for profilin self-association. Other mechanisms for profilin interactions (e.g., hydrophobic, ionic) can, however, not be excluded.

As exemplified for birch pollen profilin, circular dichroism analysis demonstrates that polymerization did not cause unfolding or a major change of the profilin secondary structure. We showed further that birch pollen profilin monomers are more stable to thermal denaturation than the homopolymers. Birch pollen profilin polymers were readily detected by a rabbit antiserum raised against the 25 C-terminal amino acids of birch profilin (et al. 1993; Fedorov et al. 1997) and IgE antibodies of profilin-allergic patients (data not shown). Mono- as well as polymeric profilin states likewise bound to actin, indicating that polymerization does not impair the interaction of profilin and actin.

Considering the role of our in vitro finding that profilins can form homopolymers via surface-exposed cysteine residues, one has to be aware that at physiological sites of profilin accumulation, i.e., in the cytoplasm of cells, reducing conditions will likely prevent polymer formation. The occurrence of profilin polymers as possible nuclei for the organization and/or accumulation of profilactin complexes even at intracellular sites close to the cell membrane [e.g., membrane protrusions of mammalian cells (Buss et al. 1992) and the surface of *Listeria monocytogenes* (Theriot et al. 1994)] is therefore rather unlikely. There is, however, evidence that plant profilins

can become available at extracellular sites, as when pollen is hydrated. During *in vitro* pollen germination substantial amounts of profilin are eluted from pollen grains and form oligomers that can be detected in nitrocellulose-blotted extracts with profilin-specific antisera (Mittermann and Valenta, unpublished data). Likewise, under pathological conditions, such as when atopic individuals come into contact with pollen or other profilin-containing sources (Valenta et al. 1991, 1992; Vallier et al. 1992), profilin is liberated and exposed to oxidizing conditions at the surfaces of mucosal membranes (in the respiratory tract). Such conditions will likely lead to profilin polymerization and affect the immunogenicity of this protein. Polymerization of profilin (e.g., birch profilin) during initial sensitization may therefore explain how a highly conserved cytoskeletal component which shares similarity with its human counterpart is able to induce significant immune responses and, perhaps, to break tolerance (Valenta et al. 1991). Our hypothesis, that polymeric profilin may represent the sensitizing agent, is indeed supported by results obtained from immunization experiments, which demonstrate that birch profilin induces IgG-subclass (IgG₂) responses in mice and rhesus monkeys which are typical for polymeric antigens (Vrtala et al. 1996).

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