Part III

Analysis of lectin activity of XSP30 with *N*-linked glycans of glycoproteins

Lectin activity of XSP30

Two animal glycoproteins (ovalbumin, asialofetuin) with standard *N*-linked oligosaccharide chains (Fig. 13) were separated by SDS-PAGE and blotted onto nitrocellulose sheets. The sheets were treated with xylem sap from cucumber roots, and XSP30 bound to glycoproteins was detected by anti-XSP30 antiserum. XSP30 reacted with ovalbumin, but only weakly with asialofetuin (Fig. 14C). When the blot was treated with *Ricinus communis* agglutinin (RCA), which has galactose-specific lectin activity (Beanziger and Fiete 1979), a much stronger signal was detected on asialofetuin than on ovalbumin (Fig. 14D).

Binding of XSP30 to galactose-removed asialofetuin

I examined the interaction of XSP30 to asialofetuin lacking its terminal galactose by treating the protein with galactosidase. XSP30 interacted with similar affinities to asialofetuin with and without galactose, whereas RCA reacted poorly with galactose-removed asialofetuin (Fig. 15, B and C).

Comparison of the lectin activities of XSP30 and concanavalin A

Binding capacity of XSP30 was also compared to that of concanavalin A (Con A), a mannose-specific lectin (Naismith *et al.* 1996), using various proteins to identify the XSP30 recognition site. High mannose-type glycoproteins, soybean agglutinin (SBA), soybean peroxidase, and γ-globulin, were analyzed by lectin blot with XSP30 or Con A (Figs. 13 and 16B). XSP30 interacted with SBA, but had little

interaction with soybean peroxidase or γ -globulin, both of which have fucosylated core GlcNAc-GlcNAc region of the *N*-linked sugar chain (Figs. 13 and 16B). Con A interacted with SBA and soybean peroxidase, but had little interaction with γ -globulin (Fig. 16C).

Inhibition of XSP30 binding with oligo-*N*-acetylglucosamine

XSP30 binding to SBA was partially inhibited by 0.1 mM and completely inhibited by 1 mM of tri-*N*-acetylchitotriose $(GlcNAc)_3$ (Fig. 17, lanes 2 and 3). Di-*N*-acetylchitobiose $(GlcNAc)_2$ did not inhibit XSP30 (Fig. 17, lanes 4 and 5).

Binding of XSP30 to cucumber leaf and stem tissue sections

I did not detect any XSP30 in either leaf or stem sections (Fig. 18, B and F). When sections were first incubated in xylem sap, anti-XSP30 antiserum detected XSP30 on leaf parenchyma cells (Fig. 18C). Weak staining was also detected on stem parenchyma cells (Fig. 18G). When proteins in section were removed by proteinase K treatment, most of the staining disappeared (Fig. 18, D and H).

Binding activity of XSP30 to leaf proteins

The ability of XSP30 to bind to proteins in the particulate fraction of cucumber leaf cells was examined (Fig. 19). The proteins were analyzed by reaction with anti-XSP30 antiserum either with (lane 3) or without (lane 2) prior treatment with xylem sap. In both cases, non-specific high molecular weight bands (arrow) were

detected. However, a smear of staining in the high molecular weight range was detected only after xylem sap treatment (lane 3).