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Drosophila position-specific antigens resemble the vertebrate fibronectin-receptor family

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The *Drosophila* position-specific (PS) antigens are a family of cell surface glycoprotein complexes thought to be involved in morphogenesis. Their overall structures and biochemical properties are similar to those of a group of vertebrate receptors, including those for fibronectin, fibrinogen and vitronectin, and also the leukocyte antigens Mac-1, LFA-1 and p150,95 and the VLA family of cell surface antigens. The N-terminal sequences of the α subunits of some of these molecules are homologous to the N-terminus of a PS antigen component. The *Drosophila* PS antigens thus appear to be homologous to these vertebrate receptors.

Key words: Drosophila/position-specific antigens/homology/fibronectin-receptor/morphogenesis

Introduction

An early event of spatial organization during the development of *Drosophila* is the subdivision of the embryo into repeat units and compartments, along the antero-posterior axis (Garcia-Bellido *et al.*, 1976). Later, in larval development, further subdivision of the anterior and posterior compartments occurs in some thoracic imaginal discs (Garcia-Bellido *et al.*, 1976; Brower *et al.*, 1981). It has been suggested that differences in cell surface properties are responsible for the segregation of cells belonging to different compartments (Morata and Lawrence, 1975). In a search for molecules conveying such properties the position-specific (PS) antigens were found (Wilcox *et al.*, 1981).

The PS antigens are present on most embryonic, larval and adult tissues of *Drosophila* (Wilcox and Leptin, 1985). Their first appearance roughly coincides with the onset of gastrulation. Changes in the spatially restricted patterns of their expression are correlated not only with compartmentalization (Wilcox *et al.*, 1981; Brower *et al.*, 1984; Brower, 1984) but also with other morphogenetic events such as the formation of folds and grooves (Brower *et al.*, 1985). This suggests a general role for these proteins in morphogenesis.

Each PS antigen is a complex of at least two glycoproteins (gps). One of these, gp110 (mol. wt ~110 kd), is found in all complexes (Brower *et al.*, 1984; Wilcox *et al.*, 1984). The other glycoproteins vary from complex to complex and we have called these molecules collectively the variable components. They fall into two sets, PS1 and PS2, defined by monoclonal antibodies. PS1 antibodies immunoprecipitate complexes consisting of gp110 and the PS1 variable components, while PS2 antibodies precipitate complexes of gp110 and the PS2 variable components. Different organs, tissues and regions of organs carry different and characteristic combinations of PS1 and PS2 complexes (Wilcox and Leptin, 1985). Antibodies recognizing the common component gp110 (PS3 antibodies) precipitate both classes of PS complexes. These antibodies also precipitate some gp110 that is not associated with either PS1 or PS2 variable components. A 92-kd doublet (gp92) is often co-precipitated with this latter gp110.

In vertebrates, the movement and attachment of cells during morphogenesis often requires their interaction with the extracellular matrix (Boucaut *et al.*, 1984; LeDouarin, 1984). Receptors for components of the extracellular matrix have been isolated from various vertebrate species and cell types (Pytela *et al.*, 1985; Knudsen *et al.*,1985; Gardner and Hynes, 1985; Giancotti *et al.*, 1985; Brown and Juliano, 1985; Akiyama *et al.*, 1986; Patel and Lodish, 1986). These receptors are multimeric complexes consisting of non-covalently linked α and β subunits. The β subunits are highly compacted by intramolecular disulfide bridges. The α subunits consist of disulfide-linked heavy and light chains. The structural and biochemical properties of the PS antigens described in this paper suggest that they are homologous to these vertebrate receptors. Thus, they may act in morphogenetic mechanisms not peculiar to *Drosophila*, but generally required for the construction of multicellular organisms.

Results

Details of the subunit structures of PS antigens

We have reported earlier (Wilcox *et al.*, 1984) that the PS antigen complexes show characteristic and unusual behaviour on two-

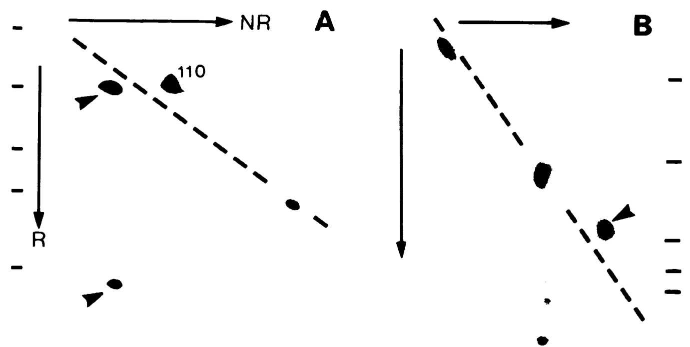

Fig. 1. Two-dimensional non-reducing/reducing gels of PS antigens. **(A)** Two-dimensional non-reducing (NR)/reducing (R) gel (first dimension 7.5%, second dimension 15%) of PS1 antigen extracted from a lysate of surface-iodinated cells of the Schneider-1 cell line. Gp110 runs above the gel diagonal (dashed line). The variable component is cleaved by 2-mercaptoethanol to yield heavy and light chains of M_r 116 and 25 kd, respectively (arrowheads). Gp110 runs at an anomalously high position in the 15% gel (2nd dimension). Mol. wt markers are 205, 116, 67, 45 and 29 kd. **(B)** Two-dimensional NR/R gel (20% both dimensions) of total PS antigen gp110 eluted from a one dimensional NR gel and subjected to CNBr degradation. One product runs above the diagonal (arrowhead; apparent mol. wt before reduction 12 kd, after reduction 19.5 kd) indicating the presence in gp110 of a small highly compacted region. Another product, probably an aggregation artefact, runs above the diagonal at the origin of the second dimension gel and is cut off in this figure. Mol. wt markers are 45, 26, 17, 12 and 8 kd.

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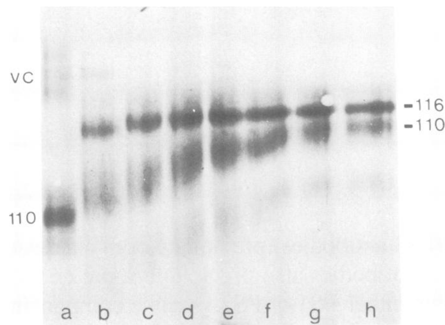

Fig. 2. The effect of reduction on the gel migration of the PS1 variable component heavy chain gp116. PS1 antigen from cells of the Emal[1] cell line was applied to a 7.5% gel in sample buffer containing 0, 0.6, 1.8, 6, 18, 60, 180 and 600 mM 2-mercaptoethanol (**lanes a–h**, respectively) and components detected using ConA/HRP. The apparent mol. wt of gp116 first drops due to the release of the light chain then increases, with increasing concentration of reductant, from ~112–114 to 116 kd. The M_r of gp110 increases from ~100 to 110 kd.

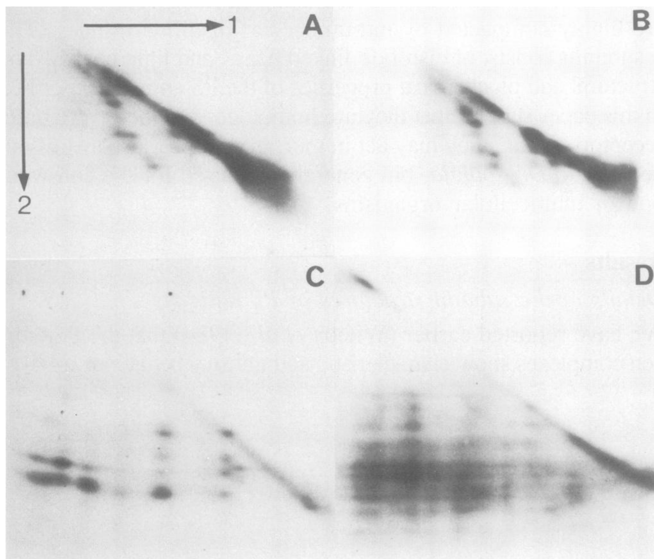

Fig. 3. CNBr/chymotrypsin peptide maps of gp110 (**A** and **B**) and the variable components (**C** and **D**) from late embryonic PS1 (A,C) and PS2 (B,D) antigens. ^{125}I-labelled antigen components were separated on two-dimensional NR/R gels and the various components eluted from the gel. The individual components were degraded with CNBr, the fragments separated on a 20% gel, each lane cut out, placed on a second 20% gel, treated with chymotrypsin and run out to generate a two-dimensional gel. The dashed line indicates the gel diagonal. The two gp110s show very similar maps, while the variable components are clearly different both from gp110 and from each other.

dimensional non-reducing/reducing SDS-polyacrylamide gels (SDS–PAGE). The common component gp110 migrates more rapidly under non-reducing than under reducing conditions, while the different variable components all run faster after reduction (Figures 1A and 4B). This property of gp110 indicates that in its native state it is held in a compact conformation by intramolecular disulfide bonds. The increase in apparent mol. wt is ~10 kd or 10%. Cyanogen bromide cleavage of gp110 releases a fragment which migrates with a mol. wt of ~12 kd on a non-reducing gel and 19.5 kd after reduction (arrowed in Figure 1B). This increase, 7.5 kd, is almost as large as that seen with the intact molecule and may suggest that a large proportion of the disulfide bonds in gp110 are concentrated in a small part of the molecule.

The increased mobility of the variable components after reduction is due to their dissociation from a light chain of mol. wt ~25 kd. This is illustrated for the main PS1 variable component, gp116, in Figure 1A. We will show later (e.g. Figure 4C) that all the variable components of both PS1 and PS2 antigens consist of a heavy chain of variable size (from ~105 to 150 kd) attached to light chains of constant size.

In addition to the disulfide bonding that links the heavy and light chain of each variable component there are also disulfide bonds within the heavy chain. When the light chain is released by very low concentrations of reducing agent (0.6 mM 2-mercaptoethanol) the heavy chain migrates faster than in its fully reduced form. In the presence of increasing amounts of reducing agent it migrates at increasingly higher apparent mol. wts (shown in Figure 2 for the PS1 variable component gp116). This suggests that the variable components in their native state are also compacted by intramolecular disulfide bonds, though to a lesser extent than gp110.

The PS1 and PS2 gp110 components are very similar

We have referred to the gp110 component of the complexes as the common component because of the identical gel migration and the apparent antigenic identity of the 110-kd molecules found in different complexes (Wilcox *et al.*, 1984).Thus, mouse antibodies recognizing gp110 in its native form in the complex (the PS3 antibodies mentioned above) precipitate both PS1 and PS2 complexes (Brower *et al.*, 1984) and a mouse antibody (DX4C8) that binds to gp110 on Western blots (Wilcox *et al.*, 1984) and a panel of rat antibodies raised against the denatured protein recognize the gp110 band in both antigens (not shown).

Comparison of peptide fragments of gp110 from PS1 and PS2 confirms that the two proteins are essentially identical. When PS1 and PS2 gp110 components are digested sequentially with cyanogen bromide (CNBr) and chymotrypsin, using a two-dimensional gel (Cleveland) procedure, the gels show almost identical patterns (Figure 3A and B).

Non-reducing/reducing two-dimensional gels of PS complexes often show a curious product which runs at ~100 kd (with gp110) under non-reducing conditions but with a mol. wt of ~180 kd after reduction (Figure 4C). The molecule is related to gp110 as it is recognized by anti-gp110 monoclonals, and CNBr/chymotrypsin digestion of it yields a pattern of fragments identical to that seen with gp110 (data not shown). Furthermore, when the 180-kd protein eluted from a two-dimensional gel is boiled in SDS, gp110 is released. We conclude that the molecule is non-covalently dimerized gp110.

The sets of PS1 and PS2 variable components are not closely related

The variable components of the PS1 complexes are antigenically unrelated to those of the PS2 complexes. PS1 monoclonal antibodies (which recognize the PS1 variable components) extract all PS1 complexes from cell lysates, leaving behind PS2 complexes that can then be precipitated by PS2 antibodies, and vice versa (Brower *et al.*, 1984). However, we had previously interpreted limited proteolytic digests as indicating a similarity between the major PS1 and PS2 variable components (Wilcox *et al.*,

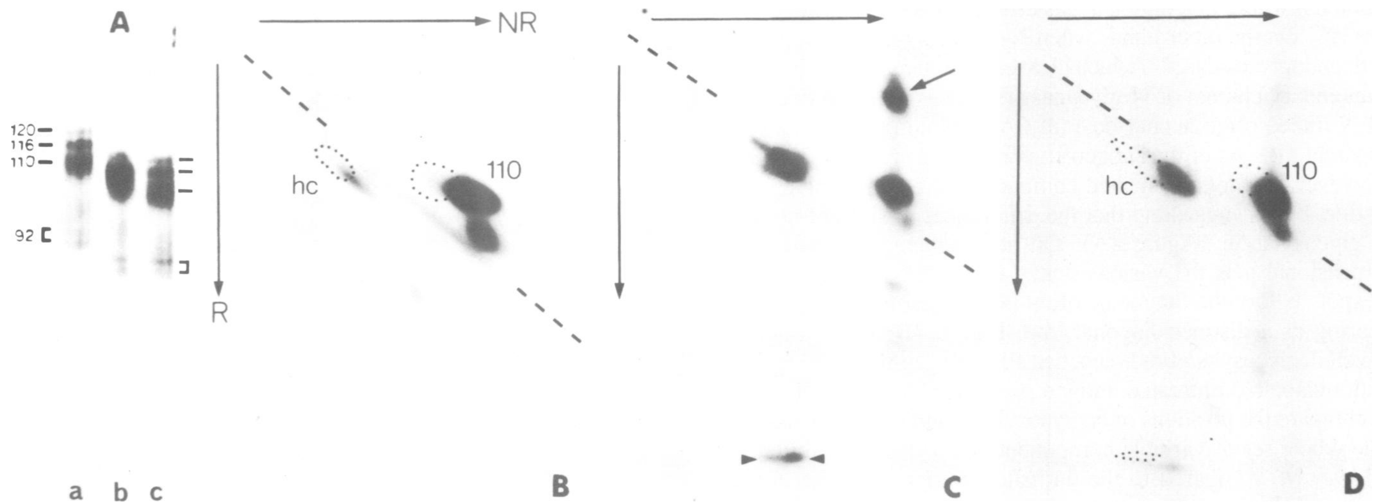

Fig. 4. The effect of N-deglycosylation on the gel mobility of PS antigen components. **(A)** One-dimensional gel of ^{125}I-labelled PS1 antigen from Emal[1] cells **(a)** untreated control; **(b)** after incubation with endoglycosidase F; **(c)** after incubation with N-glycanase. The 120- and 116-kd variable components are apparently each reduced in mol. wt by ~7 kd by treatment with either enzyme, gp110 by ~5 kd in lane b and by ~8–9 kd in lane c. The antigen in lanes b and c no longer binds ConA (not shown). **(B–D)** Two-dimensional NR/R gels on which ^{125}I-labelled antigen that had been deglycosylated with Endo F (B) or N-glycanase (D) was run together with unlabelled, untreated antigen. After electroblotting onto nitrocellulose, the untreated antigen was visualized by staining with ConA and HRP and the deglycosylated antigen by autoradiography. Shown here are the autoradiograms, and superimposed on them the outlines of the ConA-stained untreated components. The antigen in B was radiolabelled using Bolton and Hunter reagent which labels gp110 much more strongly than the variable components. Lactoperoxidase-catalysed iodination (as in C and D) gives relatively stronger labelling of the variable components. **Panel B** shows PS2 antigen from embryos, run on 7.5% gels in both dimensions. The diagonal is marked by a dashed line. Gp110 (and a breakdown product running below and to the right of gp110) above the diagonal, as well as the variable component heavy chains (hc), running as a line below the diagonal, have moved down and to the right, i.e. to lower mol. wts in both dimensions. The line of variable components has not collapsed into one spot. **Panels C** and **D** show pooled ^{125}I-labelled PS1 and PS2 embryonic antigens run on a 7.5% gel in the first and on a 5–20% gradient gel in the second dimension. Under these conditions the light chains released from the variable components are visible. **Panel C** shows untreated ^{125}I-labelled antigen as a control. Note especially the line of variable component light chains (between arrowheads) lying under the diagonal line of heavy chains; after deglycosylation of the antigen **(panel D)** they show, as expected, the same shift to the light as the heavy chains and a shift of ~2 kd down. (A 180-kd non-covalent dimer of gp110, referred to in the text, is arrowed.)

1984). This now seems unlikely, since a comparison of CNBr/chymotrypsin digests of the heavy chains of these components shows few, if any, fragments in common (Figure 3C and D). We do not know however whether there are conserved domains within the heavy chains nor whether the light chains are related.

Within each set the variable components have different polypeptide backbones

In embryos and on different larval and adult organs and tissues PS1 and PS2 antibodies each recognize a number of different complexes composed of gp110 assciated with one or more variable components. Different organs appear to express different subsets of variable components (Wilcox and Leptin, 1985). Often, tissue-specific variability in cell surface glycoproteins is due to differential glycosylation of a common polypeptide (e.g. Barclay *et al.*, 1976; Cullen *et al.*, 1981; Rothbard *et al.*, 1982). We wondered whether the same was true for PS antigens, since all their components are glycosylated [as judged by Concanavalin A (ConA) binding]. If the differences in mol. wt between variable components seen on SDS–PAGE were due to differences in the extent of glycosylation, the ladder of components should collapse into one band or spot upon removal of the glycans. Deglycosylation experiments show that this is not the case.

It is unlikely that the proteins carry O-linked glycans. Lectins specific for sugars commonly found in (vertebrate) O-glycans do not bind to PS antigens and treatment with O-glycanase had no effect on the antigen under conditions (see Materials and

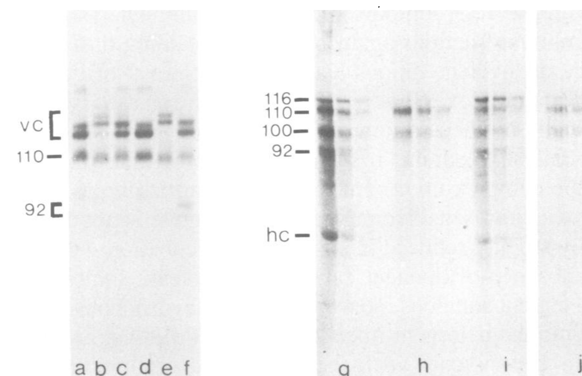

Fig. 5. Lanes a–f. Removal of Ca^{2+} leads to the release of gp92 from the PS3 complexes. Cells of the Emal[1] cell line were dialysed overnight in the presence of protease inhibitors (1 mM PMSF, 2 μg/ml pepstatin, 2 μg/ml leupeptin, 0.2 TIU/ml aprotinin) at 4°C against PBS containing 5 mM EGTA **(lanes a–c)** or 1 mM $CaCl_2$ **(lanes d–f)** prior to lysis and sequential extraction with PS1, PS2 and PS3 antibodies. Lanes d–f show the normal PS antigen patterns. After dialysis against EGTA the gp92 component is missing from the PS3 complex **(lane c). Lanes g–j**. Detergent partitioning of gp110, gp116 and gp92. Samples of total PS antigen from the Emal[1] cell line from a TX-114 biphasic separation experiment. **Lane g:** starting material (5, 2 and 1% of total volume), **lane h:** first detergent phase (50, 25, 10%), **lane i:** first aqueous phase (5, 2 and 1%), **lane j:** second detergent phase (50, 25, 10%). Gp110 (and its breakdown product gp100) partition preferentially into the detergent phase while gp92 (like contaminating immunoglobulin heavy chain, HC) remains in the aqueous phase.

methods) where glycophorin was efficiently deglycosylated (not shown). On the other hand, when N-linked sugars are removed with endoglycosidase F (which cleaves high mannose, hybrid and biantennary chains) or N-glycanase (which is believed to cleave all N-linked oligosaccharides) all ConA binding is lost and the apparent mol. wt of the antigen components drops by ~7−8 kd. However, the deglycosylated components are still resolved into distinct bands suggesting that the differences in size are not due to glycosylation (Figure 4A). On non-reducing/reducing two-dimensional gels the various heavy chains are seen as a series of spots below the diagonal, often poorly resolved but still appearing as a distinct diagonal line. Figure 4B shows a gel on which deglycosylated radiolabelled PS2 antigen was run together with unlabelled untreated antigen (visualized by ConA binding) to compare the positions of deglycosylated and untreated antigen. The whole set of variable component heavy chains is shifted to a lower M_r compared to the untreated components, i.e. to the right and downwards, but is still seen as a line of components with different mol. wts. The light chains released from the variable components show a concomitant shift to the right (Figure 4C and D). Other experiments confirm that the light chains are also glycosylated: they stain with ConA and their mol. wt drops by ~2 kd upon deglycosylation (see Figure 4D for example).

We conclude that the variability in mol. wt of the different variable component heavy chains is not due to differential N-glycosylation but reflects differences in the polypeptide chains.

gp92 is peripheral and gp110 has properties of a transmembrane protein

PS antigens are glycosylated, are found in the membrane fraction in cell fractionation experiments, are accessible to antibodies on live cells and can be labelled on intact cells by lactoperoxidase. By these criteria they are cell surface antigens.

We now show that gp92 is an extrinsic protein whose association with the PS3 complex requires calcium. Dialysis of cells or membrane fractions against EGTA-containing buffers in the absence of divalent cations leads to the removal of gp92 from the PS3 complex (Figure 5a−f). This release is blocked specifically by the addition of Ca^{2+} (but not Mg^{2+}; not shown) to the dialysis medium. It seems unlikely that the loss of gp92 from the complex upon removal of calcium is due to degradation by calcium-sensitive proteases, since gp92 is not degraded, but only slightly reduced in size, during incubation with trypsin in the absence of divalent cations and protease inhibitors.

In detergent solutions most proteins with hydrophobic domains insert into the detergent micelles while hydrophilic proteins remain in the solvent. With increasing temperature the size of detergent micelles increases until the solution becomes turbid and the micelles can be separated from the solvent by centrifugation. With Triton X-114 this occurs at room temperature which makes this detergent suitable for the analysis of proteins (Bordier, 1981). When Triton X-114 solutions of PS antigens are separated into aqueous and detergent phases, gp92, already shown to be a peripheral protein, and contaminating mouse IgG are found only in the aqueous phase (Figure 5g−j). In contrast, gp110 is enriched in the detergent phase, indicating that it has a hydrophobic domain and is therefore probably a transmembrane protein.

The PS1 variable component gp116 is usually enriched in the aqueous phase, but some is found in the detergent phase (see Figure 5h and j). Possibly the bulk of it is released from the complex during purification and partitions into the aqueous phase, with the gp116 remaining associated with the complex being drawn into the detergent with gp110. Gp116 was not removed

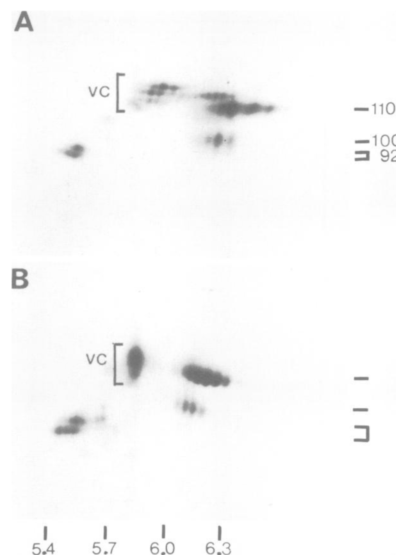

Fig. 6. Two-dimensional IEF gels of total PS antigen from Emal[1] cells. Radiolabelled antigen was analyzed by IEF/7.5% SDS−PAGE according to the procedure of O'Farrell (1975). Gel A shows untreated total PS antigen and gel B the same antigen after treatment with N-glycanase. Gp110, gp100, gp92 and the variable components are indicated. Approximate pH values of the IEF gradient are given.

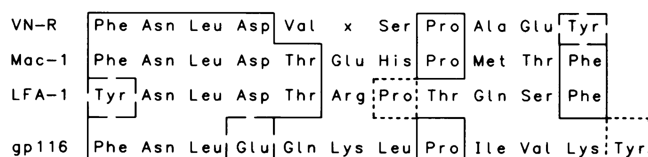

Fig. 7. Amino acid sequence of the N terminus of the variable component gp116 of the PS1 antigen from Emal[1] cells compared to the N-termini of the α subunits of three vertebrate receptors (from Springer *et al.*, 1985; Suzuki *et al.*, 1986). VN-R is the vitronectin receptor. Boxes indicate identity, dashed boxes conservative substitutions. The amino acid at position 6 in VN-R is not identified.

from the cell surface by washing at either high or low pH or in high salt buffers, suggesting that it is not a typical peripheral protein. It is possible of course that the entire length of the gp116 heavy chain is extracellular and only the light chain to which it is covalently attached (which was not analyzed in these experiments) is anchored in the membrane, as it appears to be in the case of a vertebrate receptor for the extracellular matrix component vitronectin (Suzuki *et al.*, 1986).

PS antigens are acidic proteins and show charge heterogeneity

Figure 6A shows a typical pattern for a PS antigen on an isoelectric focusing/SDS−PAGE two-dimensional gel. All the proteins are acidic and both the common component gp110 and the variable components show charge heterogeneity. Following N-deglycosylation (Figure 6B) the heterogeneity of the variable component heavy chains disappears and all show the same pI although still differing in mol. wt. They focus at a lower pI than the glycosylated components. The reason for this shift in pI is obscure, but it seems possible that the removal of glycans has exposed acidic residues.

The N-terminus of gp116 is homologous to the N-termini of vertebrate receptor α subunits

Figure 7 shows the sequence of the 12 N-terminal amino acids

of the PS1 variable component heavy chain gp116 purified from the tissue culture cell line Emal[1]. The sequence shows homology to the N-termini of the α subunits of the human vitronectin receptor and the mouse leukocyte antigens Mac-1 and LFA-1. The three vertebrate proteins have identical residues at positions 2, 3 and 4 and differ only by conservative substitutions (either one of the two aromatic amino acids) at positions 1 and 11. A proline occurs at position 8 in two of the sequences and at position 7 in the third. The remaining five residues are not conserved. All of the conserved residues are also found in gp116, or differ only by conservative substitutions (the acidic asp at position 4 is replaced by the alternative acidic residue, glu). The aromatic residue at position 11 in the vertebrate proteins is at position 12 in gp116. If gaps are introduced in the vertebrate sequences (before the pro at position 7 in LFA-1 and after position 7 in the two other sequences) the prolines will line up at position 8 and the aromatic residues at position 12 in all five sequences. Altogether, the gp116 sequence is as similar to the vertebrate sequences as the vertebrate sequences are to each other.

Discussion

The PS antigen complexes each consist of a component that is highly compacted by intramolecular disulfide bonds associated non-covalently with a somewhat larger component composed of disulfide-linked heavy and light chains. This structure is identical to that of the vertebrate receptors for molecules like fibronectin, vitronectin and fibrinogen (which we will call the fibronectin-receptor family). The PS antigen variable components correspond to the α subunits and the common component gp110 to the β subunit of the vertebrate receptors. The sizes of the individual PS antigen components also fall within the size range of the corresponding subunits of the vertebrate proteins (β subunits 100−140 kd, α subunits 115−160 kd). Although many cell surface molecules are multimeric complexes, held together by non-covalent interactions (MHC antigens) or disulfide bonds (insulin receptor, antibodies) the characteristic structure we have described for the PS antigen complexes has hitherto been found only in the fibronectin-receptor family.

While disulfide bridges are a common feature of cell surface proteins, the number and distribution of the bridges differs between families of receptors. For example, members of the immunoglobulin superfamily have few (one to six) disulfide bonds spaced evenly over the whole length of the polypeptide dividing it into domains of ~100 amino acids (reviewed by Hunkapiller and Hood, 1986). In contrast, many of the disulfide bonds in gp110 appear to be contained within a 19-kd fragment of the molecule. Similarly, 38 of the 58 cysteines in the chick fibronectin receptor, integrin, are localized within 25% of the length of the molecule (Tamkun *et al.*, 1986). While this accumulation of cysteines in only part of the polypeptide is not unique to PS antigens and fibronectin receptors [it also occurs in the EGF-receptor and the LDL-receptor for example (reviewed in Geisow, 1985)] it is another aspect of the structural similarity of PS antigens and the fibronectin-receptor family.

The PS antigens also resemble the fibronectin receptors in the following biochemical details. They are acidic proteins with similar two-dimensional IEF patterns showing charge heterogeneity of the individual components (cf. Hasegawa *et al.*, 1985; Hemler *et al.*, 1987). Both the β subunits and gp110 are resistant to trypsin on the cell surface and this resistance is lost in the presence of reducing agents (our unpublished observations; Giancotti *et al.*, 1985). The *Drosophila* variable components and vertebrate α subunits are partially resistant to trypsin: gp116 loses a fragment of ~20 kd as does the high mol. wt component of the BHK cell putative fibronectin receptor (Tarone *et al.*, 1982). Gp110 has a tendency to dimerize, as was also observed for the platelet receptor β subunit, IIIa (McGregor *et al.*, 1981; J.J.Calvete, J.L.McGregor and J.Gonzalez-Rodrigues, submitted).

Two other groups of vertebrate cell surface proteins have recently been shown to be related to the fibronectin-receptor family. The lymphocyte and macrophage antigens LFA-1 (involved in cell−cell interactions between T killer cells and their targets), Mac-1 (a complement receptor) and p150,95 consist of a common β subunit of ~95 kd and antigenically distinct α subunits between 150 and 180 kd (Sanchez-Madrid *et al.*, 1983; Springer *et al.*, 1985; Keizer *et al.*, 1985). The N-terminal amino acid sequences of the α subunits of two of them (LFA-1 and Mac-1) are homologous to the N-terminus of the vitronectin receptor α subunit (Suzuki *et al.*, 1986). Members of the other group, the VLAs, were first discovered on T lymphocytes at a late stage after activation (hence their name: very late antigens), but the family now includes molecules on many other cell types (Hemler *et al.*, 1987). Some members have the typical structure with α subunits consisting of S-S linked heavy and light chains, while others have single-chain α subunits (Hemler *et al.*, 1987). Again, the common β subunit of VLAs is found on nearly all cells analyzed, while the α subunits associated with it vary between cell types. These two families therefore share with the PS antigens the variability of their α subunits and the apparent invariability of the β subunit. It is possible that this variability in α chains is the basis for variability in receptor specificity.

The N-terminal sequence homology between gp116 and three of the vertebrate proteins is the strongest evidence for the homology between these groups of proteins. Significantly, gp116 is homologous to the α subunits of the vertebrate receptors which we had concluded from the structural similarities to be equivalent to the PS antigen variable components. We do not know the reason for this homology, which in the vertebrate receptors appears not to extend over the entire molecule (Suzuki *et al.*, 1987). The sequence could be a domain conserved independently of the rest of the protein or could have arisen by convergence, possibly reflecting structural constraints or some signalling function for the N terminus having little to do with a shared general structure or function of the molecules (like sites for calcium or nucleotide binding or for phosphorylation in otherwise unrelated proteins). However, considered together with the structural and biochemical similarities between PS antigens and the vertebrate molecules it appears more likely that the homology indicates a true evolutionary relationship of the molecules.

We do not know with which extracellular molecules the PS antigens interact. A feature common to the known ligands of the vertebrate receptors (e.g. fibronectin, laminin, vitronectin, and the serum proteins fibrinogen and von Willebrand factor) is that they all contain the amino acid sequence arg-gly-asp (RGD). The binding of the receptors to their ligands is inhibited by peptides containing this sequence (Plow *et al.*, 1985, and reviewed in Ruoslahti and Pierschbacher, 1986). In *Drosophila*, injection of such peptides into embryos inhibits gastrulation (Naidet *et al.*, 1987). Thus it appears that there are receptors involved in the interaction of cells with their environment during *Drosophila* development that recognize RGD-containing ligands. We have tested whether any molecules in *Drosophila*, in particular PS antigens, would bind to this peptide immobilized on columns but

were unable to isolate any proteins from embryo or cell line lysates, under conditions where we could purify two bands of ~115 and 125 kd (probably the vitronectin receptor — see Pytela *et al.*, 1985) from human placenta lysates (M.Leptin and M.Wilcox, unpublished results). This does not mean that PS antigens do not recognize RGD-containing ligands, since the fibronectin receptor also fails to bind to the immobilized peptide even though the peptide competes for the receptor binding to the ligand (Pytela *et al.*, 1985).

In view of the functional diversity of the ligands recognized by the vertebrate receptors it is difficult to predict what PS antigens might bind to. They might, like LFA-1, mediate direct cell–cell interactions. On the other hand they might interact with extracellular matrix components and, indeed, the localization of the antigens predominantly on the basal side of the imaginal disc epithelium, which is in contact with the basal lamina (cf. Figure 4 and 5 in Brower *et al.*, 1985), would be consistent with this. Thus, the hypothesis that compartment boundaries are set up or maintained by differential affinity of cells on either sides of the boundaries may need to be reconsidered.

Materials and methods

Antigens: sources, affinity isolation and purification

Embryonic antigens. (a) From cells. Mid–late embryos (12–18 h after egg laying; *D. melanogaster* Oregon R strain) were dechorionated and washed well with balanced salts solution (BSS; Chan and Gehring, 1971). After pelleting, the embryos were resuspended in 5 vol. ice-cold BSS and homogenized briefly (loose-fitting Dounce homogenizer, 4–5 strokes). The released cells were filtered successively through nylon filters of 150- and 50-μm mesh to remove debris and vitelline membranes and washed well with 10–20 vol. cold BSS. Cells were pelleted at 150 g for 5 min at 4°C and resuspended in BSS for surface labelling. Labelled cells were then lysed in lysis buffer for affinity isolation of PS antigens. (b) From membranes. Packed, dechorionated embryos were suspended in 10 vol. ice-cold 10× diluted Dulbecco's PBS (DPBS) and homogenized in a Dounce homogenizer. The suspension was centrifuged, first at 300 *g* for 2 min to remove debris, nuclei and vitelline membranes, then at 8000 *g* for 10 min to pellet membranes. The pellet was washed once in DPBS then either resuspended in DPBS for radiolabelling or immediately lysed in lysis buffer.

Antigens from cell lines. Tissue culture cell lines were grown as described previously (Wilcox *et al.*, 1984). Cells were harvested, washed three times in ice-cold DPBS, then resuspended in DPBS for surface radiolabelling, or in lysis buffer.

Affinity isolation and purification of PS antigens. Antigens were purified from non-ionic detergent lysates using the appropriate protein-A-purified PS monoclonal antibody coupled to Affi-Gel 10 beads (BioRad) according to procedures already described (Brower *et al.*, 1984), except that 50 mM diethylamine, pH 11, was used to elute the antigen. The antigens (after neutralization) were usually freed from non-glycoprotein contaminants by purification on lentil lectin–sepharose. Antigen eluted from antibody columns was made up in LL-buffer (20 mM Tris-HCl pH 7.4, 200 mM NaCl, 2 mM $CaCl_2$, 0.2% NP-40, 0.2% Triton X100 and protease inhibitors) and incubated with 100–150 μl lentil lectin–Sepharose beads (Pharmacia) for 2 h at room temperature. The beads were washed extensively with LL buffer and the antigen eluted with 25 mM Tris–HCl pH 7.4, 10% α-methylmannoside, 0.1% SDS.

Radiolabelling

Surface labelling. For lactoperoxidase-catalysed ^{125}I-iodination of surface proteins 1 ml washed intact cells (or a membrane fraction) were resuspended in 1 ml of DPBS. 50 μl of lactoperoxidase (Sigma L2005; 2 mg/ml), 1 mCi [^{125}I]iodide (Amersham International) and 50 μl hydrogen peroxide (0.12% in DPBS) were added and the mixture incubated for 5 min at room temperature. Then another 50 μl of hydrogen peroxide were added, and after a further 10 min incubation, the reaction was stopped by the addition of 10 μl of a solution of saturated tyrosine. The cells or membranes were then washed several times in ice-cold DPBS containing 5 mM KI and 0.1% NaN_3.

Bolton and Hunter ^{125}I-labelling of isolated PS antigens. Antigens, eluted from lentil lectin–sepharose in Tris/SDS buffers, were dialyzed and concentrated against 50 mM borate buffer, pH 8.5, then adjusted to 0.5–1% SDS, and added (in a volume of ~50–125 μl) to 0.2–0.4 mCi Bolton and Hunter reagent (Amersham) from which the solvent had been evaporated under a stream of N_2. The mixture was incubated for 20 min at room temperature under nitrogen. Residual reagent was reacted with glycine by the addition of 25 μl of a 0.2 M solution in 0.1 M borate (5 min). The protein was separated from low mol. wt label on a BioGel P10 column equilibrated with 25 mM Tris–HCl, pH 6.8, 0.1% NaN_3.

Gel electrophoresis

Antigens were analyzed by SDS–PAGE as described previously (Wilcox *et al.*, 1984). All gels were 0.7 mm thick unless stated otherwise. Details of acrylamide concentrations are given in the appropriate figure legends. For two-dimensional non-reducing/reducing gels the first dimension was run on a 0.7-mm thick gel and the second on a 1-mm thick gel. Two-dimensional IEF/SDS–polyacrylamide gels were run according to published procedures (O'Farrell, 1975).

Detection of antigens

In all cases antigens were transferred electrophoretically to nitrocellulose (Towbin *et al.*, 1979) prior to detection. Unlabelled proteins were detected histochemically following consecutive binding of ConA and horseradish peroxidase (HRP) (as described by Hawkes, 1982, except that BSA was replaced by 0.2% polyvinylpyrrolidone 360) and radiolabelled proteins by autoradiography.

CNBr and CNBr/chymotrypsin degradation

Components of radiolabelled antigens (usually from 100 μl packed cells or 500 μl packed embryos) were separated on one- or two-dimensional gels and detected by exposure of the wet gel to X-ray film. Spots were cut from the gel, the acrylamide finely crushed and protein eluted by incubation for 4 h at room temperature in 4 vol. 50 mM Tris–HCl, 100 mM NaCl, 0.1 mM EDTA, 0.2% SDS, 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 8.0. The protein was precipitated from the supernatant by 4 vol. cold acetone (4 h/–20°C), pelleted in a microfuge and dried in air. The antigen was then redissolved in 100 μl of 70% formic acid and incubated for 2 h at room temperature after the addition of a crystal of CNBr. The reaction mixture was lyophilized three times (twice after the addition of 200 μl of distilled water) and the residue taken up in gel sample buffer.

Two-dimensional non-reducing/reducing gel analysis of the degradation products was performed as described above, but on 20% gels. Two-dimensional reducing gels in which products were subjected to chymotrypsin proteolysis after fractionation in the first dimension, were performed according to a modified Cleveland procedure (Bordier and Crettol-Järvinin, 1979); pairs of strips from the first (0.7 mm thick) gel were placed on top of a 1-mm thick 20-cm gel and overlayered with 0.15 ml of a 150 μg/ml solution of crystalline chymotrypsin (Sigma) in 150 mM Tris–HCl pH 6.8, 0.1% SDS, 10% glycerol before electrophoresis in the second dimension.

Deglycosylation

O-deglycosylation. 25-μl reactions contained 50 mM sodium phosphate pH 5.8, 5 mM calcium acetate, 0.5 mM EDTA, 2.5% Nonidet P-40 (NP-40), 10 μg glycophorin and the combined PS1 and PS2 antigens immunoprecipitated from membranes isolated from 200 μl of embryos. Incubation was at 37°C for 2 h either without enzyme, with 1.5 μg neuraminidase (Sigma N-3001), with 5 μU O-glycanase (Genzyme), or with neuraminidase and O-glycanase (O-glycanase added after 1 h). After the addition of reducing sample buffer, each sample was run on two lanes of a 7.5% gel. One lane of each reaction was stained with Coomassie blue to detect glycophorin and its deglycosylation products, the other with ConA/HRP as described above to detect PS antigens.

N-deglycosylation. 25-μl reactions contained PS antigens (as indicated in the figure legends) from 200 or 400 μl packed dechorionated embryos (depending on whether the antigens were isolated from membranes or isolated cells). Reactions contained either: (i) 25 mM sodium phosphate pH 6.1, 0.2% NP-40 and 1 μl of endoglycosidase F (prepared according to the method of Elder and Alexander (1982); gift of J.Kaufman, Basel Institute for Immunology) or (ii) 50 mM sodium phosphate pH 8.6, 0.6% NP-40 and 0.1 U N-glycanase (Genzyme). Incubation was for 2 h at 37°C (i) or 30°C (ii) and reactions were stopped by the addition of an equal volume of 2× gel sample buffer.

Partitioning of PS antigen components into a non-ionic detergent phase

Radiolabelled PS antigens were prepared according to published procedures except that Triton X-114 (TX-114) was the only detergent used during the isolation, purification and labelling of the antigens. Partitioning of antigen components was carried out according to the procedure of Bordier (1981). Antigen preparations were dialysed against 20 mM Tris–HCl, 10 mM EGTA, pH 8.1 and made up to 100 mM NaCl, 1% TX-114 (with a trace of phenol red). 250-μl reactions were kept in ice for 2 h then layered onto 300 μl 6% sucrose, 0.06% TX-114, incubated at 37°C for 5 min and centrifuged at 750 *g* for 10 min at room temperature. The bottom (orange) detergent phase (~20 μl) was kept for anlaysis, the middle sucrose layer discarded and the top (pink) aqueous phase (~250 μl), after the removal of a sample for analysis and the addition of 30 μl 10% TX-114, carried through the partitioning procedure again. Samples of the two aqueous and two detergent phases were analyzed by SDS–PAGE.

Microsequencing of the gp116 α-subunit of the PS1 antigen

PS1 antigen was prepared from 22.5 ml packed cells of the Emal[1] cell line (~2×10^{10} cells) on affinity columns, yielding ~60 μg protein. A small fraction of the antigen was ^{125}I-labelled and added back to the cold protein as tracer.

The antigen components were separated on a 7.5% SDS−polyacrylamide gel (0.7 mm thick), from which they were electroblotted onto an activated glass fibre filter. Whatman GF/F glassfibre filters were covalently derivatized with trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride after etching the glass with trifluoroacetic acid as described (Aebersold *et al.*, 1986). The electroblotting was carried out in 25 mM Tris, 195 mM glycine, 0.5 mM DTT, pH 8.3 at 50 V for 2h at 0°C. The filter was dried at 50°C and autoradiographed to localize the gp116 band. To estimate the amount of protein present the blot was stained with the fluorescent dye 3,3′-dipentyloxacarbocyanine iodide and bands were visualized by u.v. illumination at 254 nm. The region of the filter containing gp116 was cut out with a razor blade and directly inserted into the cartridge of a Caltech gas phase sequenator (Hewick *et al.*, 1981).

Analysis of PTH amino acid derivatives from each cleavage was carried out as described by Hunkapiller and Hood (1983) on an IBM cyanocolumn except that 5−7% v/v tetrahydrofuran was added to buffer A and the pH was adjusted to 5.1.

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