Synaptic Cleft Glycoproteins Contain Homologous Amino Acid Sequences

Abstract. *The concanavalin A–binding glycoproteins of the rat synaptic junction were isolated by affinity chromatography. These glycoproteins had molecular weights of 160,000, 123,000, 110,000, and 95,000. The tryptic peptide maps of these glycoproteins showed that the three largest glycoproteins contained a high percentage of identical peptides. This indicates that the amino acid sequences of these glycoproteins have a high degree of homology. The 95,000-dalton glycoprotein was unrelated to the other three. These findings suggest that homologous glycoproteins may participate in synapse formation or maintenance, or both.*

The rat synaptic junction is composed of a well-defined group of polypeptides, several of which have been identified. These include myosin (*1*), actin (*2, 3*), tubulin (*3, 4*), calmodulin (*5*), the major postsynaptic density protein (*3*), and four major glycoproteins that are characterized by their affinity for the lectin concanavalin A (Con A) (*6*). These glycoproteins, which have apparent molecular weights of 160,000, 123,000, 110,000, and 95,000, have been designated Con A-I, Con A-II, Con A-III, and Con A-IV, respectively (*6*). Glycoproteins of similar size are also present in synaptic junctions prepared from the brains of chick, cow, and human (*6*). Furthermore, little difference is found in the relative amounts of these glycoproteins in synaptic junctions prepared from several different regions of the bovine brain (*6*). Cytochemical studies with rat subcellular fractions have shown that these glycoproteins are localized within the synaptic cleft on the external surface of the postsynaptic plasma membrane (*7*). They are minor glycoproteins in the synaptic plasma membranes from which the synaptic junctions originate (*6, 8*) and are found only in those subcellular fractions that contain synaptic junctions (*8*). Thus, these glycoproteins appear to be highly enriched at synapses (*8, 9*).

Glycoproteins have been implicated in a number of cell-cell interactions and recognition phenomena (*10*). Furthermore, in the central nervous system, changes in Con A receptors, as well as in other lectin receptors (glycoconjugates), have been correlated with both reactive (*11*) and developmental (*12*) synaptogenesis. Thus, it is possible that these glycoproteins have an important function in both the establishment of synaptic contacts and in the maintenance of the normal synaptic junction. We isolated the Con A–binding glycoproteins from synaptic junction preparations; we found that Con A-I, Con A-II, and Con A-III, but not Con A-IV, generate similar tryptic peptide maps. This indicates that a large degree of homology must exist among the amino acid sequences of these synaptic glycoproteins.

The Con A–positive glycoproteins of the synapse were isolated from synaptic junctions by a modification of the procedures for affinity chromatography. The total glycoprotein fraction isolated represented 1.5 to 2 percent of the total 280-nm absorbing material applied to the column. Assuming a 50 percent recovery of synaptic junctions from the homogenate and an 80 percent recovery of glycoproteins from synaptic junctions, these glycoproteins have been purified approximately 10,000-fold. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) (*8*) showed that the Con A–positive material contained three major Coomassie blue–staining polypeptides with molecular weights of 160,000, 110,000, and 95,000, in addition to polypeptides of Con A that leaked from the column (Fig. 1). The 95,000-dalton polypeptide was the major species, comprising about 50 percent of the Coomassie blue–staining material (Fig. 1). However, since Coomassie blue binds anomalously to glycoproteins (*13*), this value is not definite. The polyacrylamide gel was incubated with ^{125}I-labeled Con A and subjected to autoradiography (*8*). Three major bands and one minor band of radioactive peptides were detected. The major radioactive bands corresponded to Con A-I, Con A-III, and Con A-IV; Con A-II appeared as a minor band that migrated a little more slowly than Con A-III (Fig. 1). The number of Con A binding sites contained by these proteins is not proportional to the intensity of the Coomassie blue stain. The rank order of Con A binding sites contained by these glycoproteins is Con A-III > Con A-I > Con A-IV > Con A-II, whereas the order of intensity of Coomassie blue staining is Con A-IV > Con A-III > Con A-I > Con A-II. Since the order of Con A binding sites given above is identical to that determined for these glycoproteins in gels of synaptic junctions, the glycoprotein fraction we isolated is representative of the distribution of these glycoproteins in the intact junction (*6, 8*).

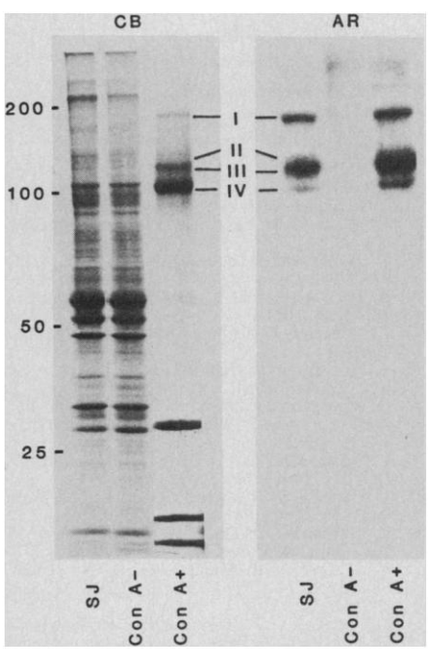

Fig. 1. Purification of the Con A–binding glycoproteins of the synaptic junction (*SJ*). Conconavalin A (Sigma Chemical Co., St. Louis, Missouri) was coupled to cyanogen bromide–activated Sepharose 4B (Pharmacia, Piscataway, New Jersey) (*18*). Synaptic junctions (4 ml, 5 mg/ml), prepared as in (*19*), were solubilized by heating for 15 minutes at 40°C in 10 m*M* tris-Cl (*p*H 7.5) containing 0.5 percent (weight to volume) SDS, 1 percent (by volume) β-mercaptoethanol, 100 μ*M* phenylmethylsulfonylfluoride, and 100 μ*M* sodium azide. The *p*H was adjusted to 6.0 with 1*M* HCl, and the solution was made 150 m*M* with respect to *N*-ethylmaleimide (Sigma). Dialysis was carried out for 24 hours against two changes of 10 m*M* tris-Cl (*p*H 7.5) containing 100 μ*M* sodium azide, and then for 24 hours against three changes of 10 m*M* tris-Cl containing 0.1 percent SDS. The solubilized synaptic junctions were then diluted with 10 m*M* tris-Cl (*p*H 7.5) to a concentration of 0.05 percent SDS and centrifuged at 100,000*g* for 30 minutes. The supernatant was removed and applied to a Con A–Sepharose column (diameter, 0.5 cm; height, 2 cm) equilibrated with 10 m*M* tris-Cl (*p*H 7.5). After the sample was applied, the column was rinsed with five column volumes of buffer [10 m*M* tris-Cl (*p*H 7.5)]. The Con A–positive fraction was eluted with 0.2*M* α-methyl-D-glucoside in 1 m*M* tris-Cl (*p*H 7.5) containing 0.01 percent SDS. This fraction was dialyzed for 48 hours against several changes of water and lyophylized. Forty-five micrograms of the solubilized synaptic junctions and the Con A–negative sample and 5 μg of the Con A–positive material were applied to 7 to 14.5 percent polyacryamide linear gels (*8*). The gels were incubated with ^{125}I-labeled Con A and autoradiographed (*8*). Because of the relative degree of enrichment, the Con A–positive glycoprotein sample was autoradiographed for ½ day. The other samples were autoradiographed for 3 days. Abbreviations: *CB*, Coomassie blue–staining proteins of these fractions; *AR*, an autoradiograph of these gels after incubation with ^{125}I-labeled Con A.

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The three major Coomassie blue–staining glycoproteins and the area of the gel that corresponds to Con A-II were removed, and ^{125}I tryptic peptide maps were prepared. Con A-I, Con A-II, and Con A-III contained a large percentage of identical patterns of labeled peptides (Fig. 2). Approximately 50 percent of the peptides were common to all three glycoproteins, as shown by comigration of the peptides in a mixture of equivalent amounts of radioactively labeled Con A-I and Con A-III (Fig. 2). Each of these three glycoproteins also contained several labeled peptides not contained in either of the other two. In addition, some of the peptides were common to only two of the three glycoproteins. Con A-IV contained no pattern of peptides in common with Con A-I, Con A-II, or Con A-III. Thus, a large percentage of the primary structure of Con A-I, Con A-II, and Con A-III must be identical, whereas Con A-IV is unrelated to the other three glycoproteins.

Several explanations are possible for the similarity observed among Con A-I, Con A-II, and Con A-III. Con A-II and Con A-III could be proteolytic fragments of Con A-I created during the preparation of the synaptic junctions. This is unlikely, however, since relative amounts of these proteins are constant from preparation to preparation. Furthermore, there is no loss of Con A-I with concomitant increase of Con A-II and Con A-III when synaptic junctions are incubated in Krebs-bicarbonate buffer at 30°C for up to 4 hours (data not shown). Thus, the amino acid sequences of the three glycoproteins, Con A-I, Con A-II, and Con A-III appear to contain a high degree of homology. The heterogeneity in both apparent molecular size and the Con A binding capacity could be due to differences in the lengths of the polypeptide chains or differences in additional moieties covalently attached to the protein, such as carbohydrate groups. Many examples of homologous proteins arising through gene duplication have been reported (*14, 15*). Usually these newly created proteins retain many of the functional features of the original protein. Since these glycoproteins are located in the same morphological structure, the synaptic junction, they may have a role in some synaptic function that is fine-tuned by the unique portions of these molecules. Alternatively, these glycoproteins may differ only in their carbohydrate sequence, composition, or the points of attachment of the carbohydrate to the protein. In this case, the specificity of each glycoprotein would be determined only by the carbohydrate structure (*16*). If these glycoproteins are recognition sites for lectin-like adhesive interactions at synapses, then carbohydrate diversity would allow a large variety of recognition sites without alteration of the polypeptide chain. This possibility is supported by the finding that the carbohydrate moieties are not identical, since these glycoproteins react to different degrees with ^{125}I-labeled Con A and ^{125}I-labeled fucose-binding protein (*17*). Synaptic junctions may contain additional glycoproteins that are unable to recognize Con A but that have a polypeptide

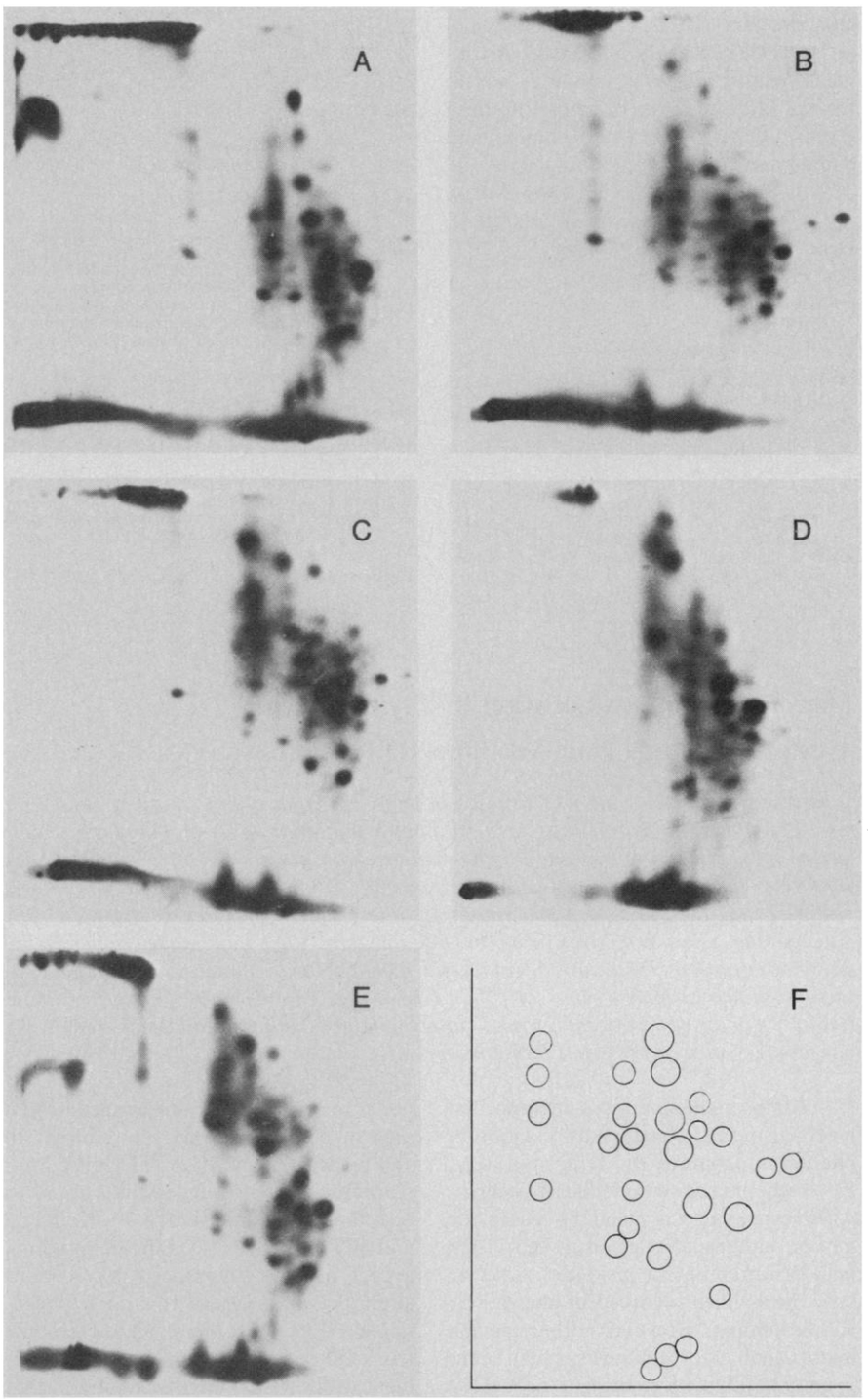

Fig. 2. Iodine-125 tryptic peptide maps of the Con A–binding glycoproteins of the synaptic junction. The bands on the gel that corresponded to the Con A–positive glycoproteins were removed and peptide maps were prepared as described (*8, 20*). The radioactive peptides were separated by electrophoresis in the *x* axis and chromatography in the *y* axis. (A) Con A-I; (B) Con A-II; (C) Con A-III; (D) Con A-IV; (E) a mixture of Con A-I and Con A-III; (F) a tracing of peptide map (E) that contains only those peptides that Con A-I, Con A-II, and Con A-III have in common. Since there is a loss of detail through photography, some of the minor peptides indicated in (F) may not be visible in (A), (B), or (C).

chain homologous to the Con A–binding glycoproteins.

Since the synapses from which these glycoproteins originated were isolated from whole brain, one type of glycoprotein may be associated with a particular class of synapses, or these glycoproteins may have different functions at the same synapse. A means of recognizing specific glycoproteins at particular synapses appears to be necessary in order to distinguish between these possibilities and to further elucidate the function of these glycoproteins in the establishment and maintenance of synaptic contacts.

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23 November 1981; revised 19 January 1982

Pheromone Source Location by Flying Moths: A Supplementary Non-Anemotactic Mechanism

Abstract. *After the wind was stopped in an insect flight tunnel, male oriental fruit moths continued to fly in zigzag fashion along a stationary pheromone plume. Their lateral excursions from the time-averaged pheromone plume were no greater without wind than in wind of 38 centimeters per second. When the pheromone plume was removed and the wind stopped, males initiated wider track reversals when they reached the pheromone-free area in still air than they had made while in the pheromone plume. This non-anemotactic mechanism of maintaining plume contact—possibly a special kind of klinotaxis—when coupled with the orthokinetic retinal velocity of apparent ground pattern motion, allowed males to reach the pheromone source area from 1 to 2 meters away without wind.*

Optomotor anemotaxis is an important mechanism in the successful location of pheromone sources by flying moths (*1*, *2*). Such pheromone-mediated steering with respect to the wind by using the motion pattern of cues from the visual field has often been considered to be the only mechanism involved in pheromone source finding. However, other mechanisms, such as orthokinesis, must occur in conjunction with steering to propel the moth over the ground toward the source (*3*). Farkas and Shorey (*4*) proposed that "aerial trail following" to maintain lateral contact with the plume occurred by means of a chemotactic turning back toward the plume; they implied that a longitudinal effect also occurred to allow males to arrive at the more concentrated area of pheromone near the source. In still air with an intact plume, pink bollworm males could successfully orient to near the pheromone source (*4*). Kennedy and Marsh (*1*) disproved the longitudinal aspect of their hypothesis by demonstrating that progress toward a pheromone source requires an optomotor feedback response to the apparent movement of the visual environment and that the males in the Farkas-Shorey experiments had already set their optomotor anemotactic courses and orthokinetic velocities before the wind stopped. To end up near the source, the moths only had to retain their general up-tunnel direction by maintaining for a short time the optical sensation of front-to-back ground pattern movement developed while they were in the wind.

We reexamined the problem of orientation to pheromone in a windless environment, focusing on the lateral component of movement that might keep males close to the plume. This type of movement has not been thoroughly examined by previous researchers (*1*, *2*, *4*). We found that males can indeed locate a pheromone source with a high success rate without the aid of wind, provided they have already established a set ground speed and direction while in the wind. We also found evidence that when the wind dropped to zero, lateral contact with the (time-averaged) plume is maintained without the aid of anemotaxis and possibly occurs through a protracted chemoklinotaxis. The wide casting movements characteristic of plume loss, called reversing anemomenotaxis when there is wind (*1*, *2*), also occur upon plume loss when there is no wind. This finding further supports a chemotactic mechanism for maintaining lateral contact with the plume.

Oriental fruit moth males at their time of peak sexual activity (*5*) were released individually in the pheromone plume from a small screen cage at the downwind end of a wind tunnel (working area, 3.7 by 1.0 by 0.9 m) (*6*). The synthetic sex pheromone source (*7*, *8*), a rubber septum impregnated with the three sex attractant components for this species, was placed on a sheet metal platform (15 by 15 by 15 cm) at a location 3.1 m up-tunnel from the males. We first tested to see whether males could locate the source without wind by allowing them to begin up-tunnel flight in the (time-averaged) plume. We then quickly stopped the wind (velocity, 38 cm/sec) (*9*). The males could locate the source much of the time without wind (44 percent, $N = 27$) and a large percentage (81 percent) could get within 10 cm of the septum, averaging a flight of 1.5 ± 0.4 m [mean ± standard deviation (S.D.)] ($N = 22$) after wind stoppage to do so. This confirms the unquantified results of a similar experiment on *Pectinophora gossypiella* (*4*). None of the males that flew to within 10 cm from the source in zero wind flew beyond it; rather, half actually landed on the platform and displayed hairpencils at the septum. If only up-tunnel momentum were involved, the moths should have proceeded past the source. The mean distance from the plume's axis during track reversal (turning back toward the plume) was not significantly greater for moths flying without wind (6.6 ± 1.9 cm, $N = 27$)

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