EVIDENCE FOR THE GLYCOPROTEIN NATURE OF THE INDUCER OF SEXUALITY IN VOLVOX

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SUMMARY: The inducer of sexuality in Volvox carteri binds to the saccharide binding site of concanavalin A. Its activity show heterogeneous and relatively low mobility during electrophoresis on sodium dodecyl sulfate polyacrylamide gels. These and other findings support the view that the inducer of sexuality is a glycoprotein.

Sexual differentiation in Volvox is induced by a species specific macromolecular inducer (1-6). In Volvox carteri, the inducer acts on the gonidia (asexual reproductive cells), causing them to cleave to form sexual individuals rather than cleaving to form asexual individuals (3,4). Thus, induction produces a change in developmental pathway from asexual to sexual. In Volvox carteri f. nagariensis, induction occurs at concentrations of about 10^{-15}M (4,7) and evidence has been presented that only two molecules of inducer are involved in each induction event (8). The inducer is sensitive to heat and Pronase digestion and has been concluded to be at least in part protein (1-6). The inducer resembles glycoproteins in being more resistant to heat or proteolysis than are most proteins, suggesting that it may be a glycoprotein (9). Partially purified inducer copurifies with glycoprotein, providing further support for this suggestion (7,10). Data presented below indicate that the inducer contains a saccharide component. In the light of earlier results, one may conclude that the inducer is a glycoprotein.

MATERIALS AND METHODS

Inducer was prepared from strain 69-1b of V. carteri f. nagariensis as described by Starr (4). Inducer assays were performed by the method of Starr using the HK 10 strain. Inducer titers were calculated as described earlier (8).

To measure possible binding of the inducer to concanavalin A, small (1.5 ml bed volume) columns of DEAE cellulose (.5 meq/gm) were prepared in pasteur pipettes. The DEAE cellulose was suspended in buffer composed of 10^{-2}M NaCl, 10^{-3}M tris, 10^{-3}M $CaCl_2$, 2 x 10^{-3}M $MnCl_2$, pH 7.2. 0.1 ml of inducer in Volvox medium (11) was added to .9 ml of the above buffer. Where concanavalin A was used, 2 mg of concanavalin A (Sigma Chemical Co.) was added to the above mixture. All such mixtures were allowed to stand for 1 hr at room temperature before being applied to the DEAE cellulose column. All columns were then washed with 5 ml of buffer (above). Following that, two additional 5 ml washes were made using buffer with or without glucose (.2 M). Material eluted from the column during each wash was assayed for inducer activity.

Inducer activity migration was studied during electrophoresis on sodium dodecyl sulfate (SDS) polyacrylamide gels by the method of Weber and Osborn (12). Gels were cut into 2 mm segments. Each segment was added to a tube containing 10 ml of sterile 10^{-2}M NaCl solution, crushed with a glass rod and allowed to elute into the solution for 4 hrs at 4°. Each tube was then assayed for inducer activity.

RESULTS AND CONCLUSIONS

A common property of glycoproteins is their ability to complex with plant lectins (13). Plant lectins bind to glycoproteins and other saccharide containing molecules but do not bind to proteins not containing saccharides. Consequently, if the inducer of sexuality is a glycoprotein it should bind to suitable lectins; if it is a protein devoid of saccharide it should not bind. Probably the most well-studied plant lectin is concanavalin A, a protein derived from jack beans (13). Concanavalin A binds to a variety of molecules containing mannose or glucose or their derivatives (14,15). Such binding is competitively inhibited by glucose.

An approach to measure possible binding of concanavalin A to the inducer was suggested by the finding that the inducer of V. carteri f. nagariensis shows little affinity for DEAE cellulose in .01M salt at neutral pH (Kidder and Pall,

unpublished) whereas the anionic concanavalin A (16) would be expected to show strong affinity for the cationic DEAE cellulose.

As shown in Table I, inducer applied to a DEAE cellulose column in the

TABLE I

	No Concanavalin A (Second and third washes with glucose)	Concanavalin A (Second and third washes with glucose)	Concanavalin A (Second and third washes without glucose)
1st Wash	55.5×10^5	1.45×10^5	2.13×10^5
2nd Wash	$.97 \times 10^5$	31.5×10^5	1.07×10^5
3rd Wash	0	3.66×10^5	1.19×10^5

Elution of inducer activity from DEAE cellulose columns. Values listed are for estimated inducer titers per wash expressed in inducer units.[7]

absence of concanavalin A is almost entirely removed from the column in a single 5 ml wash with buffer (column 1). In contrast, if inducer is mixed with concanavalin A before application to the column, less than 10% of the inducer is removed from the column in the initial 5 ml wash (columns 2 and 3). One may infer from these results that most of the inducer of sexuality is bound to the concanavalin A which in turn is bound to the column. Subsequent washes of the column with buffer containing .2M glucose removed large amounts of inducer (column 2) whereas washes with buffer devoid of glucose removed relatively little inducer (column 3). It may be concluded from these results that the concanavalin A binding to the inducer is through its specific saccharide binding site and thus is competitively inhibited by glucose. It follows that most inducer molecules contain a saccharide component probably including glucose, mannose or their derivatives. In the light of earlier studies indicating the proteinaceous character of the inducer (1-6), one may conclude that the inducer is a glycoprotein.

Further studies of the inducer were performed by studying the migration of its activity during electrophoresis on sodium dodecyl sulfate polyacrylamide gels. The mobility of polypeptides on such gels is inversely correlated to

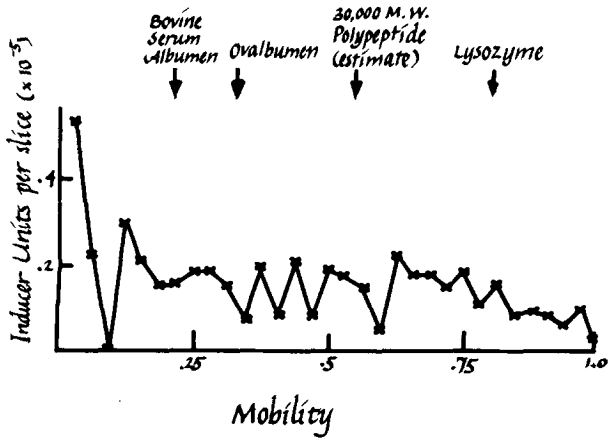

Figure 1. Mobility of inducer activity on sodium dodecyl sulfate polyacrylamide gels. The mobilities of bovine serum albumen, ovalbumen and lysozyme are marked and were used to estimate the mobility of a 30,000 molecular weight polypeptide. In this experiment, over half of the inducer activity applied to the gel appeared to be eluted from it.

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their molecular weight (12). As shown in Figure 1, the inducer activity can be eluted from such gels and shows a wide variety of mobilities. Control gels with bovine serum albumen, ovalbumen and lysozyme show that each of these proteins give a single predominant polypeptide band on such electrophoresis. Although gel filtration studies estimate the molecular weight of the inducer at 30,000 (4), more than half of the inducer activity migrates with mobility characteristic of polypeptides of larger than 30,000 molecular weight (Figure 1 and Reference 12). The sodium dodecyl sulfate would be expected to decrease rather than increase the aggregation of inducer molecules. Therefore, most of the inducer activity migrates more slowly than would be expected if it were composed only of polypeptide material. It is known that glycoproteins migrate more slowly on such polyacrylamide gels than do similarly sized polypeptides devoid of saccharide material (17). Consequently, the low mobility of most of the inducer activity on these gels supports the view that the inducer molecules contain glycoprotein with substantial amounts of saccharide or at least nonpolypeptide material.

The unexpected feature of the results shown in Figure 1 is the great heter-

ogeneity of mobility shown by the inducer activity. The inducer molecules may be very heterogeneous in their saccharide component, thus leading to the heterogeneity. Whatever the source, this heterogeneity may cause difficulties in purifying the inducer free of other materials and in demonstrating such purity.

The glycoprotein nature of the inducer helps to explain some of the properties of the inducer. Glycoproteins are generally more resistant to heat and proteolytic degradation than are other proteins (9). The inducer of sexuality in Volvox is relatively resistant to heat or proteolytic degradation (1-6). It is interesting to note that although the inducer of sexuality is Volvox carteri is cationic, it is insensitive to trypsin treatment (4). It is likely that the inducer molecules have a polypeptide core surrounded by a polysaccharide coat, the core being shielded from proteolytic attack by the polysaccharide.

Recently, Starr and Jaenicke (7) reported that highly purified inducer preparation showed properties expected of glycoprotein. If their preparation were free of macromolecules other than inducer, the concanavalin A studies reported above would not be needed to demonstrate the glycoprotein nature of the inducer. However, their protein stain and activity do not coincide on disc gels, indicating that the inducer preparation is not completely pure. In addition, although SDS gel electrophoresis of their material gives a single band stained with coomassic blue (7), it is known that some glycoproteins don't stain with this procedure (18). Consequently, this is not a good test of homogeneity when one may be dealing with glycoproteins. It would be useful to determine if the activity of the highly purified inducer migrates heterogeneously on SDS gel electrophoresis as does the activity characterized above.

Finally, the affinity of concanavalin A for the inducer reported above suggests that affinity columns containing that lectin may be useful in purifying the inducer.

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