GLYCOPROTEIN FUCOSYL TRANSFERASE IN THE ENDOPLASMIC RETICULUM OF CASTOR BEAN ENDOSPERM CELLS

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1. Introduction

Several proteins found in the major organelle fractions isolated from germinating castor bean endosperm are glycosylated [1]. The analysis of sugars released from glycopeptides derived from organelle membrane and matrix subfractions indicated that fucose was associated with proteins located in the endoplasmic reticulum (ER) membrane [2]. Although L-fucose has been found in several plant glycoproteins (for example, lima bean lectin [3]), the glycosyltransferase which incorporates this sugar into glycoproteins has not been examined in plants [4].

The intracellular localization of glycoprotein fucosyltransferase in castor bean endosperm is of particular interest. Studies with mammalian tissues have established that whereas core sugars such as *N*-acetylglucosamine and mannose are added to nascent polypeptides by enzymes located in the rough ER membrane [5,6] peripheral sugars such as fucose are added by enzymes located in the Golgi apparatus [7]. During the early post-germinative development of castor bean seedlings, the gluconeogenic endosperm cells do not divide [8] and the Golgi apparatus is not well developed in such cells [9,10].

The confinement of fucosylated glycoproteins to the ER membrane in castor bean endosperm [2] suggests that this fraction may be the major intracellular site of fucose incorporation. This has been confirmed in the present work.

2. Experimental

Castor bean (*Ricinus communis* L.) seeds were soaked overnight in running tap water and germinated in the dark in moist vermiculite at 30°C. Endosperm halves excised from 2-day-old seedlings were homogenized by chopping with a single razor blade in grinding medium [11] contained in a petri dish on ice. The particulate enzyme preparation was obtained by centrifuging the crude homogenate at 20 000 × *g* and 2°C for 20 min. The pellet was resuspended in 150 mM Tris–HCl, pH 7.5, 2 mM β-mercaptoethanol and 12% (w/v) sucrose. Cellular organelles present in the crude homogenate were separated by sucrose density gradient centrifugation as described previously [11]. After centrifugation, gradients were collected as 1.0 ml fractions using an ISCO model 185 density gradient fractionator.

Glycoprotein labelling in intact tissue was performed by incubating each of 20 excised endosperm halves with 5 μCi of [^{3}H]fucose (6.7 Ci/mmol; Radiochemical Centre, Amersham, U.K.) which was added directly to the adaxial surfaces of each half in 5 μl. After incubation at 30°C for 2 the tissue was homogenized and the organelles isolated. Trichloroacetic acid (TCA)-insoluble glycoprotein radioactivity and chloroform/methanol (2:1)-soluble lipid radioactivity was determined in each collected sucrose gradient fraction as described previously [12].

Total fucosyltransferase activity was determined by incubating 2 ml of particulate enzyme preparation of 30°C with 10 mM $MgCl_2$ and 0.05 μCi of GDP[^{14}C]fucose (24.5 Ci/mol) in a final volume of 2.5 ml. At various times samples (0.25 ml) were removed into 4 ml of chloroform/methanol (2:1). After the addition of 1.75 ml of water, radioactivity present in (a) chloroform/methanol (2:1)-soluble product, (b) chloroform/methanol/water (10:10:3)-

soluble product, and (c) TCA-insoluble product was determined as described for mannosyltransferase [12]. The subcellular distribution of fucosyltransferase was determined in a similar manner by incubating collected 1.0 ml gradient fractions with 0.05 μCi GDP[^{14}C]fucose and 10 mM $MgCl_2$. After incubation at 30°C for 1 h, the reaction was stopped by adding 1 ml of methanol. The precipitated material was collected by centrifugation and the pellet was washed twice in 50% methanol. The washed pellet was resuspended in 2 ml of chloroform/methanol/water (10:10:3) and allowed to stand for 15 min at room temperature. After centrifugation the supernatant was decanted into a scintillation vial, evaporated to dryness and its radioactivity was determined after adding 10 ml of scintillant [13]. The residue was resuspended in 10% (w/v) TCA, filtered onto a Whatman GF/A filter disc, washed in 10% TCA and its radioactivity was determined by scintillation counting.

The enzymic digestion of fucosylated membrane glycoprotein was performed using isolated microsomes prepared from [^{3}H]fucose-labelled endosperm tissue. The collected microsomes were osmotically disrupted by adding an equal volume of 50 mM Tricine, pH 7.5, containing 0.5 M KCl. The membranes were collected by centrifugation at 50 000 × *g* and 2°C for 30 min and were gently resuspended in 20% (w/v) sucrose. An aliquot of the membrane suspension was counted and the rest was divided into four equal portions. Two of these portions were mixed with an equal volume of 0.2 M citrate buffer, pH 4.5, containing 0.2 units of fucosidase supplemented in one instance with 0.5% (v/v) Triton X-100. The two remaining portions were mixed with an equal volume of 100 mM Tris–HCl, pH 7.0, containing 400 μg/ml each of trypsin and chymotrypsin, supplemented in one case with 0.5% Triton X-100. The mixtures were incubated at 30°C for up to 2 h and removed samples were centrifuged at 100 000 × *g* and 2°C for 30 min. The supernatants were counted and the percentage of the total membrane bound [^{3}H]fucose solubilized was determined.

3. Results and discussion

The ER membrane was shown to be the major particulate location of fucose-containing polymer by incubating intact tissue with [^{3}H]fucose. After organelle fractionation, microsomes, mitochondria and glyoxysomes were effectively separated and were located using the respective marker enzymes cholinephosphotransferase, fumarase and catalase [14]. [^{3}H]Fucose labelled TCA-insoluble material was largely recovered in the ER membranes (fig.1.). The capacity of the endosperm tissue to incorporate [^{3}H]fucose into TCA-insoluble product varied during seedling development, increasing over the first 3 days and subsequently declining. Gradient fractionation established that the ER membrane was the major site of TCA-insoluble radioactivity at all developmental stages (data not shown).

The [^{3}H]fucose into the ER membrane was

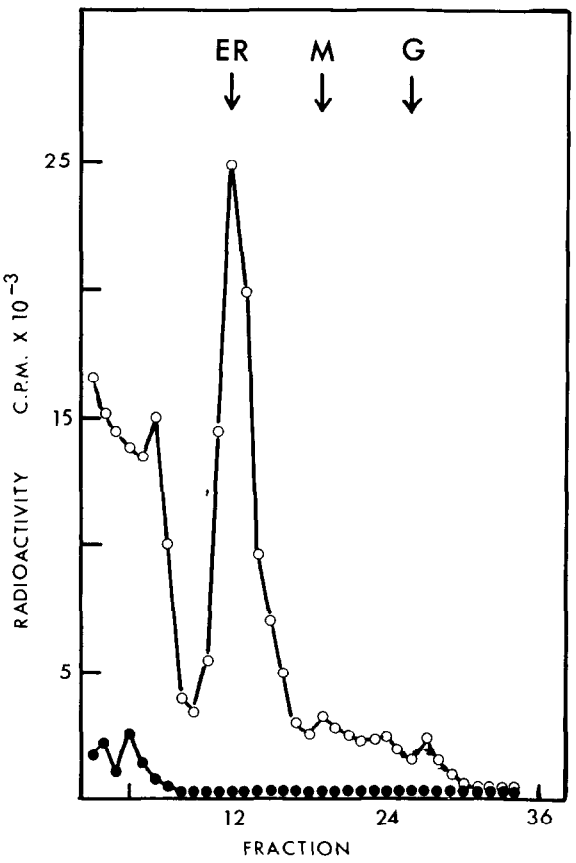

Fig.1. Distribution of TCA-insoluble radioactivity (○) and chloroform/methanol (2:1)-soluble radioactivity (●) amongst collected fractions after sucrose density gradient centrifugation of the homogenate prepared from 20 endosperm halves each incubated with 5 μCi [^{3}H]fucose for 2 h. ER, M and G designate fractions containing peak activities of marker enzymes for endoplasmic reticulum, mitochondria and glyoxysomes respectively.

probably associated with protein. Incubating labelled microsomes with either trypsin/chymotrypsin or fucosidase resulted in the solubilization of a significant proportion of the total membrane bound radioactivity (fig.2a). Further, the [^{3}H]fucose was almost completely solubilized when the microsomes were incubated with these enzymes in the presence of Triton X-100 (fig.2b) indicating that most of the protein-bound fucose was exposed on the luminal surface of the microsomal vesicle membrane. The integrity of the enzymic [^{3}H]fucose release was supported by the following observations: (a) [^{3}H]fucose was not released from the membrane to any great extent in the absence of proteases or fucosidase with or without added Triton X-100; (b) when the integral membrane proteins were labelled by incubating intact tissue with [^{35}S]-methionine [11], ^{35}S was not released to any great extent during subsequent incubation with fucosidase in either the absence or presence of Triton X-100 (data not shown).

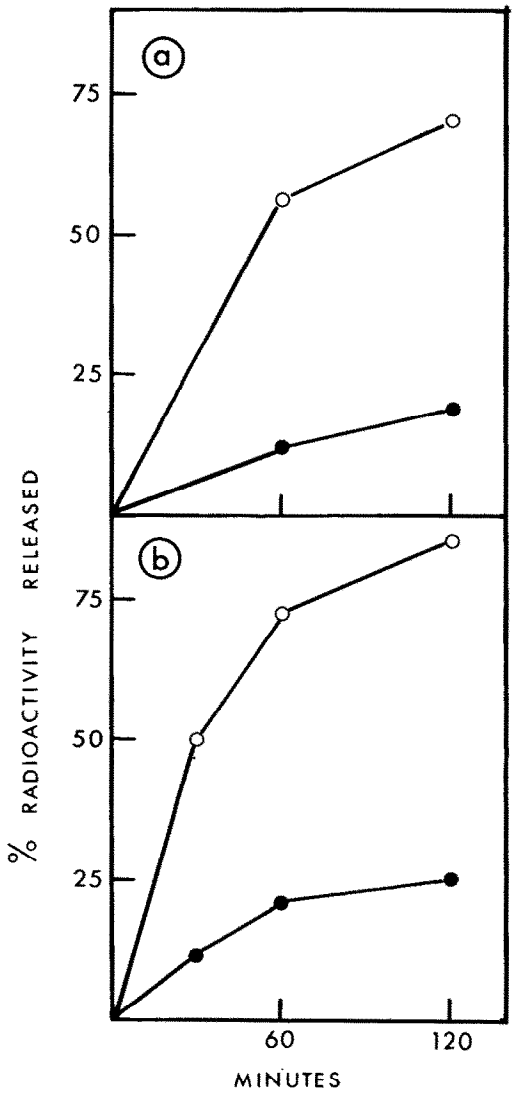

Fig.2. Release of [^{3}H]fucose from labelled ER membranes incubated with (a) trypsin/chymotrypsin or (b) fucosidase in the absence (•) or presence (○) of 0.5% Triton X-100.

The crude particulate fraction prepared from 2-day-old endosperm tissue catalysed the incorporation of [^{14}C]fucose from GDP[^{14}C]fucose into product soluble in chloroform/methanol/water (10:10:3) and into TCA-insoluble product (fig.3). Incorporation into chloroform/methanol (2:1)-soluble lipid was not observed. One possible explanation for these data is that fucose is transferred from GDP[^{14}C]fucose to an oligosaccharide lipid, possibly pre-existing and already containing *N*-acetylglucosamine and mannose, and that the oligosaccharide moiety is subsequently trans-

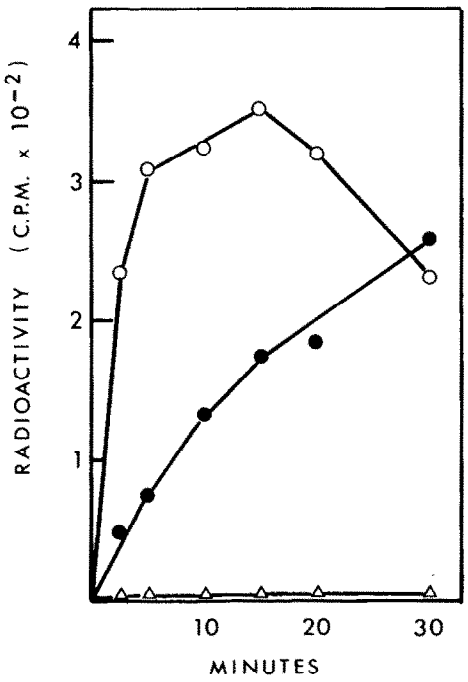

Fig.3. Time course for the incorporation of fucose from GDP[^{14}C]fucose, catalysed by the particulate fraction prepared from castor bean endosperm, into chloroform/methanol (2:1)-soluble product (△), chloroform/methanol/water (10:10:3)-soluble product (○), and the TCA-insoluble residue (•).

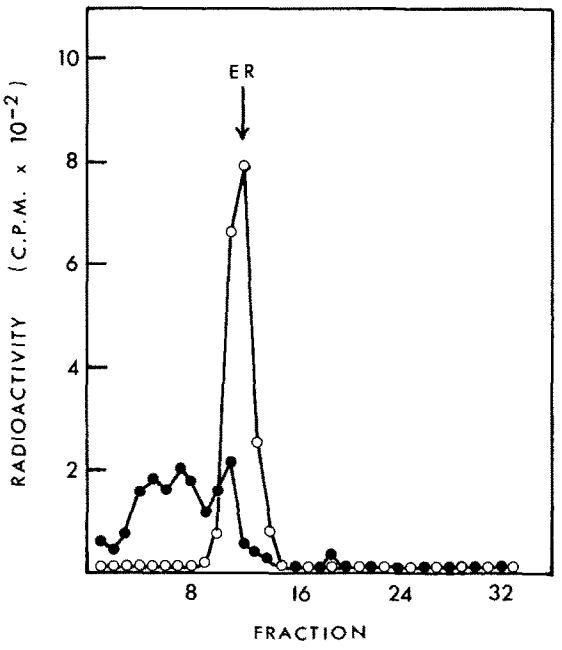

Fig.4. Incorporation of fucose from GDP[^{14}C]fucose into chloroform/methanol/water (10:10:3)-soluble product (•) and TCA-insoluble product (○) by collected fractions after sucrose density gradient centrifugation of castor bean endosperm homogenate.

ferred from the lipid carrier to a protein acceptor. The intracellular localization of enzymes catalysing the synthesis of these products was investigated by incubating collected sucrose gradient fractions with GDP[^{14}C]fucose. After a 60 min incubation time, [^{14}C]fucoprotein was the major product and the enzyme catalysing this synthesis was clearly associated with the ER membrane peak (fig.4). The chloroform/methanol/water (10:10:3)-soluble product was synthesized by enzymes peaking at lower sucrose densities.

At the present time we feel it is unlikely that the chloroform/methanol/water-soluble fucose-containing product has a functional role in protein glycosylation. This conclusion is based on the following considerations: (a) studies with mammalian systems have provided no evidence that fucose is incorporated into glycoprotein via lipid-linked intermediates [15]; (b) the enzymic activity catalysing the synthesis of the chloroform/methanol/water-soluble product is not associated with the ER, in contrast to enzymes catalysing the synthesis of the *N*-acetylglucosamine and mannose containing oligosaccharide lipid which is involved in core glycosylation [16–19]; (c) mild acid hydrolysis did not release [^{14}C]fucose from the chloroform/methanol/water-soluble product into the aqueous phase; (d) previous studies have established that mannose-containing oligosaccharide lipid formed in castor bean endosperm serves as an intermediate in the ER-catalysed core glycosylation of an appropriate acceptor protein, sulphitolysed ribonuclease A [20]. In contrast, when the [^{14}C]fucose-containing chloroform/methanol/water-soluble product was incubated with sulphitolysed ribonuclease A under identical conditions, transfer of [^{14}C]fucose to protein did not occur (data not shown).

On the basis of the above considerations, we think it most likely that fucose is transferred directly from GDP-fucose to a core glycosylated acceptor. The core glycosylation step, in which the *N*-acetylglucosamine and mannose-containing oligosaccharide is transferred from its lipid carrier to a nascent polypeptide chain, and the subsequent addition of fucose are both catalysed by enzymes located in the ER membrane [20, fig.4]. Although core glycosylation is thought to occur co-translationally [5], the addition of fucose is probably a post-translational event as it obviously is in the case of Golgi-associated fucosyltransferase in other eukaryotic cells.

The structural nature and functional role of the fucose-containing chloroform/methanol/water-soluble product are not known at present.

Acknowledgements

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