ISOLATION AND CHARACTERISATION OF A FUCOSYL TRANSFERASE ASSOCIATED WITH DICTYSOMES FROM THE BROWN ALGA *FUCUS SERRATUS* L.

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1. Introduction

Histochemical and autoradiographical studies have shown the Golgi apparatus to be involved in the synthesis of polysaccharides and glycoproteins in marine algae [1,2]. A procedure for isolating intact and enzymically active dictysome enriched fractions from the marine alga *Fucus serratus* has recently been described [3,4]. Further investigations have now shown that a fucosyl transferase linking L-fucose from the sugar nucleotide GDP-fucose to the acceptor L-fucose or the sulphated fucose polymer fucoidan is also located primarily in this fraction.

2. Materials and methods

2.1. *Plant material*

Young *F. serratus* plants were collected from a variety of sites on the Yorkshire coast and stored in the seawater system of the Zoology department, Leeds University (13°C, 300 μE $m^{-2}s^{-1}$ photon flux density, 12 h light/dark cycle, aerated 5 $L.h^{-1}$) until required.

2.2. *Chemicals*

The radiochemicals GDP-[U-^{14}C]fucose (spec. act. 117 μCi/mmol), L-[1-^{3}H]fucose (spec. act. 5.3 Ci/mmol) and L-[1-^{14}C]fucose (spec. act. 57 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, Bucks, England.

GDP-fucose was synthesized as previously described [5]. Fucoidan was purchased from K & K Laboratories Inc., New York. The enzyme α-fucosidase (EC 3.2.1.51) (1 mg/ml) was purchased from Boehringer-Mannheim.

Heat-stable endogenous acceptors were prepared as previously described [4]. All other chemicals were obtained from Sigma Chemical Company, St. Louis, Missouri. All chemicals were the highest purity commercially available.

2.3. *Organelle isolation*

Dictyosome enriched fractions were isolated as previously described [4].

2.4. *Enzyme assays*

Protein content of a 6% (w/v) TCA precipitate was assayed by the Lowry method [6].

Fucosyl transferase: The final reaction mixture, total vol. 50 μl, contained 100 nmol acceptor, 20 nmol GDP-fucose, 0.1 μCi GDP-[U-^{14}C]fucose, 2.5 μmol Tris–HCl, pH 7.5, 2.0 μmol $MgCl_2$, 0.5 μl Triton X-100 or 0.5 mg sodium deoxycholate. The reaction was initiated by adding 50 μl membrane suspension (~1 mg ml^{-1} protein) and the mixture incubated at 30°C for 30 min. The reaction was terminated by the addition of 100 μl 0.25 M EDTA.

With monosaccharides as acceptors the reaction mixture was passed through 1.0 ml Dowex-1-Cl^{-1} (200–400 mesh) ion-exchange resin, washed with 5 × 0.2 ml distilled water, eluate and washings were pooled and counted for radioactivity. With polysaccharides as acceptors, 0.8 ml ethanol was added to the reaction mixture, the suspension centrifuged (10 000 × g, 2 min), the pellet washed with 4 × 0.25 ml 70% (v/v) ethanol, the polysaccharides dissolved in 3 × 0.1 ml 2% (w/v) Na_2Cd_3 and counter for radioactivity.

For identification of reaction products 5 μCi [U-^{3}H]fucose was used as acceptor, 1 μCi GDP-[U-^{14}C]fucose as substrate, all other conditions being the same as above. 50 μl of the reaction mixture were spotted onto Whatmann 3 MM chromatography paper and eluted in a descending manner for 18 h with ethanol/1 M ammonium acetate, pH 7.5 (7:3, v/v) together with standards of GDP-[U-^{14}C]fucose and [1-^{14}C]fucose.

3. Results

3.1. *Subcellular location*

Table 1 shows the location of the fucosyl transferase in isolated membrane fractions from *F. serratus*. Activity is predominantly located in the dictyosome fraction with some activity also present in the microsomal fraction.

3.2. *Time course, pH dependence, protein concentration, temperature dependence*

Using both L-fucose and fucoidan as acceptors the enzyme reaction was linear for up to 2 h. The reaction was linear with respect to protein concentration over the range 25–250 μg protein. The pH profile was narrow based with an optimum at 7.5, the optimal temperature for the reaction was 30°C.

3.3. *Enzyme stability*

The enzyme lost 25% activity after storage at –20°C for 2 days. All assays were therefore carried out on freshly prepared material.

Table 2
Fucosyl transferase activities of Golgi-rich fraction

Acceptor	Specific activity (nmol fuc. incord. h^{-1}. mg protein^{-1})
L-Fucose	713
no acceptor	–
no Triton X-100	78
no $MgCl_2$	52
Fucoidan	135
D-Fucose	27
Galactose	56
Mannose	16
Manitol	12
Sodium alginate	19
Endogenous	121
Laminaran	–

Values are the mean for five determinations

3.4. *Effect of cations, GTP and detergent concentrations*

The enzyme showed a requirement for cations (table 2). Mg^{2+} stimulated to an optimum at 20 mM, Mn^{2+} at 40 mM. Na^+, K^+, Ca^{2+}, Zn^{2+} had no effect. GTP had no stimulatory effect on the reaction.

The enzyme required a detergent to potentiate activity. Maximum stimulation occurred with 0.2%

Table 1
Location of the fucosyl transferase in isolated membrane fractions from *F. serratus*

Cell fraction	Specific activity (nmol h^{-1} mg protein^{-1})	Total activity (nmol h^{-1})	Total protein (mg)	Relative specific activity	%Yield
Homogenate	23 ± 5	3680 ± 1400	160	–	–
2000 × *g* pellet	–	–	70	–	–
10 000 × *g* pellet	12 ± 3	576 ± 144	48	0.5	16
Golgi-rich	713 ± 85	1934 ± 250	2.5	31	52
100 000 × *g* pellet	48 ± 9	1160 ± 218	20	2.1	26
100 000 × *g* supernatant	10 ± 2	200 ± 40	20	0.4	5.4

Values are the mean ± SD for eight determinations

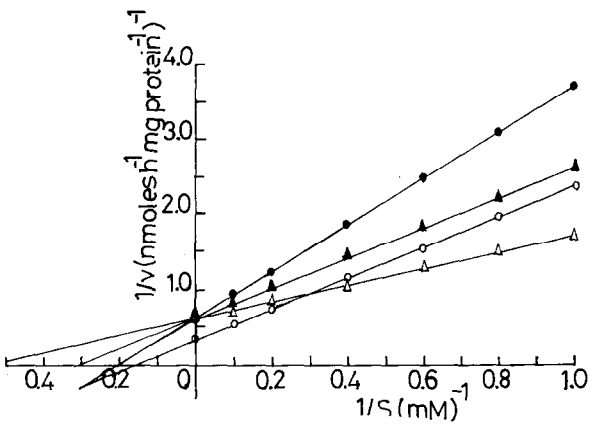

Fig.1. Lineweaver-Burk plot of fucosyl transferase. (•) 20 mM GDP-fucose. (○) 5 mM GDP-fucose. (▲) 10^{-4} M fucoidan. (△) 10^{-3} M fucoidan. The regression equations of the plot were calculated on a Burrows 102 computer, all correlation coefficients are over 90%, all values are the mean of six determinations (SD ≤ ± 25%).

(v/v) Triton X-100 and 0.4% (w/v) deoxycholate.

3.5. *Effect of substrate concentrations*

The enzyme appeared to obey Michaelis-Menten kinetics for a bisubstrate reaction with a K_m 7 mM for GDP-fucose, 3×10^{-4} M for fucose and 5 mM for fucoidan. Figure 1 shows the effect on the fucosyl transferase of varying L-fucose concentration at two different GDP-fucose levels, and the effect of varying fucoidan levels. The addition of fucoidan gives rise to a competitive inhibition plot showing that both substrates are competing at the active sites of the same enzyme.

3.6. *Characterization of reaction products*

When the products of the double labelling reaction were examined radiochromatographically a peak of radioactivity (R_F 0.37) separate from standards of L-fucose and GDP-fucose was found which when eluted and counted for ^3H and ^{14}C showed an equimolar ratio of [^{3}H]fucose and [^{14}C]fucose.

When the compound was treated with dilute acid (0.2 M HCl, 1 h, 100°C) or α-fucosidase (50 μg, 3 h, 30°C) and the reaction products examined chromatographically, a single radioactive peak co-chromatographed with standard L-fucose. When eluted and counted for radioactivity (^3H and ^{14}C) an equimolar ratio of [^{3}H]fucose and [^{14}C]fucose was found. It was therefore concluded that the reaction product was a fucose dimer linked by an α-glycosidic bond. No data is available on the site of linkage.

3.7. *Acceptor specificity*

Table 2 shows the transfer of fucose from the sugar nucleotide to various acceptors. The best substrate is L-fucose with appreciable transfer to fucoidan and heat stable endogenous acceptors.

3.8. *Sulphydryl inhibitors*

The sulphydryl inhibitors iodoacetamode at 10^{-4} M and *p*-chloro mercuribenzoate at 5×10^{-4} M completely inhibited fucosyl transferase activity. It was concluded that the sulphur amino acids cystine and/or methionine were present in the active site(s) of the enzyme and played a vital role in the reaction.

4. Discussion

A fucosyl transferase designated GDP-fucose: L-fucose α-fucosyl transferase catalysing the transfer of L-fucose from GDP-fucose to L-fucose and the sulphated fucan fucoidan [7] was located primarily in the Golgi apparatus of the brown alga *F. serratus*, though appreciable activity was also present in the microsomal fraction. The enzyme was tightly membrane-bound, needing a detergent to potentiate activity, showed a higher affinity for L-fucose (3×10^{-4} M) than for fucoidan (5 mM), a narrow-based pH optima of 7.5, an optimal temperature of 30°C and was present in sufficient amounts to account for the observed in vivo incorporation of $^{14}CO_2$ into fucoidan under sub-optimal photosynthetic conditions [8].

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