α 1 — > 2 FUCOSYLTRANSFERASE OF HUMAN BONE MARROW

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1. Introduction

Blood group antigenic determinants of H specificity i.e. L-fucosyl α1 — >2-D-galactose results from the action of the specific α1 — >2 fucosyltransferase which transfers L-fucose from GDP-L-fucose to terminal nonreducing galactosyl residues of the appropriate acceptors. The enzyme is controlled by H gene [1] and in some organs of the body also by Se gene [2, 3]. The H active structures are then the substrates for the transferases of *N*-acetylgalactosamine and galactose which are associated respectively with A and B blood group specificities [4]. Since blood group A, B, H antigens occur in human erythrocytes of secretors and non-secretors alike it could be anticipated that α1 — >2 fucosyltransferase activity of the bone marrow would not depend upon the secretor status, a situation similar to that reported by Schenkel-Brunner et al. for blood serum [5]. In the present paper we report the occurence of α1 — >2 fucosyltransferase in human bone marrow of secretors and nonsecretors of A, B, H blood group active substances. We also describe some properties of the marrow enzyme which unlike the serum enzyme is particle bound.

2. Materials and methods

Guanosine diphosphate-L-fucose was prepared by the method of Ginsburg [6]. Radioactive guanosine diphosphate [^{14}C] L-fucose, 195 mCi/mmol was a product of Radiochemical Centre, Amersham. Fucosyllactose was isolated from human milk as described previously [7]. Fucosylgalactose was prepared from fucosyllactose by the Kuhn's alkaline degradation after Shen at al. [2].

Enzyme preparations were isolated from human breast bones which were collected 20 hr after death. The method employed was the slightly modified procedure of Basu and Basu [8]. Bone marrow was homogenised with 2 vol of 0.32 M sucrose, 0.001 M EDTA and 0.0014 M mercaptoethanol, pH 7.0, filtered through cheesecloth and centrifuged at 1500 *g* for 10 min. The pellet was reextracted with 1 vol of the same solution and centrifuged as before. The 1500 *g* supernatants were pooled and centrifuged at 100 000 *g* for 90 min. The pellet was rehomogenised with 1 vol of the extraction solvent which included this time 0.6% Triton X-100, and centrifuged at 100 000 *g* for 90 min. The resulting supernatant was used as the enzyme preparation.

Fucosyltransferase activity was measured under conditions specified in the legends to figures and table. Incubation mixtures were similar to those employed by Schenkel-Brunner et al. [5]. Reactions were stopped by freezing the samples at −70°C. After thawing, incubation mixtures were passed through Dowex 2 Cl-columns and treated as described by Kobata et al. [9] for the measurement of α-galactosyltransferase.

The linkage of fucosyl residues incorporated into lactose was determined according to Munro and Schachter [10] subjecting the isolated trisaccharide reaction products to alkaline degradation.

Chromatography was performed on Whatman 3 MM paper with upper phase of ethyl acetate—pyridine—water 2:1:2 solvent mixture using fucosyllactose and fucosylgalactose as standards. Appropriate areas

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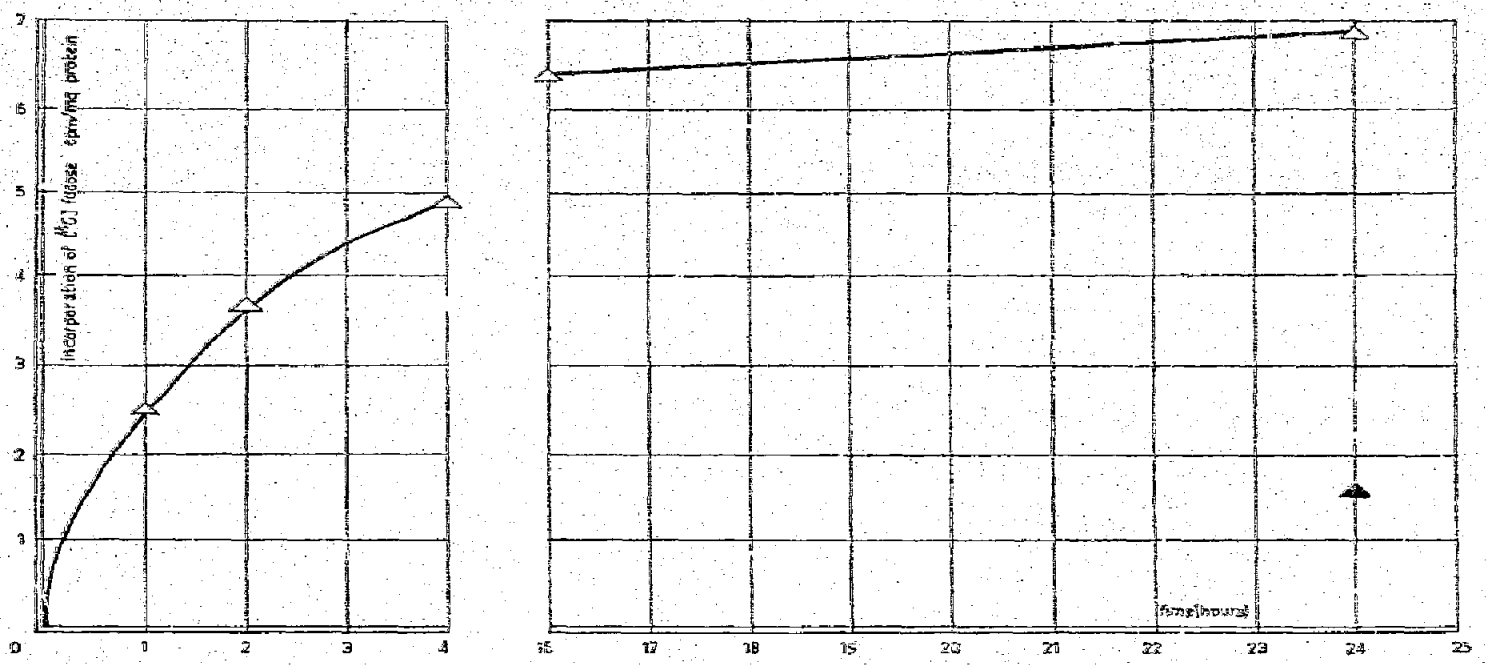

Fig. 1. Incorporation of L-[^{14}C] fucose into lactose. Reaction mixture contained in a final vol of 100 µl: GDPL-[^{14}C] fucose 1.2 nmole, 135 000 cpm; ATP, 1 µmole; buffer Tris–Cl, 2.5 µmole, final pH 6.9; $MgCl_2$, 0.75 µmole; lactose, 100 µg; NaN_3 1 µmole; and enzyme preparation, 280 µg protein, bone marrow no. 4, Le^{a-b+}. Temp. 37°C. (— ▲ —) Preincubation lasted 4 hr at 37°C, then GDPL-[^{14}C] fucose was added and the reaction run for additional 20 hr.

of chromatograms were cut off and counted in a Packard TriCarb liquid scintillation counter. The secretor status of cadavers was inferred from Lewis blood group specificity of erythrocytes. Protein was determined by the method of Lowry et al. [11].

3. Results and discussion

Enzyme preparations obtained from human marrow rapidly incorporated fucose into lactose during the first 4 hr. Thereafter the reaction slowed down considerably which might indicate enzyme instability. This indeed proved to be the most likely explanation because preincubation of the marrow enzyme for 4 hr at 37°C with all the reagents excluding GDP-L-fucose resulted in a marked slackening of the reaction (see fig. 1). The amount of disaccharide acceptor was not rate limiting since apparently undiminished amounts of lactose could be detected on chromatograms of the reaction products even after 24 hr of incubation. Thus the behavior of the bone marrow enzyme is quite different from that of serum fucosyltransferase which retains its activity for over 48 hr [5].

The pH optima for bone marrow and serum fucosyltransferase activities are respectively 7.2 and 7.5 with lactose as an acceptor (fig. 2). A similar value (pH 7.4) for serum fucosyltransferase with lactosamine used as an acceptor was reported by Schenkel-Brunner et al. [5].

The relation between protein concentration in incubation mixtures with bone marrow enzyme and the amount of fucose incorporated into lactose is close to linear up to about 600 µg of protein per 100 µl (fig. 3).

Bone marrow fucosyltransferase did not depend upon Lewis blood group of erythrocytes of the deceased (see table 1). Thus it may be assumed that it also bears no relation to the secretor status as predicted by Morgan and Watkins [12]. The activity of the enzyme can be considered as a manifestation of H gene dependent α1 — >2 fucosyltransferase because the main trisaccharide reaction product was 2′ fucosyllactose. The remaining fucose (not α1 — >2 linked)

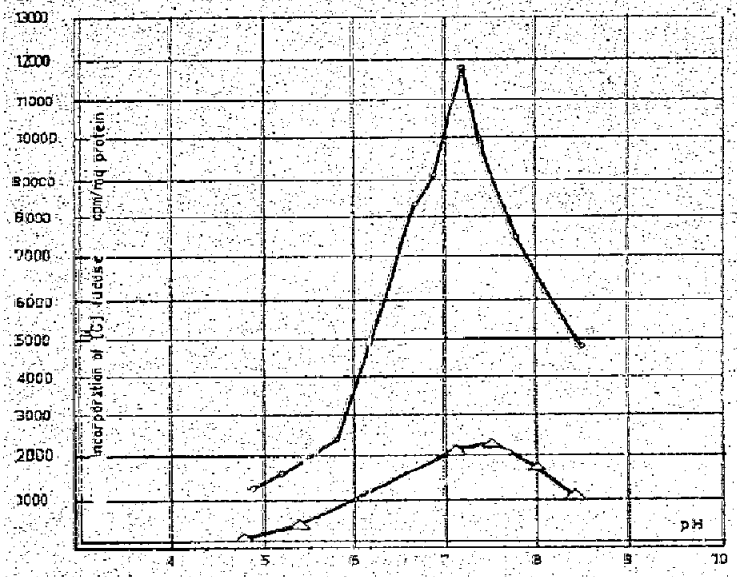

Fig. 2. The effect of pH on the incorporation of L-[^{14}C]-fucose into lactose. (– ○ –) Bone marrow enzyme, preparation no. 12, Le^{a+b-}, 370 μg protein (– △ –) Pooled serum from 20 routine blood donors, 25 μl, 1.8 μg protein. The reaction mixtures were as specified in table 1 except that 2.5 μmole of different buffers were used. pH 4.8 to 5.4, sodium acetate–acetic acid; pH 5.8 to 6.9 ammonium acetate–acetic acid; pH 7.1 and above, Tris–Cl. The pH values plotted are those of the reaction mixtures, measured after incubation. Incubation with bone marrow enzyme lasted 4 hr and with serum 48 hr, at 37°C.

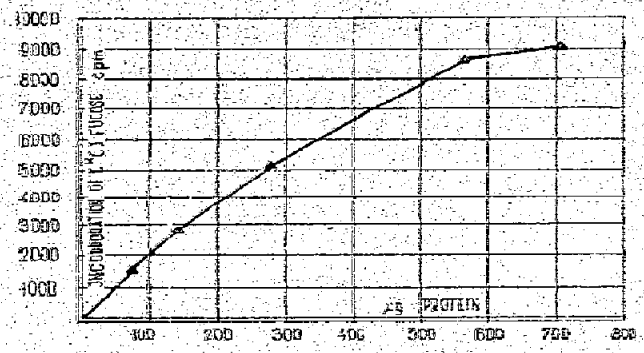

Fig. 3. Effect of the amount of enzyme preparation on the incorporation of L-[^{14}C] fucose into lactose. The reaction mixture was as in table 1. Bone marrow enzyme preparation no. 13, Le^{a-b+} was used. Incubations lasted 4 hr at 37°C.

could be transferred to lactose in α1 – >3 glycosidic linkage. The enzyme responsible for the latter reaction was found in human serum [5]. Human bone marrow fucosyltransferase is in contrary to milk [2] and serum [5] enzymes at least in part particle bound and may be solubilised with Triton X-100. Nevertheless a considerable portion (up to 50%) of the 1500 *g* supernatant activity remained soluble after the first centrifugation at 100 000 *g*. In relation to protein content this activity was however several times lower than that in the remaining particle bound fraction.

Table 1
Fucosyltransferase activity in human bone marrow of secretors and nonsecretors

Sample no.	Red cell phenotype	Radioactive products formed /cpm/mg protein/		% Fucose* α1 – >2 linked to galactose
		Fucosyllactose	Fucosylgalactose	
5	B, Le^{a+b-}	8328	4600	92
11	B, Le^{a+b-}	7971	3230	68
12	A, Le^{a+b-}	13693	6159	75
14	A, Le^{a+b-}	8290	4242	80
8	A, Le^{a-b-}	11982	5933	77
10	A, Le^{a-b+}	5870	2503	72
13	O, Le^{a-b+}	10640	4215	66
15	AB, Le^{a-b+}	5643	2378	76

* 60% Recovery of fucosylgalactose from 2' fucosyllactose was assumed. Reaction mix. contained in a final vol of 100 μ: GDPL-[^{14}C] fucose, 1.5 nmole, 180 000 cpm; ATP, 1 μmole; buffer Tris–Cl, 2.5 μmole final pH 7.2; $MgCl_2$, 0.75 μmole; lactose, 100 μg; NaN_3, 1 μmole and enzyme preparations containing from 250 μg to 700 μg protein. Incubation time 4 hr; Temp. 37°C.

We have not determined whether the fucosyltransferase activity of the soluble fraction resulted from autolysis of the bone marrow tissue.

The particle bound fucosyltransferase exhibited after solubilisation appreciably higher activity per mg of protein then that found in blood serum. Nevertheless the instability of the marrow enzyme under incubation conditions make difficult the assesment of its activity in vivo.

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