ACTIVITY OF 2-α-L-FUCOSYLTRANSFERASE IN HUMAN SERA AND RED CELL MEMBRANES

A study of common ABH blood donors, rare 'Bombay' and 'Parabombay' individuals

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1. Introduction

A large number of fucose containing substances, chemically characterized as oligosaccharides, glycoproteins or glycolipids have been isolated from cells, tissues or fluids of various animal species including those of human, bovine, pig, dog and rat [1–7].

The fucose is found to be attached to carbohydrate residues by at least three distinct types of glycosidic bonds. In the first L-fucose is α-(1→2) linked to a β-galactose unit by the action of the blood group H-dependent gene product. In human, this enzyme has been described in a soluble form in milk and serum [8–11] and in particulate form in stomach mucosa, submaxillary glands and bone-marrow [12,13]. The L-fucose residue is also found α-(1→4) linked to a subterminal *N*-acetyl-D-glucosamine. The corresponding enzyme is recognized as the Lewis gene product and was demonstrated among *Le* individuals in milk, submaxillary glands, stomach mucosa [8,12–14] but not in serum or plasma [9,10]. In the third type, L-fucose is linked to the C-3 position of the subterminal *N*-acetyl-D-glucosamine, the addition being catalysed by a 3-α-L-fucosyltransferase found up to now in all human individuals [8–12].

However, other fucosyltransferases are very likely to occur because some compounds with L-fucose linked α-(1→3) or α-(1→6) to *N*-acetylglucosamine or β-galactose residues also have been described [15–17].

In secretory tissues such as submaxillary glands or stomach mucosa, the synthesis of the H enzyme (2-α-L-fucosyltransferase) is controlled by a second gene, termed secretor gene *Se* [12]. It has been shown that milk from non-secretor *se se* individuals is devoid of H enzyme activity [8]. In serum or bone-marrow however, the H enzyme is always found, whatever the secretor status [9,13]. The only exceptions are the serum from rare 'Bombay' individuals thought to be homozygotes for a recessive *h* gene [9,18–20]. The A, B or H substances are absent from both red cells and secretions of these individuals, while their plasma always contain the anti-A, -B, and -H agglutinins. These findings are certainly among the most important which support the biosynthetic pathway of A and B blood group substances predicted by Morgan and Watkins [21] because the H substance is considered to be the biochemical precursor of A and B.

In addition to the 'Bombay' blood samples, some individuals whose red cells express A and B antigens, but no H, have been described [22–24]. Following Race and Sanger [25] these people were named 'Parabombay'. The purpose of this paper was therefore to investigate the H enzyme activity in serum and red cell membranes from 'Bombay' and 'Parabombay' individuals. When necessary, the A and B blood group glycosyltransferases were simultaneously estimated. As a preliminary step in this work, the H enzyme activity from common blood samples was also studied as a function of their ABO phenotypes.

2. Materials and methods

Sera from A_1, A_2, A_1A_2, A_1B, A_2B, B_I, B_{II}, O and

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Bombay donors were collected and stored at −20°C until used.

Red cell ghosts from normal (A_1, A_2, A_1B, B, O) and Bombay cells were prepared according to Dodge et al. [26], and the protein content of the red cell membrane suspensions was estimated with the Folin Ciocalteu reagent [27].

Guanosine diphospho-L-[U-^{14}C]fucose (153 mCi/mmol) purchased from the Radiochemical Centre (Amersham) was used in 2% ethanolic solution. UDP-*N*-acetyl-D-[1-^{14}C]galactosamine (47.2 mCi/mmol) and UDP-D-[^{14}C]galactose (274 mCi/mmol) obtained from Nen Chemicals (Frankfurt) were respectively used in 50% and 2% ethanolic solution.

Lacto-*N*-Biose I (*O*-β-D-galactopyranosyl (1→3)-*N*-acetyl-D-glucosamine) was a gift from Professors Lemieux and Gauhe. It was either chemically synthesized or extracted from human milk. 2′-Fucosyllactose (*O*-α-L-fucopyranosyl (1→2)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose) was extracted from human milk.

Enzyme assays in human sera:
A and B enzyme assays have been described [28,29]. H enzyme assays were carried out in total vol. 55 μl with the following components: serum, 25 μl; Tris–HCl, pH 7.2, 2 μmol; $MgCl_2$, 0.5 μmol; GDP-[^{14}C]-fucose, 0.92 nmol; NaN_3, 1 μmol; Lacto-*N*-Biose I, 100 μg; ATP, 0.64 μmol. The mixture was incubated for 72 h at 37°C.

Enzyme assays in RBC ghosts:
A and B enzymes in erythrocyte membrane were estimated as described by Cartron et al. [30]. The 2-α-L-fucosyltransferase from RBC was performed by adding the following components in total vol. 160 μl: 500–1000 μg erythrocyte membrane protein; Tris–HCl buffer pH 7.2, 8 μmol; $MgCl_2$, 2 μmol; GDP-[^{14}C]fucose, 184 nmol; NaN_3, 1 μmol; Lacto-*N*-Biose I, 200 μg; ATP 2.58 μmol and 5 μl of Triton X-100 10% (v/v). The mixture was incubated for 72 h at 37°C.

The α-L-fucosidase activity of normal, 'Bombay' and 'Parabombay' sera was tested as described by Ramage et al. [31].

Characterisation of H enzymatic product:
The trisaccharide α-L-Fuc (1→2) Gal β (1→3) GlNAc synthesized by addition of L-[^{14}C]fucose into Lacto-*N*-Biose I was characterized after high voltage electrophoresis in 0.1 M ammonium formate buffer, pH 3.5 (Whatman paper No. 40; 2000 V, 100 mA, 2 h) and descending chromatography in ethylacetate/pyridine/acetic acid/water (5:5:1:3). The R_{Lact} was 1.03 in this solvent.

3. Results and discussion

Lacto-*N*-Biose I was used as a convenient low molecular weight acceptor for H enzyme assays, because:
(i) The 4-α-L-fucosyltransferase activity of human sera is negligible.
(ii) This sugar is not a substrate for the very common 3-α-L-fucosyltransferase [9].
Accordingly, the L-fucose incorporated is almost exclusively α-(1→2) linked to the terminal β-galactose residue of Lacto-*N*-Biose I. This compound was identified by its chromatographic mobility.

3.1. *2-α-L-fucosyltransferase levels in blood samples*

110 sera were studied including 16 A_1, 17 A_2, 14 O, 17 A_1B, 16 A_2B, 10 B_I, 10 B_{II} and 10 A_1A_2, the latter being identified by the pH ratio dependent curve of the A gene product [32]. The enzyme activity was found in all sera either from secretor or non-secretor individuals, suggesting that the serum enzyme does not originate from mucous secretory tissues. The mean values observed within each ABO blood group are reported in table 1, but a more interesting pattern was noticed when all the results were plotted (fig.1). In spite of a large overlap between each group, the H enzyme activity was found on the average to be higher in A_1A_2, A_1, A_1B and A_2 sera than in O, A_2B or B (B_I and B_{II} are subgroups defined by Badet et al. [28] according to the level of the serum α-D-galactosyltransferase). This finding was also recently reported by Chester et al. [33]. The results suggest some kind of reciprocal relationship between the H enzyme level of the serum and the H antigenic content of the red cells. It was however verified that such a gradient of H blood group enzyme activity was not correlated with a fucosidase activity or with the A or B enzyme levels of the sera. The mean release of paranitrophenol from PNP-α-L-fucopyranoside was equal to 2.13, 2.08, 1.74, 2.37, 2.99, 2.82 and 2.23 μmol/min/litre in the A_1A_2, A_1, A_1B, A_2, O, A_2B and B sera described above. Large variations of the fucosidase activity were also noticed within a group (not shown). Moreover,

Table 1
ABH blood group enzyme activities in sera and RBC ghosts from common ABH donors

Genotype	Activity of blood group glycosyltransferases						
	In serum				In RBC ghosts		
	A_1 (pH 6.0)	A_2 (pH 7.0)	B	H	A	B	H
O	–	–	–	7044 (14)	–	–	5530 (2)
A_1[a]	28 000 (16)	–	–	9380 (16)	64 200 (1)	0	5690 (1)
A_2[b]	–	16 419 (17)	–	8491 (17)	10 800 (1)	0	4500 (1)
A_1B	25 372 (14)	–	11 480 (14)	9107 (17)	52 925 (2)	31 700 (2)	4920 (2)
A_2B	–	12 751 (16)	8248 (16)	6445 (16)	–	–	–
A_1A_2	29 198 (9)	24 548 (9)	–	11 392 (10)	–	–	–
B_I	–	–	5820 (10)	6313 (10)	–	73 430 (2)	5775 (2)
B_{II}	–	–	12 300 (10)	4876 (10)			

[a] Maximum A enzyme activity at pH 6.0 in A_1 sera
[b] Maximum A enzyme activity about pH 7.0 in A_2 sera

Enzyme activities are expressed in cpm of radiolabelled sugar incorporated into appropriate acceptors as described in Methods. Values in brackets indicate the number of samples tested

incubation at neutral pH of the purified radiolabelled H trisaccharide with several samples of sera (37°C for 72 h) did not demonstrate any release of [^{14}C]fucose. Lastly, the differences of H enzyme levels in sera were not related to A or B glycosyltransferase activities.

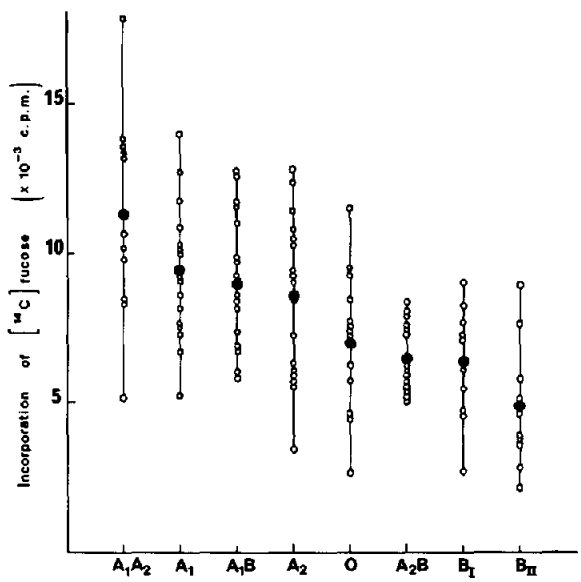

Fig.1. Incorporation of L-[^{14}C]fucose into Lacto-*N*-Biose I by serum samples from donors of different ABO groups as shown on abscissa. (•) Mean enzyme activity.

Therefore, the relationship between H enzyme activity and ABO phenotype remains unclear mainly because the origin of the serum enzyme is not known. Among the possible hypotheses one may consider that the enzyme is, for some unknown reason, partly inactivated at the site of its biological function. Such results however must be borne in mind if attempts are made for quantitative estimations of H enzyme level.

The 2-α-L-fucosyltransferase activity was more homogeneous in red cell membranes from eight donors including 2 O, 1 A_1, 1 A_2, 2 B and 2 AB individuals, among which, three were not secretors. The H enzyme is membrane bound in these preparations, and maximum activity is obtained only by addition of Triton X-100. The relationship with ABO phenotypes of RBC was not found, but only a small number of samples have been investigated.

However, the heterogeneity of H enzyme activity in sera may also result from the contributions of unidentified cells secreting transferase in the blood stream.

The A and B enzyme activities of the RBC membrane were higher than the H activity, but it is possible that better results could be obtained using other detergents for membrane solubilisation [4].

3.2. *Serum and red cell ABH glycosyltransferase in 'Bombay' and 'Parabombay' individuals*

As previously described by Race and Watkins [18,19] and Schenkel-Brunner et al. [9], the sera from 'Bombay' people O_h, O_h^A, O_h^B were devoid of H enzyme activity. We have furthermore demonstrated that the H enzyme is also deficient in the red cell stroma of the 7 individuals tested (table 2). These expected results suggest either that the hypothetical *h* gene is amorph or that it controls the synthesis of a catalytically inactive protein. The true genotype of these rare individuals may however be deduced from the simultaneous investigations of the A and/or B glycosyltransferase, always active in subjects with the appropriate blood group.

Similar results have been obtained with the nine 'Parabombay' blood samples A_h, B_h and AB_h, namely:

(i) Absence of detectable 2-α-L-fucosyltransferase activity in serum and RBC membranes.

(ii) Normal amount of A and/or B serum and RBC enzyme activity in accordance with ABO phenotype (table 2).

The results demonstrate that the weak A and/or B antigenic content of the A_h, B_h or AB_h red cells is not related to abnormal synthesis of A and/or B glycosyltransferase but rather to a limited production of the blood group H precursor. It is usually admitted that the small amount of the A and/or B antigens on Parabombay red cells is built on the few H determinants produced by poorly active variants of the H gene. In such samples, a weak H enzyme activity is expected but was not found, may be due to the rather insensitive method of assay. It is possible that the phenyl-β-galactoside would be a more suitable substrate when a low H enzyme activity is expected [33].

Some workers have suggested an alternative biosynthetic pathway which would make possible the production of A and B antigen independently of the

Table 2
ABH blood group enzyme activities in sera and RBC ghosts from 'Bombay' and 'Parabombay' individuals

Sample	Genotype[a]	In serum				In RBC ghosts		
		A_1 (pH 6.0)	A_2 (pH 7.0)	B	H	A	B	H
Bombay								
SCH	0	0[b]	0	0	0	–	–	0
MART[c]	0	0	0	0	0	–	–	–
BEN	A_1	52 700	30 300	0	0	–	–	–
GER	A_1	48 400	36 400	0	0	100 700	–	0
LEN	A_1	41 700	32 100	0	0	21 100	–	0
JOS	A_1B	24 000	20 200	11 100	0	49 900	14 600	0
AFS (No. 3)[c]	B	0	0	4200	0	–	–	–
Parabombay								
BEG[c]	A_1	20 130	11 800	0	0	–	–	–
OTT	A_1	30 700	17 275	0	0	81 300	–	0
GEO[c]	A_2	18 700	28 500	0	0	8000	0	0
JES	A_2	13 200	27 200	0	0	10 800	–	0
DIJ	A_2B	11 400	27 500	7900	0	–	–	–
MARA	A_1B	31 200	18 500	12 000	0	107 200	82 100	0
CER	B	0	0	15 000	0	–	150 000	0
TUP	B	0	0	36 120	0	–	117 800	0
PAY	B	0	0	34 070	0	–	66 200	0

[a]Genotype deduced from either family or enzyme studies
[b]No detectable enzyme activity
[c]Saliva not tested for ABH substances

Enzyme activities are expressed in cpm of radiolabelled sugar incorporated into appropriate acceptors as described in Methods

usual H precursor [23,34]. This concept is however entirely speculative and it is not up to now supported by any experimental evidence.

The study of other H variants characterized by a selective decrease of the H receptors on RBC and normal amount of salivary substances (Hm phenotypes) is actually in progress. It may provide further information both on the nature of the antigenic defect and the origin of serum 2-α-L-fucosyltransferase.

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