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Note

Inhibition of the blood group A^1 and A^2 gene-specified *N*-acetyl-α-D-galactosaminyltransferases by uridine diphosphate D-galactose*

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The *N*-acetyl-α-D-galactosaminyltransferases [UDP-2-acetamido-2-deoxy-D-galactose: *O*-α-L-fucosyl-(1→2)-D-galactose 2-acetamido-2-deoxy-α-D-galactosyltransferase] determined by the human blood-group A^1 and A^2 genes and the α-D-galactosyltransferase [UDP-D-galactose: *O*-α-L-fucosyl-(1→2)-D-galactose α-D-galactosyltransferase] determined by the blood-group *B* gene occur in soluble form in serum[1–4]. These enzymes are conveniently assayed by the transfer of radioactively labelled sugars from uridine diphosphate (UDP) *N*-acetyl-D-[^{14}C]galactosamine or uridine diphosphate (UDP) D-[^{14}C]galactose to the low molecular weight acceptor 2′-fucosyllactose [*O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose][3–4]. Whilst investigating the two transferases in the sera of blood group AB subjects, we observed that, under conditions where the concentrations of both the acceptor and donor substrates were saturating, the sum of the activities of the two enzymes assayed individually was slightly greater than the sum of the activities measured when both donor substrates were present in the reaction mixture. This prompted an examination of the effect of UDP-D-galactose on the *N*-acetyl-α-D-galactosaminyltransferases in serum samples from group A_1 and A_2 donors and the effect of UDP-*N*-acetyl-D-galactosamine on the α-D-galactosyltransferase in serum samples from group B donors. In each of the examples of A_1 and A_2 sera examined, the transfer of *N*-acetyl-D-[^{14}C]galactosamine to 2′-fucosyllactose was markedly inhibited by the presence in the reaction mixture of a concentration of UDP-D-galactose equal to that of UDP-*N*-acetyl-D-galactosamine. The transfer of D-[^{14}C]galactose to the same acceptor by the transferases in group B sera, however, was not effected by the presence of UDP-*N*-acetyl-D-galactosamine. These results suggested that UDP-D-galactose might be a competitive inhibitor in the *N*-acetyl-α-D-galactosaminyltransferase system and further kinetic studies were therefore carried out.

*Dedicated to the memory of Professor W. Z. Hassid.

The *N*-acetyl-α-D-galactosaminyltransferases in serum samples from blood group A_1, A_2, and A_1B donors were assayed at different concentrations of UDP-*N*-acetyl-D-galactosamine in the presence and absence of a fixed concentration of UDP-D-galactose. The substrate concentration curves and the derived Lineweaver–Burk plots[5] are given in Figs. 1–3. For the *N*-acetylgalactosaminyltransferases in the A_1, A_2, and A_1B sera, the inhibition by UDP-D-galactose appeared to be primarily competitive, since there is an apparent increase in K_m value but little alteration of the limiting velocity in the presence of the inhibitor. The K_m values for UDP-*N*-acetyl-D-galactosamine, and the K_i values for UDP-D-galactose, calculated from the data in Figs. 1–3, are given in Table I. The K_m and K_i values for each of the enzymes were almost identical, indicating that the *A* gene-specified transferases bind equally well to either UDP-*N*-acetyl-D-galactosamine or UDP-D-galactose, although they catalyse the transfer of only *N*-acetyl-D-galactosamine to the acceptor substrate. The transferase in the group A_2 serum differed from the corresponding enzymes in the A_1 and A_1B sera in that the K_m and K_i values for the A_2 serum enzyme were slightly higher, but the affinity for the two nucleotide sugars was again approximately equal. The transferases in A_1 sera were previously shown[6] to have considerably lower K_m values than those in A_2 sera for the low-molecular-weight acceptors 2′-fucosyllactose and lacto-*N*-fucopentaose I [α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-GlcNAc*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc]. Substrate concentration curves, in the presence and absence of UDP-*N*-acetyl-D-galactosamine, of the α-D-galactosyltransferase in B serum failed to reveal any inhibition (Fig. 4). The K_m value for UDP-D-galactose in this system (1.6×10^{-5}M) was similar to the K_m values for UDP-*N*-acetyl-D-galactosamine found for the transferases in the sera from the A_1 and A_1B donors (Table I).

In common with many other glycosyltransferases (for example, see Babad and Hassid[7]), the A^1 and A^2 gene-associated *N*-acetyl-α-D-galactosaminyltransferases and the *B* gene-associated α-D-galactosyltransferase are inhibited by UTP, UDP, and other

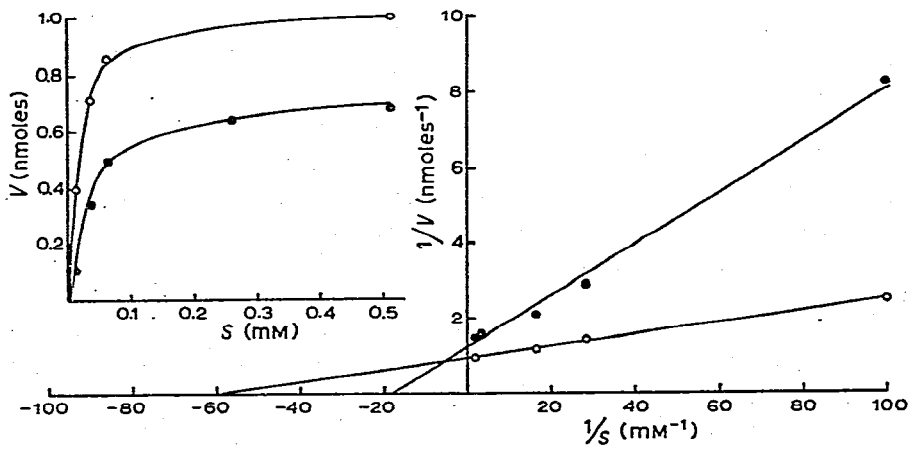

Fig. 1. Serum from blood-group A_1 donor (C.R.). Incorporation of *N*-acetyl-D-[^{14}C]galactosamine into 2′-fucosyllactose in presence (●—●) and absence (○—○) of 0.05mM UDP-D-galactose.

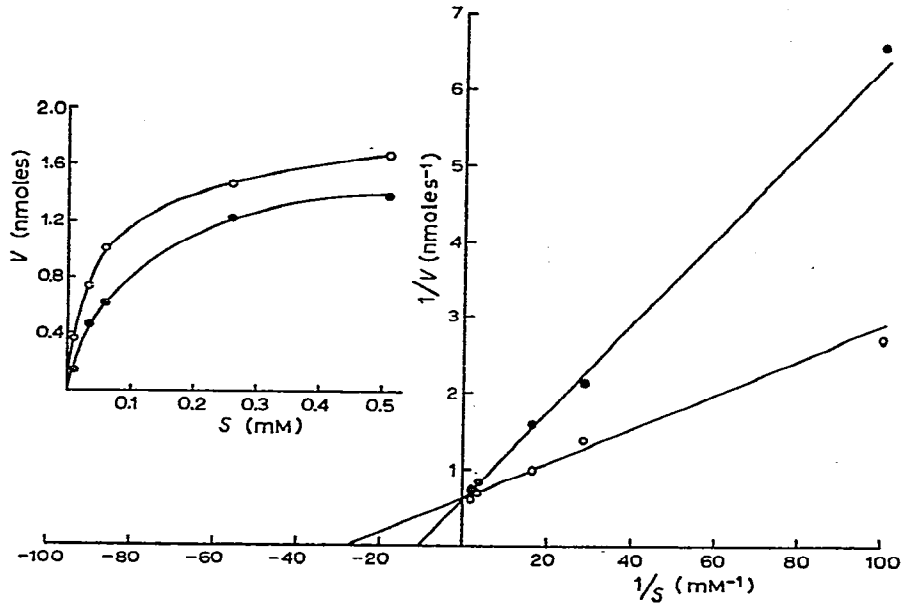

Fig. 2. Serum from blood-group A_2 donor (S.B.). Incorporation of *N*-acetyl-D-[^{14}C]galactosamine into 2′-fucosyllactose in presence (●—●) and absence (○—○) of 0.05mM UDP-D-galactose.

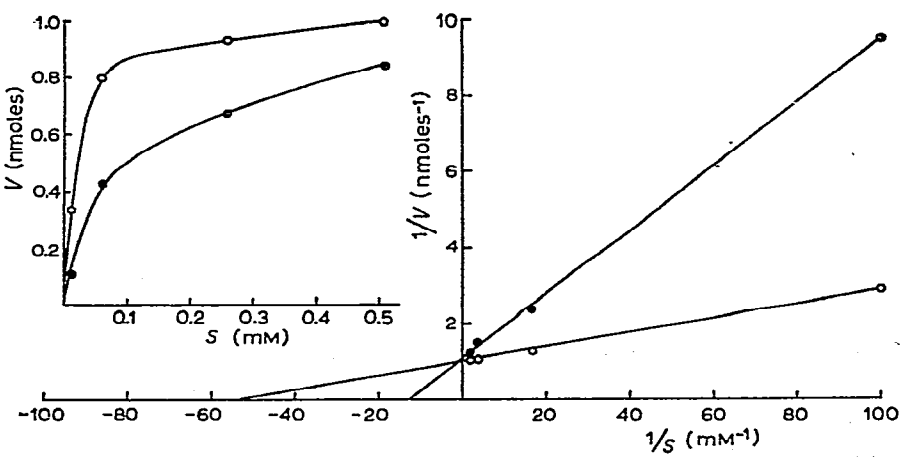

Fig. 3. Serum from blood-group A_1B donor (R.S.). Incorporation of *N*-acetyl-D-[^{14}C]galactosamine into 2′-fucosyllactose in presence (●—●) and absence (○—○) of 0.05mM UDP-D-galactose.

nucleotides[8]. The competitive inhibition of the *N*-acetyl-α-D-galactosaminyltransferases in serum from A_1, A_2, and A_1B donors by UDP-galactose, and the absence of inhibition of the α-D-galactosyltransferase in B serum by UDP-*N*-acetylgalactosamine, is, however, of interest in connection with the status of the A^1, A^2, and B genes as alternative alleles at the *ABO* genetic locus. If the glycosyltransferases are, indeed,

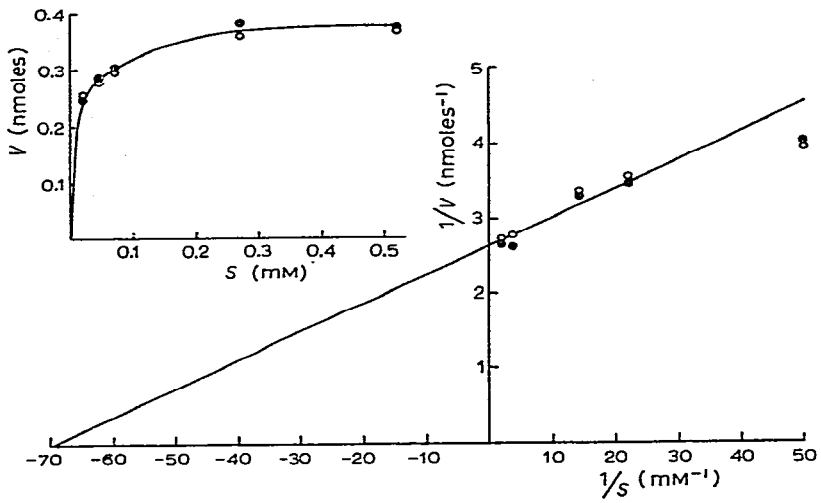

Fig. 4. Serum from blood-group B donor (B.C.). Incorporation of D-[^{14}C]galactose into 2′-fucosyllactose in presence (●—●) and absence (○—○) of 0.05mM UDP-*N*-acetyl-D-galactosamine.

TABLE I

N-ACETYL-α-D-GALACTOSAMINYLTRANSFERASES IN SERUM FROM BLOOD-GROUP A_1, A_2, AND A_1B DONORS. K_m VALUES FOR UDP-*N*-ACETYL-D-GALACTOSAMINE AND K_i VALUES FOR UDP-D-GALACTOSE

Serum		K_m *for UDP-GalNAc* (μM)	K_i *for UDP-Gal*[a] (μM)
Donor	*Blood group*		
C.R.	A_1	17	22
S.B.	A_2	37	35
R.S.	A_1B	19	16

[a]Calculated from the equation $K_i = i \cdot K_m \cdot (K_p - K_m)^{-1}$ where K_p is the apparent Michaelis constant in the presence of a fixed concentration of inhibitor i[17].

the primary protein products of the genes[9], then the mutational change that led from *A* to *B*, or *vice versa*, must be assumed to have influenced the enzymically active site of the protein, either directly, or by inducing a conformational change in the whole molecule that indirectly affects the active site. Since the nucleotide part of the molecule is the same in UDP-D-galactose and UDP-*N*-acetyl-D-galactosamine, the difference in the inhibitory power of the nucleotide sugars in the *A* and *B* gene-specified transferase systems must presumably be related to the nature of the sugar moiety. It can be postulated that the difference in the combining sites of the enzymes is such that the *A* gene-specified transferases can accommodate the smaller D-galactosyl residue in UDP-D-galactose whereas *N*-acetylgalactosamine, with the bulkier *N*-acetyl amino group at C-2 of the hexose ring, may not fit into the combining site of the *B* gene-specified α-D-galactosyltransferase.

EXPERIMENTAL

Materials. — Unlabelled UDP-D-galactose was purchased from Sigma London Chemical Company Ltd. and UDP-D-[^{14}C]galactose (240 Ci/mole) from the Radiochemical Centre (Amersham, England). Unlabelled UDP-*N*-acetyl-D-galactosamine was prepared by the method of Carlson *et al.*[10] and UDP-*N*-acetyl-D-[^{14}C]galactosamine (43 Ci/mole) was purchased from the New England Nuclear Corporation (Frankfurt, Germany). 2′-Fucosyllactose was supplied by Dr. A. Gauhe (Max-Planck-Institut für Chemie, Heidelberg, Germany).

Enzyme assays. — The experiments depicted in Figs. 1–3 were carried out under conditions where product formation was proportional to time, and rate of reaction was proportional to enzyme concentration. Fresh serum (25 µl) was incubated at 37° for 16 h with UDP-*N*-acetyl-D-[^{14}C]galactosamine, at a range of concentrations from 0.01 to 0.51mM (each containing 150,000 c.p.m.), in the presence or absence of 0.05mM UDP-D-galactose. The other constituents of the reaction mixture were: manganese dichloride (4.0 µmoles), ATP (1.0 µmole), 2′-fucosyllactose (0.5 µmole), and Tris-HCl buffer (5.0 µmoles), pH 7.5, in a total volume of 200 µl. The reaction mixtures for the experiments on the α-D-galactosyltransferase (Fig. 4) were identical, except that the serum was incubated with a range of concentrations of UDP-D-[^{14}C]galactose (each containing 150,000 c.p.m.) from 0.02 to 0.52mM in the presence or absence of 0.05mM UDP-*N*-acetylgalactosamine. At the end of the incubation, the neutral sugars were separated from the other components of the reaction mixtures by resolution on ion-exchange papers[11]. The digests were applied to strips of Whatman DE 81 paper which were stitched to strips of Whatman CM 82 paper. The neutral sugars were eluted with water, the eluate evaporated to a small volume, and applied to Whatman No. 40 paper for chromatography in ethyl acetate–pyridine–water (2:1:2, v/v, upper phase, Solvent *a*). Radioactive peaks were detected with a 7201 Packard Radiochromatogram Scanner and counted in a Nuclear Chicago Mark II Scintillation counter.

The enzymes in the A_1, A_2, and A_1B sera transferred *N*-acetyl-D-[^{14}C]-galactosamine to 2′-fucosyllactose to give a compound ($R_{lactose}$ 0.5 in solvent *a*) which corresponded in its chromatographic properties to the tetrasaccharide previously synthesised with this acceptor when group A tissues were used as the enzyme source[12]. The labelled *N*-acetylgalactosamine was released from the tetrasaccharide by an *N*-acetyl-α-D-galactosaminidase from (*Lumbricus terrestris*[13]) that was free from β-activity. In the experiments with group B serum as the enzyme source, D-[^{14}C]-galactose was transferred to 2′-fucosyllactose to give a tetrasaccharide ($R_{lactose}$ 0.4 in solvent *a*) which corresponded to that previously identified[14]. Radioactive D-galactose was released by a purified α-galactosidase from *Trichomonas foetus*[15] and was not split off by a β-galactosidase isolated from the same organism[16]. The velocity of the reaction (V) at each concentration of nucleotide sugar substrate (S) was calculated as nmoles of *N*-acetyl-D-galactosamine, or D-galactose, transferred in 16 h to the acceptor trisaccharide.

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