127. On a Le^a Substance-Decomposing Enzyme. II

Chemical Effects of the Enzyme on Water-Soluble Blood Group Substances

By Ken FURUKAWA and Shoei ISEKI

Department of Legal Medicine, School of Medicine,
Gunma University, Maebashi

(Comm. by Tanemoto FURUHATA, M.J.A. Sept. 12, 1964)

Annison and Morgan¹⁾ isolated from human ovarian-cyst fluids Le^a substance and observed that, like A substance, it was comprised of L-fucose, D-galactose, D-glucosamine, D-chondrosamine and eleven amino acids, that hydrolysis with mild acid liberated from Le^a substance almost all the fucose constituting 13% of it, while the same procedure liberated from A substance three-quarters of fucose, accounting for 18% of it, and furthermore that ratio of D-glucosamine to D-chondrosamine in Le^a substance was 3:1, whereas the ratio in A substance was nearly 1:1.

Watkins²⁾ reported that extract of *Trichomonas foetus* destroyed blood group activities of A, B, H, and Le^a substances, resulting in the production of about 20% of reducing sugars and a liberation of fucose, galactose and *N*-acetylhexosamine.

Watkins and Morgan³⁾⁻⁵⁾ clarified that reaction of anti-Le^a agglutinin was strongly inhibited by lacto-*N*-fucopentaose II and lacto-*N*-difucohexaose II, and reaction of anti-Le^b agglutinin weakly inhibited by lacto-*N*-difucohexaose I and lacto-*N*-difucotetraose, and subsequently assumed that a branched trisaccharide unit, in which α -fucosyl unit by 1→4 linkage and β -D-galactosyl unit by 1→3 linkage are attached to *N*-acetylglucosamine, would be an important part of the Le^a determinant structure, and that two α -L-fucosyl units joined as branches to each of two adjacent sugars would play a part in Le^b specificity.

It was elucidated by Watkins and Morgan³⁾ that Le^a-decomposing action of *T. foetus* extracts was inhibited by L-fucose, and that Le^a activity of lacto-*N*-fucopentaose II was destroyed by the crude *T. foetus* enzyme preparation to be completely broken down into its component sugars. According to Watkins,^{6),7)} H-decomposing enzyme from *T. foetus* splits lacto-*N*-difucohexaose I into lacto-*N*-fucopentaose II and fucose, as the result enhancing the Le^a activity, and Le^a-decomposing enzyme from *T. foetus* splits lacto-*N*-fucopentaose II into lacto-*N*-tetraose and fucose, as the result abolishing Le^a activity.

Painter *et al.*⁸⁾ isolated from A, B, H, and Le^a substances, four

common disaccharides, and Rege *et al.*⁹⁾ three common trisaccharides from them. And they⁹⁾ estimated that through combination of these sugars, two sugar sequences— β -Gal-(1→3)- β -GNac-(1→3)- β -Gal-(1→3)-GalNAc and β -Gal-(1→4)- β -GNac-(1→3)- β -Gal-(1→3)-GalNAc—would be formed in the carbohydrate chains of H and Le^a substances. They could not obtain oligosaccharide containing residues of L-fucose which are considered to be related to H and Le^a specificities, but they believed that in this case L-fucose and sialic acid residues would be attached as branching units to the sugars which make up the main carbohydrate chains.

This paper deals with chemical effects of Le^a-decomposing enzyme from *Bac. cereus* Le^{a10)} on water-soluble blood group substances.

Materials and methods. 1. *Enzyme preparations.* The Le^a enzyme preparation was prepared by the method as described in the previous paper,¹⁰⁾ and the enzyme preparation which destroys both O(H) and Le^b activities was prepared from *Bac. fulminans* by the method described by Furukawa *et al.*¹¹⁾ 2. *Analytical methods.* For the qualitative determination of sugars, paper chromatography was used; and in quantitative determination, Dische and Shettles' method was employed for fucose, Dische's method for galactose, and Blix's modification of Elson and Morgan's method for hexosamine.

Results. 1. *Enzyme action of Le^a substance:* After treatment of 0.1 per cent solution of Le^a substance for 48 hours at 37° with the Le^a enzyme preparation, the capacity of Le^a substance to inhibit anti-Le^a serum was lost as shown in Table I. And after dialysis of the mixture, fucose and galactose were demonstrated in the dialysate as simple sugars. Results of their quantitative determinations, given in Table II, indicate that these fucose and galactose respectively

Table I. Serological effects of the enzymes on Le^a and O(H) substances

Blood group substance	Treatment with enzyme	Agglutination inhibition titers		
		Anti-Le ^a rabbit serum	Anti-Le ^b rabbit serum	Anti-O(H) eel serum
Le ^a O(H)−, Le(a+b−) (0.1%)	Before	256	—	—
	Treatment with Le ^a enzyme	—	—	—
O(H) O(H)+, Le(a±b+) (0.1%)	Before	4	128	512
	I. Treatment with Le ^a enzyme	—	128	512
	II. Treatment of I with O(H) and Le ^b enzymes	32	—	—
	III. Treatment of II with Le ^a enzyme	—	—	—

Table II. Chemical effects of the enzymes on Le^a and O(H) substances

Blood group substance	Treatment with enzyme		fucose %	galactose %	hexosamine (as glucosamine) %
Le ^a	Before		6.8	24.6	16.8
O(H)-, Le(a+b-)	Treatment with Le ^a enzyme	DP	2.3	7.8	0
		NP	4.5	16.6	16.8
O(H) O(H)+, Le(a±b+)	Before		9.3	31.5	20.0
	I. Treatment with Le ^a enzyme	DP	0.3	5.9	0
		NP	8.8	24.3	20.0
	II. Treatment of I with O(H) and Le ^b enzymes	DP	3.0	0.7	0
		NP	5.8	23.6	20.0
	III. Treatment of II with Le ^a enzyme	DP	2.6	2.4	0
		NP	3.2	21.0	20.0

DP: dialyzable portion, NP: non-dialyzable portion.

amounted to about 34% and 32% of their original quantities. It can subsequently be assumed that the enzyme preparation would contain fucosidase and galactosidase. Since this preparation did not contain O(H)-decomposing enzyme, this fucosidase is considered different in activity from that of O(H) enzyme preparation from *Bac. fulminans* or *Bac. cereus* O(H); and the galactosidase is considered a kind of β -galactosidase, because it did not decompose melibiose but decomposed lactose into galactose and glucose.

2. Enzyme action on O(H) substance: After treatment of O(H)+, Le(a±b+) substance with the Le^a enzyme preparation, its slight Le^a activity disappeared, and after dialysis of this enzyme-treated substance, fucose and galactose were demonstrated in the dialysate to about 3% and 19% of their original amounts, respectively. When the non-dialyzable portion was further treated with the enzyme preparation, containing the O(H)- and Le^b-decomposing enzymes, its O(H) and Le^b activities disappeared while Le^a activity developed. After dialysis of this twice enzyme-treated substance, the dialysate contained about 32% of the original amount of fucose and about 2% of the original amount of galactose liberated as simple sugars. When the non-dialyzable fraction was thirdly treated with the Le^a enzyme preparation, the Le^a activity, which had developed as the result of the second enzymatic treatment disappeared, and after dialysis of this enzyme-treated substance, fucose and galactose were demonstrated in the dialysate to about 28% and 8% of their original amounts, respectively (Tables I and II).

Discussion. The Le^a enzyme preparation from *Bac. cereus* Le^a contains a kind of fucosidase and of β -galactosidase. This preparation, acting on Le^a substance, liberates about 34% of the total fucose, which is the determinant of Le^a specificity; and acting on O(H)+,

Le(a±b+) substance, it liberates about 3% of the total fucose. Furthermore, when allowed to act on the substance which has developed Le^a activity as the result of treatment of Le^a enzyme-treated O(H) substance with O(H)- and Le^b-decomposing enzyme preparation, it sets free about 28% of the original amount of fucose. The amount of fucose, liberated from Le^a substance by the Le^a enzyme preparation equals to the sum of fucoses liberated by this enzyme preparation from O(H)+, Le(a±b+) substance and from the substance obtained by the treatment of Le^a enzyme-treated O(H) substance with O(H) and Le^b enzyme preparation. It is considered that the above fucosidase, different from that contained in the O(H)- and Le^b-decomposing enzyme preparation derived from *Bac. fulminans* or *Bac. cereus* O(H), liberates only the fucose which is the determinant of Le^a specificity, and that it is the similar one to the fucosidase in the Le^a-decomposing enzyme preparation from *Trichomonas foetus*, which was reported by Watkins⁷⁾ to split α-L-fucosyl unit with 1→4 linkage.

The O(H)- and Le^b-decomposing enzyme preparation from *Bac. fulminans*, acting on O(H) substance, liberates about 32% of the total fucose, which is the determinant of the O(H) and Le^b specificities.

The Le^a-decomposing enzyme preparation from *Bac. cereus* Le^a contains also a kind of β-galactosidase besides fucosidase. Since, however, the Le^a activity of the Le^a and O(H) substances does not correspond with the amount of galactose liberated from these substances by the action of the enzyme preparation, this β-galactosidase is hardly considered to liberate only the galactose related to the determinant of the Le^a specificity, but assumed to split also the other β-galactosyl unit.

Conclusion. Le^a enzyme preparation from *Bac. cereus* Le^a contains a kind of fucosidase, which is different from that of O(H) and Le^b enzyme preparation from *Bac. fulminans* or *Bac. cereus* O(H), and in addition a kind of β-galactosidase. When the enzyme preparation is incubated with Le^a substance, about 34% of the total fucose and about 32% of the total galactose are liberated as simple sugars.

When the enzyme preparation is incubated with O(H)+, Le(a±b+) substance, about 3% of the total fucose and about 19% of the total galactose are liberated as simple sugars, accompanied by disappearance of slight Le^a activity in this substance. When this Le^a enzyme-treated O(H) substance is further incubated with O(H)- and Le^b-decomposing enzyme preparation, O(H) and Le^b activities disappear, while Le^a activity reappears. When this twice enzyme-treated substance is further subjected to the action of the Le^a enzyme preparation, about 28% of the original amount of fucose and about 8%

of the original amount of galactose are liberated as simple sugars.

References

- 1) Annison, E. F., and Morgan, W. T. J.: Studies in immunochemistry. 10. The isolation and properties of Lewis (Le^a) human blood-group substance. *Biochem. J.*, **50**(4), 460-471 (1952).
- 2) Watkins, W. M.: Enzymes of *Trichomonas foetus*. The action of cell-free extracts on blood-group substances and low-molecular-weight glycosides. *Biochem. J.*, **71**(2), 261-274 (1959).
- 3) Watkins, W. M., and Morgan, W. T. J.: Specific inhibition studies relating to the Lewis blood-group system. *Nature*, **180**(4594), 1038-1040 (1957).
- 4) —: Further observations on the inhibition of blood-group specific serological reactions by simple sugars of known structure. *Vox Sang.*, **7**(2), 129-150 (1962).
- 5) —: Possible genetical pathways for the biosynthesis of blood group mucopolysaccharides. *Vox Sang.*, **4**(2), 97-119 (1959).
- 6) Watkins, W. M.: Changes in blood group specificity induced by enzymes. *Bull. Soc. Chim. Biol.*, **42**(12), 1599-1605 (1960).
- 7) —: Changes in the specificity of blood-group mucopolysaccharides induced by enzymes from *Trichomonas foetus*. *Immunol.*, **5**(2), 245-266 (1962).
- 8) Painter, T. J., Rege, V. P., and Morgan, W. T. J.: A comparison of the disaccharides obtained from human blood-group A, B, H and Le^a substances by partial acid hydrolysis. *Nature*, **199**(4893), 569-570 (1963).
- 9) Rege V. P., Painter, T. J., Watkins, W. M., and Morgan, W. T. J.: Three new trisaccharides obtained from human blood-group A, B, H and Le^a substances: Possible sugar sequences in the carbohydrate chains. *Nature*, **200**(4906), 532-534 (1963).
- 10) Furukawa, K., Fujisawa, K., and Iseki, S.: On a Le^a substance-decomposing enzyme. I. Serological action of the enzyme on blood group substances. *Proc. Japan Acad.*, **39**(5), 325-329 (1963).
- 11) —: Changes in Lewis blood group specificity induced by enzymes. *Proc. Japan Acad.*, **39**(7), 540-545 (1963).