Inhibition of Rat Small-Intestinal α -(1 $\rightarrow$ 2)-Fucosyltransferase

By AGUSTIN BELLA, JUN., and YOUNG S. KIM

Gastrointestinal Research Laboratory, Veterans Administration Hospital, San Francisco, Calif. 94121,
 and Department of Medicine, University of California, San Francisco, Calif. 94122, U.S.A.

(Received 12 October 1971)

In a recent paper (Bella & Kim, 1971) we reported the isolation of an α -(1 $\rightarrow$ 2)-fucosyltransferase from the mucosa of the rat small intestine. The activity observed with this enzyme is represented by the reaction:

where R represents the inner carbohydrate portion of the acceptor molecule. The enzyme is capable of transferring fucose to acceptors having a terminal β -galactosyl residue. Glycoproteins and oligosaccharides having this terminal structure are good acceptors for the enzyme.

The product of the reaction, GDP, was found by kinetic determination to be a competitive inhibitor

Table 1. Purine and pyrimidine nucleotides tested for the inhibition of α -(1 $\rightarrow$ 2)-fucosyltransferase

Details of the assay procedure are given in the text. The concentration of each compound in the reaction mixture is 0.89 mM. The substituents R and R' refer to the basic purine nucleotide structure:

The enzyme activity is expressed as activity relative to that of an incubation mixture containing no added nucleotide.

Compound	R	R'	Phosphate	Relative activity
1. Guanine	OH	NH ₂	None	102
2. Guanosine	OH	NH ₂	None	82
3. Guanosine + P _i	OH	NH ₂	P _i	82
4. 2':3'-Cyclic GMP	OH	NH ₂	2':3'-Mono	74
5. dGMP	OH	NH ₂	5'-Di	3
6. GMP	OH	NH ₂	5'-Mono	5
7. GDP	OH	NH ₂	5'-Di	1
8. GTP	OH	NH ₂	5'-Tri	4
9. IMP	OH	H	5'-Mono	69
10. IDP	OH	H	5'-Di	26
11. XMP	OH	OH	5'-Mono	45
12. XDP	OH	OH	5'-Di	33
13. AMP	NH ₂	H	5'-Mono	112
14. ADP	NH ₂	H	5'-Di	110
15. ATP	NH ₂	H	5'-Tri	120
16. CDP	*	*	5'-Di	110
17. CTP	*	*	5'-Tri	112
18. UDP	*	*	5'-Di	115
19. UTP	*	*	5'-Tri	120

* Not applicable.

of the enzyme (Bella & Kim, 1971). Similar inhibition exhibited by GMP, GTP and GDP-mannose suggests that the guanosine moiety might be involved in the attachment of the substrate to the enzyme.

The present study concerns the structural requirement for inhibition of the enzyme. Analogues and derivatives of GDP were studied for their ability to inhibit the fucosyltransferase reaction.

The GDP-[^{14}C]fucose ($193\mu\text{Ci}/\mu\text{mol}$) was obtained from Amersham/Searle, Arlington Heights, Ill., U.S.A.) and its purity checked before use (Bella & Kim, 1971). The following companies are the sources of the biochemicals listed in Table 1: Calbiochem, San Diego, Calif., U.S.A., nos. 1-8, 16 and 17; P-L Biochemicals Inc., Milwaukee, Wis., U.S.A., nos. 9-12; Sigma Chemical Co., St Louis, Mo., U.S.A., nos. 13-15.

Pig submaxillary-gland mucin treated to remove sialic acid and fucose as previously described (Bella & Kim, 1971) was used as the acceptor for the enzyme. In the standard assay the enzyme preparation (0.2mg of protein) was incubated at 20°C for 5min with the following components: GDP-[^{14}C]fucose ($1.04\mu\text{M}$), cacodylate-acetate buffer, pH 6.0 (0.2mM), magnesium acetate ($40\mu\text{M}$), Triton X-100 (0.1%), pig submaxillary-gland mucin (0.5mg) and the inhibitor to be tested. The reaction was terminated by addition of 3ml of cold 1.0% (w/v) phosphotungstic acid in 0.5M-hydrochloric acid. Complete precipitation was obtained after standing the tubes in ice-cold water for 30min. The precipitate was centrifuged at $2500g$ for 5min and the pellet washed three times with the phosphotungstic acid solution. Radioactivity was measured in a Packard Tri-Carb liquid-scintillation counter with a toluene phosphor after solubilization of the pellet with 0.5ml of NCS solubilizer (Packard Instrument Co., Downers Grove, Ill., U.S.A.).

Results listed in Table 1 show the relative inhibition by analogues of guanosine and by

pyrimidine compounds. The most effective inhibitor of the enzyme is GDP. GMP and GTP are also very effective. The presence of a phosphate residue on C-5' of guanosine seems to be a requirement for good inhibition, since guanosine, guanosine+ P_i and 2':3'-cyclic GMP are only slightly inhibitory. The integrity of the ribose moiety apparently is not a prerequisite for inhibition, since dGDP was a potent inhibitor.

Modification of the purine ring by removal of an amino group from C-2 or substitution with a hydroxyl group, as in the inosine phosphates and the xanthosine phosphates respectively, diminishes the inhibition to a significant degree. Substitution of the hydroxyl group on C-6 with an amino group completely abolished the inhibiting ability of the purine nucleoside phosphates. It appears that the presence of a hydroxyl group on C-6 of the purine is an important requirement for inhibition. The pyrimidine nucleotides tested did not inhibit the enzyme.

The inhibition of a fucosyltransferase by GTP is unusual, since Grollman, Hall & Ginsburg (1965), Grollman & Marcus (1966) and Jabbal & Schachter (1971) reported fucosyltransferase preparations that were stimulated by the presence of this nucleotide.

The present results show that the most effective inhibitor of the enzyme requires a purine nucleotide with a hydroxyl group on C-6, an amino group on C-2 and a covalently linked phosphate on C-5'.

This study was supported in part by VA-RE-TR Grant and VA Grant from the Veterans Administration.

- Bella, A. B., jun. & Kim, Y. S. (1971). *Archs Biochem. Biophys.* **147** (in the Press).
Grollman, A. P., Hall, C. W. & Ginsburg, V. (1965). *J. biol. Chem.* **240**, 975.
Grollman, A. P. & Marcus, D. M. (1966). *Biochem. biophys. Res. Commun.* **25**, 542.
Jabbal, I. & Schachter, H. (1971). *J. biol. Chem.* **246**, 5154.