Specificity, Inhibition, and Synthetic Utility of a Recombinant Human α-1,3-Fucosyltransferase

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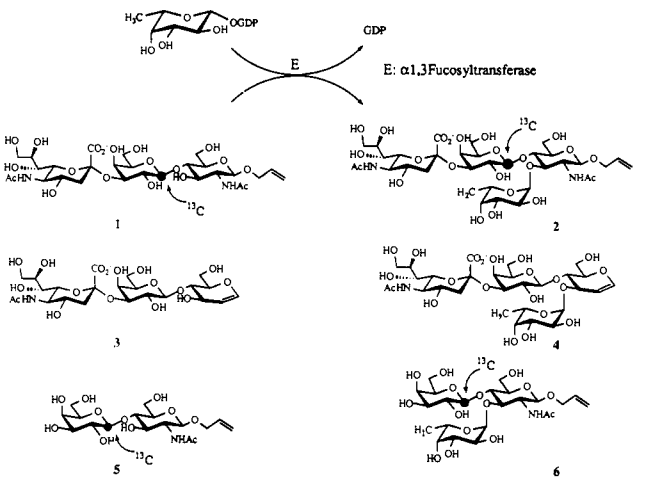
Received May 1, 1992

Many biologically important oligosaccharides are fucosylated[1] at the late stage[2] with the enzyme fucosyltransferase (FucT).[3] Of particular interest is the FucT that catalyzes the transfer of L-fucose from GDP-Fuc to the 3-OH of GlcNAc.[4] At least seven different α1,3FucT in mammalian sources have been reported.[5] The human α1,3FucT responsible for the production of the sialyl Le^x oligosaccharide has recently been cloned and overexpressed.[6] Sialyl Le^x is a ligand of ELAM-1 (endothelial leucocyte adhesion molecule 1) and is involved in inflammatory processes and tumor developments.[7] Sialyl Le^x and derivatives, or inhibitors of α1,3FucT, are therefore potentially useful as antiinflammatory or antitumor agents.

The cloned α1,3FucT showed relatively broad acceptor specificity (Table I).[8] The enzyme accepts Galβ1,4GlcNAc and preferentially NeuAcα2,3Galβ1,4GlcNAc as substrates. It also accepts Galβ1,3GlcNAc and lactose at relatively high concentrations. The regioisomer NeuAcα2,6Galβ1,4GlcNAc and the glycal-containing disaccharide Galβ1,4glucal (lactal) are poor substrates; however, sialylated lactal **3** is a relatively good substrate.

Several compounds were examined as inhibitors of FucT. Lacking the critical 3-OH of GlcNAc, compound GalB1,4(3-deoxy)GlcNAc is, as expected, a weak inhibitor, consistent with previous studies on deoxygenated oligosaccharides for glycosyltransferases.[9] Although Galβ1,4(5-thioGlc) is a good substrate, the pseudodisaccharide Galβ-1,4-deoxynojirimycin is an inhibitor.

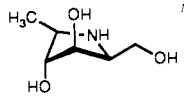
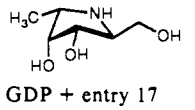
Scheme I



The nucleoside diphosphate GDP, a byproduct of the enzymatic fucosylation reaction, is also an inhibitor, indicating the problem of product inhibition and the need for the in situ regeneration of GDP-Fuc in the enzymatic synthesis on large scales. Another interesting observation is the synergistic inhibition of FucT with GDP and the aza sugars (Table I), indicating that GDP and the aza sugar may form a complex in the active site to mimic the transition state of the fucosyl-transfer reaction.

Several fucosylated oligosaccharides were then prepared on 15-30-mg scales using the cloned α1,3FucT (Scheme I). A typical experimental procedure was as follows: A solution of **1**[10] (23 mg, 31 μmol) and GDP-Fuc[11] (70 mg, 105 μmol) in HEPES buffer (1 mL, 200 mM, pH 7.4) containing Mn^{2+} (20 mM) and the α1,3FucT solution (1 mL, 0.01 U) was stirred gently under an argon atmosphere at room temperature for 5 days. The reaction progress was monitored by TLC (1 M NH_4OAc/*i*PrOH, 1:2.4). The mixture was concentrated and chromatographed on a silica gel column with EtOAc/*i*PrOH/water (2:2:1). The tetrasaccharide-containing fractions were concentrated and passed through a BioGel P-2 column and then a Dowex 50W-X8 [H^+] column, both eluted with water. Neutralization with 1 N NaOH and lyophilization gave the sialyl Le^x **2** (18 mg).[12] A similar procedure was applied to the synthesis of ^{13}C-labeled **2**, the sialyl glycal **4**, and Le^x (**6**).[12] The ^{13}C-labeled saccharides are useful

Table I. Substrates or Inhibitors for α-1,3-Fucosyltransferase

entry	substrates	K_m (mM)	V_{rel}[a]	entry	inhibitors	IC_{50}[b] (mM)
1	Galβ1,4GlcNAc	35	100	11	Galβ1,4(3-deoxy)GlcNAcβOallyl[c]	710
2	Galβ1,4Glc	500	150	12	Galβ1,4deoxynojirimycin[c]	8
3	Galβ1,4(5-thioGlc)[c]	12	51	13	Galβ1,3GalNAc	>100
4	Galβ1,4GlcNAcβOallyl[c]	16	64	14	GlcNAcβ1,4GlcNAc	NI[h]
5	Galβ1,3GlcNAc	600	130	15	GDP	0.05[i]
6	Galβ1,4Glucal[c,d]	34	10	16	GDP-Man	2
7	NeuAcα2,3Galβ1,4GlcNAc[e]	100	620	17		34
8	NeuAcα2,3Galβ1,4GlcNAcβOallyl[f]	280	380	18		80
9.	NeuAcα2,3Galβ1,4Glucal[f]	64	330	19	GDP + entry 17	*l*
10	NeuAcα2,6Galβ1,4GlcNAc[g]	70	13			

[a] Relative maximal velocities with 0.1 mM GDP-Fuc and 10 mM $MnCl_2$. [b] Inhibitor concentration required to give 50% inhibition with 0.1 mM GDP-Fuc. [c] Gautheron-Le Narvor, C.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 1130. [d] Haworth, W. N.; Hirst, E. L.; Plant, M. M. T.; Reinolds, R. J. W. *J. Chem. Soc.* **1930**, 2644. [e] Purchased from Oxford GlycoSystems, Inc., Rosedale, NY. [f] Prepared enzymatically using an α-2,3-sialyltransferase from Cytel Co., San Diego, CA. [g] Ichikawa, Y.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 4698. [h] No inhibition observed up to 50 mM concentration. [i] K_{ii} = 0.13 mM and K_{is} = 0.16 mM. [j] Kajimoto, T.; Chen, L.; Liu, K. K-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6679. [k] Prepared via fuculose-1-P aldolase reaction with (*S*)-2-azidopropanal followed by reductive amination with Pd-C. (Wang, Y. F.; Dumas, D. P.; Wong, C.-H., submitted for publication.) [l] A profound synergistic inhibition was observed at 0.05 mM GDP and 34 mM aza sugar; ~90% of the enzyme activity was inhibited in the presence of 1 mM each of ^{14}C-GDP-Fuc and LacNAc.

0002-7863/92/1514-7321$03.00/0

for conformational study,[13] and the glycal **4** could be converted to a number of sialyl Le^x derivatives on the basis of chemistry developed by Danishefsky and others.[14]

In summary, the recombinant α1,3FucT, like α(1,3/1,4)FucT,[3f] accepts a number of galactosides and sialosides as substrates and is useful for the synthesis of sialyl Le^x and related compounds. Coupled with in situ regeneration of UDP-Gal, CMP-NeuAc, and GDP-Fuc,[15] it is now possible to carry out large-scale enzymatic syntheses of sialyl Le^x and analogs. Work is in progress to investigate the synergistic inhibition of FucT with GDP and aza sugars.

Acknowledgment. Work at Scripps was supported by the NIH (GM44154) and Cytel and that at Michigan was supported by the NIH (DK38482). We thank Dr. J. C. Paulson at Cytel for helpful discussion and the kind gifts of α,1,3FucT and α2,3NeuT.

Supplementary Material Available: A listing of ^{1}H NMR spectral data for compounds **2**, **4**, and **6** (2 pages). Ordering information is given on any current masthead page.

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(8) The assay conditions were essentially the same as described previously.[3f] The specific activity of the enzyme was 2.6 U/mg (1 U = 1 μmol of GDP-Fuc consumed per hour).
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(10) Compounds **1** and **3** were prepared from allyl βLacNAc and lactal, respectively, using a cloned Galβ1,3/4GlcNAc α-2,3-sialyltransferase (Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, S.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.*, submitted for publication). The glycals **3** and **4** were first prepared chemically by Danishefsky et al. (Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koeski, K.; Oriyama, T.; Griffith, D. A.; Wong, C.-H.; Dumas, D. P. *J. Am. Chem. Soc.*, in press).
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(12) Compounds **2**, 4, and **6** were characterized with ^{1}H NMR spectroscopy (see supplementary material)
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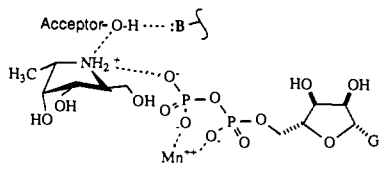

Model Studies on the Radical Induced DNA Strand Cleavage

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Received February 5, 1992

Diynene antitumor antibiotics like calicheamicin[1] or esperamicin[2] are radical generators that induce the cleavage of DNA **1** via hydrogen atom abstraction.[3] An important intermediate in this DNA strand scission is the deoxyribosyl radical **2** with the radical center at the 4'-position. This deoxyribosyl radical either reacts with oxygen[3] or decomposes directly.[4] Under anaerobic conditions ketoaldehydes **3a,b** are formed as the major products. To attain a deeper insight into the mechanism of this radical induced DNA strand cleavage under anaerobic conditions we selectively generated radicals **5a,b** by addition of benzenethiyl radicals to the dinucleotide derivatives **4a,b**.[5] Dinucleotide **4a** is cleaved quantitatively into fragments **6a** and **7a**.[6] Hydrolysis of **6a** exclusively yields ketoaldehyde **3c**.[7] It is therefore reasonable to assume that the anaerobic cleavage of DNA via deoxyribosyl radical **2** could initially lead to an enol ether of structure **6** which hydrolyzes to ketoaldehyde **3**. The rate of solvolysis depends upon the base. Thus radical addition to thymidine dimer **4b** in methanol/water (10:1) at 30 °C gives within 20 min directly the ketoaldehyde **3c** (45%) and the thymidine derivative **7b** (85%). Presumably, intermediate **6b** is hydrolyzed so rapidly that it is not built up during the reaction.

Kinetic experiments revealed that the fragmentation rate of **5a** is larger than 10^8 s^{-1} (30 °C). Using an excess of benzenethiol the mononucleotide derivative **8** yielded mainly fragment **6a** and a small amount of the addition product **10**. Under pseudo-first-order conditions a rate ratio $k_{6a}/k_{10} = 6.4$ was measured.[8] Thus, the rate coefficient of the fragmentation **9** → **6a** is larger than that of the hydrogen abstraction **9** → **10**. This is a remarkable result as benzenethiol is one of the most effective hydrogen donors, reacting with alkyl radicals with rate coefficients of about 10^8 M^{-1} s^{-1} (25 °C).[9]

The analogous benzoate **11** yielded only addition product **13**, the fragmentation product **6a** was not observed. This means that

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0002-7863/92/1514-7322$03.00/0