Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 571–573

Inhibition kinetics of carba- and *C*-fucosyl analogues of GDP-fucose against fucosyltransferase V: implication for the reaction mechanism

Andrew J. Norris,[a,b] Julian P. Whitelegge,[c] M. Jane Strouse,[b] Kym F. Faull[c] and Tatsushi Toyokuni[a,*]

[a]*Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at University of California, Los Angeles, CA 90095, USA*
[b]*Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90096, USA*
[c]*Pasarow Mass Spectrometry Laboratory, Department of Psychiatry and Biobehavioral Sciences and the Neuropsychiatric Institute, University of California, Los Angeles, CA 90024, USA*

Received 22 October 2003; revised 22 November 2003; accepted 2 December 2003

This paper is dedicated to Professor Seiichiro Ogawa on the occasion of his retirement from Keio University, Japan

Abstract—Inhibition kinetics of two isosteric analogues of GDP-fucose (GDP-Fuc) were investigated against fucosyltransferase V using electrospray ionization mass spectrometry coupled to multiple reaction monitoring. The carba-Fuc analogue was found to be a competitive inhibitor with a K_i value of 67.1 ± 9.8 μM, similar to the K_m value for GDP-Fuc (50.4 ± 5.5 μM), while the *C*-Fuc analogue exhibited significantly weak competitive inhibition with a K_i value of 889 ± 93 μM.

Fucosyltransferases (Fuc-Ts) transfer fucose (Fuc) from GDP-Fuc to oligosaccharide acceptors with inversion of the anomeric configuration (i.e., β→α), completing the biosynthesis of fucosylated oligosaccharides. Since the fucosylated oligosaccharides play pivotal roles in cell–cell recognition phenomena, Fuc-T's have been the subject of considerable interest in glycobiology.[1,2] Among the known Fuc-T's, Fuc-T V is the most thoroughly characterized enzyme and responsible for the biosynthesis of the Lewis×determinant [Galβ1-4(Fucα1-3)GlcNAcβ1-R].[3] The Fuc-T V reaction is believed to follow an ordered sequential Bi–Bi kinetic mechanism with GDP-Fuc binding first and the product GDP being released last.[4] Based on isotopic studies it is proposed that the transition state of the Fuc-T V reaction involves considerable oxocarbenium ion character of the Fuc moiety.[4]

To date a number of GDP-Fuc analogues that resemble the Fuc moiety in either the ground state or the putative transition state have been synthesized as potential inhibitors of Fuc-Ts.[5,6] The GDP-Fuc analogues, comprised of carba-Fuc and *C*-Fuc (i.e., **1** and **2**, respectively), are of particular interest due to their isosteric nature to GDP-Fuc and their inherent stability towards enzymatic cleavage[7–10] (Fig. 1). Thus, both **1** and **2** can be a valuable tool for understanding the molecular interaction between GDP-Fuc and Fuc-Ts. However, to the best of our knowledge, little biological data have been reported and hence their biological significance remains uncertain.[5,11] Accordingly, we have undertaken the kinetic characterization of **1** and **2** against commercially available Fuc-T V using a mass spectrometry-based assay method, which we developed recently.[12,13]

The isosteric analogues **1** and **2** were synthesized as previously described,[7,8] purified by reversed-phase HPLC[14] and used as their disodium salts. Their ^{1}H NMR spectra confirmed the conformational similarity of the carba- and *C*-Fuc moieties to the Fuc moiety of GDP-Fuc (i.e., $^1C_4$ conformation).[15] Detailed kinetic studies of **1** and **2** were carried out using electrospray ionization

* Corresponding author at present address: LA Tech Center, Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, 6140 Bristol Parkway, Culver City, CA 90230, USA. Tel.: +1-31-670-8695; fax: +1-31-670-8428; e-mail: ttoyokuni@mednet.ucla.edu

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.12.003

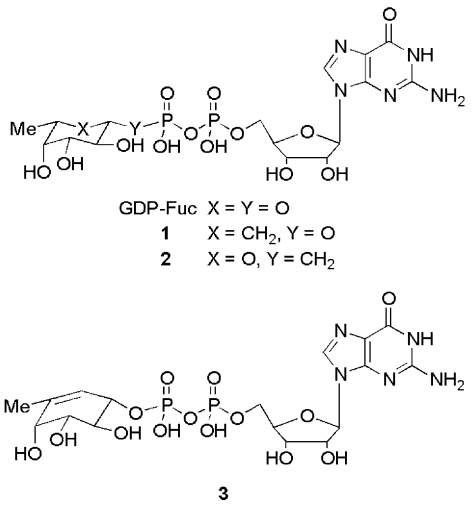


Figure 1. Structure of GDP-Fuc and its carba- and *C*-Fuc analogues (**1** and **2**, respectively) as well as the unsaturated carba-Fuc analogue **3**.

mass spectrometry coupled to multiple reaction monitoring (ESI-MS/MRM) as reported previously.[12,13] ESI-MS/MRM serves as a rapid and highly accurate method for the kinetic characterization of enzymes and inhibitors. The enzyme reactions were performed using Fuc-T V (0.085 milliunit) and 20 mM *N*-acetyllactosamine in 20 mM Bis-Tris (pH 6.8) containing 10 mM $MnCl_2$ and 2 mM dithiothreitol at 37 °C.[16] After termination of the reactions by addition of MeOH containing 2′-deoxyguanosine 5′-diphosphate (dGDP) as an internal standard, each sample was diluted with MeCN–H_2O–Et_3N (35/65/0.2, v/v/v) and analyzed by ESI-MS in negative ion mode. MRM analysis was performed to monitor the temporal progress of the reactions. Thus, the reaction product transition *m*/*z* 442 [(GDP–H)$^-$]→*m*/*z* 159 [(P_2O_6H)$^-$] was monitored with reference to the internal standard transition *m*/*z* 426 [(dGDP–H)$^-$]→*m*/*z* 159 [(P_2O_6H)$^-$]. The standard calibration curve was used to obtain a quantitative value of the enzyme velocity expressed in units of concentration of GDP per min. In addition, the GDP-Fuc transition *m*/*z* 588 [(GDP-Fuc–H)$^-$]→*m*/*z* 442 [(GDP–H)$^-$] was monitored to insure that less than 10% of the substrate was consumed during the course of the reaction, and the transitions for **1** *m*/*z* 586 [(**1**–H)$^-$]→*m*/*z* 362 [(GMP–H)$^-$] and for **2** *m*/*z* 586 [(**2**–H)$^-$]→*m*/*z* 362 [(GMP–H)$^-$] were used to verify the stability of **1** and **2** during the reaction.

Dixon analysis of **1** and **2** showed a signature plot for competitive inhibition (Fig. 2) with a $K_i = 67.1 \pm 9.8$ μM and 889 ± 93 μM, respectively.[17] The K_i value for **1** is considered moderate and similar in magnitude to the K_m value for GDP-Fuc (50.4 ± 5.5 μM).[12] The results indicate that the ring oxygen of Fuc is not critical for recognition of GDP-Fuc by the enzyme, however, it is essential for transfer to occur. In contrast, the data from **2** represents a greater than one order of magnitude change in affinity caused by the replacement of the glycosidic oxygen with a methylene group. This indicates that the glycosidic oxygen plays an important role in GDP-Fuc binding to the enzyme. A similar magnitude of change in affinity was observed with GDP when the

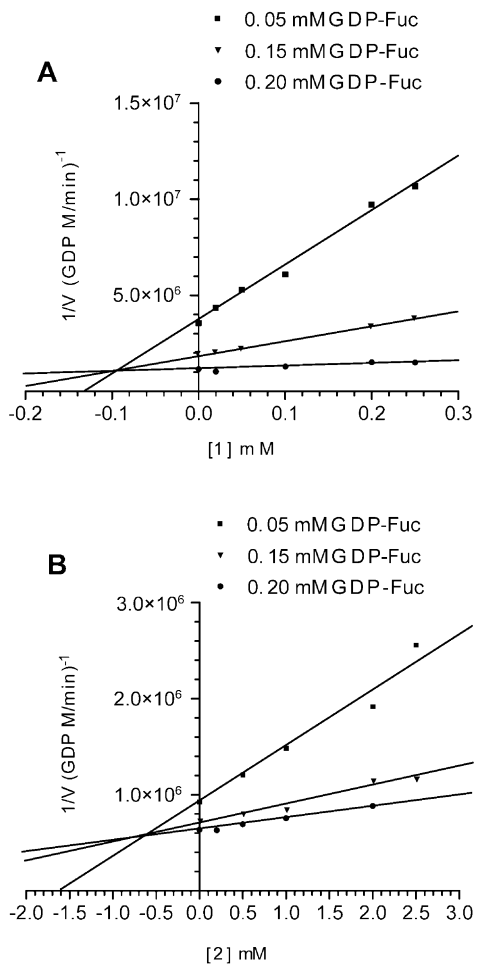


Figure 2. Dixon analysis showing a signature plot for competitive inhibition for **1** (A) and **2** (B) at the indicated concentrations of GDP-Fuc.

β-phosphate oxygen was replaced with a methyl group.[18] Interestingly, divalent metal ions are known to participate in leaving group activation by direct coordination to the departing atom.[19] In the case of galactosyltransferase (Gal-T) reactions, a Mn^{2+} is reported to leave the enzyme as a UDP-Gal·Mn^{2+} complex.[20,21] Since Fuc-T V is reported to be Mn^{2+}-dependent,[4] it is conceivable that the coordination of a metal cofactor (Mn^{2+}) to the glycosidic oxygen might be an important interaction between GDP-Fuc and Fuc-T V. Such a coordination phenomenon is absent in the currently available X-ray crystal structures of glycosyltransferase in complex with a sugar nucleotide donor, where a metal cofactor is coordinating with the negative charges of the pyrophosphate group of the sugar nucleotide.[22] However, this is not surprising since the Mn^{2+}-glycosidic oxygen interaction is most likely to occur when the second substrate (sugar acceptor) is bound, hence promoting activation (transition state).

We previously reported the K_i value for the unsaturated carba-Fuc analogue **3** (25.6 ± 2.8 μM),[13] which, as compared to the K_i value for **1**, indicates that Fuc-T V has a preference towards a half-chair conformation (i.e., **3**) over the stable $^1C_4$ conformation (i.e., **1**). The conformational distortion of the Fuc moiety from the ground state $^1C_4$ to a half-chair (or a boat) conformation

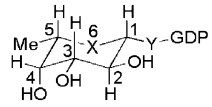
results in a quasi-equatorial (or quasi-axial) orientation for the glycosidic bond. This conformation places the glycosidic bond antiperiplanar to the sp^3 lone pairs on the ring oxygen, which is a stereoelectronically favorable arrangement for aglycon departure.[23,24] Accordingly, in addition to the leaving group activation by a Mn^{2+}, the substrate distortion toward the catalytically favorable conformation within the enzyme active site may possibly be an important factor for lowering the activation energy in the Fuc-T V catalyzed reaction.[25]

Acknowledgements

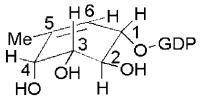
We thank Professors Jon M. Fukuto, Arthur K. Cho and Kendall N. Houk at UCLA for helpful discussion and Professor Jacques Maddaluno at Université de Rouen (France) for comments on this manuscript. The W. M. Keck Foundation provided support toward MS instrument purchase. The NMR data were based upon work supported by the National Science Foundation under equipment grant No. CHE-0116853.

References and notes

1. Becker, D. J.; Lowe, J. B. *Glycobiology* **2003**, *13*, 41 R.
2. deVries, T.; Knegtel, R. M. A.; Holmes, E. H.; Macher, B. A. *Glycobiology* **2001**, *11*, 119 R.
3. Weston, B. W.; Nair, R. P.; Larsen, R. D.; Lowe, J. B. *J. Biol. Chem.* **1992**, *267*, 4152.
4. Murray, B. W.; Takayama, S.; Schultz, J.; Wong, C.-H. *Biochemistry* **1996**, *35*, 11183.
5. Compain, P.; Martin, O. R. *Bioorg. Med. Chem.* **2001**, *9*, 3077.
6. Mitchell, M. L.; Tian, F.; Lee, L. V.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3041.
7. Cai, S.; Stroud, M. R.; Hakomori, S.; Toyokuni, T. *J. Org. Chem.* **1992**, *57*, 6693.
8. (a) Luengo, J. I.; Gleason, J. G. *Tetrahedron Lett.* **1992**, *33*, 6911. (b) Norris, A. J.; Toyokuni, T. *J. Carbohydrate Chem.* **1999**, *18*, 1097.
9. Reviews on carba-sugars: (a) Suami, T.; Ogawa, S. *Adv. Carbohydr. Chem.* **1990**, *48*, 21. (b) Ogawa, S., In *Carbohydrate Mimics, Concepts and Methods*; Chapleur, Y., Ed.: Wiley-VCH: Weinheim, 1998, p 87.
10. A review on *C*-glycosides: Postema, M. H. D. *C-Glycoside Synthesis*; CRC: Boca Raton, 1995.
11. The carba-sugar and *C*-glycoside analogues of other sugar nucleotides have been synthesized as potential inhibitors of the corresponding glycosyltransferases. However, very few biological data have been reported, except for the carba-Gal analogue of UDP-Gal. See: Yuasa, H.; Palcic, M. M.; Hindsgaul, O. *Can. J. Chem.* **1995**, *73*, 2190.
12. Norris, A. J.; Whitelegge, J. P.; Faull, K. F.; Toyokuni, T. *Biochemistry* **2001**, *40*, 3774.
13. Norris, A. J.; Whitelegge, J. P.; Faull, K. F.; Toyokuni, T. *Anal. Chem.* **2001**, *73*, 6024.
14. Analytical HPLC was carried out using a C-18 column (Phenomenex Aqua, 5 μ, 250×10 mm) with a linear gradient elution of 0–5% MeCN in 0.05M $NH_4{\cdot}HCO_3$ over 13 min at 3 mL/min. The effluent was monitored by absorbance at 254 nm. The GDP-Fuc analogues **1**, **2** and **3** eluted at 12.5 min, 11.0 min and 13.0 min, respectively.
15. The ^1H NMR spectra (D_2O) of **1**, **2**, **3** and GDP-Fuc were obtained on a Bruker Avance 600 spectrometer. The ^1H spectrum of **3** was obtained with ^{31}P decoupling. The vicinal coupling constants of ring protons are in concordance with those for a chair conformation (for GDP-Fuc, **1** and **2**) and for a distorted half-chair-like conformation (for **3**)



GDP-Fuc X = Y = O
1 X = CH_2, Y = O
2 X = O, Y = CH_2



3

	$J_{1,2}$ Hz	$J_{2,3}$ Hz	$J_{3,4}$ Hz	$J_{4,5}$ Hz	$J_{5,6ax}$ Hz	$J_{5,6eq}$ Hz	$J_{1,6}$ Hz
GDP-Fuc	8.3	9.4	3.5	<1.0	-	-	-
1	8.8	9.3	3.0	<1.0	13.0	4.1	-
2	8.9	9.5	3.1	<1.0	-	-	-
3	7.5	11.0	4.2	-	-	-	2.3

16. See refs 11 and 12 for the experimental detail.
17. The K_i values were determined by non-linear regression of the data fit to the equation for competitive inhibition: $v = V_{max}[\text{GDP-Fuc}]/\{K_m(1 + [\text{Inhibitor}]/K_i) + [\text{GDP-Fuc}]\}$. See: Motulsky, H. J.; Ransnas, L. A. *FASEB J.* **1987**, *1*, 365.
18. Schuster, M.; Blechert, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1809.
19. Horton, N. C.; Perona, J. J. *Nat. Struct. Biol.* **2001**, *8*, 290.
20. Tsopanakis, A. D.; Herries, D. G. *Eur. J. Biochem.* **1978**, *83*, 179.
21. (a) Bovine β-1,4-galactosyltransferase (Gal-T1) is believed to possess two closely spaced (18 Å) metal binding sites. See: O'Keeffe, E. T.; Hill, R. L.; Bell, J. E. *Biochemistry* **1980**, *19*, 4954. (b) The crystal structure of Gal-T1 co-crystallized with UDP-Gal and $MnCl_2$ supports these metal binding sites. One metal seems to be required for UDP-Gal binding and catalysis and the other for catalysis in addition to aiding acceptor binding. See: Ramakrishnan, B.; Balaji, P. V.; Qasba, P. K. *J. Mol. Biol.* **2002**, *318*, 491.
22. (a) Lariviére, L.; Gueguen-Chaignon, V.; Moréra, S. *J. Mol. Biol.* **2003**, *330*, 1077. (b) Pedersen, L. C.; Darden, T. A.; Negishi, M. *J. Biol. Chem.* **2002**, *277*, 21869. (c) Ünligil, U. M.; Zhou, S.; Yuwaraj, S.; Sarkar, M.; Schachter, H.; Rini, J. M. *EMBO J.* **2000**, *19*, 5269.
23. Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: New York, 1983.
24. Kirby, A. J. *Acc. Chem. Res.* **1984**, *17*, 305.
25. X-ray crystallographic studies of enzyme-bound substrates of several cellulases have shown that the glycoside ring is distorted into a boat/skew conformation prior to cleavage. See: (a) Davies, G. J.; Mackenzie, L.; Varrot, A.; Dauter, M.; Brzozowski, A. M.; Schülein, M.; Withers, S. G. *Biochemistry* **1998**, *37*, 11707. (b) Sulzenbacher, G.; Driguez, H.; Henrissat, B.; Schülein, M.; Davies, G. J. *Biochemistry* **1996**, *35*, 15280.