Substrate flexibility of a 2,6-dideoxyglycosyltransferase†

David L. Jakeman,*[ab] Charles N. Borissow,[a] Cathy L. Graham,[a] Shannon C. Timmons,[b] Taryn R. Reid[a] and Ray T. Syvitski[a]

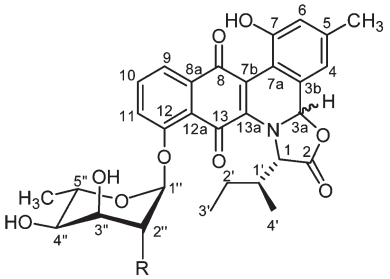
Received (in Cambridge, MA, USA) 21st June 2006, Accepted 18th July 2006
First published as an Advance Article on the web 2nd August 2006
DOI: 10.1039/b608847c

We report the first 2,6-dideoxysugar-*O*-glycosyltransferase with substrate flexibility at the 2 position, confirm the function of a putative NDP-hexose 2,3-dehydratase in the jadomycin B biosynthetic gene cluster and deduce the substrate flexibility of downstream enzymes in L-digitoxose assembly, enabling reprogramming of biosynthetic gene clusters to modify sugar substituents.

Glycosyltransferases play a critical role in the biosynthesis of therapeutically relevant natural products.[1] Glycosylation significantly affects bioactivity, either through altered interactions with the target binding site, or by altering absorption, metabolic, pharmacokinetic or pharmacodynamic properties of the natural product.[2] As a result of the potential to alter these properties and thus develop improved therapeutic agents, there is intense interest in the mechanistic, structural and functional roles of glycosyltransferases.[3–5] Central to this endeavor is the inherent substrate specificity of glycosyltransferases responsible for natural product biosynthesis, and development of methods to examine and broaden their substrate specificity. Preliminary reports of glycosyltransferase substrate specificity led to a hypothesis that, in general, the enzyme class maintained stringent donor and acceptor requirements[6,7] unlike their hydrolytic counterparts the glycosidases.[8,9] Recently, the substrate specificity of glycosyltransferases involved in natural product biosynthesis has been examined through *in vitro*[10–13] and *in vivo*[14–16] assays, and significant flexibility in both the donor and acceptor substrates for several enzymes has been observed. Glycosyltransferases are presently grouped into over 80 different families based on their sequence homology, which dictates their three dimensional fold and the stereochemical outcome of catalysis at the anomeric centre.[17] The family 1 glycosyltransferases involved in natural products biosynthesis frequently attach deoxysugars. One of the factors affecting the stability of a glycosidic linkage is the presence or absence of hydroxyl functionalities on the sugar.[18] The presence of a hydroxyl functionality at position 2 contributes more significantly to the stability of the anomeric glycosidic linkage than any other hydroxyl functionality on the sugar, thus, the stability of deoxysugar-containing natural products may be improved by placing a hydroxyl substituent at position 2.

[a]*College of Pharmacy, Dalhousie University, 5968 College St., Halifax, Nova Scotia, Canada B3H 3J5. E-mail: david.jakeman@dal.ca; Fax: 01 902 494 1396; Tel: 01 902 494 7159*
[b]*Department of Chemistry, Dalhousie University, 6274 Coberg Rd., Halifax, Nova Scotia, Canada B3H 4J3*
† Electronic supplementary information (ESI) available: characterization data (NMR/ESI/MS/MS) for ILEVS1080. See DOI: 10.1039/b608847c

In this communication we report the flexible substrate specificity of enzymes in the dideoxysugar biosynthetic pathway culminating with JadS, a family 1 2,6-dideoxy-α-L-ribohexopyranosyltransferase from *Streptomyces venezuelae* ISP5230 that is responsible for glycosylating jadomycin precursors.[19] The jadomycins are a unique family of bioactive secondary metabolites with a distinguishing oxazolone ring formed through non-enzymatic condensation of a polyketide-derived intermediate with amino acids in the defined growth media.[20,21] Evidence indicates that attachment of L-digitoxose proceeds after formation of the oxazolone ring,[22] although this has yet to be unambiguously determined.



Jadomycin B, R = H
ILEVS1080, R = OH

The *jad* genes responsible for assembly of the dideoxysugar in jadomycin B have been sequenced previously, and *jad*-disrupted strains were shown, in a preliminary analysis by HPLC, to produce only the jadomycin B aglycone.[19] However, analysis of a homologous glycosyltransferase, UrdGT2, involved in the synthesis of urdamycin A, demonstrated a remarkable donor sugar flexibility by transferring not only the natural activated substrate D-olivose but activated L- and D- rhodinoses.[16] This implies that interactions between C4, C5 and C6 of the carbohydrate and UrdGT2 are not critical for catalytic activity.[16] In light of the flexible substrate specificity of UrdGT2, we investigated the transconjugant strain VS1080 in which the wild-type *jadO* gene of the jadomycin producer had been disrupted with apramycin. Based on sequence homology, JadO has been putatively assigned as a nucleoside diphosphate (NDP)-hexose 2,3-dehydratase. Herein, the isolation and structural elucidation of a novel jadomycin analogue (ILEVS1080) from strain VS1080 grown on L-isoleucine as the sole nitrogen source is reported and confirms the function of JadO. The isolation of this analogue demonstrates a flexible activated donor sugar specificity of the JadS glycosyltransferase. Using MS/MS to analyze a culture extract of VS1080 grown on L-isoleucine we observed, in the enhanced product ion scan mode, fragmentation of a parent ion peak (m/z = 565) to form the jadomycin B aglycone (m/z = 419). The loss of 146 Da

was entirely consistent with loss of a 6-deoxyhexosyl moiety, since all jadomycin B analogues examined previously have shown a loss of 130 Da, consistent with loss of the L-digitoxosyl moiety.[23,24] The jadomycin B aglycone ion subsequently fragmented to the phenanthroviridin (m/z = 305) as expected. The observed loss of a 6-deoxyhexose was consistent with the predicted role of JadO in the dideoxysugar biosynthetic pathway, since inactivation of the dehydratase gene *jadO* should result in an NDP-sugar containing a hydroxyl functionality at C2. From a 3 L fermentation of *S. venezuelae* ISP5230, using our recently improved conditions for the production of jadomycin B,[25,26] we obtained 43 mg of crude ILEVS1080. This was purified by reversed-phase chromatography on C18 media followed by normal-phase chromatography on silica gel to yield 2.6 mg of ILEVS1080. Rohr and co-workers, using wild-type *S. venezuelae* ISP5230, obtained 12 mg L^{-1} of pure jadomycin B.[20] We attribute the lower isolated mass of ILEVS1080 to be a result of the alteration in kinetic parameters of the enzymes downstream of JadO involved in dideoxysugar assembly since they are acting upon a non-natural substrate. NMR analysis of the signals for H-3a revealed that ILEVS1080 exists in two diastereomeric forms (3a*S*/3a*R*) in a ratio of 65/35, consistent with the ratio observed for jadomycin B.[20] The diagnostic signals for the H-2″ methylene protons of either form of jadomycin B were not present (Fig. 1). However, additional signals were observed at 4.20 and 4.77 ppm. Through analysis of COSY and NOESY spectra these signals were identified as H-2″ and 2″-OH, respectively, for both the 3a*R*/3a*S* diastereomers of the jadomycin B analogue. A comparison of the $^3J_{H-H}$ coupling constant data

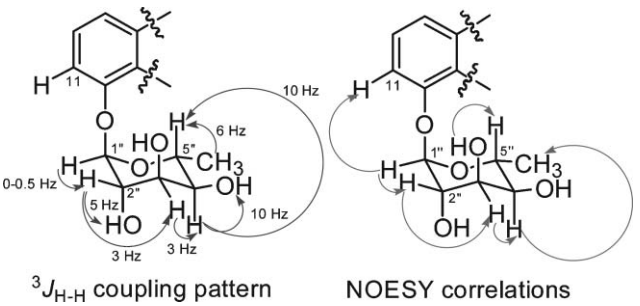


Fig. 2 $^3J_{H-H}$ coupling patterns and NOESY correlations describing a 6-deoxy-α-L-altropyranoside.

(Fig. 2) to that obtained for L-digitoxose confirmed the axial orientation of the 2″-OH substituent. A 0–0.5 Hz coupling was observed between H-1″ and H-2″$_{eq}$ and a 3.0 Hz coupling was observed between H-2″ and H-3″, entirely consistent with the H-1″ → H-2″$_{eq}$ → H-3″ couplings observed for the L-digitoxose moiety in jadomycin B.[20] The remaining ^{1}H NMR signals corresponded well with those observed for jadomycin B. Thus, the NMR data strongly suggest that the deoxyhexose attached to the jadomycin B aglycone is 6-deoxy L-altrose in a $^1C_4$ conformation.

It is also interesting to note that for glycoside hydrolysis by retaining glycosidases the 2 position is critical for transition state stabilization, contributing 18 or 22 kJ mol^{-1} to the glycosylation and deglycosylation of the glycosidase.[27] Clearly, the presence or absence of a hydroxyl substituent at position 2 does not critically influence the transition state stabilization

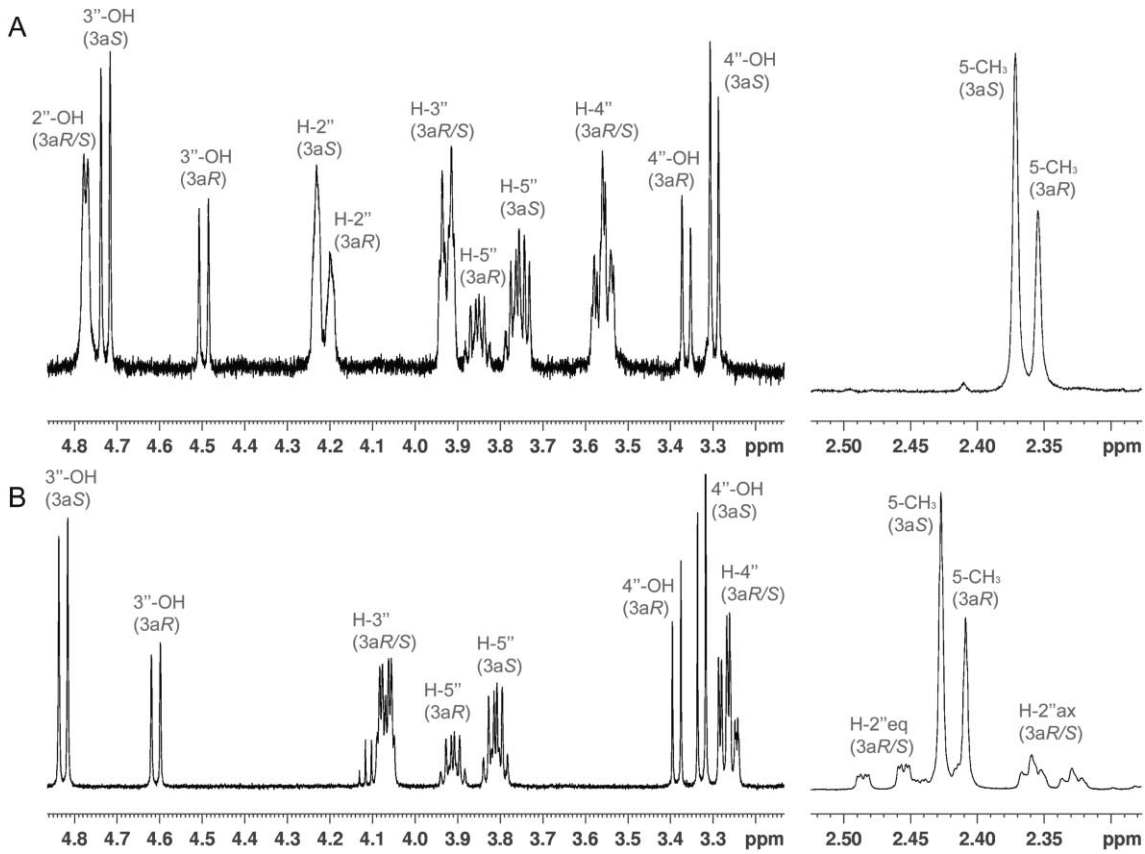


Fig. 1 Regions of the ^{1}H NMR spectra of ILEVS1080 (A) and jadomycin B (B) recorded in acetone-d_6.

required for glycosyl transfer by the family 1 inverting glycosyltransferase JadS.

Structural analysis of ILEVS1080 definitively establishes that gene products downstream of *jadO*, namely *jadP*, *jadU*, *jadV* and *jadS*, based on the proposed biosynthetic pathway for L-digitoxose,[19] are flexible in their substrate specificity, do not have an absolute requirement for a NDP-2,6-dideoxyhexose, and will transform a NDP-6-deoxyhexose. From the publicly available published sequence data on *S. venezuelae* ISP5230 there does not appear to be a cryptic glycosyltransferase outside the jadomycin B biosynthetic gene cluster to account for transfer of the 6-deoxyhexose, which is supported by the knowledge that only one polyketide biosynthetic gene cluster was observed when a *S. venezuelae* ISP5230 genomic library was initially probed by southern hybridization.[28]

In summary, we have demonstrated that the 2,6-dideoxyhexosyltransferase, JadS, will process a NDP-6-deoxysugar as substrate. This is the first report of a 2,6-dideoxysugar-*O*-glycosyltransferase with substrate flexibility at the 2-position. It has significant implications for the study of the numerous family 1 glycosyltransferases involved in the biosynthesis of natural products containing 2-deoxyhexoses, particularly in an *in vitro* environment, where syntheses of activated 2-deoxyhexose-1-phosphates,[29] and their corresponding NDP derivatives, have been synthetically challenging due to their instability.[30] Our *in vitro* studies on the structure, function and mechanism of JadS will be presented in due course.

This work was supported in part by grants from the Canadian Foundation for Innovation, the Canadian Institutes of Health Research, and the Atlantic Chapter of the Canadian Breast Cancer Foundation. RTS would like to acknowledge the Cancer Research Training Program for financial support. TRR would like to acknowledge a Merck Foundation Summer Student Award. We thank Dr Leo C. Vining for helpful discussions and access to strain VS1080.

Notes and references

1 J. S. Thorson, T. J. Hosted, J. Q. Jiang, J. B. Biggins and J. Ahlert, *Curr. Org. Chem.*, 2001, **5**, 139–167.
2 C. Walsh, C. L. F. Meyers and H. C. Losey, *J. Med. Chem.*, 2003, **46**, 3425–3436.
3 D. Bowles, E. K. Lim, B. Poppenberger and F. E. Vaistij, *Annu. Rev. Plant Biol.*, 2006, DOI: 10.1146/annurev.arplant.57.032905.105429.
4 A. Luzhetskyy, A. Vente and A. Bechthold, *Mol. BioSyst.*, 2005, **1**, 117–126.
5 P. G. Hultin, *Curr. Top. Med. Chem.*, 2005, **5**, 1299–1331.
6 D. H. G. Crout and G. Vic, *Curr. Opin. Chem. Biol.*, 1998, **2**, 98–111.
7 M. M. Palcic, *Curr. Opin. Biotechnol.*, 1999, **10**, 616–624.
8 D. L. Zechel and S. G. Withers, *Acc. Chem. Res.*, 2000, **33**, 11–18.
9 D. J. Vocadlo and S. G. Withers, *Glycosidase-catalysed oligosaccharide synthesis*, in: , *Carbohydrates in chemistry and biology*, ed. B. Ernst, G. W. Hart and P. Sinay, Weinheim, Germany, Wiley-VCH GmbH, 2000. pp. 723–844.
10 C. Albermann, A. Soriano, J. Q. Jiang, H. Vollmer, J. B. Biggins, W. A. Barton, J. Lesniak, D. B. Nikolov and J. S. Thorson, *Org. Lett.*, 2003, **5**, 933–936.
11 M. Oberthur, C. Leimkuhler, R. G. Kruger, W. Lu, C. T. Walsh and D. Kahne, *J. Am. Chem. Soc.*, 2005, **127**, 10747–10752.
12 M. Yang, M. Brazier, R. Edwards and B. G. Davis, *ChemBioChem*, 2005, **6**, 346–357.
13 C. L. F. Meyers, M. Oberthur, J. W. Anderson, D. Kahne and C. T. Walsh, *Biochemistry*, 2003, **42**, 4179–4189.
14 A. P. Salas, L. Zhu, C. Sanchez, A. F. Brana, J. Rohr, C. Mendez and J. A. Salas, *Mol. Microbiol.*, 2005, **58**, 17–27.
15 M. Perez, F. Lombo, L. L. Zhu, M. Gibson, A. F. Brana, R. Rohr, J. A. Salas and C. Mendez, *Chem. Commun.*, 2005, 1604–1606.
16 D. Hoffmeister, G. Drager, K. Ichinose, J. Rohr and A. Bechthold, *J. Am. Chem. Soc.*, 2003, **125**, 4678–4679.
17 P. M. Coutinho, E. Deleury, G. J. Davies and B. Henrissat, *J. Mol. Biol.*, 2003, **328**, 307–317.
18 M. N. Namchuk, J. D. McCarter, A. Becalski, T. Andrews and S. G. Withers, *J. Am. Chem. Soc.*, 2000, **122**, 1270–1277.
19 L. Wang, R. L. White and L. C. Vining, *Microbiology*, 2002, **148**, 1091–1103.
20 U. Rix, J. Zheng, L. L. Remsing Rix, L. Greenwell, K. Yang and J. Rohr, *J. Am. Chem. Soc.*, 2004, **126**, 4496–4497.
21 R. T. Syvitski, C. N. Borissow, C. L. Graham and D. L. Jakeman, *Org. Lett.*, 2006, **8**, 697–700.
22 U. Rix, C. C. Wang, Y. H. Chen, F. M. Lipata, L. L. R. Rix, L. M. Greenwell, L. C. Vining, K. Q. Yang and J. Rohr, *ChemBioChem*, 2005, **6**, 838–845.
23 D. L. Jakeman, S. Farrell, W. Young, R. J. Doucet and S. C. Timmons, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1447–1449.
24 D. L. Jakeman, C. L. Graham and T. R. Reid, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5280–5283.
25 D. L. Jakeman, C. L. Graham, W. Young and L. C. Vining, *J. Ind. Microbiol. Biotechnol.*, 2006, DOI: 10.1007/s10295-006-0113-4.
26 C. N. Borissow, C. L. Graham, R. T. Syvitski, T. R. Reid, J. Blay and D. L. Jakeman, *Manuscript in preparation*, 2006.
27 M. N. Namchuk and S. G. Withers, *Biochemistry*, 1995, **34**, 16194–16202.
28 L. Han, K. Yang, E. Ramalingam, R. H. Mosher and L. C. Vining, *Microbiology*, 1994, **140**, 3379–3389.
29 M. D. Percival and S. G. Withers, *Can. J. Chem.*, 1988, **66**, 1970–1972.
30 M. Oberthur, C. Leimkuhler and D. Kahne, *Org. Lett.*, 2004, **6**, 2873–2876.

