Biosynthesis of 3,6-Dideoxyhexoses: New Mechanistic Reflections upon 2,6-Dideoxy, 4,6-Dideoxy, and Amino Sugar Construction[1]

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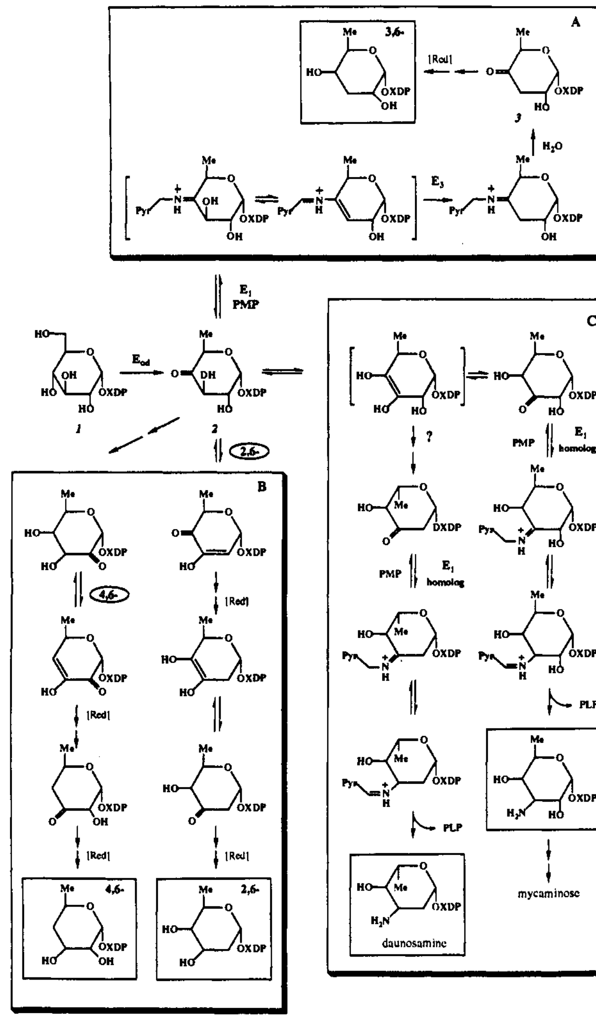
The deoxy sugars are found ubiquitously in nature as constituents of glycoproteins, bacterial cell walls, and numerous secondary metabolites.[2] Particularly notable are the 2,6- and 4,6-dideoxyhexoses found in a broad range of bioactive compounds. Although the biological significance of these dideoxyhexoses has been well recognized, attempts to control and/or mimic their functions have been hampered by the lack of knowledge about the biosynthesis of these unusual sugars.

Currently the only dideoxyhexose pathway being studied at the enzymatic level is that of the formation of 3,6-dideoxyhexoses found in the lipopolysaccharides of *Yersinia*. Their production, exemplified by the biosynthesis of ascarylose (Scheme IA),[3] is initiated by the CDP-D-glucose 4,6-dehydratase (E_{od})-catalyzed conversion of CDP-D-glucose (**1**) to 6-deoxy-D-*glycero*-L-*threo*-4-hexulose (**2**).[4] Subsequent C-3 deoxygenation is accomplished in two consecutive reactions catalyzed by CDP-6-deoxy-L-*threo*-D-*glycero*-4-hexulose-3-dehydrase (E_1), a PMP-dependent iron-sulfur-containing enzyme,[5] and CDP-6-deoxy-$\Delta^{3,4}$-glucoseen reductase (E_3), a [2Fe-2S]-containing flavoprotein.[3b,6] The final steps from **3** to the various 3,6-dideoxyhexoses include a stereospecific C-4 reduction in conjunction with a possible C-5 epimerization. On the basis of the ascarylose precedence, a similar pathway for 2,6-dideoxyhexose formation has been proposed.[7] An analogous mechanism can also be envisioned for 4,6-dideoxyhexose formation.

For 2,6- and 4,6-dideoxyhexose formation to follow the above postulated routes, one crucial prerequisite is the coexistence of E_1 and E_3 equivalents in the host biosynthetic systems. Although isolation of the corresponding enzymes would be desired, due to the uncertainty of the nature of the substrates and thus the complication of their preparation, an approach relying on sequence comparison of the corresponding biosynthetic gene clusters appears to be more propitious at this time. To address this issue and study the mechanism of 3,6-dideoxyhexose formation, we have cloned the entire ascarylose (*asc*) gene cluster from *Yersinia pseudotuberculosis*.[8] Interestingly, searches[9] of the public and

Scheme I



our local data bases utilizing the obtained *asc* query sequences revealed the lack of an E_3 homolog in the 2,6-dideoxyhexose (daunosamine **4**) containing daunorubicin[10] and the 2,6- as well as 4,6-dideoxyhexose (cladinose **5** and desosamine **6**) containing erythromycin.[11] Since E_3, which catalyzed electron transfer from NADH to reduce the E_1 product,[3b] is an integral part of the C-3 deoxygenation in the 3,6-dideoxyhexose pathway, the lack of E_3 homology within these antibiotic gene clusters implies that the postulated mechanisms of 2,6- and 4,6-dideoxyhexose formation can no longer be based on 3,6-dideoxyhexose formation in gram-negative bacteria.[12]

Although an E_3 homolog is absent, significant E_1 (*ascC*) consensus was found with genes in daunorubicin (*dnrJ*),[10b] erythromycin (*eryC1*),[11c] and tylosin (*tylB*)[13] clusters (Figure 1). These E_1 homologs may code for the long-sought dehydrases

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(9) Searches were performed using FASTDB (Brutlag, D. L.; Dautricourt, J.-P.; Maulik, S.; Relph, J. *Comput. Appl. Biosci.* **1990**, *6*, 237), TFASTA, and FASTA (Devereux, J.; Haeberli, P.; Smithies, O. *Nucleic Acids Res.* **1984**, *12*, 387).

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(12) Although the possibility that not all deoxy sugar biosynthetic genes within the erythromycin pathway have been elucidated cannot be ruled out, available data strongly suggest that the entire cluster of daunosamine genes have been located, sequenced, and identified.

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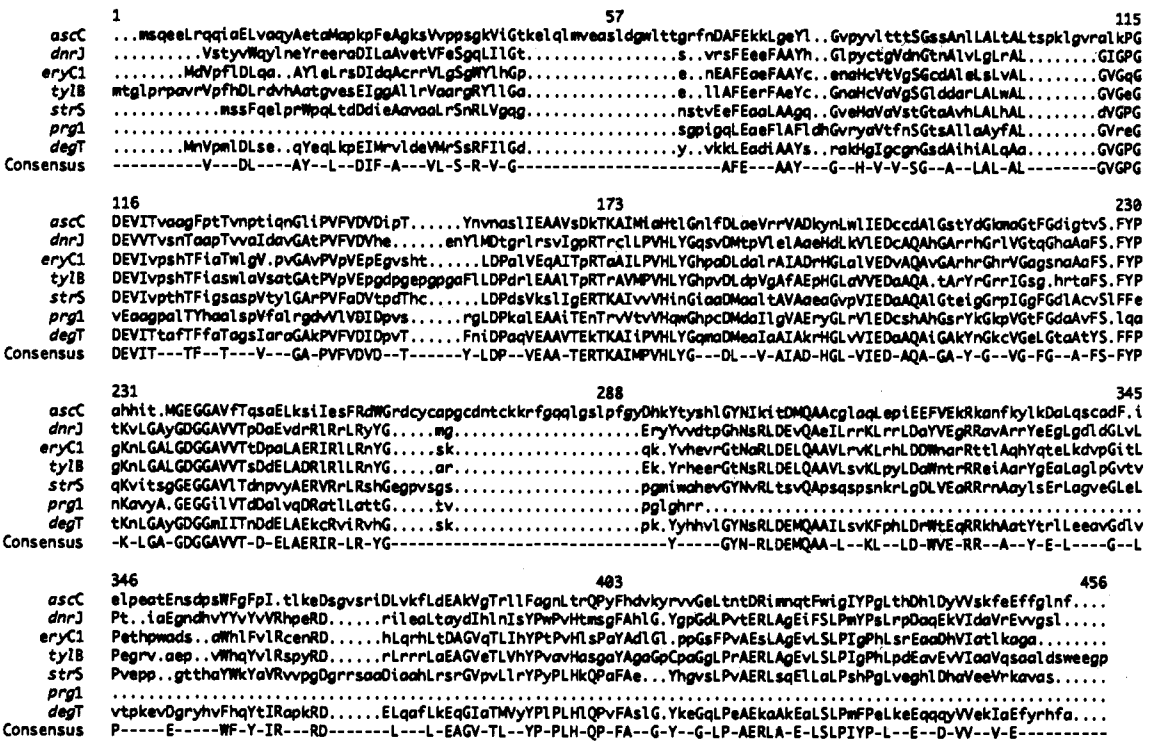
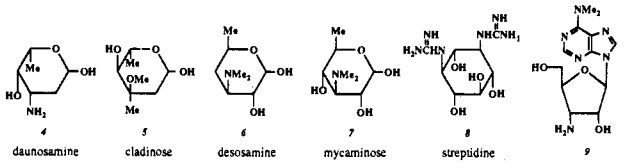


Figure 1. Alignment of the deduced amino acid sequences from the ascarylose (*ascC*, coding for E_1), daunorubicin (*dnrJ*), erythromycin (*eryC1*), tylosin (*tylB*), streptomycin (*strS*), puromycin (*prg1*), and *Bacillus subtilis* (*degT*) clusters (derived from the GENALIGN and PILEUP programs).[9] Capital letters represent identical residues or conservative replacements.

essential for the biosynthesis of 2,6- and 4,6-dideoxyhexoses. The catalysis may involve a sequence of tautomerization and elimination (Scheme IB) similar to the dehydration catalyzed by 6-phosphogluconate dehydratase[14] and dihydroxy acid dehydratase.[15] However, the lack of a presumed iron–sulfur binding sequence, expected among the α,β-dihydroxy acid dehydratases,[16] in *dnrJ*, *eryC1*, and *tylB* suggested an alternate role for their translated products. Interestingly, E_1 homologs were also found in gene clusters of streptomycin (*strS*)[17] and puromycin (*prg1*);[18] both of these antibiotics are known not to contain 2,6- or 4,6-dideoxyhexoses. Our data bank searches also revealed similarity between *ascC* and the deduced product of the *Bacillus subtilus degT* gene, which seems to have several biological functions.[19] All six of these genes (*dnrJ*, *eryC1*, *tylB*, *strS*, *prg1*, and *degT*) have been previously assigned regulatory roles within their respective pathways,[10b,11c,13,17-20] largely on the basis of the pleiotropic behavior of the *degT* gene.[19,20] However, since E_1 plays an indispensable part in C-3 deoxygenation, the significant homologies illustrated in Figure 1 suggest that the above proteins provide a catalytic rather than a regulatory role.

Based on their strong homologies with E_1, the individual roles of these proteins may exhibit an ancestral relationship to the E_1 PMP-dependent catalysis. One common trait shared among these antibiotics is their possession of an amino sugar as part of their structural entities: daunosamine (**4**) in daunorubicin, desosamine (**6**) in erythromycin, mycaminose (**7**) in tylosin, streptidine (**8**) in streptomycin, and the aminonucleoside moiety (**9**) of puromycin.



Thus, a more likely role, consistent with vitamin B_6-dependent catalysis, can be postulated for the *dnrJ*, *eryC1*, *tylB*, *strS*, and *prg1* gene products, in which they all function as an aminotransferase within their respective pathways (Scheme IC). This biosynthetic proposal is appealing since only a slight mechanistic divergence from the E_1-catalyzed dehydration would lead to a normal PLP/PMP-dependent transamination. The fact that modest residue congruence was found between these genes and the available PLP-dependent aminotransferases provides encouraging support for this postulation.[21] Moreover, transaminases believed to assist in amino sugar formation in *Escherichia coli*, *Salmonella*, and *Pasturella* have been previously identified as PLP/L-glutamate-dependent enzymes.[22]

Although the function of these E_1 homologs remains to be verified, our results have furnished strong evidence supporting a new catalytic role for these proteins previously proposed to function merely in a regulatory capacity. Therefore, not only have studies done on the biosynthesis of 3,6-dideoxyhexoses afforded a more lucid understanding of this specific sugar formation, but, more importantly, sequence comparisons based on the *asc* cluster have infused refreshing mechanistic insights into the general biosynthetic routes of the production of 2,6-dideoxy, 4,6-dideoxy, and 4-amino sugars.

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