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基础研究和新技术

大肠杆菌O77中*wabD*基因编码的*β*-1,3-甘露糖基转移酶产物结构的质谱表征

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摘要

关键词

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Structure Elucidation of the Product of *β*-1,3-Mannosyltransferase Encoded by *wabD* Gene in Escherichia coli O77 Using Mass Spectrometry

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Abstract The O77 antigens of Escherichia coli contains a Man-*β*-1,3-GlcNAc linkage within the repeating unit. A synthetic substrate analog of the natural acceptor substrate undecaprenol-pyrophosphate-lipid [GlcNAc-α-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl] was used as an acceptor and GDP-Man as a donor substrate. Electrospray ionization tandem mass spectrometry(ESI-MS/MS) is applied for the detailed structural characterization of the enzyme product. A systematic study was conducted on enzyme product to allow rationalization of the fragmentation processes. The major fragments observed in the ESI-MS/MS spectra result from cleavage of glycosidic bond and diphosphate moiety. The fragment originating from the nonreducing end of the product yields information on sequence. Cross-ring cleavages, which are very informative of the linkages of the monosaccharide residues constituting the product, and ‘internal’ cleavage ions which are derived from elimination of substituents from around the pyranose ring, were also observed. This extensive fragmentation shows the expected Man-*β*-1,3-GlcNAc linkage in the product, confirming that wabD is form of GDP-Man: GlcNAc-pyrophosphate-lipid *β*-1,3-mannosyltransferase.

Key words Mannosyltransferase _ electrospray ionization mass apectrometry(ESI) _ gene function

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