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Crystal Structures and Enzyme Mechanisms of a Dual Fucose Mutarotase/Ribose Pyranase

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Escherichia coli FucU (*Fuc*ose *U*nknown) is a dual fucose mutarotase and ribose pyranase, which shares 44% sequence identity with its human counterpart. Herein, we report the structures of *E. coli* FucU and mouse FucU bound to L-fucose and delineate the catalytic mechanisms underlying the interconversion between stereoisomers of fucose and ribose. *E. coli* FucU forms a decameric toroid with each active site formed by two adjacent subunits. While one subunit provides most of the fucose-interacting residues including a catalytic tyrosine residue, the other subunit provides a catalytic His-Asp dyad. This active-site feature is critical not only for the mutarotase activity toward L-fucose but also for the pyranase activity toward D-ribose. Structural and biochemical analyses pointed that mouse FucU assembles into four different oligomeric forms, among which the smallest homodimeric form is most abundant and would be the predominant species under physiological conditions. This homodimer has two fucose-binding sites that are devoid of the His-Asp dyad and catalytically inactive, indicating that the mutarotase and the pyranase activities appear dispensable in vertebrates. The defective assembly of the mouse FucU homodimer into the decameric form is due to an insertion of two residues at the N-terminal extreme, which is a common aspect of all the known vertebrate FucU proteins. Therefore, vertebrate FucU appears to serve for as yet unknown function through the quaternary structural alteration.

Keywords: FucU; fucose; ribose; structure; enzyme mechanism

Introduction

Mutarotases catalyze the interconversion between a pair of isomers (α- and β-anomers) that are different in the position of the hydroxyl group at the C1 carbon atom of monosaccharides in a cyclic conformation. GalM of *Escherichia coli* is the first identified mutarotase that catalyzes the anomeric interconversion of hexose sugars.[1,2] Later, two other *E. coli* proteins have been verified to be L-rhamnose mutarotase (YiiL) and L-fucose mutarotase (FucU).[3,4] Through structure-inspired biochemical analyses, a hypothetical yeast protein, YMR099cp, was characterized as D-hexose 6-phosphate mutarotase.[5] Recently, RhaU of *Rhizobium leguminosarum*, which exhibits weak sequence homology with YiiL, was characterized as an L-rhamnose mutarotase.[6] In addition, RbsD, which exhibits 25% sequence identity with FucU, was shown to catalyze the interconversion between the five- and six-membered ring forms of D-ribose.[4] Since the spontaneous conversion between the α- and β-anomers of a monosaccharide in solution is usually slow (e.g., 0.015 min^{-1} for glucose),[7] the mutarotase activities appear to be critical for the supply of specific stereoisomers of sugar substrates to sugar-metabolizing enzymes when the concentration of their substrate is low. Consistently, a *YiiL*-null *E. coli* strain exhibited a longer lag phase, slow growth, and reduced maximum growth compared with wild-type strain

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Abbreviations used: SD NMR, saturation difference nuclear magnetic resonanc; CPA, carboxypeptidase A; PDB, Protein Data Bank.

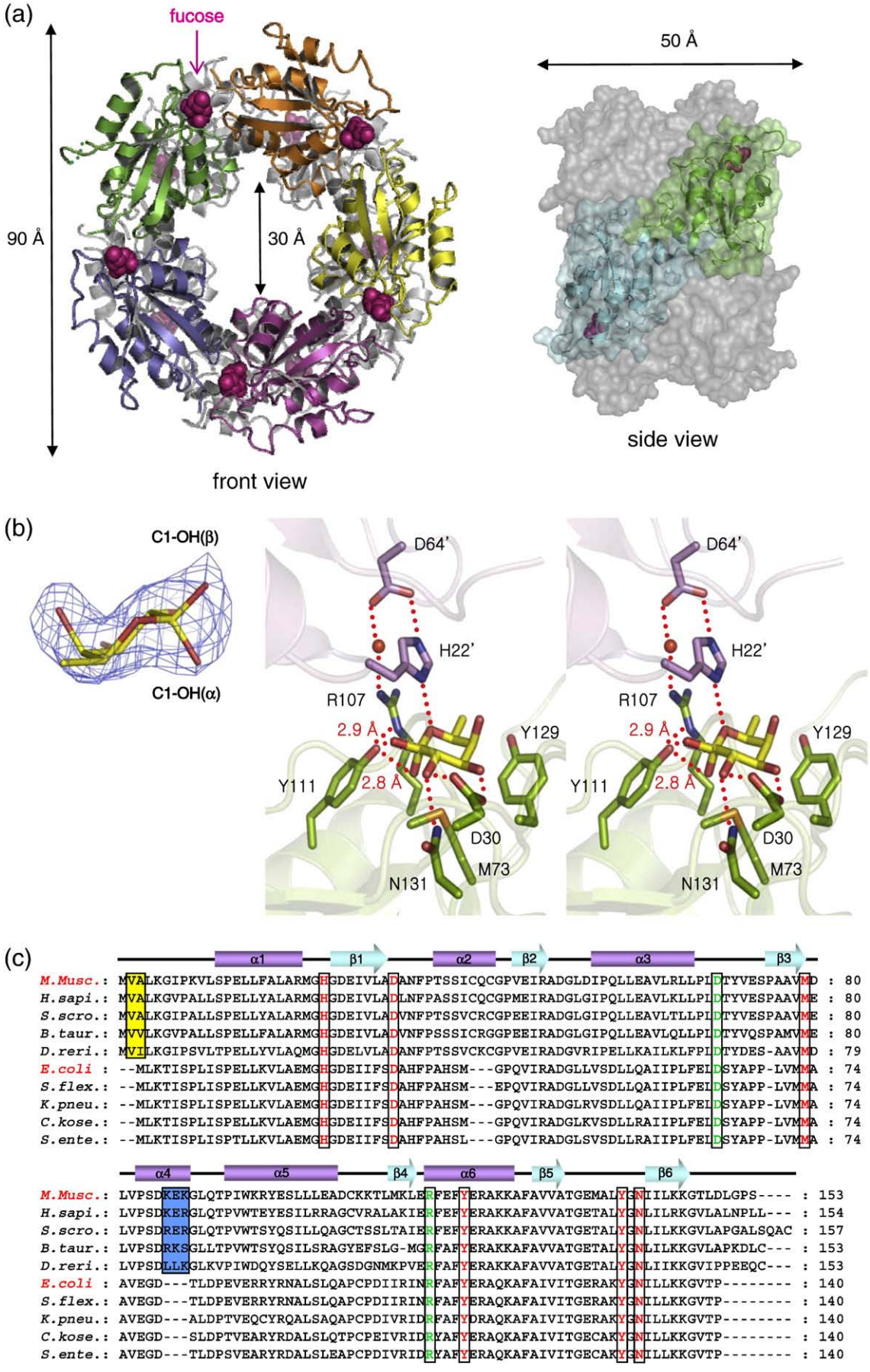

Fig. 1 *(legend on next page)*