Structure/function study of Lewis alpha3- and alpha3/4-fucosyltransferases: the alpha1,4 fucosylation requires an aromatic residue in the acceptor-binding domain.

All vertebrate alpha3- and alpha3/4-FUTs possess the characteristic acceptor-binding motif VxxHH(W/R)(D/E). FUT6 and FUTb enzymes, harboring R in the acceptor-binding motif, transfer fucose in alpha1,3 linkage, whereas FUT3 and FUT5 enzymes with W at the candidate position can also transfer fucose in alpha1,4 linkage—FUT3 being more efficient than FUT5. To determine the involvement of the W/R residue in acceptor recognition, we produced 34 variants of human FUT3, FUT5, FUT6, and ox FUTb Lewis enzymes. Among the FUT3 variants where W(111) was replaced by the other amino acids, only enzymes with an aromatic residue at the candidate position kept about 50% of alpha1,4 activity and showed no changes in K(m) values for GDP-Fuc donor and H-type 1 acceptor substrates. All other substitutions produced enzymes with less than 20% of the alpha1,4 activity. Thus the ability of alpha3/4-FUTs to recognize type 1 substrates involves the aromatic character of W in the acceptor-binding domain. The alpha1,3 activity of FUT6 and FUTb significantly decreased when their R residue was substituted by basic or charged residues. Moreover, FUT3 and FUT5 variants with W→R substitution had a better affinity for H-type 2 substrate and higher alpha1,3 activities. Therefore the optimal fucose addition in alpha1,3 linkage requires the R residue in the acceptor-binding motif of Lewis FUTs.

UMR 1061 Universite-INRA, GDR-CNRS 2590, Institut des Sciences de la Vie et de la Sante, Faculte des Sciences et Techniques, 87060 Limoges, France.