

L-Fucosylated glycoconjugates, in particular those bearing the disaccharide sequence L-Fuc- $\alpha(1\rightarrow2)$ -D-Gal, are of central importance in mammalian biosystems, and three enzymes of the albumen gland from the snail *Helix pomatia* were used to develop a viable methodology for the fast and efficient synthesis of a range of oligosaccharides bearing this moiety.

During these investigations, the  $\alpha(1\rightarrow2)$ -L-galactosyltransferase ( $\alpha(1\rightarrow2)$ -L-GalT), which *in vivo* transfers L-Gal from GDP-L-Gal to terminal, non-reducing D-Gal residues of an oligosaccharide, was given greatest priority, because this enzyme also exhibits high  $\alpha(1\rightarrow2)$ -L-fucosyltransferase activity. The  $\alpha(1\rightarrow2)$ -L-galactosylation and  $\alpha(1\rightarrow2)$ -L-fucosylation with  $\alpha(1\rightarrow2)$ -L-GalT were optimised, and the enzyme was then used *in vitro* to transfer L-Fuc from GDP-L-Fuc to a series of acceptor substrates under the optimised reaction conditions. The site of interglycosidic linkage was seen to be of little or no consequence, as the homologous series D-Gal- $\beta(1\rightarrow n)$ -D-Glc $\beta$ OMe ( $n = 2, 3, 4$  and  $6$ ) was synthesised and  $\alpha(1\rightarrow2)$ -L-fucosylated on a milligram scale, with pleasing yields of 65-70% of peracetylated trisaccharides **115** – **118**, which reflect the reaction yields of the corresponding enzymatic glycosylations. The anomericity of the terminal-subterminal interglycosidic linkages is of central relevance, with  $\alpha$ -linkages not being accepted, as seen for the examples of methyl melibioside and D-Gal- $\alpha(1\rightarrow4)$ -D-Gal. If the acceptor's terminal-subterminal interglycosidic linkage is of  $\beta$ -configuration to an axial hydroxyl group, as in the case of D-Gal- $\beta(1\rightarrow4)$ -D-Gal, the enzyme does not recognise the acceptor. The overall substrate flexibility of this enzyme, combined with its reactivity and easy, inexpensive availability, makes it a very powerful synthetic tool.

In addition to  $\alpha(1\rightarrow2)$ -L-GalT, the *Helix pomatia* albumen gland contains two potent and rare D-galactosyltransferases: a  $\beta(1\rightarrow6)$ -D-galactosyltransferase ( $\beta(1\rightarrow6)$ -D-GalT-I), which transfers D-Gal from UDP-D-Gal to oligosaccharide subterminal D-Gal residues, and a second  $\beta(1\rightarrow6)$ -D-galactosyltransferase ( $\beta(1\rightarrow6)$ -D-GalT-II), which is activated by the presence of  $Mn^{2+}$  ions, and transfers D-Gal from UDP-D-Gal to terminal, non-reducing D-Gal residues of an oligosaccharide. Each of these three enzymes are easily and independently activated by incubation with the appropriate nucleotide sugar donor (GDP-L-Fuc and UDP-D-Gal) and/or the presence of  $Mn^{2+}$  ions. This means that the crude enzyme mixture contained in the albumen gland sediment, which is very convenient to prepare, can be used as a veritable oligosaccharide synthesis toolbox. Sequential incubation of the acceptor **8** with the albumen gland sediment and the appropriate donor lead to the facile synthesis of the oligosaccharide series **22**, **128** and **130**. Incubation of trisaccharide **22** with UDP-D-Gal and the albumen gland sediment in the presence of  $Mn^{2+}$  ions lead to a tetrasaccharide **149**, which after peracetylation could be unambiguously identified as compound **150**. The employment of the three galactosyltransferases is by no means limited to smaller oligosaccharides.

Thus, the albumen gland sediment represents an extremely useful synthetic potential, offering three very reactive glycosyltransferases, which are either rare or otherwise difficult or expensive to access. The snail *Helix pomatia* is commonly found in Northern Europe, and if the albumen gland is harvested in early spring, the snails represent a convenient and inexpensive enzyme source. With the recent decrease in price of GDP-L-Fuc and UDP-D-Gal, and the easy availability of the  $\alpha(1\rightarrow2)$ -L-GalT,  $\beta(1\rightarrow6)$ -D-GalT-I and  $\beta(1\rightarrow6)$ -D-GalT-II, there is no reason not to exploit the synthetic methods described herein.

