Carbohydrate Polymers 93 (2013) 65–72

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Enzymatic synthesis of oligo- and polysaccharide fatty acid esters

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ARTICLE INFO

Article history:
Received 29 November 2011
Received in revised form 10 May 2012
Accepted 16 May 2012
Available online 26 May 2012

Keywords:
Lipase
Protease
Organic solvents
Ionic liquids
Supercritical carbon dioxide

ABSTRACT

Amphiphilic oligo- and polysaccharides (e.g. polysaccharide alkyl or alkyl-aryl esters) form a new class of polymers with exceptional properties. They function as polymeric surfactants, whilst maintaining most of the properties of the starting polymeric material such as emulsifying, gelling, and film forming properties combined with partial water solubility or permeability. At present carbohydrate fatty acid esters are generally obtained by chemical methods using toxic solvents and organic and inorganic catalysts that leave residual traces in the final products. Enzymatic reactions offer an attractive alternative route for the synthesis of polysaccharide esters. In this review the state of the art of enzymatic synthesis of oligo- and polysaccharides fatty esters has been described.

1. Introduction

Nowadays, the interest for synthesis of modified oligo- and polysaccharides is increasing. Amphiphilic polysaccharides can function as polymeric non-ionic surfactants, whilst maintaining most of the properties of the starting polymeric materials such as emulsifying, gelling, and film forming properties combined with partial water solubility or permeability. Their functional properties can be fine-tuned by adjusting the ratio between the hydrophilic parts, the oligo- or polysaccharide, and the hydrophobic (lipophilic) parts, the amount and the chain length of the alkyl (fatty acid) residue. Derivatives with a low degree of substitution (DS) can exhibit insufficient amphiphilic character, whilst esters with a too high DS become water insoluble. Due to their unique properties, amphiphilic oligo- and polysaccharides can find multiple applications, such as structural components in food products due to their impact on texture of food and their flavour and/or bioactive release characteristics (Chang & Shaw, 2009). They can also be used in topical formulations, to increase the solubility of insoluble or poorly soluble drugs, where they replace synthetic polymers such as poloxamer (a copolymer of polyoxoethylene–polyoxopropylene), poly(vinyl ethers), or poly(metacrylic acids). Moreover, fatty acid esters of polysaccharides can be applied for delivery and controlled release of agrochemicals (fungicides and insecticides), since due to their lipophilic character they enhance the adhesion of the formulation to the hydrophobic surface of plant leaves and decrease the wash ability.

At present, esters of carbohydrates are commercially manufactured by chemical esterification with acid chlorides, in organic solvents (e.g. dimethyl sulfoxide) using organic and inorganic catalysts (pyridine, tionyl chloride, K_2CO_3, celite) that leave residual traces in the final products. Generally, esterification reactions require high temperatures which, in combination with the catalysts used, might induce partial degradation of the polysaccharide chains and discoloration. Currently solvent-free chemical processes for esterification of starch are under development, but they do not eliminate the problem of chain degradation and high volume of wastes produced in the downstream processing step. Chemical esterification is non-selective, and both primary and secondary hydroxyl groups are substituted, making this process suitable only for the production of carbohydrate esters with a high degree of substitution (DS). This is, however, a severe limitation when products with low DS are targeted, as in the case of amphiphilic polysaccharides. Since chemical esterification results in complex mixtures of mono-, di- and tri-esters with random distribution of the ester groups both on the carbohydrate monomer and the polymer backbone.

Enzymatic processes offer an attractive alternative route for the synthesis of oligo- and polysaccharide esters. Selective processes catalysed by enzymes may be performed under mild conditions of temperature and pressure, thereby avoiding polymer degradation. Application of enzymes for modification of polysaccharides will bring the advantage of the high specificity and

Abbreviations: CFAE, carbohydrate fatty acid esters; CLEAs, carrier-bound cross-linked enzyme aggregates; CMC, critical micelle concentration; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DS, degree of substitution; ILs, ionic liquids; OAT, bis(2-ethylhexyl)sodium sulfosuccinate; $scCO_2$, supercritical carbon dioxide.

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http://dx.doi.org/10.1016/j.carbpol.2012.05.051

regioselectivity of the reaction, which will generate products with controlled structure and functionality. The formation of ester linkages is thermodynamically favoured in low water content media and therefore alternative enzymatic procedures should employ solvent free, organic solvents, or organic solvent mixtures as reaction medium. Also media like ionic liquids (ILs) and supercritical fluids, in particularly supercritical carbon dioxide ($scCO_2$), can be used for enzyme catalysed reactions.

This minireview has been focused on the enzymatic synthesis of oligo- and polysaccharide fatty acid esters referred to in the text as carbohydrate fatty acid esters (CFAE). The esterification of mono- and disaccharides is not included since this topic is extensively addressed in literature (Allen & Tao, 1999; Chang & Shaw, 2009; Gumel, Annuar, Heidelberg, & Chisti, 2011; Kennedy et al., 2006; Polat & Linhardt, 2001).

2. Enzymes

Lipases (EC 3.1.1.3), esterases (EC 3.1.x.x), proteases (EC 3.4.21.x), and peptidases (EC 3.4.x.x) have been applied for the synthesis of CFAE. All these enzymes are highly stereo- and regioselective catalysts that usually operate under mild reaction conditions, are robust and easy to handle and are commercially available for industrial applications (Table 1). They are efficient catalysts for the hydrolysis of esters, but in the absence of water in neat organic solvents, they catalyse the reverse reaction, i.e. the syntheses of esters. Common to these hydrolases is the catalytic triad serine–histidine–aspartic acid in the active centre, although the residues occur in a different order in each protein sequence (e.g. Ser105-His224-Asp187 in *Candida antarctica* B lipase and Ser195-His57-Asp102 in trypsin). The coupling of fatty acids to carbohydrates can take place by esterification and/or transesterification reactions (Fig. 1). The esterification reaction (reaction A in Fig. 1) is an equilibrium, that is thermodynamically controlled using non-activated, free carboxylic acids as substrates and requires the continuous removal of the water produced in the reaction to shift the equilibrium and increase the ester yield. Strategies for water removal are discussed in Section 3. Transesterification (reaction B in Fig. 1) is preferred above esterification, due to (1) the higher reactivity of the ester group as compared to a non-activated carboxylic acid residue and (2) the easier removal of the alcohol by-products from the reaction mixture as compared to water, the secondary product in the esterification reaction. Methyl, ethyl, and vinyl esters are generally used as acyl reagents in the transesterification reaction. Vinyl esters are favoured acyl reagents in most studies, since the vinyl alcohol formed in the reaction tautomerises to the volatile acetaldehyde that is easily removed from the reaction mixture thus driving the reaction to completion. However, due to its high reactivity, acetaldehyde could react with the free amino groups of the lysine residues in the protein resulting in enzyme inactivation. It has been reported that some lipases (e.g. from *Candida rugosa*) loose most of their activity when exposed to acetaldehyde (Weber, Stecher, & Faber, 1995).

Proteases from *Bacillus licheniformis* and *Bacillus subtilis* have been used for the enzymatic synthesis of carbohydrate fatty acids esters (Table 2). However, esterases and in particularly lipases are preferred above proteases due to the high substrate specificity of the latter that narrows the range of carboxylic acids and alcohol substrates (Bordusa, 2002). Like esterases, proteases have a preference for short and medium chain fatty acids, whereas lipases are the most promising biocatalysts for long chain fatty acids (Plou et al., 2002).

Subtilisin, a serine protease from *B. subtilis*, appeared to be highly efficient for esterification of the primary OH-group of carbohydrates (Bruno, Dordick, Kaplan, & Akkara, 1998). Lipases from *C. antarctica* (lipase CA) and *Thermomyces lanuginosus* (lipase TL), and an alkaline protease from *B. licheniformis* have been used to synthesize CFAE using vinyl laurate as acyl reagent. Melezitose, raffinose, stachyose, and kestose were used as oligosaccharides. Although different reaction media were used, different regioselectivity for the acylation of the primary hydroxyl groups was observed for lipases as compared to subtilisin (Perez-Victoria & Morales, 2006a). Both lipase CA and lipase TL showed the highest regioselectivity for the 6-OH galactosyl of raffinose and stachyose and 6-OH glucosyl of melezitose, whilst subtilisin modified preferentially the primary hydroxyl of the fructose residues of the oligosaccharides, the 1′-OH fructosyl in raffinose and stachyose, 6-OH fructosyl in melezitose and 1‴-OH fructosyl in kestose. For the acylation of secondary hydroxyl groups thermolysin a metalloprotease from *Bacillus thermoproteolyticus* can be used (Perez-Victoria & Morales, 2006b). Thermolysin has been used also for the esterification of cyclodextrin with fatty acids in dimethyl sulfoxide (DMSO). The regioselectivity of thermolysin was mainly against the hydroxyl group in position 2 of the glucopyranose unit although the hydroxyl group at positions 3 and 6 were also partially esterified leading to multiple-substituted esterified cyclodextrins (Choisnard et al., 2011; Pedersen et al., 2005). Lipases have been used also to synthesize lipidyl-cyclodextrins by amidation. Mono-6-amino-permethylated β-cyclodextrin was used as substrate and ethyl caprylate or vinyl laurate as acyl donor. As all the OH-groups were methylated the lipases acylated the amine group to synthesize mono-substituted methylated cyclodextrin with only one fatty acid moiety (Favrelle, Bonnet, Sarazin, & Djedaini-Pilard, 2007; Favrelle et al., 2010). Lipase from *C. rugosa* was used for transesterification of dextran with vinyl decanoate in DMSO. Using a pH-imprinted enzyme, the extent of dextran modification (49%) was increased 16 fold as compared when native lipase was used (3%) (Kaewprapan, Tuchinda, Marie, Durand, & Inprakhon, 2007). It was also observed that the degree of modification was depending on the commercial enzyme preparation used. Three factors were mentioned as possible reasons (1) relative proportion of isoenzymes present, (2) amount of water in the lyophilized enzyme preparation, and (3) the amount of lipase protein present. In a follow up study it was noticed that addition of 18-crown-6-ether before lyophilisation of the pH-imprinted enzyme resulted in even a higher extent of dextran modification. Furthermore it was observed that the regioselectivity was equalled between the hydroxyl group at position 2 and 3 of the glucose residue when saturated fatty acids were used as acyl donor. In the case of unsaturated acyl donors a preference for the hydroxyl group at position 2 of the glucose residue was observed, whereas vinyl pivalate, a sterically hindered acyl donor, had a preference towards the hydroxyl group at position 3 (Kaewprapan et al., 2011). In Table 2 an overview is given of the different carbohydrates that have been esterified with fatty acids using enzymes. In general long incubation times (20–120 h) are needed to obtain CFAE. An exception is the esterification of starch with fatty acids from coconut oil using micro wave heating and lipase TL. In this case the reaction was performed in minutes instead of hours (Rajan, Prasad, & Abraham, 2006).

For the enzymatic reactions often immobilized enzymes have been used because immobilization enhances the stability of the enzyme in organic solvents. The immobilized enzymes can be generally classified into two groups. Namely the group of carrier supported immobilized enzymes (e.g. adsorption on polypropylene or macroporous acrylic resin, covalent attachment to eupergit C or magnetic particles, silica-granulation, and sol–gel encapsulation), and the other group is the carrier-free immobilized enzymes like bound cross-linked enzyme aggregates (CLEAs). For example lipase from *C. rugosa* was encapsulated into a polyacrylamide gel and showed improved stability in the transesterification reaction between dextran and vinyl decanoate in anhydrous DMSO (Ge, Lu,