ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 3 (1997) 189–192

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Comparative studies on the sugar esters synthesis catalysed by *Candida antarctica* and *Candida rugosa* lipases in hexane

C. Tsitsimpikou, H. Daflos, F.N. Kolisis *

Biosystems Technology Lab., Div. IV, Chemical Engineering Dept., National Technical University of Athens, Zographou campus, 15700 Athens, Greece

Abstract

The regioselective acylation of monosaccharides, such as glucose, fructose, mannose and arabinose with lauric acid (C12) has been investigated in hexane using commercial *Candida rugosa* lipase (EC 3.1.1.3) and *Candida antarctica* (NOVOZYME) lipase immobilised on anionic resin. In order to overcome sugars insolubility in hexane, sugars were introduced in the reaction mixture preabsorbed on silica gel. The chemical structure of the products has been determined by ^{1}H NMR analysis. The two enzymes tested seemed to prefer hexoses rather than pentoses as the sugar substrate. They exhibited different water activity optimums as well as different optimum sugar/acyl donor molar ratios. Water activity control throughout the reaction and temperature increase from 30 to 50°C enhanced sugar ester production using both lipases.

Keywords: Sugar–fatty acid esters; Lipase; Water activity

1. Introduction

Fatty acid esters of carbohydrates constitute an interesting group of non-ionic surfactants, which are widely used in the pharmaceutical, cosmetic, petroleum and food industries. They are an integral part of many industrial, agricultural and food processes [1,2].

Methods to acylate sugars chemically are always characterised by extensive, tedious hydroxyl protection and deprotection steps and use of harmful solvents. Alternatively, the enzymatic approaches with lipases in non-polar organic media or solvent-free systems under nearly anhydrous conditions, which favour lipase-catalysed processes, seem to be more suitable for such purposes. Nevertheless, the problem of the carbohydrates solubility in these media creates difficulties in choosing the appropriate conditions to perform the reaction.

In this report, the acylation of monosaccharides, such as glucose, fructose, mannose and arabinose with lauric acid (C12) has been investigated in hexane using commercial *Candida rugosa* lipase (EC 3.1.1.3) and *Candida antarctica* (NOVOZYME) lipase immobilised on anionic resin. Various parameters affecting enzyme activity, such as temperature and water activity, along with the influence of sugar/acyl donor molar ratio on the production of glucose laurate have also been extensively studied.

* Corresponding author.

PII S1381-1177(96)00059-8

2. Experimental

2.1. Materials

Commercial *Candida rugosa* lipase was purchased from Sigma and used without any further purification. Immobilised lipase from *Candida antarctica* Novozyme™ was a gift from Novo Nordisk (Denmark). Glucose, fructose, arabinose, mannose, methyl glucoside, lauric acid (dodecanoic acid) and silica gel (mesh 60) were of > 99% purity and purchased from Sigma (USA). All solvents were purchased from Merck.

2.2. Sugars absorption procedure [3]

Silica gel (2 × wt of sugars) was added to a methanolic solution of the substrate (2 mmol). Methanol was evaporated under continuous agitation at reduced pressure. The homogeneous powder obtained was dried over P_2O_5 for 72 h.

2.3. Bioconversions in hexane

A typical reaction mixture consisted of 83 mM of sugar, 200 mM of lauric acid and 12.5 mg/ml enzyme preparation which were added to 4 ml hexane after a 24 h preincubation at various water activities (a_w) using the appropriate saturated salt solutions. Water activity was kept constant throughout the reaction [4]. The reaction was carried out at 40°C. At different time intervals, hexane was evaporated under reduced pressure and the reaction mixture was solubilised in the HPLC solvent.

2.4. Analytical methods

Qualitative analysis of products was made by TLC on silica gel 60 plates (Merck) using an elution system of $CH_3COOC_2H_5$:CH_3OH:H_2O = 17:6:2. Plates were developed with a 5% ethanolic solution of H_2SO_4 and 10 min incubation at 150°C.

Quantitative analysis of samples was made by HPLC on a μ-Bondapack C_{18} column using a refractive index monitor. Elution was conducted with CH_3CN:CH_3OH:H_2O = 50:15:35 and a flow rate of 1 ml/min.

Products were identified with ^1H NMR in $CDCl_3$ with TMS as internal standard.

^1H NMR spectral data for glucose laurate is as follows: 0.89 (t, 3H), 1.28 (m, 18H), 1.62 (m,

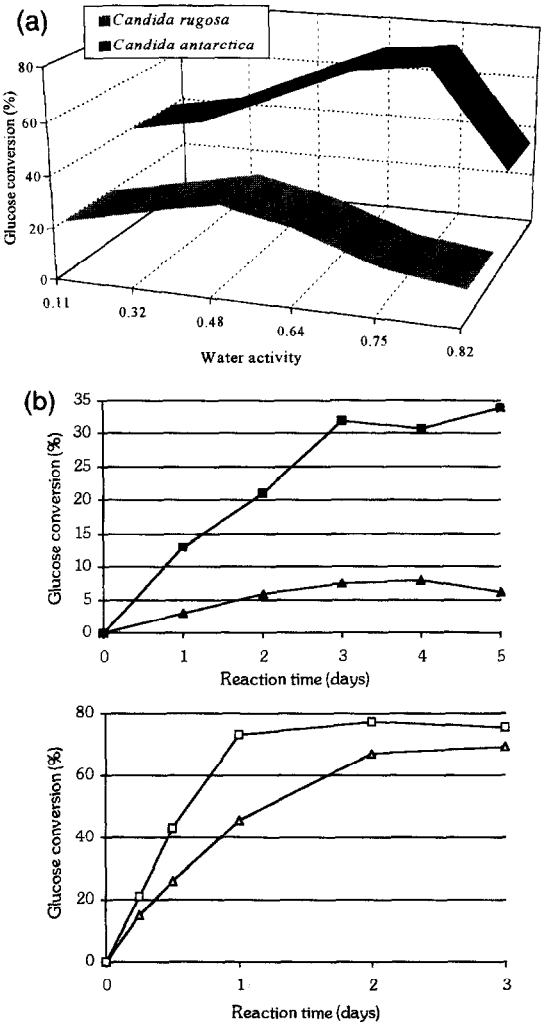

Fig. 1. (a) Effect of water activity (a_w) on the esterification of glucose with lauric acid using *Candida rugosa* (reaction time 3 days) and *Candida antarctica* (reaction time 2 days) in hexane. T = 40°C. (b) Enhancement of glucose laurate production using *Candida rugosa* (filled symbols) and *Candida antarctica* (open symbols) lipases in hexane by controlling the water activity throughout the reaction. (■, □) water activity set and controlled at the optimum value, (▲, △) without water activity control. T = 40°C.

2H), 2.36 (t, 2H), 3.34 (t, 1H, H_4), 3.51 (dd, 1H, H_2), 3.75 (m, 1H), 3.77 (m, 1H, H_5), 4.28 (dd, 1H, H_{6b}), 4.45 (dd, 1H, H_{6a}) and 4.88 (d, 1H, H_1).

3. Results and discussion

The effect of water activity (a_w) on glucose laurate production through glucose esterification with lauric acid in hexane using *Candida rugosa* and *Candida antarctica* lipases was studied. As indicated in Fig. 1a, both enzymes are active even at low water activities [5]. A different water activity optimum was observed for each enzyme (0.53 and 0.75 for *Candida rugosa* and *Candida antarctica*, respectively). Valivety et al. [6] tried to correlate the effect of a_w on enzyme activity with the molecular structure, in the case of 5 lipases whose sequences had been determined. They found that the higher the degree of homology between the enzymes sequences, the more similar the enzymes behaved at a certain a_w. Accordingly the difference observed at the water activity optimum of the two lipases tested in our study, could be due to the structural differences of the two enzyme molecules. It is worth noting that glucose laurate production is significantly enhanced by controlling the water activity throughout the reaction at the optimum for each enzyme value. When the reaction was performed with the initial water content of the system, without controlling the water activity, lower glucose laurate production rates were observed (Fig. 1b). The water content of the enzyme preparations were 2.5 and 19.6 mg H_2O per 100 mg of enzyme preparation of *Candida rugosa* and *Candida antarctica* lipase, respectively.

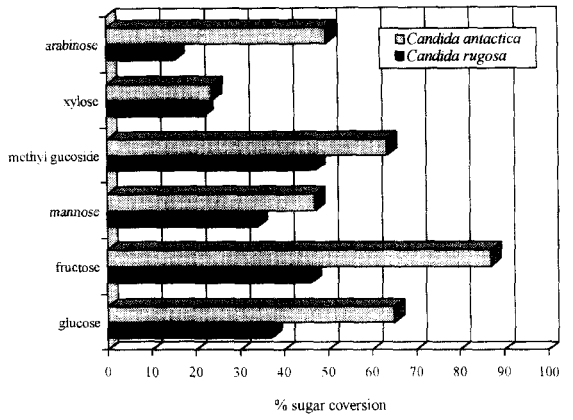

Fig. 2. Esterification of various sugars with lauric acid using *Candida rugosa* and *Candida antarctica* lipases in hexane. T = 40°C. Reaction time 4 days. Water activity was set and controlled at the optimum for each enzyme value (*Candida rugosa* a_w = 0.53, *Candida antarctica* a_w = 0.75).

The esterification of various hexoses and alkyl glucosides, such as glucose, fructose, mannose, methyl glucoside and pentoses, such as xylose and arabinose, has been performed using *Candida rugosa* and *Candida antarctica* lipases in hexane (Fig. 2). Both enzymes seem to prefer hexoses rather than pentoses as the sugar substrate. Sugar acylation proved, by ^{1}H NMR

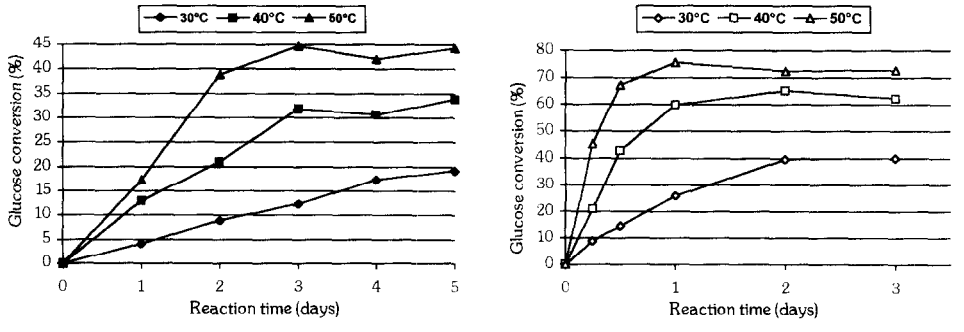

Fig. 3. Effect of temperature on the time course of glucose esterification with lauric acid using *Candida rugosa* (filled symbols) and *Candida antarctica* (open symbols) lipases in hexane. Water activity was set and controlled throughout the reaction at the optimum for each enzyme value (*Candida rugosa* a_w = 0.53, *Candida antarctica* a_w = 0.74).

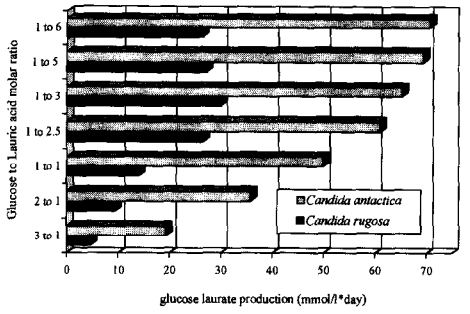

Fig. 4. Effect of glucose to lauric acid molar ratio on the glucose laurate production in hexane. $T = 40°C$. Water activity was set and controlled throughout the reaction at the optimum for each enzyme value (*Candida rugosa* $a_w = 0.53$, *Candida antarctica* $a_w = 0.75$).

spectra, to be regioselective and the 6-O-sugar-laurate was exclusively produced. Methyl glucoside is a very good substrate for both enzymes giving 47 and 63% product yield with *Candida rugosa* and *Candida antarctica*, respectively. Alkyl glucoside fatty acid esters constitute a unique class of surfactants with interesting properties [7,8]. As previously reported [9], the non-specific *Candida* enzymes catalyse the esterification of alkyl glycosides quite efficiently.

The effect of temperature on the time course of glucose esterification with lauric acid is shown in Fig. 3. Temperature increase from 30 to 50°C led to both higher reaction rates and final product yield.

Finally, in order to study the influence of sugar to acyl donor molar ratio on the glucose laurate production, seven different glucose to lauric acid molar ratios were used (Fig. 4). The two enzymes presented a different optimum, while *Candida antarctica* seems to be more efficient in this type of reaction. For *Candida rugosa* the best initial molar ratio was 1/2.5. Above this value a decrease of both the production and reaction rate was observed. In the case of *Candida antarctica* lipase the optimum value was 1/6. Such high excess of fatty acid has also been reported for the esterification of fructose with palmitic and oleic acid using *Mucor miehei* and *Candida antarctica*, respectively [10,11].

Acknowledgements

Part of this work was supported by the European Union in the frame of AIR PROJECT (AIR3-CT94-2291).

References

[1] H. Seino, T. Uchibori, T. Nishitani and S. Inamasu, J. Am. Oil Chem. Soc. 61 (1984) 1761.
[2] J.F. Shaw and A.M. Klibanov, Biotechnol. Bioeng. 29 (1987) 648.
[3] M.B. Berger, K. Laumen and M.P. Schneider, Biotechnol. Lett. 14 (1992) 553.
[4] G. Ljunger, P. Adlercreutz and B. Mattiasson, Enz. Microb. Technol. 16 (1994) 751.
[5] R.H. Valivety, P.J. Halling and A.R. Macrae, FEBS Lett. 301 (1992) 258.
[6] R.H. Valivety, P.J. Halling, A.D. Peilow and A.R. Macrae, Biochim. Biophys. Acta 1122 (1992) 143.
[7] O. Andersen and O. Kirk, Proc. Carbohydrate Bioengineering Meeting, No. 34, Elsinore, Denmark, 23–26 April (1995).
[8] C.C. Akoh and B.G. Swanson, J. Am. Oil Chem. Soc. 66 (1989) 1295.
[9] C.C. Akoh and L.N. Mutua, Enz. Microb. Technol. 16 (1994) 115.
[10] C. Scheckermann, A. Schlotterbeck, M. Schmidt, V. Wray and S. Lang, Enz. Microb. Technol. 17 (1995) 157.
[11] D. Coulon, M. Girardin, B. Rovel and M. Ghoul, Biotechnol. Lett. 17 (1995) 183.