

Leuconostoc mesenteroides glucansucrase synthesis of flavonoid glucosides by acceptor reactions in aqueous-organic solvents

Anne Bertrand,^a Sandrine Morel,^a François Lefoulon,^b Yves Rolland,^c
Pierre Monsan^a and Magali Remaud-Simeon^{a,*}

^aLaboratoire Biotechnologie-Bioprocédés UMR CNRS 5504, UMR INRA 792, INSA DGBA, 135 avenue de Rangueil,
31077 Toulouse Cedex 04, France

^bTechnologie SERVIER, 25/27 rue Eugène Vignat, 45000 Orléans, France

^cLes Laboratoires SERVIER, 22 rue Garnier, 92200 Neuilly-sur-Seine, France

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Abstract—The enzymatic glucosylation of luteolin was attempted using two glucansucrases: the dextranucrase from *Leuconostoc mesenteroides* NRRL B-512F and the alternansucrase from *L. mesenteroides* NRRL B-23192. Reactions were carried out in aqueous-organic solvents to improve luteolin solubility. A molar conversion of 44% was achieved after 24 h of reaction catalysed by dextranucrase from *L. mesenteroides* NRRL B-512F in a mixture of acetate buffer (70%)/bis(2-methoxyethyl) ether (30%). Two products were characterised by nuclear magnetic resonance (NMR) spectroscopy: luteolin-3'-O- α -D-glucopyranoside and luteolin-4'-O- α -D-glucopyranoside. In the presence of alternansucrase from *L. mesenteroides* NRRL B-23192, three additional products were obtained with a luteolin conversion of 8%. Both enzymes were also able to glucosylate quercetin and myricetin with conversion of 4% and 49%, respectively.

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1. Introduction

Flavonoids are polyphenolic compounds existing widely throughout the plant kingdom. These natural compounds are used in the food, cosmetic and pharmaceutical industries and have recently been shown to possess potential pharmacological properties. They were first described to play a role in the maintenance of blood capillary wall integrity and capillary resistance.^{1,2} A number of studies have demonstrated that, depending on their structure, flavonic compounds may also exhibit anti-inflammatory, anti-allergic, anti-oxidant or anti-tumoural activities, conferring to these polyphenols a potential protective function against cardiovascular and coronary heart diseases and against certain forms of cancer.^{1–7}

One major drawback of flavonoids is their poor water solubility, which limits their practical applications. Thus, in order to improve the flavonoid hydrophilicity, enzymatic glucosylation has been reported on various phenolic compounds: (+)-catechin,^{8–14} hesperidin,^{15,16} neohesperidin,¹⁶ naringin^{16,17} and rutin.^{18,19} The glucosylated catechin has been shown to be 100-fold more soluble than catechin,¹³ while the glucosylated naringin is 1000-fold more soluble than naringin.¹⁶ An example is the capillary protectant activity of rutin (quercetin-3-O-rutinoside). Enzymatic glucosylation produced 4'-O- α -D-glucopyranosyl-rutin, which is 30,000-fold more soluble than rutin and thus leading to an improved biological activity.^{18,19}

Glucansucrases (GS, EC 2.4.1) are a family of enzymes, which catalyse two kinds of reaction: (1) the synthesis of glucan from sucrose and (2) the acceptor reaction. The acceptor reaction corresponds to the transfer of an α -D-glucopyranosyl unit from the donor

* Corresponding author. Fax: +33 5615 59400; e-mail: remaud@insa-toulouse.fr

(sucrose) onto either a mono or disaccharide to form low molecular weight oligosaccharides, or onto a non-carbohydrate hydroxylated compound, which leads to the formation of a glycoconjugate.

Dextranase (E.C. 2.4.1.5) from *Leuconostoc mesenteroides* NRRL B-512F produces a glucan called dextran, which consists of 95% of α -(1 $\rightarrow$ 6) osidic linkages and 5% of α -(1 $\rightarrow$ 3) branched linkages.^{20–22} Another enzyme of particular interest is the alternansucrase (E.C. 2.4.1.140) from *L. mesenteroides* NRRL B-23192. This enzyme synthesises from sucrose an alternating α -(1 $\rightarrow$ 6) and α -(1 $\rightarrow$ 3) linked D-glucan, called alternan.^{23,24}

Glucansucrases have been widely used for the glucosylation of saccharides²⁵ used as acceptor molecules, primarily resulting in the formation of oligosaccharides^{26–28} and for the glucosylation of unusual saccharides like cellobiose,²⁹ acarbose,³⁰ methyl hexopyranosides,³¹ alkylglucosides,³² alditols, aldulose, sugar acids³³ and salicin.³⁴ Due to the enzymatic nature of these reactions and the solubility properties of these acceptors, the reaction medium of choice was water.

However, only three studies have focused on the glucosylation of non-saccharide molecules: phenol acting as acceptor for B-1299CB-BF563 dextranase,³⁴ salicyl alcohol as acceptor for B-742CB dextranase,³⁵ catechol and its derivatives acting as acceptors for *Streptococcus mutans* GS-5 glucosyltransferase-D.^{11,12} Various water-miscible organic solvents were used to facilitate the transglucosylation of catechol derivatives. These results have shown that the synthetic potential use of these enzymes is not restricted to conventional saccharides. Only one study has reported the activity and the stability of glucansucrase in the presence of water-miscible organic solvents.³⁶

The aim of our work was to investigate the ability of glucansucrases to glucosylate non-water soluble flavonoids and to determine the effect of glucosylation on the water solubility of the resulting glucosylated flavonoid. Conditions enabling the enzymatic glucosylation of one particular flavonoid, namely luteolin were first established. Products were characterised and subsequently tested for their water solubility. This approach was then applied to five other flavonoids in order to develop the potential use of glucansucrases to glucosylate flavonoids.

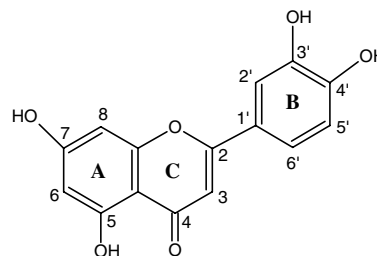


Figure 1. Structure and chemical numbering of luteolin (3',4',5,7-tetrahydroxy-flavone or 2-(3',4'-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one).

2. Results and discussion

Glucosylation of luteolin (Fig. 1) was chosen as the model reaction with glucansucrases.

2.1. Influence of organic solvent on substrate solubility and glucansucrase activity

Luteolin acceptor was slightly soluble (14 μ M) in the buffered medium usually employed for dextranase or alternansucrase (sodium acetate buffer 20 mM, pH 5.2). As luteolin was poorly soluble in the reaction medium, various organic solvents were tested to find a solvent able to solubilise both luteolin and sucrose. As shown in Table 1, bis(2-methoxyethyl) ether (MEE) and dimethyl sulfoxide were the only solvents in which both acceptor and donor could be solubilised.

The effect of these solvents on the glucansucrase activity was then investigated (see Table 2). It appears that

Table 1. Solubility of luteolin and sucrose in sodium acetate buffer and in organic solvents

	[Luteolin] _{max} (mM) ^a	[Sucrose] _{max} (mM) ^a
NaOAc buffer (20 mM, pH 5.2)	0.014	2000.0
Acetonitrile	1.5	—
1,4-Dioxane	8.9	0
Methanol	10.6	6.4
Ethyl acetate	12.7	—
MEE (bis[2-methoxyethyl] ether)	456.5	0
Dimethyl sulfoxide	560.8	120.0

^a Luteolin and sucrose concentrations were calculated from HPLC peaks area after calibration with standards.

Table 2. Substrate solubility and glucansucrase activity measured in a mixture of AcONa buffer and organic solvents

		Substrate solubility (mM)		Glucansucrase activity (%)	
		Sucrose	Luteolin	Dextranase B-512F	Alternansucrase B-23192
AcONa Buffer (20 mM, pH = 5.2)		2000	0.014	100	100
AcONa Buffer (70)	MEE (30)	1316	11	55	70
AcONa Buffer (70)	Me ₂ SO (30)	1297	10	59	58
AcONa Buffer (60)	MEE (40)	1186	15	8	50
AcONa Buffer (60)	Me ₂ SO (40)	1114	13	27	32
AcONa Buffer (50)	MEE (50)	977	90	5	28
AcONa Buffer (50)	Me ₂ SO (50)	902	15	3	15

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