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ARTICLE

Ionic Liquid Effects on the Activity of β-Glycosidase for the Synthesis of Salidroside in Co-solvent Systems

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Abstract: The preparation of salidroside was successfully carried out in fourteen ionic liquids (ILs)-containing systems using β-glycosidase from black plum seeds for the first time. The optimum conditions were determined for $C_6MIm{\cdot}BF_4$, pH, phosphate buffer content, and molar ratio of tyrosol to D-glucose to be 1% (v/v), 5.9, 20% (v/v), and 8:1, under which the initial reaction rate and yield were 3.3 mmol/(L·h) and 24.5%, respectively. Moreover, the effects of 1-alkylimidazolium-based ILs possessing different alkyl chain lengths from C_2 to C_{10} and a variety of anions including BF_4^-, PF_6^-, Cl^-, Br^-, and I^- on enzyme activity in co-solvent systems were investigated. The results indicate that the optimal chain length of the alkyl substituent on the imidazolium ring of the cation was C_6.

Key words: ionic liquid; salidroside; glucosylation; black plum seed meal; co-solvent

Salidroside, a phenylpropanoid glycoside isolated from the root of the Chinese medicinal plant *Rhodiola sachalinensis* A. Bor., shows many pharmacological properties for the treatment of fatigue, inflammation, anoxia, cardiovascular disease, and cancer [1,2]. However, *R. sachalinensis* is on the edge of extinction due to the over-exploitation. In this sense, several strategies for preparing salidroside were explored [3–5]. Fortunately, enzymatic synthesis of salidroside has been proven to a favorable and practicable option for this purpose owing to its simplicity, high selectivity, simple product isolation, and environmental friendliness compared with the use of cell/tissue culture and organic synthesis [6,7]. Recently, Xu and co-workers [8–11] synthesized salidroside using β-glycosidases from several fruit seeds in co-solvent mixtures with good yields of 13.0%–15.8%.

Ionic liquids (ILs), as a potential replacement for organic solvents, have gained increasing attention for various enzymatic synthetic processes over the past decade as they are known to improve the specificity of the enzyme [12]. Since the first biocatalysis in ILs was reported in 2000, there are numerous examples of biocatalytic reactions that have been successfully carried out in such neoteric media [13]. The great enthusiasm for this methodology may be attributed to the "green" and "designable" properties of the ILs [14].

In many cases, enzymes showed a comparable or even higher activity, stability, and selectivity in IL-containing systems compared with conventional organic solvents [14]. However, very little work has been done on β-glycosidase-mediated preparation of β-D-glucopyranosides in such IL-containing systems. Encouraged by this, the enzymatic synthesis of salidroside was selected as a model reaction to elucidate the characteristics of the β-glycosidase from black plum seeds in a $C_6MIm{\cdot}BF_4$-containing system for the first time. Moreover, the influence of the cations and anions in combination with ILs has been examined (Scheme 1).

1 Experimental

1.1 General procedure for the enzymatic synthesis of salidroside

The different seed meals were prepared as described by Yu et al. [10]. The reaction was performed in a 10 ml Erlenmeyer shaking flask capped with a septum containing 1.6 ml dioxane,

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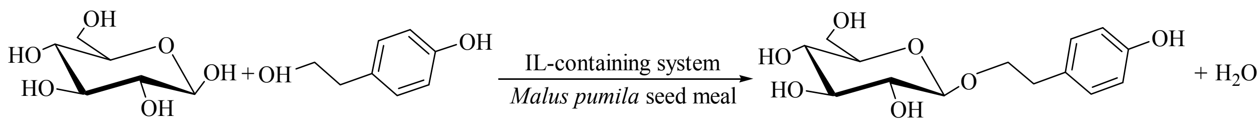

Scheme 1. Enzymatic synthesis of salidroside.

0.4 ml phosphate buffer (pH 6.8, 100 mmol/L), 0.5 mmol D-glucose, 5 mmol tyrosol, and different fruit seed meal at 50 °C and 200 r/min. Aliquots were withdrawn at specified time intervals from the reaction mixture, and then diluted 50 times with the corresponding mobile phase prior to HPLC analysis. One unit of β-glycosidase activity (U) was defined as the amount of enzyme required to produce 1 μmol salidroside in the first hour under the above conditions. The specific activities of the black plum seed meal, apple seed meal, peach seed meal, bitter almond seed meal, and prune seed meal were 27.6, 23.4, 25.9, 13.5, and 19.7 U/g. 1-Ethyl-3-methylimidazolium tetrafluoroborate ($C_2MIm·BF_4$), 1-butyl-3-methylimidazolium tetrafluoroborate ($C_4MIm·BF_4$), 1-hexyl-3-methylimidazolium tetrafluoroborate ($C_6MIm·BF_4$), 1-ethyl-3-methylimidazolium hexafluorophosphate ($C_2MIm·PF_6$), 1-butyl-3-methylimidazolium hexafluorophosphate ($C_4MIm·PF_6$), 1-hexyl-3-methylimidazolium hexafluorophosphate ($C_6MIm·PF_6$), 1-octyl-3-methylimidazolium hexafluorophosphate ($C_8MIm·PF_6$), 1-butyl-3-methylimidazolium chloride ($C_4MIm·Cl$), 1-butyl-3-methylimidazolium bromide ($C_4MIm·Br$), 1-ethyl-3-methylimidazolium iodide ($C_2MIm·I$), 1-propyl-3-methylimidazolium iodide ($C_3MIm·I$), 1-butyl-3-methylimidazolium iodide ($C_4MIm·I$), 1-hexyl-3-methylimidazolium iodide ($C_6MIm·I$), 1-decyl-3-methylimidazolium iodide ($C_{10}MIm·I$), and standard salidroside were purchased from Sigma-Aldrich. Black plum seeds were obtained from a food processing company. Tyrosol was from Aladdin. All other chemicals were from commercial sources and of the highest purity available.

1.2 Reusability

To assess the stability of the enzyme for repeated use, batch synthesis of the salidroside was conducted by the addition of 7.0 U black plum seed meal to 2 ml of a co-solvent system containing 0.02 ml $C_6MIm·BF_4$, 0.4 ml phosphate buffer (pH 5.9, 100 mmol/L), and 1.58 ml dioxane at 50 °C and 200 r/min. The enzyme was recovered by filtration after reaching the maximum product yield, washed with fresh reaction mixture, and then dried. The residual activity was determined for the next batch reaction under the same conditions.

1.3 HPLC analysis

The reaction mixture was analyzed by RP-HPLC on a 4.6 mm × 250 mm (5μm) Zorbax SB-C18 column (Agilent Technologies Industries Co., Ltd., USA) using an Agilent G1311A pump and a UV detector at 275 nm. The mobile phase is a mixture of water and methanol at a flow rate of 1.0 ml/min. The volumetric ratio of water to methanol and the retention time for salidroside were 40/60 and 2.47 min.

2 Results and discussion

2.1 Effect of reaction medium

Several typical organic solvents and $C_6MIm·BF_4$ were examined as alternative media for the synthesis of salidroside by using β-glycosidase from black plum seeds. Table 1 showed that *N*,*N*-dimethylformamide and dimethyl sulfoxide had an inhibitory effect on the activity of the enzyme due to their hydrophilicity in nature. Of the six organic solvents tested, dioxane proved to be the most favorable one with respect to the product yield (14.9%). Interestingly, the enzyme performances were significantly affected in $C_6MIm·BF_4$-containing systems as compared with the other media. For instance, the product yield was substantially enhanced with an increasing concentration of $C_6MIm·BF_4$ up to 1.0%, and a further increase in $C_6MIm·BF_4$ content resulted in a poor outcome. A similar observation was found for the preparation of *N*-acetyllactosamine by β-galactosidase from *Bacillus circulans* [15]. This may be explained by the fact that the high concentration of hydrophilic $C_6MIm·BF_4$ tends to strip more water molecules from the reaction system to hydrate itself, thus leading to a dehydrated enzyme and lower enzyme flexibility [16]. The high ionic strength, substrate ground-state stabilization and poorer mass transfer caused by a high con-

Table 1 Effect of solvents on the enzymatic synthesis of salidroside

Solvent	V_0/ (mmol/(L·h))	Time (h)	Yield (%)
Dimethyl sulfoxide	0.1	48	0.6
Dioxane	1.3	72	14.9
Acetone	1.0	64	4.2
N,*N*-Dimethylformamide	0.1	48	0.4
tert-Butanol	0.8	86	12.3
Tetrahydrofuran	0.3	72	4.2
Dioxane/$C_6MIm·BF_4$ (0.5%, v/v)	1.3	72	16.3
Dioxane/$C_6MIm·BF_4$ (1.0%, v/v)	1.5	72	20.2
Dioxane/$C_6MIm·BF_4$ (1.5%, v/v)	1.4	72	18.9
Dioxane/$C_6MIm·BF_4$ (2.0%, v/v)	1.4	72	18.2
Dioxane/$C_6MIm·BF_4$ (2.5%, v/v)	0.9	80	16.5
Dioxane/$C_6MIm·BF_4$ (3.0%, v/v)	0.6	86	14.2

Reaction conditions: 0.5 mmol D-glucose, 4.0 mmol tyrosol, 7.0 U black plum seed meal, 2.0 ml solvent containing 0.2 ml phosphate buffer (pH 6.8, 100 mmol/L) and various amounts of $C_6MIm·BF_4$, 50 °C, 200 r/min.