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Utility of ionic liquid for improvement of fluorination reaction with immobilized fluorinase

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The utility of immobilized fluorinase derived from fluorinase, KF, SAM and water-absorbing polymer for the improvement of biological fluorination, giving 5′-fluoro-5′-deoxyadenosine from *S*-adenosyl-*L*-methionine, was described. Further, the contribution of ionic liquids in the fluorination reaction with immobilized fluorinase was mentioned.

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1. Introduction

Studies of recyclable catalysts and/or reusable media are having an important impact on organic reactions, and various kinds of reactions in those fields have been reported until now [1–5]. Further, there is a growing interest in the development of the greener chemical processes that are required both now and in the future. The reaction using biocatalysts in ionic liquids has also been developed [6–9]. In our papers [10], we have reported that the advantages of using an ionic liquid as a solvent are the easy separation of the product from the reaction mixture, the easy handling the reaction media and the recyclable use of the reaction system.

In the fluorine chemistry, the fluorination process with fluorinase (5′-fluoro-5′-deoxyadenosine synthase), which was found from *Streptomyces cattleya* (*S. cattleya*), has been having an important impact on the biological synthesis to introduce the fluorine atom onto the carbon atom [11,12]. However, it is not easy to handle in applications because of low reactivity and stability of fluorinase [13]. In view of the development of more advanced processing systems, the challenge to develop practical processes, reaction media and/or conditions is one of the most important issues in biological methodology for the synthesis of fluorinated materials.

In this paper, to increase the conversion yield from SAM to 5′-FDA, we would like to describe the utility of ionic liquid in the fluorination reaction with immobilized fluorinase.

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2. Results and discussion

In the fluorination reaction with fluorinase (200 μl, 1 mg/ml)–Tris–HCl buffer solution (pH 8.0) in the presence of KF (1 mM in H_2O, 2 μmol), the conversion yield from *S*-adenosyl-*L*-methionine (SAM; 50 nmol) to 5′-fluoro-5′-deoxyadenosine (5′-FDA) is 5.7%.

To improve the conversion yield in the above reaction, we have examined the fluorination reaction with fluorinase in an ionic liquid instead of Tris–HCl buffer solution based on our reports for the utility of ionic liquid in organic reactions [10]. However, the fluorination reaction in ionic liquids did not proceed in the absence of water. In our recent papers [14], we have reported the stabilizing effect of the enzyme by keeping water around it using water-absorbing polymer and the utility of ionic liquid in the case of the immobilized biocatalyst. Therefore, in attempting to increase the conversion yield from SAM to 5′-FDA, we have used the immobilized fluorinase (immobilized fluorinase was prepared from fluorinase in Tris–HCl buffer solution (200 μl, 1 mg/ml), KF 2 μmol, SAM 50 nmol, and water-absorbing polymer 20 mg) in ionic liquid (800 μl) or Tris–HCl buffer solution (800 μl). After the fluorination reaction was carried at 37 °C, 5′-FDA was extracted with diethyl ether (10× 10 ml). The product peak and yields were determined by HPLC analyses according to a previous report [11]. As it is well known that it is possible to reuse an ionic liquid as the reaction medium in the same reaction, we have examined to reuse the above reaction system.

After removing 5′-FDA from the reaction mixture with diethyl ether (10× 10 ml), the remained diethyl ether in the reaction mixture was removed under dynamic vacuum, and the residue (from second to third cycles) was incubated for 1 h at 37 °C as shown in

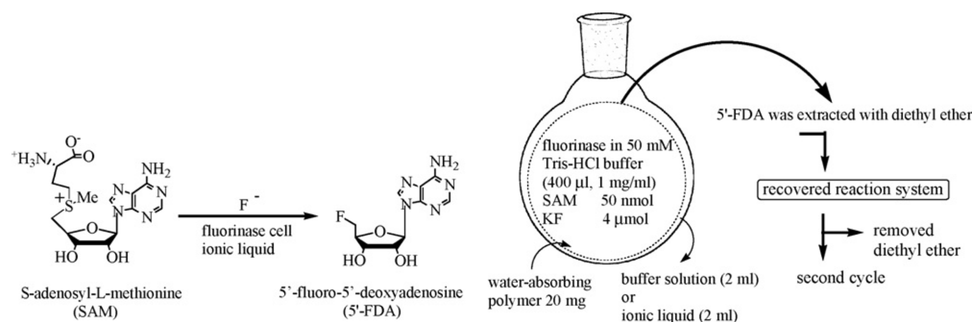

Fig. 1. Fluorination reaction with immobilized fluorinase in solvent.

Table 1
Utility of an ionic liquid with fluorinase (200 µg).

Entry	IL[a] or buffer	Conversion yield (%)[b]			Total	Ratio[c]
		First	Second	Third		
1	[emim][BF_4]	1.7	1.0	0.7	3.4	87
2	[amim][BF4]	1.6	1.2	0.6	3.4	87
3	[bmim][BF_4]	2.8	2.1	0.6	5.5	141
4	[DEME][BF_4]	2.9	1.8	1.2	5.9	151
5	[DEME][BF_4][d]	3.2	1.1	2.0	6.3	162
6	[hexylmim] [BF_4]	4.9	3.3	0.9	9.1	233
7	[bmim][PF_6]	2.7	0.3	0.1	3.1	79
8	[octylmim][PF_6]	5.5	–[e]	–[e]	5.5	141
9	[emim][OTf]	1.3	0.9	0.4	2.6	67
10	PP13 TFSA	1.8	–[e]		1.8	46
11	[Bu_3P(C12)][BF_4]	–[e]				
12	[bmim][TFSA]	–[e]				
13	[DEME][TFSA]	–[e]				
14	Tris–HCl buffer	3.4	0.5	–[e]	3.9	100

[a] [emim][BF_4]: 1-ethyl-3-methylimidazolium tetrafluoroborate; [amim][BF_4]: 1-allyl-3-methylimidazolium tetrafluoroborate; [bmim][BF_4]: 1-butyl-3-methylimidazolium tetrafluoroborate; [DEME][BF_4]: *N*,*N*-diethyl-*N*-methyl-*N*-(2-methoxyethyl) ammonium tetrafluoroborate; [hexylmim][BF4]: 1-hexyl-3-methylimidazolium tetrafluoroborate; [bmim][PF_6]: 1-butyl-3-methylimidazolium hexafluorophosphate; [octylmim][PF_6]: 1-octyl-3-methylimidazolium hexafluorophosphate; [emim][OTf]: 1-ethyl-3-methylimidazolium trifluoromethanesulfonate; PP13 TFSA: *N*-methyl-*N*-propylpiperidinium bis(trifluoromethanesulfonyl)amide; [Bu_3P(C12)][BF_4]: tributyl(dodecyl)phosphonium tetrafluoroborate; [bmim][TFSA]: 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)amide; [DEME][TFSA]: *N*,*N*-diethyl-*N*-methyl-*N*-(2-methoxyethyl) ammonium bis(trifluoromethanesulfonyl)amide.

[b] Reaction condition: immobilized cell was prepared from fluorinase (1 mg/ml, 200 µl), 2 µmol KF, SAM 50 nmol, and water-absorbing polymer 20 mg, 37 °C, 6 h, Tris–HCl buffer or ionic liquid (800 µl).

[c] Ratio of total conversion yield of ionic liquid/Tris–HCl buffer.

[d] Reaction condition: immobilized cell was prepared from fluorinase 400 µg (1 mg/ml, 400 µl), 4 µmol KF, SAM 50 nmol, and water-absorbing polymer 20 mg, 37 °C, 6 h, ionic liquid (2 ml).

[e] 5′-FDA was not isolated.

Table 1. From the results shown in Table 1, we have found that some ionic liquids are the convenient for the improvement of total conversion yields to increase more than that of non-immobilized fluorinase, and that the ratio (fluorinase, SAM, KF, ionic liquid and Tris–HCl buffer solution) is an important factor as shown in Table 1 (entries 4, 5 and 14). Further, in the case of ionic liquids (entries 11–13), it is impossible to detect 5′-FDA in the mixture solution of ionic liquid and product by HPLC.

In the next step, we have examined to use twice amount of fluorinase (400 µg) and KF (4 µmol) for the preparation of immobilized fluorinase. The fluorination reaction using one of the immobilized fluorinases prepared from fluorinase in Tris–HCl buffer solution (400 µl, 1 mg/ml), KF 4 µmol, SAM 50 nmol, and water-absorbing polymer 20 mg, was carried in ionic liquid (2 ml) and/or Tris–HCl buffer solution (2 ml) at 37 °C for 1 h shown as in Fig. 1.

Table 2
Utility of an ionic liquid in fluorination with fluorinase (400 µg).

Entry	IL or buffer	Conversion yield (%)[a]					Total	Ratio[b]
		First	Second	Third	Fourth	Fifth		
15	[DEME][BF_4]	2.3	2.5	1.4	1.0	1.3	8.5	90
16	[hexylmim][BF_4]	4.7	3.2	1.8	1.3	1.5	12.5	133
17	[octylmim][BF_4][c]	4.5	3.8	2.6	1.1	0.7	12.7	135
18	[octylmim][PF_6]	19.8	2.6	0.4	–[d]	–[d]	22.8	243
19	[hexylmim][PF_6][c]	12.9	2.7	0.3	–[d]	–[d]	15.9	163
20	Tris–HCl buffer	2.5	3.5	2.7	0.5	0.2	9.4	100

[a] Reaction condition: immobilized cell was prepared from fluorinase in Tris–HCl buffer solution (1 mg/ml, 400 µl), 4 µmol KF, SAM 50 nmol, and water-absorbing polymer 20 mg, 37 °C, 1 h, ionic liquid (2 ml).

[b] Ratio of total conversion yield of ionic liquid/Tris–HCl buffer.

[c] [octylmim][BF_4]: 1-octyl-3-methylimidazolium tetrafluoroborate; [hexylmim][PF_6]: 1-hexyl-3-methylimidazolium hexafluorophosphate.

[d] 5′-FDA was not isolated.

In the above immobilized fluorinase system, it is possible to recycle the reaction system for the increase of the conversion yield of fluorination reaction up to 9.4% as shown in Table 2. In the fluorination with the immobilized fluorinase in ionic liquids [octylmim][PF_6] and [hexylmim][PF_6], we have found that the conversion yield was increased up to 2.4 and/or 1.6 times more than that of Tris–HCl buffer solution (pH 8.0). Furthermore, we have found that the ionic liquids ([hexylmim][BF_4], [octylmim][BF_4], [octylmim][PF_6] and/or [hexylmim][PF_6], entries 16–19) are convenient for the improvement of total conversion yields to increase up to 2.2–4 times more than that of non-immobilized fluorinase. In the above system, it seems that the transformation of 5′-FDA from the immobilized fluorinase to ionic liquid layer such as the immiscible hydrophobic ionic liquids ([octylmim][PF_6] and [hexylmim][PF_6]), is the driving force to increase the conversion yield. Further, in the use of more miscible ionic liquids or Tris-buffer solution, it seems that the transformation of SAM from the immobilized fluorinase to ionic liquid layer is more easier than that of the immiscible hydrophobic ionic liquids. Therefore, the higher concentration of SAM in the immiscible hydrophobic ionic liquids may result in the higher conversion yield in the first run.

3. Conclusion

In conclusion, we have established an improvement of the conversion yield for the fluorination with the immobilized fluorinase derived from fluorinase, KF, SAM, water-absorbing polymer and Tris–HCl buffer solution (pH 8.0) in an ionic liquid, giving 5′-fluoro-5′-deoxyadenosine from *S*-adenosyl-*L*-methionine. This is the first example for the improvement biological fluorination with the immobilized fluorinase in an ionic liquid.

4. Experimental

4.1. Materials and methods

4.1.1. Bacterial strains and plasmids

Escherichia coli BL21(DE3) pLysS were used for fluorinase production. The fluorinase gene (*flA*) was amplified from the *S. cattleya* NBRC14057 genome using primers reported previously [11]. Only a single, silent mutation in *flA* was detected by CEQ8000 DNA analysis (Beackman Coulter). The *flA* was cloned in pET28b+ (Novagen) and consequently fused to the His-tag-containing peptide in the N-terminal of the enzyme, termed pETflA. The *flA* protein was purified by Ni^{2+}-affinity column.

4.1.2. First cycle

The *flA* protein (400 μl, 1 mg/ml) in 50 mM Tris–HCl buffer (pH 8.0), 50 nM of *S*-adenosyl-*L*-methionine and KF in water (1 mM in H_2O, 4 μl) were mixed, and then the water-absorbing polymer (BL-100, 20 mg) was added into the above mixture and then stirred quickly. An ionic liquid (2 ml) was added to the resulting immobilized *flA* protein, and then the mixture was stirred for 1 h at 37 °C, and then the 5′-FDA was extracted with diethyl ether (10× 10 ml), and analyzed by HPLC (column; shim-pack VP-ODS 150L x 4.6; SHIMADZU). After the remained diethyl ether in the reaction system was removed under dynamic vacuum, the recovered reaction system was used in the second cycle.

4.1.3. Second cycle

After the recovered reaction system was stirred for 1 h at 37 °C, the product (5′-FDA) was extracted with diethyl ether (10× 10 ml), and analyzed by HPLC. After the system was worked up similarly, the recovered reaction system was reused in the third cycle.

The third, fourth and/or fifth cycle was worked up similarly.

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