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# An insight into the solvent effect on the positional selectivity of the immobilized lipase from *Burkholderia cepacia* in 1,3-diolein synthesis†

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The solvent effect on the positional selectivity of the immobilized lipase from *Burkholderia cepacia* in 1,3-diolein synthesis was investigated for the first time. The results indicated that the preferential selectivity to *sn*-1 hydroxyl of the glycerol molecule over *sn*-2 hydroxyl was weaker in solvents with higher log *P* values.

The consumption of 1,3-diacylglycerol oil has beneficial effects on suppressing the accumulation of body fat and preventing the increase of body weight.[1–4] The enzyme-mediated 1,3-diacylglycerol preparation has attracted more and more attention because of its mild reaction conditions and safe products.[5–9] However, the positional isomer 1,2-diacylglycerol may be generated simultaneously. Consequently, the positional selectivity of the enzyme employed is important for high yield of 1,3-diacylglycerol.

Since the reaction medium could exert a profound influence on the enzyme selectivity, a great many research articles have focused on the effect of the solvent physico-chemical properties on the enzyme selectivity. It has been demonstrated that, among all the reported physico-chemical properties, log *P* (the logarithm of the partition coefficient of a solvent in the standard octanol–water two-phase system) was found to be more influential.[10–15]

As far as we know, an insight into the influence of log *P* of the solvent on the positional selectivity of the immobilized lipase from *Burkholderia cepacia* (PSL-C) during 1,3-diolein synthesis by the esterification of oleic acid with glycerol has not been reported. In light of this, we innovatively present here the study of the effect of the reaction media on the positional selectivity of PSL-C.

Different solvents with log *P* ranging from −0.23 to 4.5 were screened as the reaction media for PSL-C-catalyzed esterification of oleic acid with glycerol with the aim of preparing 1,3-diolein. Diolein yield, including 1,3-diolein and 1,2-diolein, was presented in Table 1 column 3. As can be seen, although the reaction can proceed in all the solvents employed, the diolein yield markedly depended on the reaction medium. The lowest diolein yield (58.8%) was obtained in acetone the log *P* value of which was the minimum (−0.23). The moderate diolein yields were observed in the relatively hydrophilic solvents such as tetrahydrofuran, *t*-butanol, 4-methyl-2-pentanone and chloroform. Six tested relatively hydrophobic solvents (toluene, tetrachloromethane, cyclohexane, *n*-hexane, *n*-heptane and *n*-octane) showed higher diolein yields. The best results with 88.5% yield of diolein were achieved when *n*-heptane and *n*-octane were used as the reaction media. The higher enzyme activity involved in this case might be attributable to the fact that the solvents with higher log *P* value could retain the microenvironment moisture around the catalytic active site of PSL-C more, which can maintain the original conformation of the enzyme, thus preventing its deactivation due to the loss of essential water.[16–20]

However, it was worth noting that, although the ratio values of 1,3-diolein to 1,2-diolein which were listed in Table 1 column 4 were also affected by the reaction media remarkably, the trend was reversed compared with the one of the diolein yields with the increasing log *P* of the solvent. The highest ratio values of 1,3-diolein to 1,2-diolein (31.1) was achieved in acetone which was the most hydrophilic one among all the solvents used. And then the ratio of 1,3-diolein to 1,2-diolein descended dramatically along with the increase of the log *P*. The minimal ratio of 1,3-diolein to 1,2-diolein was 7.1 which happened in the most hydrophobic solvent *n*-octane. In particular, the ratio value of 1,3-diolein to 1,2-diolein was still affected by the solvent noticeably in six relatively hydrophobic solvents (toluene, tetrachloromethane, cyclohexane, *n*-hexane, *n*-heptane and *n*-octane), in which the diolein yield was influenced slightly. It is universally acknowledged that the ratio of 1,3-diolein to

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Table 1 Solvent effect on the enzymatic 1,3-diolein synthesis

| Solvent | log *P* | Diolein yield (%) | 1,3-Diolein /1,2-diolein | $k_1$ | $k_3$ | $k_9$ | $k_{11}$ | $k_1/k_9$ | $k_3/k_{11}$ |
|---|---|---|---|---|---|---|---|---|---|
| Acetone | −0.23 | 58.8 | 31.1 | 0.691 | 0.754 | 0.048 | $4 \times 10^{-5}$ | 14.40 | 18 850 |
| Tetrahydrofuran | 0.49 | 75.3 | 25.8 | 1.004 | 1.036 | 0.097 | $8 \times 10^{-4}$ | 10.35 | 1295 |
| *t*-Butanol | 0.8 | 81.1 | 22.6 | 1.142 | 1.251 | 0.121 | 0.003 | 9.44 | 417.0 |
| 4-Methyl-2-pentanone | 1.31 | 82.5 | 17.8 | 1.156 | 1.384 | 0.145 | 0.008 | 7.97 | 173.0 |
| Chloroform | 2.0 | 85.0 | 12.1 | 1.212 | 1.423 | 0.161 | 0.016 | 7.53 | 88.9 |
| Toluene | 2.5 | 87.3 | 10.2 | 1.307 | 1.466 | 0.229 | 0.024 | 5.71 | 61.1 |
| Tetrachloromethane | 3.0 | 87.9 | 8.9 | 1.395 | 1.701 | 0.311 | 0.034 | 4.49 | 50.0 |
| Cyclohexane | 3.2 | 88.2 | 8.7 | 1.485 | 1.932 | 0.476 | 0.041 | 3.12 | 47.1 |
| *n*-Hexane | 3.5 | 88.3 | 8.1 | 1.553 | 1.994 | 0.632 | 0.046 | 2.46 | 43.3 |
| *n*-Heptane | 4.0 | 88.5 | 7.6 | 1.571 | 2.016 | 0.846 | 0.049 | 1.86 | 41.1 |
| *n*-Octane | 4.5 | 88.5 | 7.1 | 1.692 | 2.268 | 0.918 | 0.059 | 1.84 | 38.4 |

1,2-diolein was subject to the preferential selectivity of PSL-C towards *sn*-1 hydroxyl over *sn*-2 hydroxyl of the glycerol molecular and the acyl-migration between *sn*-1 hydroxyl and *sn*-2 hydroxyl (which was neglected because of the little contribution, data not shown). As reported, the secondary structure of PSL-C varied with the organic solvents used, which could affect the access of the substrates to the active site of PSL-C.[19,20] Accordingly, the secondary structure changes of PSL-C may be contributed to the positional selectivity variations based on medium engineering. Additionally, when six relatively hydrophobic solvents served as the reaction media, diolein yields were about the same, while the ratios of 1,3-diolein to 1,2-diolein were changed significantly. Especially, the ratios of 1,3-diolein to 1,2-diolein were higher in the solvents with ring structure and lower in the hydrocarbon solvents with longer carbon chain. This observation suggested that the molecular constitution of the organic solvent may also play an important role in affecting the positional selectivity of PSL-C, which agreed with that reported by Liu *et al.*, who demonstrated that the structures and functional groups of the employed solvents exerted an influence on the behavior of PSL-C as well.[19,20]

The reaction kinetics was an efficient tool for understanding the influence of solvent on the positional selectivity of lipase which has been proved by our previous work.[14] Hence, an interesting investigation on the solvent effect on the positional selectivity of PSL-C from the points of view of kinetics was performed subsequently.

The enzymatic reaction rate constants were identified by solving the rate equations described in ESI with an adaptive step-size Runge–Kutta method within a nonlinear regression procedure using the Levenberg–Marquardt algorithm, with the aim of obtaining the best fit between the experimental data and the results calculated.[14] The rate constants which were relevant to the positional selectivity of PSL-C were presented in Table 1.

From Table 1 columns 5–8, it can be clearly seen that $k_1$, $k_3$, $k_9$ and $k_{11}$ were increased with the increasing log *P* of the solvent. It was account for that the diolein yield was enhanced obviously along with the increase of the log *P*. $k_1$ was much bigger than $k_9$ for all the solvents used. It was indicated that the selectivity of PSL-C to *sn*-1 hydroxyl of the glycerol molecular was much higher than the one to *sn*-2 hydroxyl in each solvent. But we must pay attention to the fact that the increasing times of $k_1$ were much smaller than the ones of $k_9$ with the increasing log *P* of the solvent. As a result, $k_1/k_9$ was decreased from 14.40 to 1.84. It was demonstrated that the preferential selectivity of PSL-C to *sn*-1 hydroxyl over *sn*-2 hydroxyl decreased with the increase of the log *P*. While the solvent were *n*-heptane and *n*-octane, $k_1/k_9$ values were only 1.86 and 1.84, respectively. Since *sn*-1 hydroxyl amount was twice *sn*-2 hydroxyl in the glycerol molecular, when $k_1/k_9$ value was close to 2.00, it can be considered that the selectivity of PSL-C to *sn*-1 hydroxyl and *sn*-2 hydroxyl was almost equivalent. It is of great benefit to preparing 2-monoolein and 1,2-diolein which are usually used as the emulsifiers and surfactants in the food industry.

Moreover, although the increasing times of $k_3$ were much smaller than the ones of $k_{11}$ with the increasing log *P* of the solvent, the values of $k_3$ were still much bigger than the corresponding ones of $k_{11}$, which resulted in that $k_{11}$ can be approximately ignored. It was indicated that the selectivity of PSL-C to *sn*-1 hydroxyl of the 1-monoolein molecular (the intermediate for 1,3-diolein synthesis) was much higher than the one to *sn*-2 hydroxyl for each solvent. Additionally, an interesting observation was that the values of $k_3/k_{11}$ were much bigger than the ones of $k_1/k_9$ in all the tested solvent. It could be suggested that when one *sn*-1 hydroxyl of the glycerol molecular was acylated forming 1-monoolein, the preferential selectivity of PSL-C to the other *sn*-1 hydroxyl over *sn*-2 hydroxyl would be increased markedly.

In view of all the results acquired above, it can be concluded that when the solvent with higher log *P* value served as the reaction medium, the achieved diolein yield was higher, while the preferential selectivity of PSL-C to *sn*-1 hydroxyl of the glycerol molecular over *sn*-2 hydroxyl was weaker which led to the lower ratio of 1,3-diolein to 1,2-diolein. Therefore, the relatively hydrophilic solvent was considered as the most suitable reaction medium for PSL-C-catalyzed synthesis of 1,3-diolein after scrutinizing the data tabulated in Table 1.

Compared with the results obtained by Novozym 435 in our previous work,[14] the maximal diolein yield reduced by 2.7% (88.5% *vs.* 91%) and the highest ratio value of 1,3-diolein to 1,2-diolein was decreased by 3.7% (31.1 *vs.* 32.3). Although both values were a little lower, PSL-C was still considered as an excellent and promising biocatalyst which could be beneficial for the rational production of 1,3-diolein.

## Conclusions

In summary, this work presented an insight into the solvent effect on the positional selectivity of PSL-C during 1,3-diolein synthesis by esterification of oleic acid with glycerol. It may be very helpful for investigating the influence of the environment on the enzyme selectivity in biocatalysis field.

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