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Phospholipase A_2-Catalyzed Transesterification of Phosphatidylcholine with Nervonic Acid in Organic Solvent

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Abstract The phospholipase A_2-catalyzed transesterification of phosphatidylcholine (PC, 95%) with nervonic acid (NA, 95%) was successfully carried out in an organic solvent. The maximum yield after 48 h was 10.3% (w/w) at 50°C with an initial water activity (a_w) of 0.16, and a molar ratio of NA to PC of 20 in 5 ml ethyl acetate.

Key words: Phospholipase A_2, transesterification, nervonic acid, phosphatidylcholine

Many researchers have reported that the fatty acids incorporated into phosphatidylcholine (PC) at the *sn*-2 position are more easily digested by the human body than the fatty acids incorporated into triglycerides at the *sn*-2 position [4]. Based on this premise, the incorporation of polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into PC was attempted by esterification by phospholipase A_2 (PLA_2) [4]. However, the esterification process is known to have a few drawbacks, such as a two-phase process and the high cost of lysophosphatidylcholine (LPC). In order to circumvent these drawbacks, we successfully transesterified PC with EPA ethyl ester using PLA_2 [5].

Nervonic acid (NA), one of the major fatty acids in brain sphingolipid, affects development of the brain and the central nerve system [3] and is related to multidrug-resistant tumors and cancer cells [2]. In this study, we tried to optimize reaction conditions for the direct transesterification of PC with NA using PLA_2 in the organic solvent system. Details of materials and methods have been described by Park *et al.* [5].

The PLA_2-catalyzed transesterification of PC with NA was carried out in various organic solvents including toluene, *n*-hexane, butyl acetate, diethyl ether, and ethyl acetate. The PLA_2 was found to be quite stable in various solvents,

Table 1. Transesterification yield of phosphatidylcholine with nervonic acid in 5 ml organic solvents as reaction media.

Solvent	Transesterification yield % (w/w)
Butyl acetate	1.52±0.2[a]
Diethyl ether	1.73±0.1
Ethyl acetate	2.31±0.1
n-Hexane	N.S.[b]
Toluene	N.S.

The reaction conditions were 30 mg PC, 20 mg NA, 10 mg PLA_2, 50°C, 24 h, and 300 rpm.
[a]S.D. based on triplicate samples.
[b]N.S. designates 'Not Significant'.

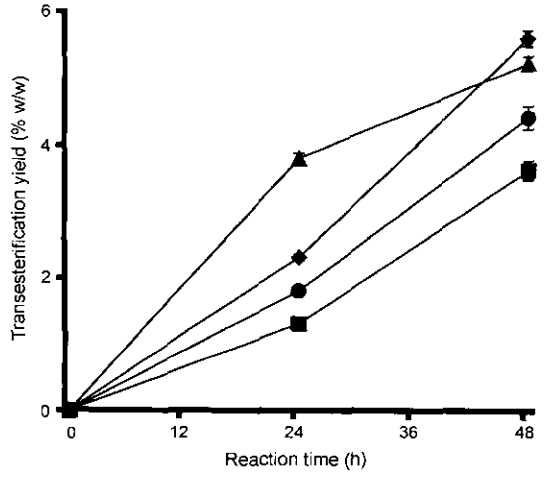

Fig. 1. Effect of reaction temperature on incorporation of 20 mg nervonic acid (NA, 95%) into 30 mg phosphatidylcholine (PC, 95%) at 24 h, 10 mg (11 U/mg) phospholipase A_2, 300 rpm in 5 ml ethyl acetate. (■, 40°C; ●, 45°C; ◆, 50°C; ▲, 55°C)

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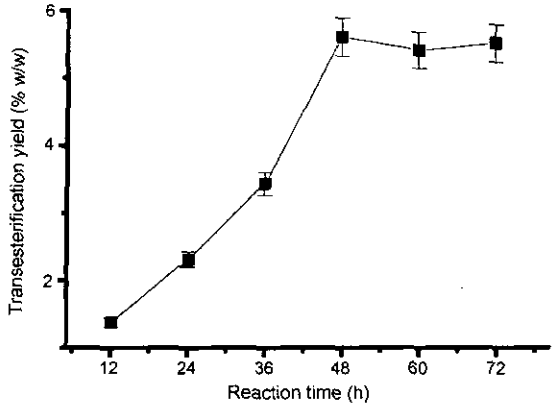

Fig. 2. Effect of reaction time on transesterification of 30 mg phosphatidylcholine (PC, 95%) with 20 mg nervonic acid (NA, 95%) by phospholipase A_2 (PLA_2, 10 mg) at 50°C, 300 rpm in 5 ml ethyl acetate.

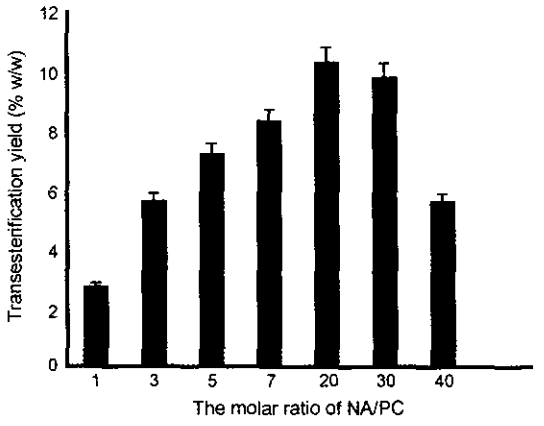

Fig. 4. Effect of the molar ratio of phosphatidylcholine to nervonic acid on synthesis of *sn*-2 NA-containing PC. (PC 3.0×10^{-5} mol constant).

as reported by Pernas *et al.* [6], and ethyl acetate was proved to be the most suitable reaction medium among the organic solvents tested (Table 1). Although polar organic solvents are generally known to remove enzyme-associated water, which is very important to the enzyme structure and activity [7], our results demonstrated that a higher transesterification yield was obtained with a more polar solvent, such as ethyl acetate. Goldberg *et al.* [1] suggested that it is absolutely necessary to consider the polarity of every substrate in transesterification because of its ability to modify the water partition between the enzyme phase and the substrate and product phase, thereby resulting in favorable changes in the enzyme activity.

The effect of reaction temperature on the transesterification of PC with NA in ethyl acetate was also investigated (Fig. 1). The results indicated that the maximum reaction rate and yield were at 50°C after 48 h of reaction. The decrease in the reaction rate and yield above 50°C was due to decrease in the enzyme stability (data not shown), as in the case of our previous study on the transesterification of PC with EPA ethyl ester [5].

We also studied the effect of the reaction time on the PLA_2-catalyzed transesterification of PC with NA in ethyl acetate at 50°C (Fig. 2). The incorporation of NA increased as the reaction time increased up to 48 h, and thereafter remained constant.

Water activity is one of the most important factors on the enzymatic synthesis in the organic solvent. Figure 3 shows that a water activity of 0.16 was optimal. At zero water activity, no products were formed (data not shown), thereby indicating that water was essential for enzyme stability and activity. Above an a_w of 0.16, the incorporation yield decreased due to the hydrolysis of PC.

To investigate the optimal reaction molar ratio of the acyl donor (NA) to the acyl acceptor (PC), the molar ratio was varied in each reaction (Fig. 4). The results showed that the reaction rate and yield gradually increased as the molar ratio of NA to PC increased up to 20, and thereafter decreased. The reason for this decrease was possibly due to substrate (NA) inhibition [4].

In conclusion, 10.3% (w/w) of *sn*-2 NA-containing PC was obtained at 50°C, after 48 h, with an initial a_w of 0.16 and an NA to PC molar ratio of 20.

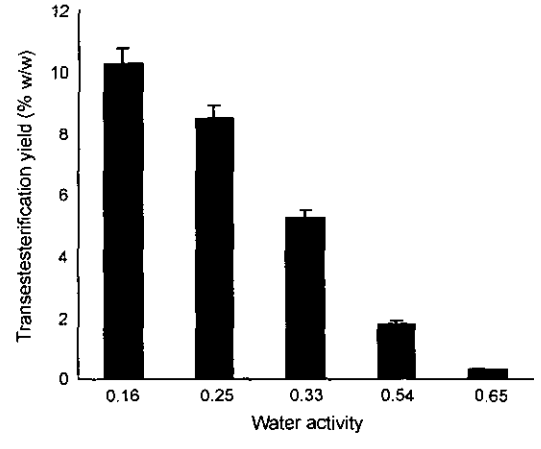

Fig. 3. Incorporation of 20 mg nervonic acid (NA, 95%) into 30 mg phosphatidylcholine (PC, 95%) as a function of water activity at 48 h, 50°C, 300 rpm in 5 ml ethyl acetate.

Acknowledgments

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