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Properties of the Microinterface formed by Phosphatidylcholine and 1-Butanol as Reaction Media of Hydrolysis of Phosphatidylcholine

Keiju Yamazaki*†, Masanao Imai and Isao Suzuki**
Course in Bioresource Utilization Sciences,
Graduate School of Bioresource Sciences,
Nihon University
1866 Kameino, Fujisawa 252-8510
JAPAN
****Phone & Fax: +81-466-84-3978**
***E-mail: gr46046f@brs.nihon-u.ac.jp**

Abstract

Microinterface of W/Omicroemulsion prepared by phosphatidylcholine was used as reaction media of hydrolysis of phosphatidylcholine by phospholipaseA_2. Phosphatidylcholine was used as an amphiphile and was acted as a substrate. Organic phase of W/Omicroemulsion in this study was prepared by mixed organic solvents i.e. 2,2,4-trimethylpentane (isooctane) as a main solvent and 1-butanol as a co-solvent. The effect of added 1-butanol was remarkable not only on reaction beginning but also on high reaction rate. The hydrolysis reaction was dramatically initiated when 1-butanol was injected into the running isooctane/PC system. The enhancement by 1-butanol addition into single organic solvent was our original finding compare with previous conventional organic solvent. The reaction rate was elevated by the added amount of 1-butanol. The enhanced reaction rate was about 150-folds. This enhancement was speculated as 1-butanol adsorption on the microinterface. The adsorbed 1-butanol improved the properties of microinterface, especially its mobility was increased by difference of the chain length between phosphatidylcholine and 1-butanol. PhospholipaseA_2 molecules were located on the microinterface due to modified mobility of microinterface. Located phospholipaseA_2 on the microinterface reacted easily with phosphatidylcholine molecule. As a result high reaction rate was obtained. Microinterfacial properties were successfully improved by adsorbed 1-butanol molecule, and were favorable to appear higher reactivity of phospholipaseA_2.

1. Introduction

Phosphatidylcholine (PC: 1,2-diacyl-*sn*-phosphoglycerides) has been widely utilized as a biocompatible emulsifier for food additive and cosmetics. PC has an unsaturated fatty acid chain at the *sn*-2-position which is oxidized easily. Chemical modification to stabilize of PC molecules is important subject for wide application and utilization.

In this investigation, PC was modified using phospholipaseA_2 (PLA_2) which catalyze at the *sn*-2-position of PC molecule. PC was known as a water immiscible substrate[1]. So, it needed to catalyze in organic phase instead of water phase.

Enzymatic reaction e.g. lipase and phospholipases using water immiscible

†: Research Fellow of the Japan Society for the Promotion of Science

substrate was carried out in W/Omicroemulsion system[2-4]. The organic phase of presented papers was used several kinds of organic solvents e.g. Hexane, Diethylether and 2,2,4-trimethylpentane (isooctane). Especially, isooctane was often used as an organic phase, because of its higher reactivity[5].

For the hydrolysis reaction of PC by PLA_2, isooctane was used as an organic phase. In most of presented papers, PC was hydrolyzed in W/Omicroemulsion with additive amphiphile[3] e.g. AOT and Span to obtain higher reaction rate. Reaction rate was increased when the amphiphile was added into the reaction media.

In this investigation, reaction media without additive amphiphile was designed by isooctane as a main solvent, alcohols as a co-solvent and PC as not only substrate but also amphiphile. The improvement of the microinterfacial property by adding 1-butanol was investigated, and the correlation between the microinterfacial property and the reaction rate was considered.

2. Materials and Methods

Hog pancreas PhospholipaseA_2 (568 U/mg) was purchased from Fluka (Buchs). The molecular weight was 14kDa. EPIKURON 200 was used not only substrate of phospholipaseA_2 but also amphiphile. It was distributed by Lucas Meyer (Hamburg). The assay of phosphatidylcholine demonstrated an effective up to 95% purified. Phosphatidylcholine acted as a substrate and an amphiphilic component, forming the W/O microemulsion. No other artificial amphiphilic component was added in this investigation.

The organic phase in the W/O microemulsion was prepared by isooctane (C_8*) as the main solvent and 1-butanol (C_4) as the co-solvents. Water phase was employed 0.05M Tris-HCl buffer (0.1M $CaCl_2$)(pH8.0 at 313K). These were analytical grade reagents purchased from Wako Pure Chemical, Ltd. (Osaka). Experimental organic solvents were produced by mixing a molar ratio of main solvent to co-solvent of 11:X.

3. Result and Discussion

3-1. Design of the reaction media employing isooctane and 1-butanol

Hydrolysis reaction of phosphatidylcholine by PLA_2 was performed in isooctane/PC system which was alcohol-free system. The system had very slight increase of hydrolysis production (Fig.1), and the solution was very high viscosity. On the other hand, when 1-butanol was directly injected into the running isooctane/PC system after waiting 30 minutes for the hydrolysis to advance, then the hydrolysis initial reaction rate was enhanced, and the solution became lower viscosity. By injecting 1-butanol, hydrolysis was successfully initiated and the reaction rate was dramatically enhanced.

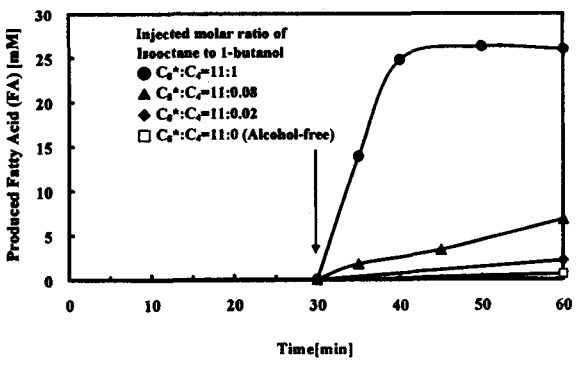

Fig. 1 Time course of hydrolysis of phosphatidylcholine by phospholipaseA_2.

3-2. Determination of the suitable mixed molar ratio of isooctane to 1-butanol

Initial reaction rate was remarkably enhanced in the molar ratio of isooctane:1-butanol(X)=11:0.05 to 0.2 (Fig.2).

More than the molar ratio of butanol=0.2, the increasing of initial reaction rate was leveled off. It reached ca. 150-folds. These findings strongly suggested that alcohol is a necessary co-solvent for quick initiation, even in small addition amounts. Furthermore, the minimum added amount of 1-butanol to enhance hydrolysis reaction rate was obtained. This initiation and enhancement was speculated as the improvement of the microinterface by 1-butanol adsorption onto the microinterface[6].

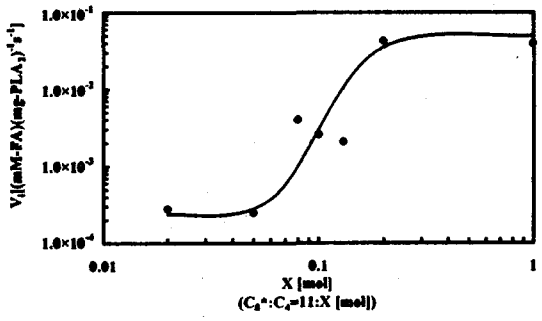

Fig. 2 Effect of the molar ratio of isooctane to 1-butanol on initial reaction rate.

3-3. The property changing of the microinterface mobility by adding 1-butanol

Fig.3 showed correlation between initial reaction rate and the molar fraction of 1-butanol to PC. Initial reaction rate was dramatically enhanced from the fraction of 1 to 4. Initial reaction rate was leveled off when the molar fraction of 1-butanol/PC was larger than 4. These results agreed well with the speculation about 1-butanol adsorbed onto the microinterface.

It was suggested that in less than the fraction was1.0, major component on microinterface was PC molecule. In this condition, PC made microemulsion droplets formed as rod like. And microemulsion solution had high viscosity. Mobility of the local condition on microinterface became lower. Therefore, PLA_2 could not locate on the interface. As a result, lower initial reaction rate was obtained. On the other hand, when the molar fraction of 1-butanol/PC was from 1 to 4, Mobility of the microinterface gradually improved. According to improvement of mobility, the initial reaction rate was enhanced. Finally, when the molar fraction was larger than 4, mobility became sufficiently high, the initial reaction rate was enough higher. The change of microinterfacial property was caused by the difference of the hydrophobic chain length between acyl chain of phosphatidylcholine and carbon chain of 1-butanol. By improving the properties of microinterface, enzymatic reactivity would be enhanced not only phospholipaseA_2 but also lipase.

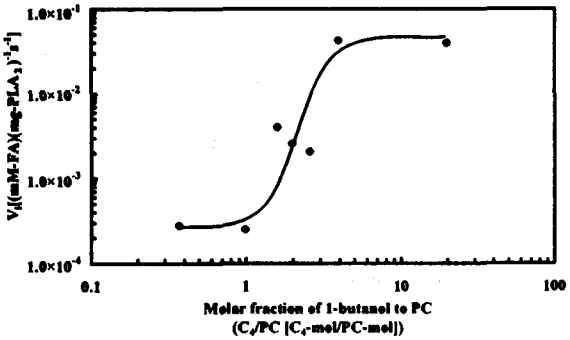

Fig. 3 Correlation between initial reaction rate and molar fraction of 1-butanol to PC.

4. Conclusion

Property of the microinterface formed by phosphatidylcholine and 1-butanol as reaction media of hydrolysis of Phosphatidylcholine was investigated.

Quick initiation and very successfully reaction rate of hydrolysis of phosphatidylcholine by phoshpolipaseA_2 could be observed in isooctane/1-butanol/PC system contrast to isooctane/PC alcohol-free system. By injecting 1-butanol into isooctane/PC system, W/Omicroemulsion as reaction media formed by phosphatidylcholine was improved. More effective amount of added 1-butanol was

determined more than X>0.2. By addition of 1-butanol, it adsorbed onto the microinterface which was formed by phosphatidylcholine. Then, mobility of the microinterface was improved, and PLA_2 could locate on the microinterface. As a result, higher reaction rate was obtained. As a concluding remarks, the enhancement of the reaction rate was caused by the property of the mobility changing of the microinterface which was formed by phosphatidylcholine.

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Notation

C_4: 1-butanol
C_8*: 2,2,4-trimethylpentane (isooctane)
FA: Produced free fatty acid
PC: Phosphatidylcholine
(1,2-diacyl-*sn*-phosphoglycerides)
PLA_2: PhospholipaseA$_2$
V_i: Initial reaction rate [mM-FA(mg-PLA_2)$^{-1}$s^{-1}]

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