Polyacrylate-Graft Silica Gel as a Support of Lipase Interesterifying Triacylglycerol in Organic Solvent

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Synopsis

Butyl acrylate was graft polymerized onto silica gel, which had been doubly modified with γ-aminopropyltriethoxysilane and 4,4′-azobis(4-cyanovaleric acid). 2-(Methoxycarbonyl)ethyltrichlorosilane was treated on another silica gel. The ester groups on both silica gel surfaces were transformed to azide to immobilize lipase. The activities of the immobilized lipase for interesterification of triacylglycerol in isopropyl ether were examined and compared with that of adsorbed lipase on silica gel, and the polyacrylate-graft silica gel was found to be an effectual support for the enzymatic interesterification. An apparent shift of the reaction equilibrium by removing water as an azeotrope of isopropyl ether is also described.

INTRODUCTION

Lipolytic enzymes immobilized with a small amount of water have attracted increasing attention, as they catalyze nonhydrolytic specific reactions.[1] In these enzymatic reaction systems, several kinds of inorganic powders were examined as a dispersing aid of enzyme to catalyze the reaction effectively. Repeated uses of the immobilized enzyme, however, caused desorption from the surface. Attempts to diminish the desorption were made by coating with polyoxyethylene networks.[2]

Biphasic reaction system of water/water-immiscible organic solvent has been incorporated into enzymatic reaction.[3] Feasibility of the reaction equilibrium shift was pointed out by Martinek et al.,[4] who demonstrated that an apparent equilibrium constant for enzymatic ester formation in a biphasic system depends on the volume ratio of the aqueous and organic solvent. Therefore, the microenvironmental effects of immobilized enzyme on equilibrium shift have been of interest to us.

In this article, procedures to immobilize lipase on three kinds of silica gels are presented. Characteristics about triacylglycerol interesterification by the immobilized lipases will also be discussed in correlation with apparent equilibrium shift.

EXPERIMENTAL

Ultraviolet (UV) and infrared (IR) spectroscopic analyses were conducted with a Hitachi UV spectroscopy U-202 and with a Japan Spectroscopic IR spectrometer 320. Scanning electron microscopic observation was made with a

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JEOL electron micrograph model 650. Reaction products were determined with a Hitachi gas chromatograph 163 using FID detector and 2 m OV-17 packed column for tributanoylglycerol reaction system or 0.5 m OV-1 packed column for trioctanoylglycerol reaction system. Column temperature was increased from 100 to 250°C at 10°C/min.

Materials

Lipase (EC 3.1.1.3) derived from *Rhizopus delemar* (Seikagaku Kogyo Co., Ltd., 600 units/mg) was commercially obtained and used without further purification.

Silica gel of a chromatography grade was employed as a surface-modifying substrate. The particle size was 74–149 μm diameter.

Silane coupling agents of γ-aminopropyltriethoxysilane (APS) and 2-(methoxycarbonyl)ethyltrichlorosilane (MES) were obtained and used without purification.

Butyl acrylate, isopropyl ether, hexanol, and tetrahydrofuran (THF) were purified by distillation. Tributanoylglycerol, trioctanoylglycerol, octanoic acid, and decanoic acid were purified by passing through silica gel column with ethyl ether-containing hexane (5%), followed by evaporation of the solvent. Their purities were confirmed by GC.

Procedure

Surface Modification of Silica Gel with Silane Coupling Agent

The silica gel (10 g) was treated with 50 mL of sodium hydroxide solution (2%) for 1 h and successively washed with 100 mL of water and 50 mL of ethanol. The air-dried silica gel was placed in 50 mL of ethanolic solution of APS (4%) overnight and the supernatant solution was discarded. The resulting silica gel was dried at 105°C for 3 h, washed with acetone, and redried to afford an APS-modified silica gel. Modification with MES was similarly carried out in 50 mL of benzene.

Graft Polymerization of Butyl Acrylate on Silica Gel

About 5.0 g of the APS-modified silica gel was added to the reaction mixture of THF (40 ml), 4,4′-azobis(4-cyanovaleric acid) (ACVA, 1.0 g), triethylamine (0.8 mL), and ethyl chloroformate (0.5 mL) at −78°C. The vessel was maintained under stirring at this temperature for 15 min and refrigerated overnight. The precipitated solid was filtered out, washed with water and THF, and dried *in vacuo* to give a radical initiator-carrying silica gel.

Graft polymerization of butyl acrylate was accomplished by stirring a suspension of the ACVA-modified silica gel (0.5 g) and butyl acrylate (5.0 mL) under nitrogen at 70°C for 2 h. The reaction mixture was discharged into methanol and the resulting solid was collected, dried, and weighed to determine the monomer conversion (15.6%). Nongraft polymer was removed by Soxhlet extraction with benzene for 12 h.

Immobilization of Lipase

On Nonmodified Silica Gel. The nonmodified silica gel (1.0 g) was repeatedly washed with 20 mL of phosphate buffer (0.1 M, pH 6.0), filtered out, and aspirated. To the moist silica gel was added 2.6 mg of dry lipase and the powder was mixed gently and kept at 4°C overnight.

On Polyacrylate-graft or MES-modified Silica Gel. The poly(butyl acrylate)-graft silica gel (1.0 g) was treated with 1.0 mL of hydrazine in 40 mL of THF at room temperature for 1 h and filtered out. The isolated silica gel was successively treated in 50 mL of hydrochloric acid (2%) with sodium nitrite solution (3.0%, 5 mL) at 5°C for 30 min. The silica gel carrying azide groups was filtered out and immediately transferred to 5 mL phosphate buffer containing 11 mg of lipase. The immobilizing reaction was allowed to proceed at 5°C for 1.5 h. The lipase-immobilized silica gel was filtered, repeatedly washed with the phosphate buffer, and used for interesterification reactions. Yields of the immobilization was determined by UV spectroscopic analysis (280 nm) of the filtrate (1.7 mg-lipase/g-SiO_2).

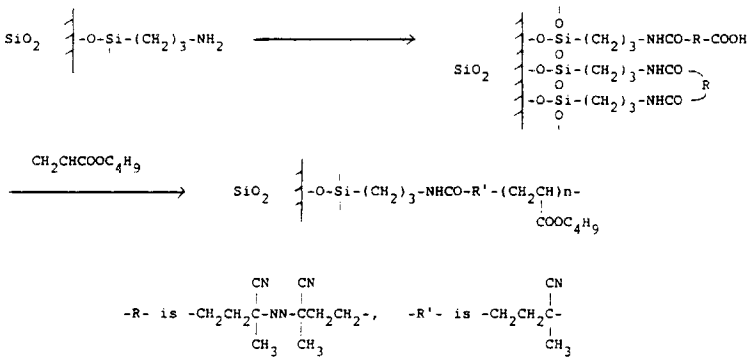
Lipase was similarly immobilized on the MES-modified silica gel with a yield of 2.5 mg-lipase/g-SiO_2.

Interesterification of Triacylglycerol by Immobilized Lipase

To a 50 mL test tube containing 1.0 g of the lipase-immobilized silica gel, was added a mixture of isopropyl ether (2.07 mL), trioctanoylglycerol (0.53 mL), decanoic acid (0.36 g), and eicosane (0.057 g), which was an internal standard of gas chromatographic (GC) analysis. The reaction vessel was shaken at 105 cycle/min in an incubator thermoregulated at 40°C. Small portions of the reaction mixture were intermittently taken for GC analysis. After the end of the reaction, the lipase-immobilized silica gel was washed three times with isopropyl ether by decantation and reused for the next batch reaction. GC peaks were assigned by characterization of the isolated products, as explained below.

It has been reported that *Rhizopus delemar* lipase hydrolyzes or interesterifies 1 and/or 3 ester groups of triacylglycerols in emulsion[5] as well as in biphasic reaction system.[2] For simplicity, 1 and/or 3 acyl-exchanged or hydrolyzed glycerols in the interesterification between trioctanoylglycerol and decanoic acid are abbreviated as follows; trioctanoylglycerol (O_3G), 1,2-dioctanoyl-3-decanoylglycerol (O_2DG), 1,3-didecanoyl-2-octanoylglycerol (OD_2G), 1,2-dioctanoylglycerol (O_2G), 1-decanoyl-2-octanoylglycerol (ODG), 2-octanoylglycerol (OG).

Products of the enzymatic hydrolysis of trioctanoylglycerol (O_3G) were isolated by column chromatography of silica gel and ethyl ether-gradient (5–25%) hexane. The isolated products of O_2G and OG were characterized by IR and GC. OG: IR 3466, 2927, 1744, 1168, 1104, 1042 (cm^{-1}); GC retention time 4.0 min. O_2G: IR 3460, 2926, 1741, 1164, 1106, 1052 (cm^{-1}); GC retention time 9.7 min. O_3G: IR 2930, 1744, 1159, 1109 (cm^{-1}); GC retention time 14.0 min. ODG and OD_2G were prepared by the reaction of OG, decanoyl chloride, and triethylamine. O_2DG was obtained similarly from O_2G. IR data of ODG were almost identical with those of O_2G, and OD_2G and O_2DG IR peaks



Scheme I. Graft polymerization of butyl acrylate on radical initiator-carrying SiO_2.

corresponded to O_3G peaks. GC retention times for ODG, O_2DG, and OD_2G were 11.2, 15.3 and 16.6 min, respectively.

RESULTS AND DISCUSSION

Surface Modification of Silica Gel and Graft Polymerization

In our earlier work,[6] it has been demonstrated that the introduction of radical initiator on calcium carbonate surface and subsequent graft polymerization onto the modified surface is an effective approach to introduce polymers onto the inorganic powder surface. From the same view point, graft polymerization onto the surface of initiator-carrying silica gel was examined.

As is illustrated in Scheme I, silica gel surface was treated with APS, and successively with ACVA to afford an active azo compound-modified silica gel. The resulting silica gel could initiate polymerization of vinyl monomers of styrene, butyl acrylate, acryl amide, and methyl methacrylate, and grafted them in high yields. For example, as described in the Experimental section, butyl acrylate was graft polymerized with a graftivity of 36% and a graft efficiency of 84%. The parameters of graftivity and graft efficiency stand for a weight percentage of graft polymer to graft polymer-silica gel composite and a weight percentage of graft polymer to total polymer produced, respectively.

The yields of the surface modification and graft polymerization are summarized in Table I. The content of APS, MES, ACVA, and poly(Bu Ac) were calculated on the data of TG weight losses. The three results of thermal gravimetric analysis, elemental analysis, and graftivity measurement were found to be in a good agreement.

Diffusive reflectance infrared spectroscopic analysis of every step of the silica gel modification (Fig. 1) has made it clear that the surface modification and graft polymerization proceeded as illustrated in Scheme I. APS-modified silica gel exhibited peak at about 2900 cm^{-1} in addition to the peaks of original silica gel. Treatment of ACVA produced new peaks at 3500–3300, 1700–1660, and 1520 cm^{-1}, indicating amide bond formation between the ACVA and the APS. A broadening of the amide I peak to 1700 cm^{-1} suggests that some free carboxylic acid was included. After graft polymerization of butyl acrylate, three peaks of 2920, 1740, and 1460 cm^{-1}, which are ascribable

TABLE I
Elemental and Thermal Gravimetric Analyses, and Graftivity Measurement of Surface Modifiers on Silica Gel Substrate

Surface modifier	MES	APS	APS + ACVA	APS + ACVA + Poly-BuAc
Elemental analysis				
Total carbon content (%)	4.00	3.05	4.77	28.39
Weight increase after graft polymerization (graftivity, %)				36
Weight loss in TG analysis (%)				
at 100–640°C	7.00	4.47	8.09	41.8
Calculated results for TG analysis (mg/g-SiO_2)				
Thermally decomposed organic group[a]	75	47	88	715 [d]
from APS or MES (carbon content)[b]	75 (42)	47 (29)	47 (29)	47 (29)
from ACVA (carbon content)[b]			41 (24)	41 (24)
from Poly-BuAc (carbon content)[b]				627 [d] (412)
Total carbon content	42	29	53	465
Calculated total carbon content from TG (%)[c]	4.0	2.7	4.9	27.1

[a] Total organic group decomposed was calculated from TG weight loss (%) by eq. of 1000 × TG (%)/(100TG(% −)).

[b] Carbon content was obtained by multiplying the following factor to the organic group: 0.552, 0.621, 0.585, 0.658 for MES, APS, ACVA, and Poly-BuAc, respectively. The factor means the weight ratio of carbon in the organic group.

[c] Total carbon content (TCC, %) was calculated from TCC (mg/g-SiO_2) by: 100 × TCC (mg/g-SiO_2)/(1000 + TCC (mg/g-SiO_2)).

[d] Graftivity calculated from TG analysis was (627/(1000 + 715)) × 100 = 37 (%).

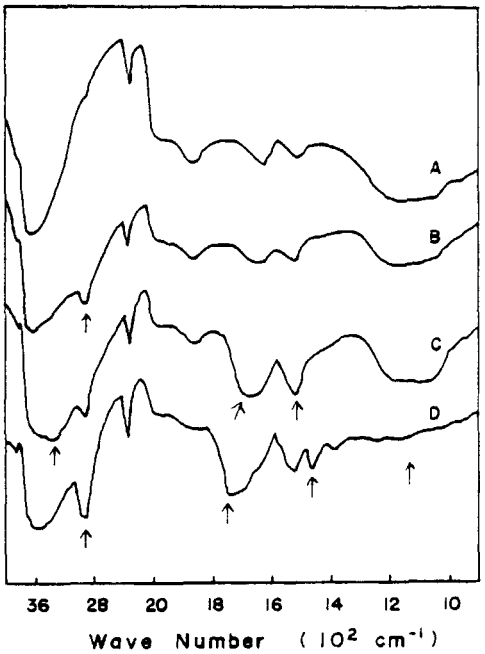


Fig. 1. Diffusive reflectance infrared spectra of (A) nonmodified, (B) APS-modified, (C) APS- and ACVA-modified, and (D) poly(Bu Ac)-graft silica gels. The silica gels were diluted by KBr to 10%.

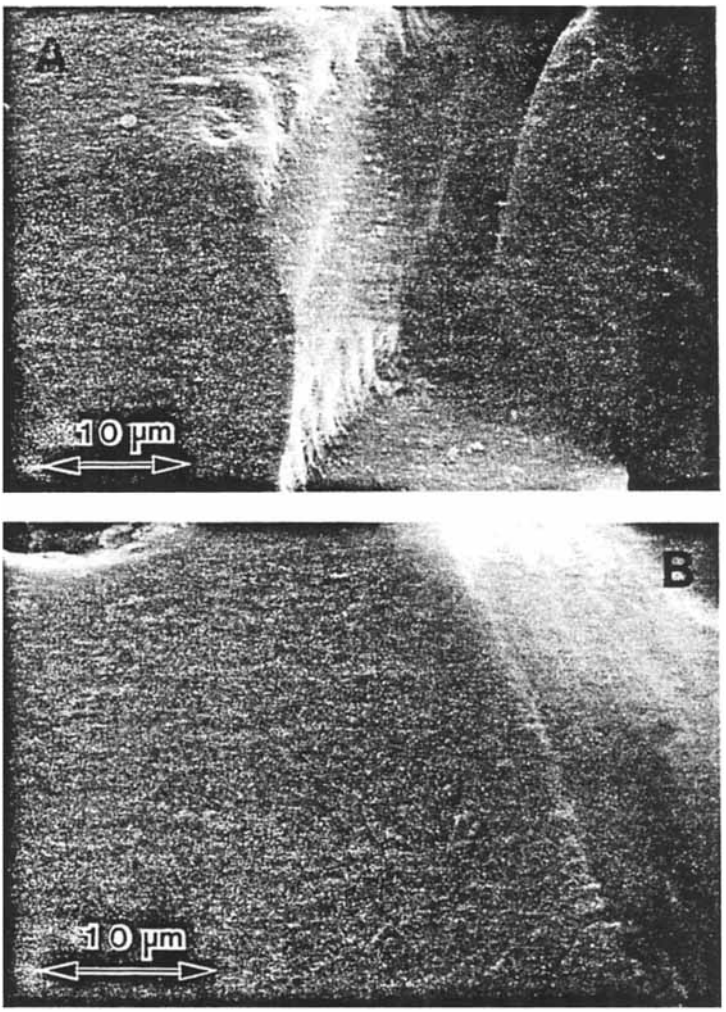


Fig. 2. Scanning electron micrographs of (A) nonmodified, (B) MES-modified, and (C) poly(Bu Ac)-graft silica gels. The graftivity was 36%.

to the graft poly(butyl acrylate), became significant and a broad peak at 1200–1050 cm^{-1} (Si-O-Si) had diminished.

Scanning electron micrography revealed the morphology of the surfaces of original, MES-modified, and poly(butyl acrylate)-graft silica gels (Fig. 2). Although the MES-modified silica gel attained hydrophobicity, morphological changes due to MES modification were not clear [(Fig. 2B)]. After graft polymerization, polyacrylate is recognizable around the silica gel, and found on the edge in an ample amount [(Fig. 2C)].

Elemental and thermal gravimetric analyses of MES-modified silica gel were similarly undertaken and some results are shown in Table I, together with those of APS-modified silica gel. An infrared peak was observed at 1735 cm^{-1}, which is ascribable to the MES ester group introduced on the silica gel.

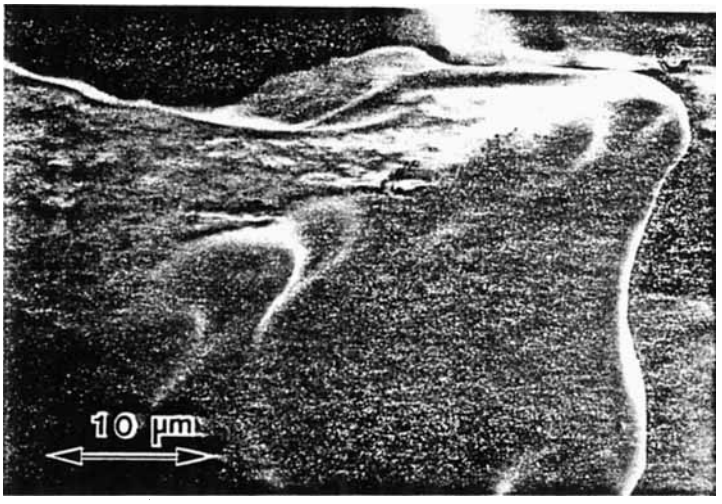
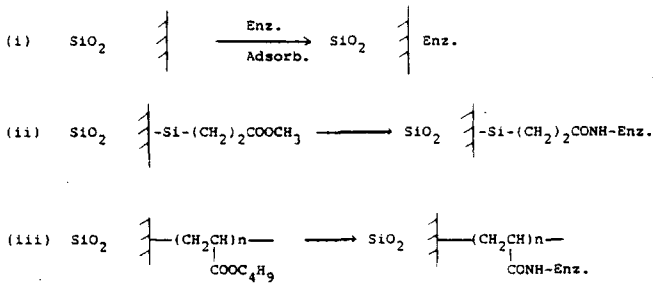


Fig. 2. (*Continued from the previous page.*)



Scheme II. Lipase immobilization of surface-modified silica gel.

Immobilization of Lipase on Silica Gel

In Scheme II are shown procedures employed to immobilize lipase on the three kinds of silica gels; (i) nonmodified, (ii) MES-modified, and (iii) polyacrylate-graft silica gels.

It has already been reported that the use of an appropriate solvent is essential to derivatize graft polymer in a high yield.[7] High conversion of graft poly(butyl acrylate) to hydrazide derivative was attained in THF. No significant reaction proceeded in an aqueous medium. Further derivation of the graft polymer to azide and lipase immobilization, however, should be performed in an aqueous solvent.

Yields of lipase immobilization by the two methods are compared with each other in Table II. The lower yield of the lipase immobilization on the polyacrylate-graft silica gel, in spite of the more ester bonds, suggests that the enzyme could not enter into the inner side of the graft polymer, probably due to crosslinking of the graft polymer with introduced enzyme and to the poor solvation of the azide-containing graft polymer with water.

TABLE II
Yields of Lipase Immobilization on Silica Gel (1.0 g)

Support (1.0 g)	MES-modified SiO_2	Poly(Bu Ac)-graft SiO_2
Yield of immobilization (mg)	2.4	1.7
Amount of MES attached (mg)[a]	75	
Amount of Poly(Bu Ac) grafted (mg)[a]		627
Amount of ester bond (mEq)	0.86	4.9
Efficiency of Immobilization (mg/mEq-ester bond)	2.8	0.35

[a] The amounts of attached MES and graft poly(Bu Ac) on silica gel were represented from Table I.

TABLE III
Interesterification of Trioctanoylglycerol with Decanoic Acid in Isopropyl Ether[a]

	Reactant (mmol) recovered		Product (mmol)					
Repetition batch no.	Decanoic acid	O_3G[b]	Octanoic acid	OG[b]	O_2G[b]	ODG[b]	O_2DG[b]	OD_2G[b]
Lipase-1[c]								
1	1.75	0.07	1.65	0.36	0.27	0.13	0.14	0.05
2	1.94	0.17	1.37	0.24	0.43	0.07	0.08	0.01
3	2.00	0.36	1.04	0.15	0.41	0.03	0.08	trace
Lipase-2[d]								
1	1.76	0.13	1.34	0.41	0.27	0.09	0.16	0.05
2	1.72	0.06	1.71	0.32	0.28	0.20	0.11	0.05
3	1.79	0.13	1.50	0.26	0.33	0.10	0.15	0.04
4	2.02	0.54	0.54	0.06	0.34	0.03	0.06	trace
Lipase-3[e]								
1	1.72	0.05	1.74	0.35	0.27	0.17	0.12	0.06
2	1.48	0.11	1.35	0.11	0.27	0.16	0.26	0.11
3	1.47	0.29	0.91	0.02	0.19	0.10	0.33	0.10
4	1.57	0.32	0.89	0.02	0.20	0.07	0.32	0.07

[a] Initial reaction conditions were as follows: Trioctanoylglycerol 0.53 mL (1.02 mmol), decanoic acid 0.36 g (2.10 mmol), isopropyl ether 2.07 mL, and eicosane 0.057 g. Reaction temperature 40.0°C. Reaction time 4 h.

[b] Glycerol derivatives were abbreviated as follows: O_3G, Trioctanoylglycerol: OG, 1-octanoylglycerol; O_2G, 1,2-dioctanoylglycerol; ODG, 1-decanoyl-2-octanoylglycerol; O_2DG, 1,2-dioctanoyl-3-decanoylglycerol; OD_2G, 1,3-didecanoyl-2-octanoylglycerol.

[c] 2.6 mg of lipase was adsorbed on nonmodified silica gel (1.0 g).

[d] 2.4 mg of lipase was immobilized on MES-modified silica gel (1.0 g).

[e] 1.7 mg of lipase was immobilized on poly(Bu Ac)-graft silica gel (1.0 g).

Interesterification of Trioctanoylglycerol with Decanoic Acid

Lipase from *Rhizopus delemar* is known to have 1,3-regiospecificity in hydrolysis or interesterification of triacylglycerols. The yields of 1 and/or 3 acyl-exchanged or hydrolyzed acylglycerols produced in the reaction of trioctanoylglycerol and decanoic acid in isopropyl ether are summarized in Table III. As the interesterification consists of fast hydrolysis and slow ester reformation[8], the octanoic acid hydrolyzed was plotted against the reaction

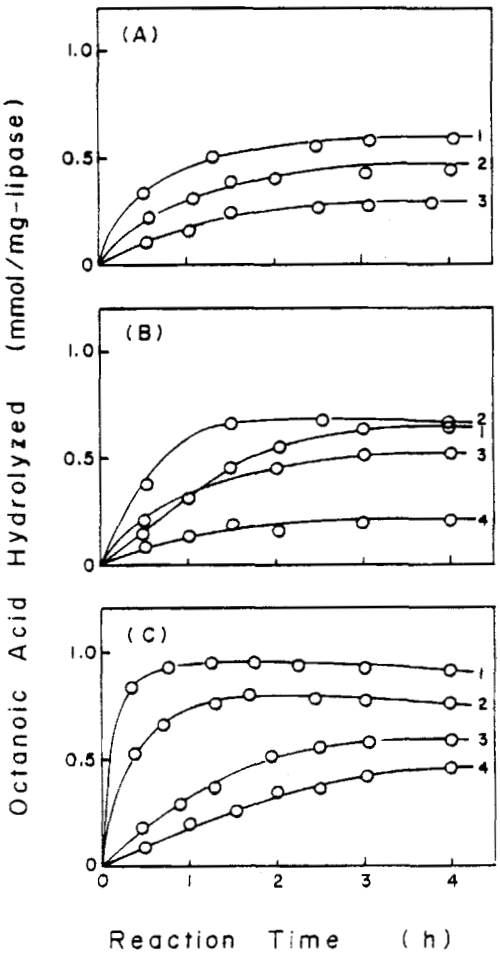


Fig. 3. Interesterification of trioctanoylglycerol with decanoic acid catalyzed with (A) lipase (2.6 mg) adsorbed on silica gel (1.0 g), (B) lipase (2.4 mg) immobilized on MES-modified silica gel (1.0 g), and (C) lipase (1.7 mg) immobilized on poly(Bu Ac)-graft silica gel (1.0 g). Numbers in the figures are reaction batch numbers.

time to estimate the enzymatic activity (Fig. 3). The overall hydrolysis rates at their initial reaction stages, that is, apparent enzymatic activities were found to be in the following order except in the first batch reactions: lipase on the graft polyacrylate (lipase-3) > lipase on the MES (lipase-2) > lipase adsorbed on the silica gel (lipase-1). The phenomenon of the lipase-2 activation after the first batch reaction has not been elucidated.

Regarding the results in Figure 3, it is also recognizable that apparent enzymatic activities generally decreased as batch reaction repeated. This finding is kinetically explicable by the exclusion of water from the reaction system. Under the experimental conditions, the substrate of water was gradually removed from the system as an azeotrope of isopropyl ether, which condensed on the upper cool part of the reaction vessel. Water in the reaction

system was also consumed after each batch reaction by yielding byproducts of mono and diacylglycerols. The water content, for example, of the lipase-3 prior to the first batch reaction and that after the fourth batch reaction were determined by Karl Fischer method and shown to decrease from 10.7 to 1.2 wt% of the lipase-immobilized silica gel. The loss of the water resulted into lowering the hydrolysis rate.

It is indicated in Table III that lipase-3 produced more acyl-exchanged triacylglycerols, O_2DG and OD_2G, after the first batch reaction than in the first batch reaction, and that the yields of octanoic acid, OG, O_2G, and ODG decreased with an increase in batch reaction number. These tendencies can be accounted for thermodynamically by the reduced quantity of water; the less water in the reaction system, the more the triacylglycerols and the less carboxylic acids are yielding by shifting the following equilibrium.

$$\text{Mono or Diacylglycerol} + \text{carboxylic acid} \rightleftharpoons \text{Triacylglycerol} + \text{water} \quad (1)$$

Similar thermodynamical effects had been expected for the interesterification by lipase-1 or 2. However, the yields of acyl-exchanged triacylglycerols were lower for repeated batch reactions. Lipase-1 and 2 were probably less stable under such water-deficient conditions.

Esterification of 2-Monooctanoylglycerol with Decanoic Acid

Equilibrium shift by removing water from the reaction system as an azeotrope of isopropyl ether was thought to be effectively applied to the enzymatic synthesis of OD_2G from OG and decanoic acid. Yields of the expected products, diacylglycerols or triaclylglycerols, are indicated to increase as the reaction batch number increased, however, the values of the yields were generally small (Table IV).

The consumption rate of the OG in the synthetic reaction was depicted in Figure 4, where monoacylglycerol (OG) is consumed at an almost identical

TABLE IV
Esterification of Monooctanoylglycerol with Decanoic Acid[a]

Repetition batch no.	Reactant recovered (mmol)	Product (mmol)				
	OG[c]	O_2G[c]	ODG[c]	O_3G[c]	O_2DG[c]	OD_2G[c]
1	0.10	0.03	0.11	trace	0.02	0.03
2	0.09	0.04	0.14	trace	0.03	0.07
3	0.08	0.04	0.17	trace	0.03	0.07
4	0.08	0.04	0.18	trace	0.03	0.08
5[b]	0.11	0.10		0.02		

[a] Initial reaction conditions were as follows: 2-Monooctanoylglycerol 0.81 mmol, decanoic acid 1.62 mmol, Isopropyl ether 1.84 g, and eicosane, 0.102 mmol. Reaction temperature 40.0°C. Reaction time 4 h. One mg of the lipase was immobilized on poly(bu Ac)-graft silica gel (1.0 g).

[b] The fifth batch reaction was carried out with 2-monooctanoylglycerol 0.61 mmol and isopropyl ether 2.0 mL and no decanoic acid was used.

[c] Abbreviations of the products are the same as those in Table III.

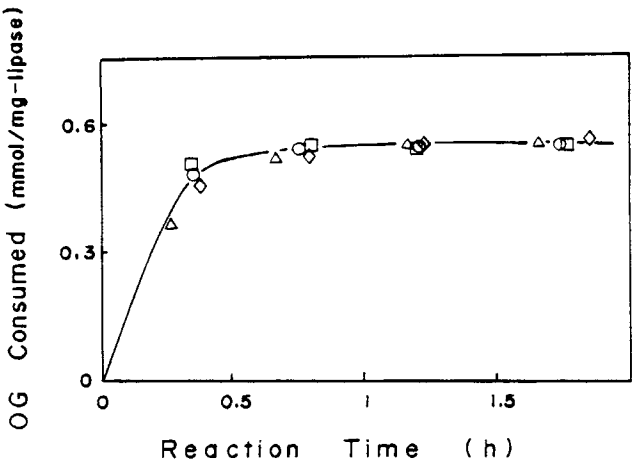


Fig. 4. Consumption of 2-monooctanoylglycerol (OG) in esterification of OG (0.81 mmol) and decanoic acid (1.62 mmol). Batch numbers of the reaction are 1; ○, 2; □, 3; ◇, and 4; △

rate in every batch reaction. This finding is quite different from the triacylglycerol interesterification [(Fig. 3C)]. In the latter reaction, water constituted one of the reaction substrates and apparent deactivation of enzyme was observed. Therefore, it is reasonably assumed that the exclusion of water by evaporation of water-isopropyl ether azeotrope scarcely deactivated the immobilized lipase.

Although the *rhizopus* lipase has 1,3-regiospecificity, the octanoyl group of 2-octanoylglycerol was found to enzymatically react and afford di or triacylglycerols (O_2G or O_3G in fifth batch reaction of Table IV). (Without lipase no significant reaction was observed under the same reaction conditions.) This observation suggests that a direct enzymatic interesterification without hydrolysis between OG proceeded.

$$2OG \rightarrow G + O_2G \quad (2)$$

In the presence of decanoic acid, the above-described 1,3-regiospecific esterification proceeded simultaneously. The concentrations of the reactants and products in the fourth reaction batch in Table IV were plotted against reaction time (Fig. 5). In the early stage of esterification, O_2G was formed in a considerable amount, although a quite small amount of octanoic acid was hydrolyzed. This supports the direct interesterification of E.q. (2). The low conversion from OG to ODG or OD_2G could be explained by the high yield of glycerol, which was sparingly soluble in isopropyl ether and consequently a quite poor substrate.

Interesterification of Tributanoylglycerol with Hexanol

In enzymatic ester synthesis from alcohols and carboxylic acids, water-soluble carboxylic acids increases the acidity of the reaction mixture and deactivates lipase.[9] A similar phenomenon was observed in the hydrolysis of tributanoylglycerol or in the interesterification of the tributanoylglycerol with

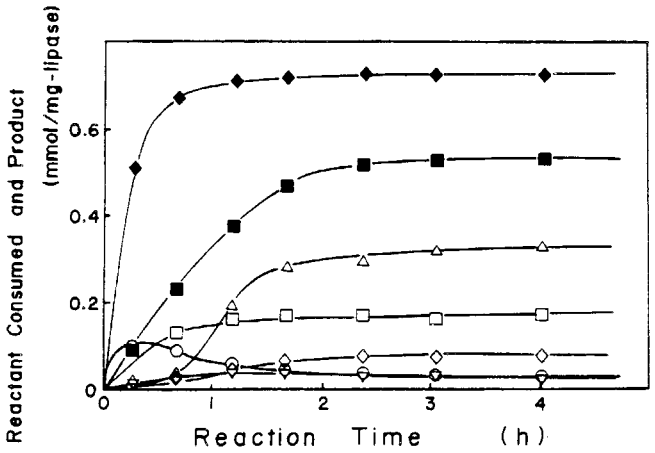


Fig. 5. Time courses of reactants consumed (OG; ◆, decanoic acid; ■) and products (ODG; □, O_2G; ○, OD_2G; ◇, O_2DG; ▽, octanoic acid; △) in the esterification of 2-monooctanoylglycerol (OG) and decanoic acid.

octanoic acid. However, in the interesterification between tributanoylglycerol and hexanol, no appreciable deactivation of lipase was observed. In Table V, the hydrolyzed product of butyric acid is shown to be drastically reduced when the batch reaction was repeated, although the alcoholized product of hexyl butyrate was produced in almost constant yields. Alcoholysis by the immobilized lipase could proceed well in such water-deficient conditions to suppress the hydrolysis. Direct alcoholysis of the tributanoylglycerol is assumed as follows:

Tributanoylglycerol + Hexanol ⇌

Mono or Dibutanoylglycerol + Hexyl Butyrate (3)

TABLE V
Interesterification of Tributanoylglycerol with Hexanol in Isopropyl Ether

Repetition batch no.	Reactant (mmol) recovered		Product (mmol)			
	Hexanol	Tributanoyl-glycerol	Hexyl butyrate	Butyric acid	2-Mono-butanoyl glycerol	1,2-Di-butanoyl glycerol
1	1.09	0.34	0.98	1.01	0.26	1.50
2	0.54	0.55	1.51	0.28	0.23	1.32
3	0.40	0.60	1.62	0.05	0.21	1.24
4	0.39	0.64	1.65	0.05	0.22	1.28
5	0.42	0.62	1.66	0.03	0.22	1.26

Reaction conditions were as follows: Tributanoylglycerol 0.61 mL (2.10 mmol), hexanol 0.27 mL (2.07 mmol), isopropyl ether 2.12 mL. Reaction temperature 40.0°C. Reaction time 24 h. Lipase (0.9 mg) immobilized on 0.51 g of polymer-graft SiO_2 was used.

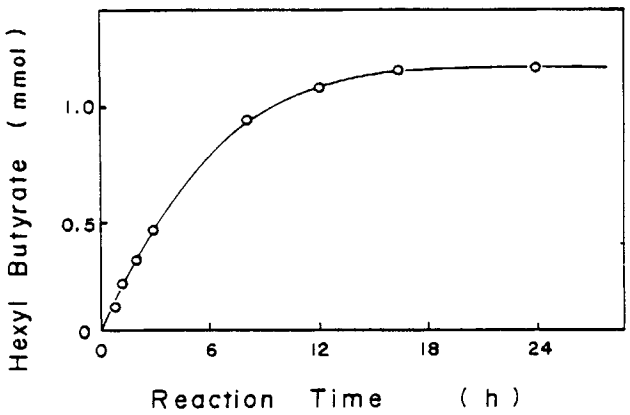


Fig. 6. Interesterification of tributanoylglycerol and hexanol catalyzed by lipase (0.9 mg) immobilized on poly(Bu Ac)-graft silica gel (0.5 g).

Reaction time course of the interesterification is presented in Figure 6, showing that the reaction time to reach the equilibrium was much longer in the alcoholysis than in hydrolysis.

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References

1. (a) B. Cambou and A. M. Klibanov, *J. Am. Chem. Soc.*, **106**, 2687 (1984). (b) A. Zaks and BA. M. Klibanov, *Science*, **224**, 1249 (1984). (c) R. A. Wisdom, P. Dunnill, M. D. Lilly, and A. Macrae, *Enzyme Microb, Technol.*, **6**, 443 (1984). (d) A. Macrae, *J. Am. Oil Chem. Soc.*, **60**, 291 (1983).

2. K. Yokozeki, S. Yamanaka, K. Takinami, Y. Hirose, A. Tanaka, K. Sonomoto, and S. Fukui, *Eur. J. Appl. Microbiol. Biotechnol.*, **14**, 1 (1982).

3. (a) S. Fukui and A. Tanaka, *Enzyme Eng.*, **6**, 191 (1982). (b) G. Bell, J. A. Blain, J. D. E. Patterson, and R. Todd, *J. Appl. Chem. Biotechnol.*, **26**, 582 (1976).

4. K. Martinek, A. N. Semenov, and I. V. Berezin, *Biochim. Biophys. Acta*, **658**, 76 (1981).

5. M. Iwai and Y. Tsujisaka, in *Lipase* B. Borgstroem, and H. L. Brockman, eds., Elsevier, North Holland, 1984, p. 443.

6. T. Nakatsuka, H. Hawasaki, S. Yamashita, and S. Kohjiya, *J. Colloid Interface Sci.*, **93**, 277 (1983).

7. T. Nakatsuka and S. Yamashita, *J. Appl. Polym. Sci.*, **28**, 3549 (1983).

8. T. Tanaka, E. Ono, M. Ishihara, S. Yamanaka, and K. Takanami, *Agric. Biol. Chem.*, **45**, 2387 (1981).

9. Y. Tsujisaka, S. Okumura, and M. Iwai, *Biochim. Biophys. Acta*, **489**, 415 (1977).

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