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# Immobilization of Prostaglandin Synthetase by Hydrophobic Adsorption

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## ABSTRACT

In this article, the immobilization of prostaglandin synthetase on *n*-alkyl or aryl amino-agar beads by hydrophobic adsorption is reported. The effects of different hydrophobic groups in the agar beads, pH of buffer, concentration of salts on the adsorption of prostaglandin synthetase, and the properties of immobilized prostaglandin synthetase were also studied. The results showed that 20–35 mg of microsome containing PG synthetase (protein content 8–15 mg) could be adsorbed on each gram of *n*-dodecylamino-agar beads after suction drying the gel in the buffer of pH 5.5 (containing 0.5 mol/L KCl), 0.1 mol/L citric-phosphate at 4°C. The remaining immobilized enzyme activity was over 80%. The optimum pH of immobilized PG synthetase is 8.0, similar to that of the native enzymes. The thermostability of immobilized PG synthetase in the buffer containing 0.5 mol/L KCl was increased. Immobilized PG synthetase was used as a catalyst of synthesis of prostaglandin $E_1$. The preservation of activity after 10 working cycles was 86.2%.

**Index Entries:** Prostaglandin synthetase; immobilization; hydrophobic adsorption; prostaglandin $E_1$.

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## INTRODUCTION

Prostaglandins (PG) are one of several classes of pharmacologically active $C_{20}$ compounds derived from polyenic acids (dihomo-r-linolenic, arachidonic, and 5,8,11,14,17 eicosapentraenic acid)—an essential fatty acid for human beings, widely known as an intracellular regulator of many physiological processes and as promising drugs for medicine and veterina by practice. Clinical uses of PG presently being investigated include the induction of labor and abortion, contraception, and the treatment of asthma, bronchitis, ulcers, high blood pressure, and thrombosis (*1,2*). One of the methods of producing PG is their synthesis from polyenic acids (dihomo-r-linolenic, arachidonic, and 5,8,11,14,17 eicosapentraenic acid) catalyzed by the prostaglandin synthetase complex, which is from the vesicular glands of sheep or bovine (*3,4*). The poor source and bad stability of the enzyme limited its use in large-scale application. Therefore, an important problem of biotechnology is the development of its most effective utilization. With the development of immobilization techniques, many enzymes have been applied in industry. However, conventional immobilization methods applied to PG synthetase resulted in preparations of very low activity and poor stability (*5,6*). We have previously reported on the successful immobilization of L-aspartase through hydrophobic interaction between the enzyme and *n*-alklyamino-agar beads (*7*). Microsome containing PG synthetase has been adsorbed into such a gel.

The purpose of the present investigation was to develop methods for immobilization of PG synthetase and to study the properties of the immobilized enzyme complex.

## MATERIALS AND METHODS

### Materials

In the experiments, we used vesicular glands from sheep stored in below −20 to −40°C and dihomo-r-linolenic acid (cis-8,11,14-eicosatrienoic acid [ETA] [purity of the preparation 95% according to the data of gas–liquid chromatography]) obtained from Pharmaceutical Research Institute of Jilin Province. Prostaglandin $E_1$ was obtained from Sigma Chemical Company, St. Louis, MO. Reduced glutathione was from Fiukachemie (AG, Switzerland). Agar power, hydroquinone, Tris, and other reagents were domestic products of the analytical grade.

### Methods

#### *Preparation of PG Synthetase*

The enzyme preparation was produced according to the previously described methods (*3*). The product was directly lyophilized.

### *Synthesis of N-Alkyl or Aryl Agar Beads*

The synthesis of *N*-alkyl or aryl agar beads carriers was according to our previously published method (*7*).

### *Immobilization of PG Synthetase*

The adsorption of prostaglandin synthetase to *N*-alkyl or aryl amino-agar beads was carried out at 4°C. The suction-dried gel (0.5 g) was suspended in 4 mL of citric-phosphate or phosphate buffer (0.1 mol/L) in the pH range 4.5–8.0, containing 0–3 mol/L KCl and 15 mg enzyme power (containing 6.0 mg protein). The mixture was gently shaken for 2 h, then the original solution was removed through filtration, and the immobilized enzyme was subsequently washed with 5 mL of pH 8.0 Tris-HCl buffer. Protein content in solution and the activity of immobilized enzyme were determined according to the following methods. Protein content was determined according to the method of Bradford using BSA as a standard (*8*).

### *Assay of Enzyme Activity*

In the experiments, prostaglandin synthetase power (15 mg) or immobilized enzyme (0.5 g) was solubilized or suspended in 5 mL of pH 8.0, 0.05 mol/L Tris-HCl buffer, 0.3 mL of 10 mmol/L hydroquinone, and 3.07 mg reduced glutathione was added. After being incubated for 2 min at 37°C, 1 μL of dihomor-r-linolenic acid (solubilized in 0.2 mL of alcohol) was added, and then subject to another incubation for 10 min at 37°C under magnetic stirring. At the end of the incubation period, the reaction solution was acidified by 2 mol/L citric acid to pH 2.0–3.0. $PGE_1$ produced in the reaction mixture was extracted directly with 25 mL of diethyl ether in a separator. The solution was then removed with a rotary evaporator, and the residue was dissolved in 10 mL of methanol. $PGE_1$ was converted to prostaglandin $B_1$ ($PGB_1$) by the addition of 0.5 mL of 3 mol/L KOH (dissolved in methanol) and 2.5 mL methanol, with further incubation at 50°C for 30 min. The concentration of $PGB_1$ in the methanol was determined by spectrophotometer at 278 nm. The determination of $PGE_1$ content was conducted as described by Bygdeman and Samuelson (*9*).

## RESULTS AND DISCUSSION

### Effects of the Different Alkyl Amino-Agar Beads on the Immobilization of PG Synthetase

Prostaglandin synthetase from the vesicular glands of the sheep is one of the membrane-bound enzymes. It has been demonstrated that it is associated with the lipids by hydrophobic interaction. When *n*-alkyl amino-agar beads are applied to the immobilization of PG synthetase, the results of immobilization depended on the hydrophobicity of the groups

Table 1
Immobilization of PG Synthetase on the Different Hydrophobic Carriers[a]

| Hydrophobic group | Yield of adsorbed protein, % | Yield of $PGE_1$, μg | Relative activity, % |
|---|---|---|---|
| *n*-Hexyl- | 86.6 | 317.4 | 90.8 |
| *n*-Dodecyl- | 87.1 | 320.0 | 92.4 |
| α-Naphthyl- | 80.9 | 325.6 | 93.4 |
| β-Naphthyl- | 80.3 | 334.6 | 95.9 |
| *n*-Butylphenyl- | 66.0 | 176.5 | 50.0 |
| Biphenyl- | 57.3 | 200.0 | 57.5 |
| Benzyl- | 64.1 | 197.5 | 56.4 |

[a]0.5 g *n*-alkyl or aryl lamine-agar beads (suction-dried), 15 mg enzyme power (protein content 6.0 mg, activity: 16.4 μ/mg protein), 4 mL of pH 5.5, 0.5 mol/L KCl, 0.1 mol/L phosphate-citric buffer were shaken for 2 h at 4°C.

on the agar beads. The different *n*-alkyl amino-agar beads may affect the immobilization of the enzyme. In Table 1, the results showed that the hydrophobic adsorption of PG synthetase onto the alkyl amino-agar beads is governed by the different alkyl groups. The *n*-dodecylamino- and α-naphthylamino agar, which have strong hydrophobicity, have high activity and high capacity for immobilization of PG synthetase.

It is illustrated that the *n*-dodecylamino- and α-naphthylamino-groups present on the surface of agar used in this study ensure that sufficient hydrophobic interaction will occur, making the adsorption process virtually irreversible. Such strong associations are necessary for an immobilized enzyme to be able to retain catalytic activity.

## Effects of pH on the Immobilization of PG Synthetase

The effects of pH on the adsorption of PG synthetase on the *n*-dodecylamino-agar beads were examined. The results obtained are presented in Fig. 1. As shown in the figure, the amount of protein adsorbed and the activity of the immobilized enzyme at pH 5.5 were the highest. This pH is near the isoelectric point (pI) of the microsome containing PG synthetase (pH 4.8–5.0). Thus, the charges of enzyme surface decreased, which enhanced the hydrophobic interaction between microsome and *n*-dodecylamino-agar beads. There was a significant difference in the amount of protein adsorbed and the activity of the immobilized enzyme in different pH solutions. The enzyme retains different charges at different pH values that might affect hydrophobicity of the enzyme surface.

## Effects of the Concentration of Salt (KCl) on the Immobilization of PG Synthetase

The hydrophobic effect is generally enhanced by the presence of salt in high concentration (*10*). Figure 2 demonstrates the influence of the

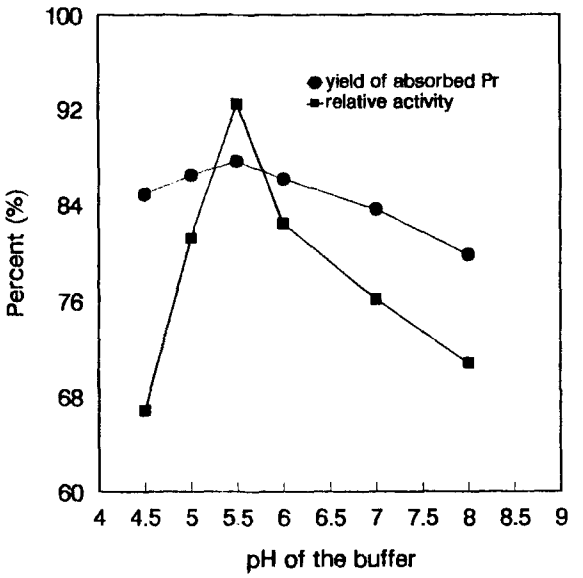


Fig. 1. Effects of pH of the buffer on the immobilization of PG synthetase. The *n*-dodecylamino-agar beads (suction-dried gel 0.5 g), 4 mL of the different pH buffer (containing 0.5 mol/L KCl), and 15 mg enzyme power were shaken for 2 h at 4°C.

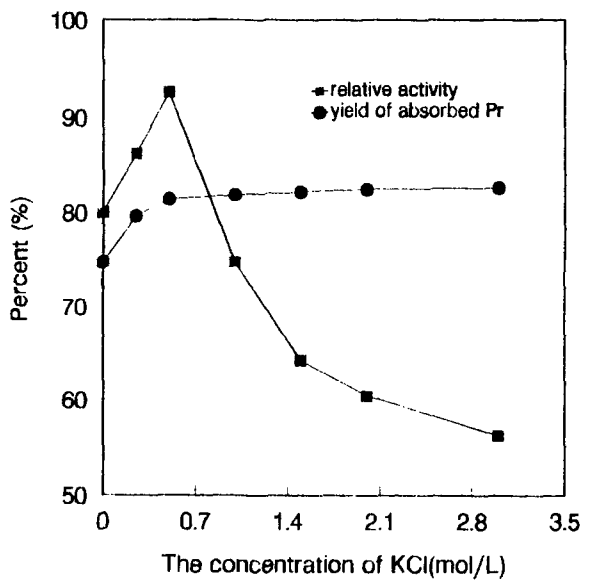


Fig. 2. Effects of the concentration of KCl on the immobilization of PG synthetase. The gel (0.5 g), 4 mL of buffer (pH 5.5), containing different concentrations of JCL, and 15 mg enzyme power were shaken for 2 h at 4°C.

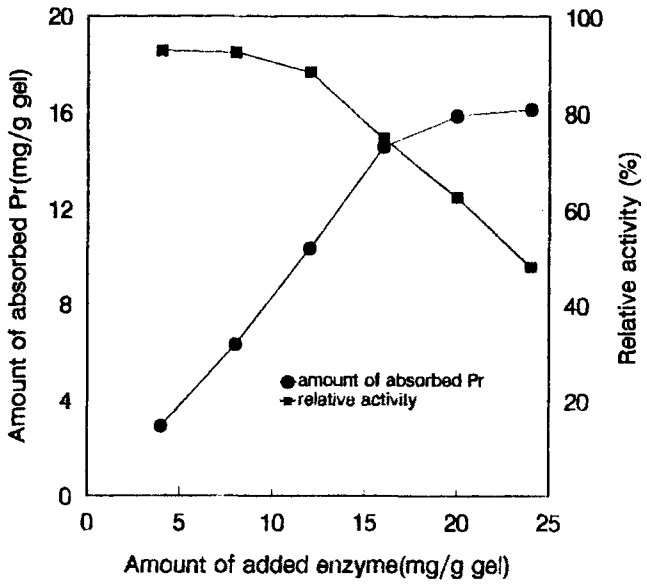


Fig. 3. Effects of the amount of added enzyme on the immobilization of PG synthetase. The gel (0.5 g), 4 mL of buffer (pH 5.5), 0.5 mol/L KCl, and different amounts of enzyme power (15 mg power contains 6 mg protein) were shaken for 2 h at 4°C.

medium salt concentration on adsorption of PG synthetase. With increasing salt concentration, hydrophobicity of the ligand on the agar beads increased. The amount of protein adsorbed was enhanced. The activity of immobilized enzyme in 0.5 mol/L KCl was the highest. Addition of salt to the surrounding medium may well affect the tertiary structure of the protein or microscome in some way, resulting in the decrease of the immobilized enzyme activity in the high concentration of KCl.

## Effects of Amounts of Added Enzyme on the Immobilization PG Synthetase

The optimal amounts of the enzyme to adsorb on the *n*-dodecylamino-agar beads were determined. As shown in Fig. 3, with increasing the amount of added enzyme (or microsome), the amounts of protein adsorbed on the carrier increased. Protein was bound to the *n*-dodecylamino-agar beads to the maximum levels at a charge of 16 mg protein/g of carrier, but the relative activity of immobilized enzyme inversely decreased.

## The Properties of Immobilized PG Synthetase

### *Optimal pH*

The effect of pH on the activity of immobilized and native PG synthetase is shown in Fig. 4. The optimal pH was found to be in the range of pH 7.5–8.5, similar to that of the native enzyme.

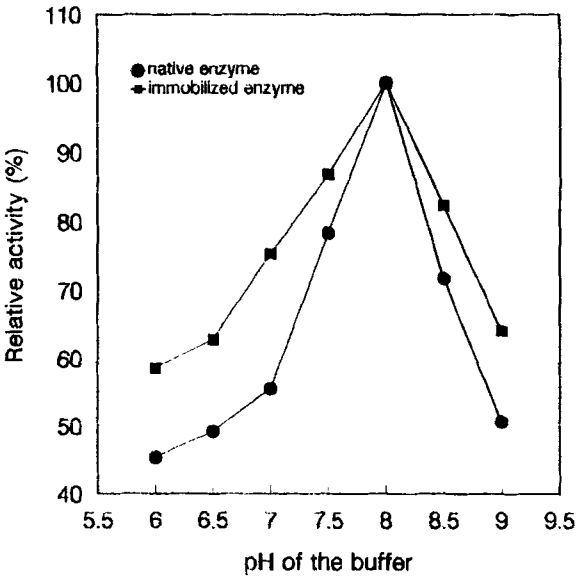


Fig. 4. Effects of pH on the activity of immobilized PG synthetase.

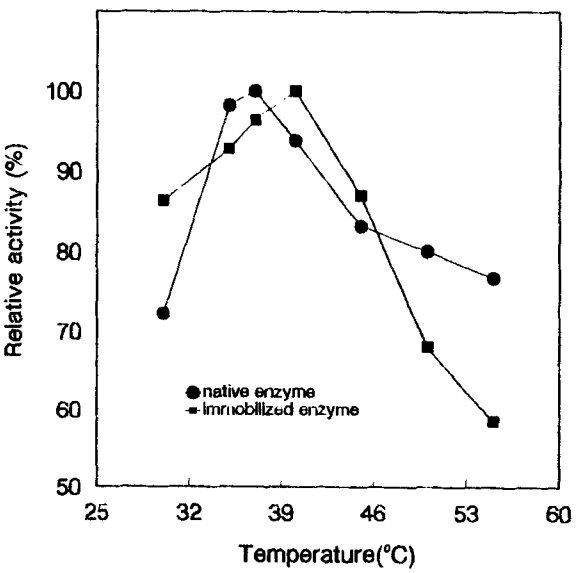


Fig. 5. Effect of temperature on the activity of immobilized enzyme.

### *Optimal Temperature*

Figure 5 shows the effect of temperature on the activity of the immobilized and native PG synthetase. The maximum temperature for activity of the immobilized enzyme shifts to the region of higher temperature (40–45°C) as compared to that of the native enzyme (35–40°C). With the continuous rise of temperature, the activity of the immobilized enzyme inversely decreased, which is possibly because of the dissociation of microsomes from the carriers.

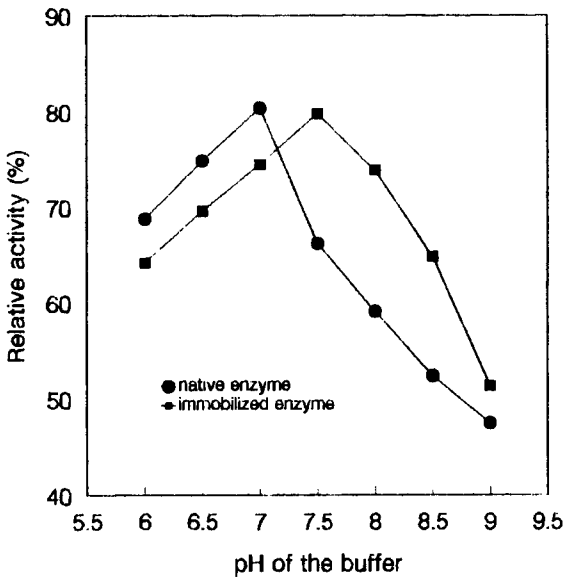


Fig. 6. The pH stability of immobilized PG synthetase.

### *The pH Stability*

The effect of pH on the stability of immobilized and native enzyme is shown in Fig. 6. After immobilized and native PG synthetase was stored in the different pH buffers at 4°C for 5 h, their enzyme activities were determined. The results showed that the stable pH of immobilized enzyme was found to be in the range of 7.0–8.0, but shifts to the region of high pH compared to that the native enzyme.

### *Thermostability*

The immobilized PG synthetase and soluble enzyme were incubated at 40 and 45°C, respectively, with shaking in the buffer at pH 8.0, 0.05 mol/L Tris-HCl or in the buffer of pH 8.0, 0.5 mol/L KCl, 0.05 mol/L Tris-HCl. Then their enzyme activities were determined. The results showed that thermostability of the immobilized PG synthetase considerably increased in the presence of 0.5 mol/L KCl, in comparison to the soluble enzyme (Fig. 7).

### *Stability in Storage at 4°C*

The immobilized PG synthetase in a buffer of pH 8.0, 0.05 mol/L Tris-HCl is stored in the cold at 4°C. After 100 d of storage of the immobilized enzyme, 65.4% of the original enzymatic activity is retained, and the soluble enzyme can only remain at 50% activity after 44 h of storage in the same conditions.

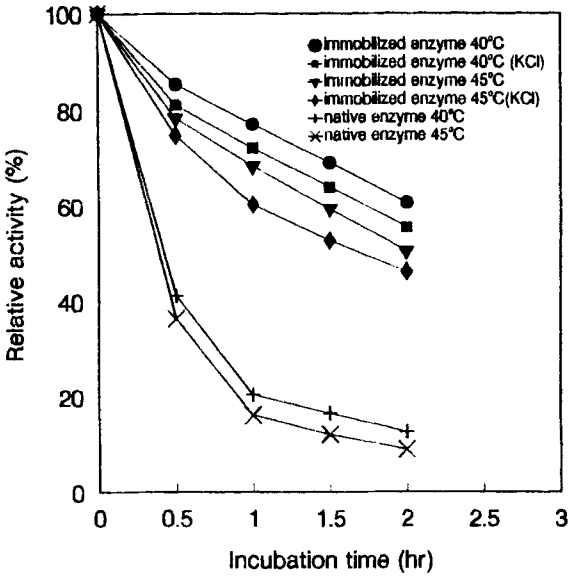


Fig. 7. Thermostability of immobilized enzyme.

### *Effects of Cofactor on the Synthesis of $PGE_1$*

The effects of the concentration of reduced glutathione and hydroquinon on the synthesis of $PGE_1$ catalyzed by the immobilized enzyme were also studied. The results showed that the optimal concentration of reduced glutathione and hydroquinon in the synthesis of $PGE_1$ did not change, which was still 2.0 and 5.4 mmol/L, respectively.

### *Effect of Concentration of ETA on the Synthesis of $PGE_1$*

In the synthetic reaction $PGE_1$ catalyzed by immobilized PG synthetase, when the concentration of ETA was 0.63 mmol/L, the yields of $PGE_1$ were found to be highest, and with the continuous increasing ETA concentration, the yields of $PGE_1$ decreased. It is illustrated that the PG synthetase complex was possibly inhibited by the substrate (Fig. 8).

### *Operational Stability*

The operational stability of immobilized PG synthetase was also studied. Figure 9 illustrates the dependence of the yield of $PGE_1$ on the multiplicity of use ($n$) of a microsomal preparation of prostaglandin synthetase, immobilized on the $n$-dodecylamino-agar beads. The PG synthetase immobilized on the $n$-dodecylamino-agar beads was used as a catalyst of the synthesis of prostaglandin $E_1$. In the case of repeated introduction of the immobilized catalyst into the reaction, the yield of $PGE_1$ is decreased. The preservation of activity after 10 15-min working cycles is 86.2%. When immobilized PG synthetase (10 g) was used to catalyze the synthesis of $PGE_1$, 54.11 mg

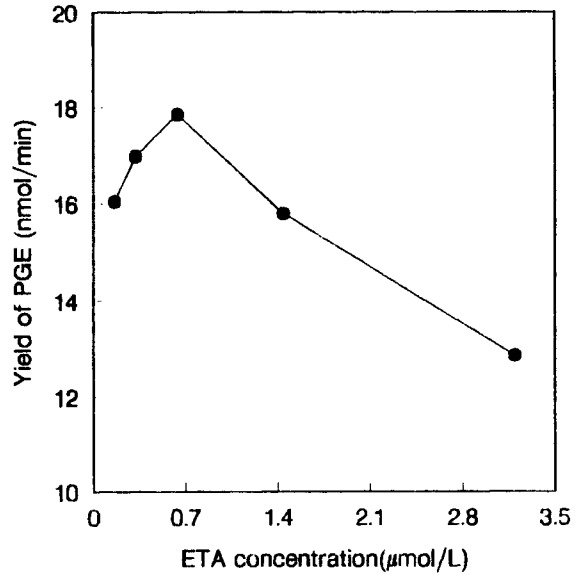


Fig. 8. Effect of the concentration of ETA on the synthesis of $PGE_1$. The immobilized enzyme (0.5 g) reacted with the different concentration of ETA in the reaction condition.

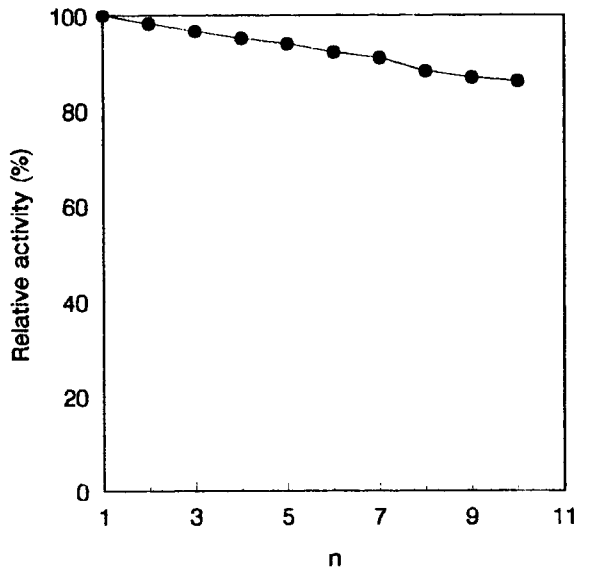


Fig. 9. The operational stability of immobilized PG synthetase. The immobilized PG synthetase (10 g) was carried out at 100 mL of Tris-HCl buffer (pH 8.0, 0.05 mol/L), the concentration of reduced glutathione and hydroquinon, 2.0 and 5.4 mmol/L, respectively, 0.63 mmol/L ETA, incubated for 15 min at 37°C under the stirring per use ($n$).

$PGE_1$ were produced after 10 cycles, but 6.21 mg $PGE_1$ were synthesized by soluble PG synthetase. The results showed that the ability of the synthesis of $PGE_1$ catalyzed by the immobilized PG synthetase increased 30-fold in comparison to that of the soluble microsomes.

Thus, the immobilization of prostaglandin synthetase by hydrophobic adsorption to *n*-alkyl amino-agar beads is a very simple and acceptable procedure, which is distinguished by a good yield of activity of the immobilized enzyme and the ability to produce $PGE_1$. It can be used in industrial-type reactors for the production of PG.

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