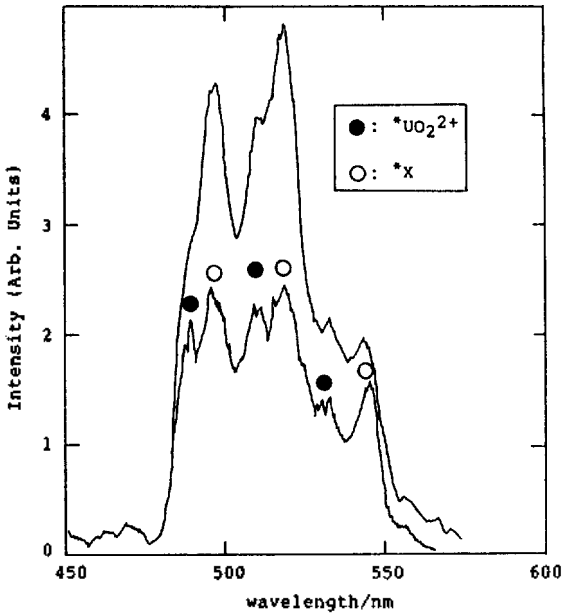


**Figure 2.** Emission spectra of uranyl ion in aqueous perchlorate solutions at 77 K: $[UO_2^{2+}]_T$=0.01 M; pH=3.32 and 3.58 (upper) at 25℃.

The emission spectra measured at 77 K, though they were of low signal-to-noise ratio, were well resolved (Figure 2). Each band observed at ambient temperature was splitted into two bands. The main bands at shorter wavelengths were at almost the same positions (488, 510 and 533 nm) as the emission bands of the excited uranyl ion, which were measured in strongly acidic solutions at ambient temperature. Therefore, the bands are assigned to those of $*UO_2^{2+}$, while the other bands at longer wavelengths to those of *X in question. Comparison of the emission spectra of $*UO_2^{2+}$ and *X shows that the band at 533 nm red-shifts to a greater degree than those at 488 and 510 nm. The emission spectrum of *X is similar to that reported by Formosinho *et al.*,[5] which was obtained from the comparison of the emission spectra at low and high temperatures. The proportion of $(UO_2)_2(OH)_2^{2+}$ is expected to be higher at 23℃ than at 4℃ under their experimental conditions because hydrolysis reactions of $UO_2^{2+}$ ion are endothermic.[9]

It may be concluded on the basis of the previous discussion that two emitting species responsible for the biexponential decay of uranyl luminescence are $*UO_2^{2+}$ and $*(UO_2)_2(OH)_2^{2+}$ ions. Tomiyasu *et al.* also assigned *X to $*(UO_2)_2(OH)_2^{2+}$ ion in their study on rates of luminescence decay of various uranyl complexes in aqueous solutions.[7] The question now arises as to why the luminescence intensity of $*(UO_2)_2(OH)_2^{2+}$ is stronger than that of $*UO_2^{2+}$. This question may be solved by considering the following facts: upon the electronic excitation the transition probabilities of hydrolysis products are higher than that of $UO_2^{2+}$ ion[12] and $*(UO_2)_2(OH)_2^{2+}$ is more long-lived than $*UO_2^{2+}$.

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## Multienzyme-Catalyzed Synthesis of D-Fructose-1,6-Diphosphate

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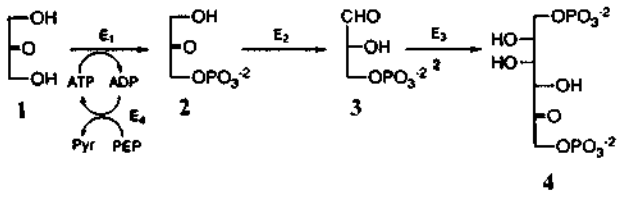
Enzymes are now widely accepted as useful catalysts for enantioselective transformations.[1-4] Much of the work concerned with enzymes as synthetic catalysts has centered on reactions involving one or two enzymes, partly because they are operationally simple and straightforward. Examples are the reactions catalyzed by lipases,[5] esterases,[6] acylase I,[7] alcohol dehydrogenases,[8] glycerol dehydrogenase,[9] lactate dehydrogenase,[10] glycerol kinase,[11] and aldolases.[12] In these reactions the enzymes transform their substrates with high stereoselectivity and provide optically active products. Thus the transformations by these enzymes offer alternative routes to a wide variety of chiral molecules.

On the other hand, less effort[13] has focused on reactions involving three or more enzymes cooperating in sequence or in parallel. Although they look more complex and less practical, multienzyme systems offer opportunity to carry out multistep processing of substrates in one pot with advantage that processes of seaperation and purification or protection




**Scheme 1.** Multienzyme-Catalyzed Synthesis of D-Fructose-1,6-diphosphate (FDP). $E_1$, glycerol kinase (GK); $E_2$, triose phosphate isomerase (TPI); $E_3$, FDP aldolase; $E_4$, pyruvate kinase (PK).

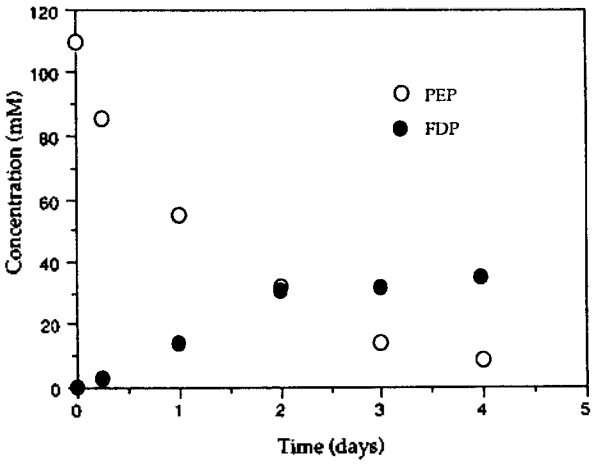



**Figure 1.** The progression of the multienzyme-catalyzed synthesis of FDP.

and deprotection are minimized. As an effort to develop enzymes as the catalysts useful in the synthesis of complex molecules, we has initiated a research program to explore the utility of multienzyme systems in carbohydrate synthesis. We herein describe the preliminary results.

Scheme 1 illustrate a multienzyme system assembled for the preparation of D-fructose-1,6-diphosphate (FDP). In this system, four enzymes cooperate to transform dihydroxyacetone (DHA) to FDP. They include glycerol kinase (EC 2.7.1.30, GK), pyruvate kinase (EC 2.7.1.40, PK), triosephosphate isomerase (EC 5.3.1.1, TPI), and FDP aldolase (EC 4.1.2.13). ATP and phosphoenolopyruvate (PEP) are employed as the phosphorylating reagents. The synthesis of FDP starts with the phosphorylation of DHA to dihydroxyacetone phosphate (DHAP) by ATP that is present in a catalytic amount, followed by the isomerization of DHAP to D-glyceraldehyde-3-phosphate (GAP) and then by the aldol condensation between DHAP and GAP to FDP. The three-step reaction is catalyzed by GK, TPI, and FDP aldolase in sequence. ATP consumed in the first step is regenerated by the reaction of ADP with PEP catalyzed by pyruvate kinase. Overall, this synthesis illustrates the cooperation of four different enzymes for the transformation of two equiv of 3-carbon unit, which is inexpensive, to one equiv of hexose diphosphate, which is relatively expensive and difficult to prepare chemically, coupled with the conversion of two equiv of PEP to two equiv of pyruvate.

In a typical experiment, the enzymes (GK, PK, TPI, and FDP aldolase) enclosed together in a small dialysis membrane were added to an aqueous solution (pH 7.5) containing DHA (1 equiv), ATP (0.01 equiv), PEP (1.1 equiv) and $MgCl_2$ (0.2 equiv). The reaction was run at r.t. The progression of reaction was followed by enzymatically assaying consumed PEP and produced FDP (Figure 1). The reaction was stopped when the production of FDP reached maximium (70% conversion). The enzyme-containing bag was isolated and the products were precipitated as the barium salts. The yield was 64%. The reaction was repeated and the same results were obtained.

This procedure has advantage and disadvantages. All the enzymes and the starting materials (DHA) used are commercially available and inexpensive. PEP is rather expensive but can be prepared readily on large scales.[14] Enzymes are used enclosed in dialysis membranes:[15] this allows the simultaneous manipulation of several enzymes with high efficiency. The conversion yield is not high, presumably, due to the partial decompositions of the reaction intermediates: DHAP and GAP are known to be relatively at r.t. Overall, this demonstrates that several enzymes can be used in combination to achieve one-pot synthesis of a complex molecule that would require multi-step chemical processes.

In summary, we has described an example for the applicatios of multiezyme systems in carbohydrate synthesis. The multienzyme system described here can be modified for the synthesis of other sugars by replacing or adding one or more enzymes. Further investigations directed toward this end are now in progress.

## Experimental

**Synthesis of FDP.** To a 240 mL 3-neck round flask equipped with an electrode, septa, and a stir bar was added an aqueous solution (100 ml, pH 7.5) containing DHA (10 mmol), PEP (11 mmol), ATP (0.1 mmol), and $MgCl_2$ (0.2 mmol). The solution was deoxygenated by bubbling nitrogen gas through the solution. Glycerol kinase (100 U), pyruvate kinase (200 U), triosephosphate isomerase (500 U), and FDP aldolase (100 U) dissolved in a small aliquot (2 mL) of the reaction solution were transferred into a small dialysis tubing. The enzyme-containing bag was submurged in the aqueous mixture to initiate the reaction. The solution mixture was gently stirred and maintained under a nitrogen atmosphere and at room temperature. Progress of reaction was monitored by enzymatically determining the concentrations of PEP and FDP.[16] The production of FDP reached maximium (35 mM, 70% conversion) in 4 days. The reaction then was stopped by removing the enzyme-containing bag. FDP was isolated as the barium salts by adding an aqueous $Ba(OAc)_2$ solution.[17] Yield 4.2 g (3.22 mmol, 64%; purity, 60% based on $Ba_2 \cdot FDP \cdot 6H_2O$).

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## Phosphatidylethanolamine-N-methyltransferase in Rat Brain: Subcellular Distribution and High Specific Activity in Nuclear Envelopes

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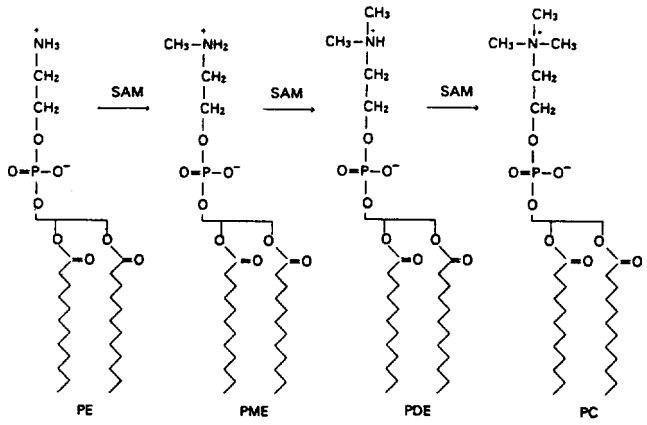
Phosphatidylethanolamine N-methyltransferase (PMT) is a membrane bound enzyme which catalyzes the methylation of phosphatidylethanolamine (PE) to phosphatidyl-choline (PC) by way of phosphatidyl-N-monomethylethanolamine (PME) and phosphatidyl-N,N-dimethylethanolamine (PDE) with S-adenosylmethionine (SAM) serving as the methyl donor. the presence and characteristics of this enzyme have been reported in various sources such as rat brain,[1,2] rat hepatocytes,[3] bovine adrenal medulla,[4] and yeast.[5] The physiological role of this enzyme has been studied in many ways.[6-8] It is thought that this enzyme plays a role related to signal transmission.[6] The sequential methylation reactions increase membrane fluidity,[7] which aids the coupling of receptor, transducer, and amplifier. For example, when D-isoproterenol binds to the β-adrenergic receptor, PMT is stimulated and




subsequent increase of membrane fluidity facilitates the coupling of β-receptor and adenylate cyclase.[8] In view of paramount importance of PMT as a possible modulator for certain biological signal transduction, many reports relating various membrane phenomena to the phospholipid methylation in brain tissue have been forwarded, including effects of aldosterone,[9] nerve growth factor,[10] histamine,[11] and protein kinase C.[12] As part of our effort for studying signal transduction system, we are also investigating enzymological aspect of PMT in rat brain tissue.

Initially Crews *et al.*[1] described the presence of phospholipid methylating enzymes in the brain. They identified two methyltransferases, PMT I and PMT II. The first enzyme (PMT I) was found to methylate PE to form PME and to have a high affinity to the methyl donor SAM. The second enzyme (PMT II) methylated PME twice to form PC and had a low affinity to SAM. They also reported that the most of methyltransferase activity in the brain was localized in particulate fraction, especially highly localized in synapatosomal plasma membranes. However, when the total activity recovered in each particulated subfraction was compared, nuclear fraction ($P_1$) had almost one half of the whole homogenate, meanwhile crude mitochondrial fraction ($P_2$) which contained synaptosomes had only a one-third and microsomal fraction ($P_3$) had less than a one-fifth of the total activity. In view of this report of a large activity found in $P_1$ fraction, we looked for the PMT activity in the nuclear subfraction in detail. We now present a revised subcellular distribution of PMT activity in rat brain, in which the highest specific activity is localized in nuclear envelopes. Recently we published a report on the characterization of PMT activity in rat brain myelin.[2]

Upon reinvestigating the subcellular distribution of PMT, we reoptimized the assay condition of PMT in nuclear fraction extensively. The methylation of phospholipid was measured by the incorporation of radioactive methyl group from SAM into lipids extracted by chloroform-methanol. Preliminary experiment revealed, however, that added exogenous substrates improved markedly the incorporation of methyl group into the products. Therefore the standard assay condition included exogenous substrate PME and PDE. The incubation medium was consisted of 100 μl of solution containing 10 mM carbonate buffer (pH 10.0), 10 mM MgCl, 100 μg of PME, 100 μg of PDE, 0.2-0.4 mg protein of nuclear suspension, and 100 μM of [H-methyl]-SAM (specific activity, 300 μCi/μmol). After maintaining uniform lipid environment