# GLYCEROL KINASE ACTIVITY IN ADENOMA ALVEOLAR TYPE II CELLS

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## 1. Introduction

Although several in vivo [1–3] and whole-cell [4–12] studies have demonstrated lung tissue can incorporate glycerol into the glycerol backbone of lipids, the presence of glycerol kinase has not been conclusively demonstrated in the tissue [2,13–15]. We have been particularly interested in the biosynthesis of dipalmitoyl-*sn*-glycero-3-phosphocholine, which is the major component of pulmonary surfactant and is synthesized by the alveolar type II cells. In previous studies [12,16–18], pulmonary adenomas induced by urethan were demonstrated to be a useful model for studies of lipid metabolism in alveolar type II cells. These benign adenomas consist almost exclusively of type II cells that contain lamellar bodies [19,20] and synthesize surfactant as normal type II cells [12,16]. However, surfactant biosynthesis is more active in adenoma type II cells than in whole lung [12,18] where type II cells comprise only 10% of the cells [21]. We found that adenomas incubated in Krebs-Ringer phosphate buffer incorporated [2-$^{3}$H, 1,3-$^{14}$C]glycerol into the glycerol moiety of phosphatidylcholine and other lipids [12]. This finding implied glycerol kinase is present and led us to examine the type II cells for activity. In the studies reported here, we demonstrate the conversion of labeled glycerol to glycerophosphate by glycerol kinase in cell-free preparations from adenoma type II cells.

## 2. Materials and methods

[U-$^{14}$C]Glycerol (131 mCi/mmol) and *sn*-[1-$^{14}$C] glycero-3-phosphate (120 mCi/mmol) were obtained from New England Nuclear Corp. Trizma (pH 7.4) and glycine were obtained from Sigma Chemical Co. ATP was obtained from P-L Biochemicals and glycerol kinase (*Candida mycoderma*) was purchased from Boehringer Mannheim.

Adenomas were harvested from the lungs of female BALB/c mice that had been injected i.p. with urethan (ethyl carbamate, 1 mg/g body weight on 3 consecutive days) approximately 6 months earlier [16]. The harvested adenomas were suspended in 0.25 M sucrose (1 g/5 ml) containing 0.1 mM dithiothreitol and homogenized at 0–4°C. Subcellular fractionations were done by centrifugation [22]. The preparations were stored at −20°C. Protein was determined by the procedure of Lowry et al. [23].

The assay conditions were similar to those employed earlier [24,25]. Standard incubations at pH 7.4 were carried out at 37°C in total vol. 0.3 ml and contained Tris–HCl (80 mM, pH 7.4), dithiothreitol (0.1 mM), glycine (80 mM), ATP (20 mM), $MgCl_2$ (6.6 mM), NaF (80 mM), and [$^{14}$C]glycerol (0.5 μCi, 3.8 nmol). The enzyme preparations were added and allowed to preincubate for 5 min before [$^{14}$C]glycerol was added to initiate the reaction. Under the conditions of the assay, a linear response was obtained with commercial glycerol kinase.

The glycerophosphate synthesized was routinely assayed by plating 20 μl of the incubation mixture directly on Silica Gel G thin-layer plates that were then developed in chloroform/methanol/ammonium hydroxide (65:35:8, v/v/v). In this solvent system, glycero-3-phosphate remained at the origin while glycerol migrated with an $R_F$ of 0.4. The distribution of radioactivity was determined by liquid scintillation spectrometry [26,27]. The glycerophosphate synthesized was further identified by paper chromatography

in ethyl acetate/acetic acid/water (3:3:1, v/v/v) [28]. The paper (5 X 35 cm) was cut into 1 cm strips and the distribution of radioactivity determined by liquid scintillation counting. Authentic *sn*-glycero-3-phosphate and glycerol migrated with $R_F$-values of 0.34 and 0.8, respectively, in this system.

## 3. Results and discussion

The conversion of [U-$^{14}$C]glycerol to [$^{14}$C]glycerophosphate was linear with protein concentrations from 20–200 μg protein/vial but at concentrations greater than 300 μg a deviation in linearity occurred. In fact, the total activity observed decreased at concentrations of homogenate greater than 300 μg protein until only 23% as much [$^{14}$C]glycerophosphate was synthesized using 6 mg protein/vial as with 200 μg/vial.

The reaction was linear with time for up to 20 min under our assay conditions. The specific activity observed in the homogenate was 44 pmol glycerophosphate synthesized/min/mg protein. The apparent $K_m$ for glycerol (at 20 mM ATP concentrations) in the system was 1.3 μM. Subcellular fractionations revealed most of the glycerol kinase activity is in the soluble fraction (table 1).

The reaction was ATP-dependent (table 1) and the [$^{14}$C]glycerophosphate synthesized was identified by paper and thin-layer chromatography as described in Materials and methods. Only two labeled compounds were detected in the products from the incubations: glycerophosphate and glycerol.

The radioassay used here is more sensitive than the conventional spectrophotometric assay for glycerol kinase [13,24] and was carried out at pH 7.4 with no hydrazine in the assay mixture. The spontaneous loss of activity upon storage was arrested by the addition of dithiothreitol; this action of dithiothreitol implies the enzyme may have a reactive sulfhydryl group as do other glycerol kinases [24].

The failure of earlier studies to demonstrate glycerol kinase activity in whole lungs may be attributable to the conditions and sensitivity of the assay systems used. On the other hand, the alveolar type II cells comprise only 10% of the cells in normal lung [21] but may contain the bulk of the glycerol kinase activity. The high levels of protein from other cell types may have made it difficult to detect its activity, for in the present study protein concentrations greater than 1 mg/ml decreased the amount of glycerophosphate synthesized.

Although adenomas incorporate labeled glycerol into lipids at a higher rate than does minced lung, the labeling profiles of dipalmitoyl phosphatidylcholine and other lipids by glycerol are the same with the two tissues [12]. The demonstration of glycerol kinase in the type II cells explains the route by which

Table 1
Subcellular distribution and ATP-dependence of glycerol kinase in adenoma alveolar type II cells[a]

| Fraction | ATP (20 mM) | [$^{14}$C]Glycerophosphate synthesized (pmol/min/mg protein) |
|---|---|---|
| Total homogenate | + | 60 |
| Total homogenate | – | 0.36 |
| Mitochondria (15 000 X *g* pellet) | + | 1.0 |
| Microsomes (100 000 X *g* pellet) | + | 0.39 |
| Soluble fraction | + | 180 |

[a] Incubations were carried out for 20 min as described in Materials and methods. The following amounts of protein were added per vial: Total homogenate, 336 μg; mitochondria, 96 μg; microsomes, 44 μg; and soluble fraction 100 μg. The values presented are averages obtained from duplicate incubations.

glycerol was incorporated into surfactant in the earlier whole-cell studies and indicates glycerol can be utilized directly by the lung for lipid synthesis without prior phosphorylation by other tissues.

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