# BIOSYNTHESIS OF SPIN-LABELLED PHOSPHOLIPIDS. ENZYMATIC INCORPORATION OF SPIN-LABELLED STEARIC ACID INTO PHOSPHATIDIC ACID

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**Received October 18, 1971**

SUMMARY

Guinea pig liver microsomes were tested for their capability to catalyze the incorporation of spin-labelled stearic acid into phospholipids. A system utilizing sn-glycero-3-phosphate-2-$^{14}$C, 12-doxyl-stearic acid, and microsomal fractions isolated from guinea pig liver yielded a phospholipid, identified as phosphatidic acid containing $^{14}$C and spin label. These experiments established the capability of a mammalian enzyme system to utilize spin-labelled stearic acid in the biosynthesis of a phospholipid.

## INTRODUCTION

Recent applications of spin-labelled probes to biological systems have demonstrated the usefulness of the approach in the study of molecular organization of biological membranes (1,2). In particular, spin-labelled steroids and fatty acids have been found to be sensitive indicators of the degree of organization and fluidity of the lipid components of membranes (3-10). Although it has been shown that Neurospora crassa (11) and

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Mycoplasma laidlawii (12) can utilize a spin-labelled fatty acid for the formation of lipid(s), conclusive evidence for the ability of a mammalian enzyme system to incorporate a spin-labelled substrate into phosphatides is lacking.

We report here experimental evidence that guinea pig liver microsomes can catalyze the incorporation of a spin-labelled fatty acid into a phospholipid, identified as phosphatidic acid, which is a key intermediary in the biosynthesis of complex phospholipids.

## EXPERIMENTAL AND DISCUSSION

The incubation system, similar to that described as favorable for the enzymatic synthesis of phosphatidic acid (13) contained: 2.5 µmoles of sn-glycero-3-phosphate-2-$^{14}$C (prepared, isolated, and characterized as described previously (14) with a specific activity of $8.04 \times 10^5$ dpm/µmole); 2.0 µmoles of the N-oxyl-4',4'-dimethyloxazolidine derivative of 12-keto-stearic acid, henceforth referred to as 12-doxyl-stearic acid (see Fig. 2), prepared, purified, and characterized as described (3); 50.0 µmoles of phosphate buffer (pH 7.0), 6.0 µmoles of $MgCl_2$, 50.0 µmoles of NaF, 10.0 µmoles of L-cysteine, 4.0 µmoles of ATP, 0.1 µmole of CoA, 2.0 mg of Triton-X100 and 0.5 ml of guinea pig liver microsomes (prepared as described (13) and containing 7.1 mg of protein) in a final volume of 1.2 ml. The reaction mixture was incubated for 30 minutes at 23°. After termination of the incubation and isolation of lipids according to Bligh-Dyer (15), 35.9 nmoles/mg protein of the biosynthesized labelled lipid was obtained.

The purification and characterization of labelled lipid thus obtained was carried out as follows:

Partial purification of biosynthesized lipid(s) was achieved by silicic acid column chromatography. After washing with chloro-

form, acidic lipid was eluted with chloroform:methanol (1:1). When 2.5 μmoles of the partially purified labelled lipid was subjected to DEAE-cellulose column chromatography as described (14), a single labelled compound exhibiting paramagnetism was eluted with linear gradient of $NH_4$-acetate as shown in Fig. 1. The

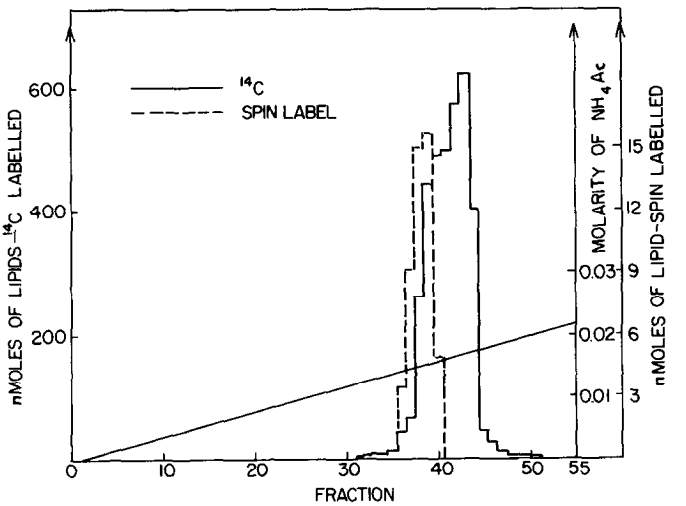


Figure 1. DEAE-cellulose column chromatography of spin-labelled, $^{14}C$-labelled lipid, obtained by a large scale preparation. The preparation of labelled lipid and the chromatographic procedures are described in the text.

recovery of the $^{14}C$ from the column was 93.2%. An aliquot of eluted compound when subjected to thin-layer and silicic acid impregnated paper (SG-81) chromatography in four solvent systems (16), gave a radioactive spot (representing more than 90% of applied radioactivity) indistinguishable from the standard of phosphatidic acid (obtained from egg-yolk lecithin with phospholipase D). Mild alkaline hydrolysis (14) of the labelled compound gave in 97.7% yield a water soluble product which was identified as 3-glycerophosphate. These experiments established that sn-glycero-3-phosphate-2-$^{14}C$ was converted with the microsomal

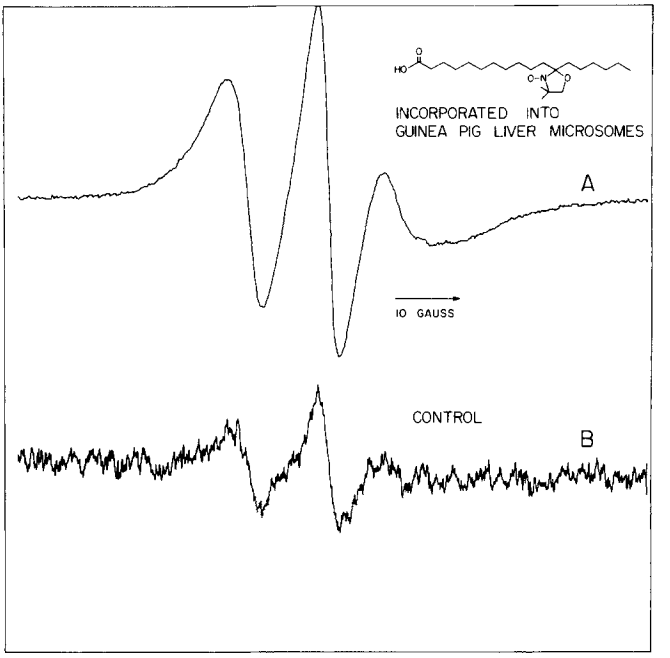


Figure 2. (A) ESR spectra of buffer solutions of guinea pig liver microsomes biosynthetically spin-labelled in the lipid portion with 12-doxyl-stearic acid.
(B) Control (with heat treated guinea pig liver microsomes).

enzyme system into phosphatidic acid, also containing 12-doxyl-stearic acid.

Electron spin resonance spectra were taken on a Varian E3 instrument using techniques described (17). Spectra of the washed microsomes, and of microsomes inactivated by heat treatment, after incorporation, are compared in Figs. 2(A) and (B). It is obvious that by non-enzymatic processes only a minimal amount of spin label can be retained, estimated to be less than 1% of the amount incorporated biosynthetically. Spectra of the 12-doxyl-stearic acid and of the purified spin-labelled phospholipid extracted from microsomes are shown in Figs. 3(A) and (B).

The ESR spectrum of the spin-labelled microsomes is indicative of rapid, effectively isotropic, motion of the spin label with a correlation time of approximately $3 \times 10^{-9}$ seconds.

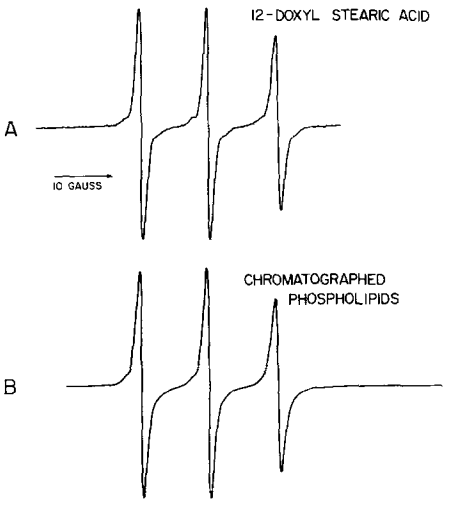


Figure 3. ESR spectra of chloroform:methanol:water (1:2:0.8 by volume) solutions of:
(A) 12-doxyl-stearic acid, $10^{-4}$ M.
(B) Intact spin-labelled lipid purified on the DEAE-cellulose column (Fig. 1, fraction 38).

## CONCLUSION

Data presented in this communication have established for the first time that spin-labelled fatty-acid (12-doxyl-stearic acid) can serve as the substrate for the enzymatic formation of phosphatidic acid in a mammalian system. Moreover, they have demonstrated that the introduction of a nitroxy-spin label into a substrate of fatty acid nature is a perturbation acceptable to the enzymatic catalysis of a mammalian system.

Studies in progress are designed to gain more experimental detail regarding the application of spin-labelled phosphatidic acid as precursor in the biosynthesis of complex phospholipids in biological membranes.

## ACKNOWLEDGEMENTS

This study was supported in part by grants from the Medical Research Council of Canada and the Ontario Heart Foundation to

N.Z. Stanacev, and by a National Research Council-Conselho Nacional de Pesquisas fellowship to S. Schreier-Muccillo.

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