STUDIES ON THE LIPIDS OF SHEEP RED BLOOD CELLS. IV. THE IDENTIFICATION OF A NEW PHOSPHOLIPID, N-ACYL PHOSPHATIDYL SERINE

Gary J. Nelson
Bio-Medical Division, Lawrence Radiation Laboratory,
University of California, Livermore, California 94550

Received November 12, 1969

Summary: A new phospholipid has been isolated from the erythrocytes of sheep. It has been tentatively identified as N-acyl phosphatidyl serine. It amounts to between 2.0 and 4.0% of the total phospholipids in sheep erythrocytes. This phospholipid has also been detected in the red cells of other ruminants, such as the cow and goat.

In earlier reports from this laboratory, the presence of an unidentified phospholipid in the erythrocytes of sheep and other ruminants was noted[1, 2, 3]. This compound has now been tentatively identified by its infrared spectrum, elemental analysis and hydrolysis products as N-acyl phosphatidyl serine (APS). A phospholipid with this structure has not previously been reported.

The compound was first detected as a distinct spot in two-dimensional, thin-layer chromatograms (TLC) of the total lipid extracts of sheep erythrocytes[1]. (See Fig. 1.) The substance migrated near phosphatidyl ethanolamine. It was isolated by a combination of column chromatography and preparative TLC[4, 5]. Phosphorus analysis of the purified material indicated that it contained only 2.8% P, rather than the 3.8 to 4.2% P found in most phospholipids of known structure. However, its infrared spectrum was almost identical to phosphatidyl serine (PS). (Typical spectra are shown in Fig. 2 for both PS and APS.) Yet it did not react with ninhydrin reagent, and its TLC migration did not mimic PS in any solvent system.

When the purified material was subjected to acid-catalyzed transmethylation, more than 80% of its initial weight was recovered

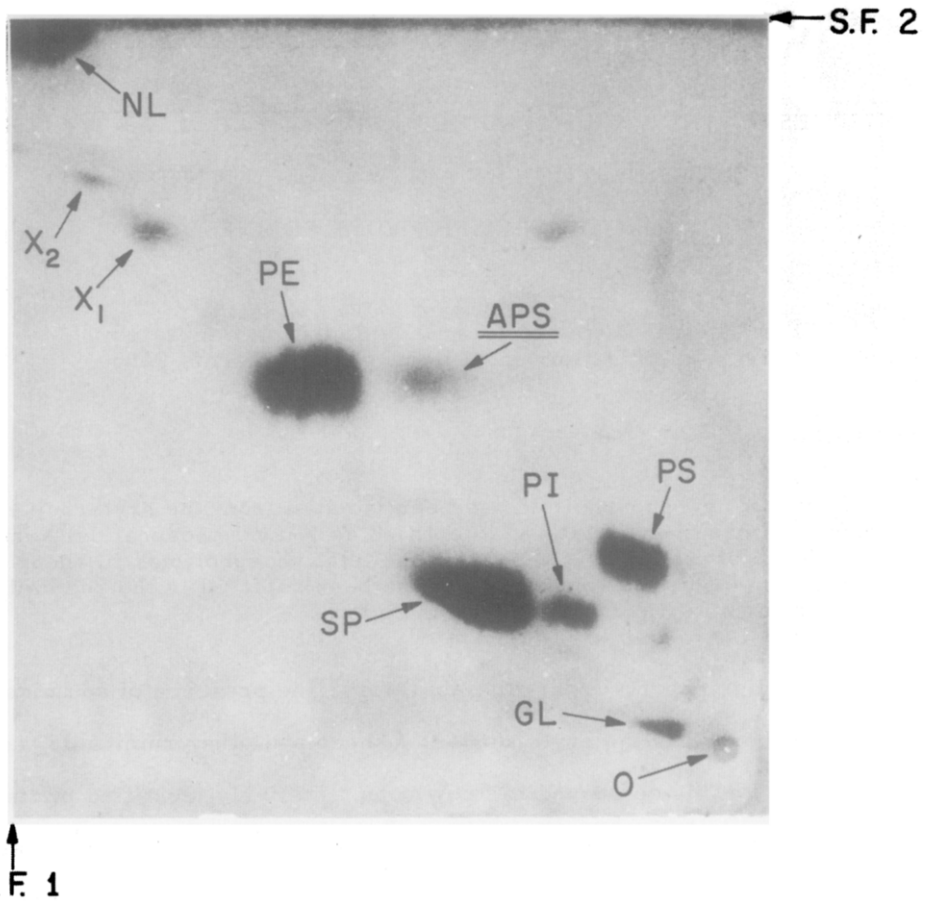


Fig. 1 A two-dimensional TLC separation of sheep erythrocyte phospholipids. Solvents: 1, $CHCl_3$ — MeOH — aq. NH_3, 65:25:5, (V/V/V); 2, $CHCl_3$ — MeOH — Acetone — HAc — H_2O, 5:2:1:1:0.5, (V/V/V/V/V). Abbreviations: O, origin; GL, glycolipid; SP, sphingomyelin; PI, phosphatidyl inositol; PS, phosphatidyl serine; APS, N-acyl phosphatidyl serine; PE, phosphatidyl ethanolamine; NL, neutral lipids; X_1, X_2, unidentified phospholipids.

---

as methyl esters. Typical diacyl phospholipids yield less than 75% of their initial weight as methyl esters upon transmethylation. Aqueous hydrolysis in acid indicated the parent compound contained only phosphorus, serine, glycerol and fatty acids, along with mono- or divalent cations. The molar ratios of the hydrolysis product were close to 1:1:1:3 for phosphorus, serine, glycerol, and fatty acids, respectively. Table 1 presents the results of these analyses along with the carbon, hydrogen and nitrogen composition of this material. Table 1 also gives

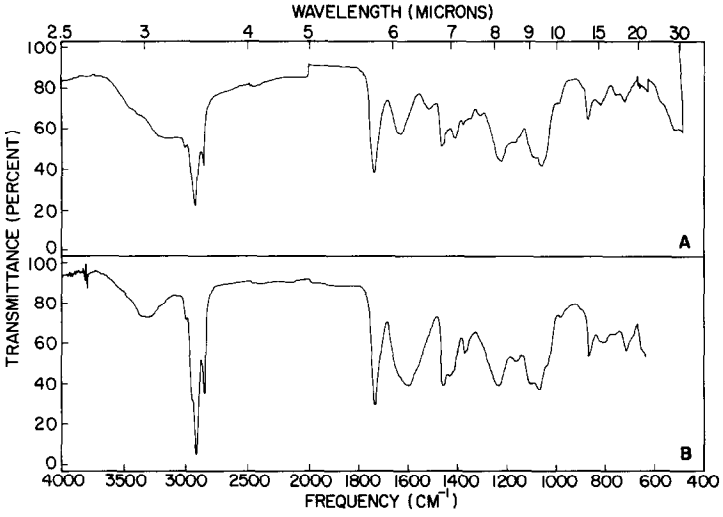


Fig. 2 Infrared spectra of purified phospholipids isolated from sheep red cells. Curve A, phosphatidyl serine; curve B, N-acyl phosphatidyl serine. Spectra obtained on thin films of samples spread on NaCl plate. The spectrophotometer was a Perkin-Elmer Model 521.

Table 1

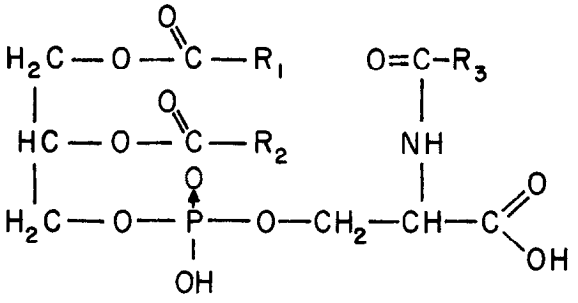
Chemical Composition and Molar Ratios of Constituents for N-Acyl Phosphatidyl Serine Isolated from Sheep Erythrocytes

	Weight Percents						
	Elemental Analysis				Hydrolysis Products		
	C	H	N	P	Serine	Glycerol	Fatty Acid
Sample	62.60	9.94	1.41	2.79	8.86	8.41	76.24
Theoretical*	62.30	9.90	1.30	2.86	9.73	8.53	79.05

	N/P	Serine/P	Glycerol/P	Fatty Acid/P
Molar Ratios	1.12	0.93	1.01	2.97

*Assuming approximate fatty acid composition reported in Ref. 3, and the dipotassium salt form of APS.

the theoretical values for the dipotassium salt form of APS for comparison to the experimental values. The fatty acid composition of the sheep red cell APS has been reported in a recent publication[3]. From these data and the fact that the compound does not have a free amino group, it most likely contains an amide-linked fatty acid and has the following structure:



in which R_1, R_2 and R_3 are long chain alkyl groups.

The proposed structure is tentative, primarily because no synthetic APS is available to compare with the natural product. APS appears to be very susceptible to auto-decomposition when isolated in pure form. It is unlikely that APS is an artifact of the extraction or isolation procedure, because identical methods applied to red cells of other species did not show the presence of this substance[1].

While APS has not previously been reported in tissue, a closely related substance, N-acyl phosphatidyl ethanolamine, has been reported by Bomstein[6] and Dawson, Clarke and Quarles[7,8] to occur in wheat flour, pea seeds and other plant seeds in small amounts. The average amount of APS in sheep erythrocyte studied in this laboratory varied between 1.4 and 4.8% of the total phospholipids[4]. The metabolism and relationship of APS to PS remains to be illuminated.

Acknowledgments

This work was performed under the auspices of the U.S. Atomic Energy Commission. The author is grateful to Dr. V. Shore for the serine analysis, and to Mr. R.A. Booth for technical assistance.

References

1. Nelson, G.J., Biochim. Biophys. Acta 144, 221 (1967).
2. Nelson, G.J., Lipids 3, 267 (1968).
3. Nelson, G.J., Lipids 4, 350 (1968).
4. Nelson, G.J., in Proceedings of the Third International Congress on Drugs Affecting Lipid Metabolism, ed. by R. Paoletti and W.L. Holmes, Plenum Press, New York, 1969, p. 350.
5. Rouser, G., Kritchevsky, G., Heller, D. and Lieber, E., J. Am. Oil Chem. Soc. 40, 425 (1963).
6. Bomstein, R.A., Biochem. Biophys. Res. Commun. 21, 49 (1965).
7. Quarles, R.H., Clarke, N. and Dawson, R.M.C., Biochem. Biophys. Res. Commun. 33, 964 (1968).
8. Dawson, R.M.C., Clarke, N. and Quarles, R.H., Biochem. J. 114, 265 (1969).