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Note

Resolution of the choline plasmalogens of various vertebrate tissues by thin-layer chromatography

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We recently have reported on the thin-layer chromatographic (TLC) resolution of the ethanolamine plasmalogens into two molecular species groups one of which is newly recognized and is, at least quantitatively, uniquely correlated with vertebrate myelin sheath[1]. Because of the relatively high content of choline plasmalogen in vertebrate cardiac muscle[2], we have explored the possibility that *this* phosphatide can be similarly resolved. The present report deals with our observations on extracts from a number of vertebrate tissues and does indeed show that cardiac muscle from several animal species contain at least two chromatographically resolvable groups of choline plasmalogen.

MATERIALS AND METHODS

Fresh, frozen-dried samples of various tissues, *e.g.* liver, pancreas, kidney, heart etc. as indicated in the results, were extracted with chloroform–methanol (2:1) at a ratio of 2 ml per 100 mg dry weight of tissue. A volume of 10 to 20 μl as used for chromatographic analysis (*cf.* ref. 1) using Schleicher & Schuell F-1500 silica gel plastic back sheets developed in chloroform–methanol–water (CMW) (100:25:3) for resolution of the phosphatidyl cholines (PC) and chloroform–ethanol–water (CEW) (65:25:3) for resolution of the phosphatidyl ethanolamines (PE). The PC was specifically detected by spraying with the Dragendorff choline reagent and PE by spraying with the fluorescent amine reagent Fluram (Roche, Nutley, N.J., U.S.A.) in acetone. The plasmalogens were then revealed by dipping in the Feulgen leuco–fuchsin aldehyde reagents[3]; the remaining lipids were revealed by Rhodamine 6G. Verification of the identity of these lipids was assisted by co-chromatography with appropriate commercially available authentic standard phosphatides.

RESULTS

With the exception of cow and cat heart (*cf.* Fig. 1a and b) the other heart specimens examined (guinea pig, rabbit, rat, mouse, armadillo) had essentially no PE-2 plasmalogen (CEW chromatograms) although some had small amounts of a Rhodamine staining PE-2 as did liver, kidney and pancreas of all the species examined.

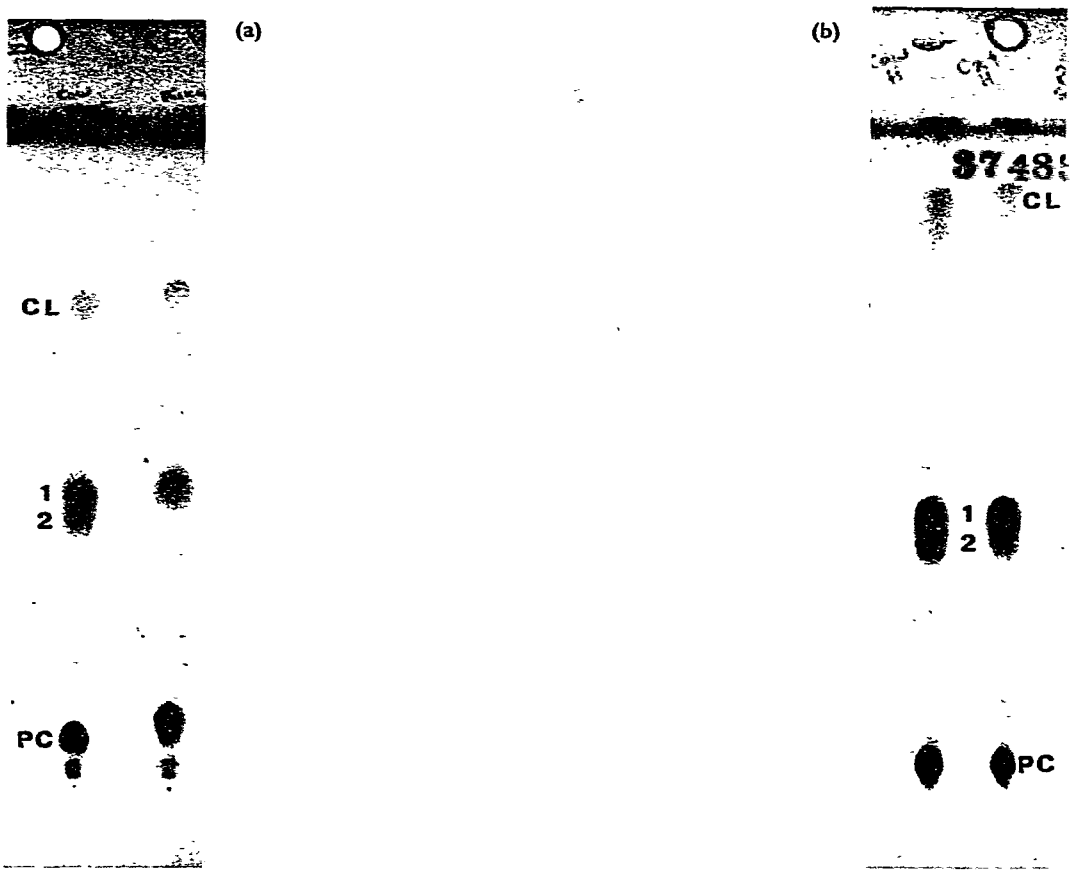

Fig. 1. (a) CEW chromatogram stained by the plasmal reaction with Rhodamine 6G counterstaining showing the phosphatides in two cardiac muscle extracts. The sample at the left is from cow, with PE resolved into two components and the sample at the right is from the spotted king snake and shows the more common single PE plasmalogen (1). CL = cardiolipin; PC = phosphatidyl choline. (b) A similar chromatographic preparation with cow heart at the left and adult cat heart at the right, each showing PE-1 and PE-2 as plasmalogen.

Cardiac muscle of these various animals was notable, in the adult, for the relatively abundant PC-1 and PC-2 plasmalogen (A and B, respectively in Fig. 2 and 3). Although PC-1 and PC-2 were present, in about equal amounts, in new-born cat and dog heart they were essentially negative for plasmalogen. Already by one week postnatal both PC-1 and PC-2 contained steadily increasing amounts of a plasmalogen component with perhaps PC-2 the greater. The choline plasmalogen of non-cardiac tissue was, on the other hand, quantitatively relatively minor although one or both PC-1 and PC-2, as Rhodamine staining spots, were observed in such tissues as liver, kidney, adrenal, placenta, egg yolk, human blood serum etc. Sometimes there appeared to be a third PC spot, mostly Rhodamine positive, between PC-1 and PC-2. As with the PE-1, PE-2 set the PC-1 appears to be the more osmiophilic and PC-2 more slowly stainable by the plasmalogen reagents.

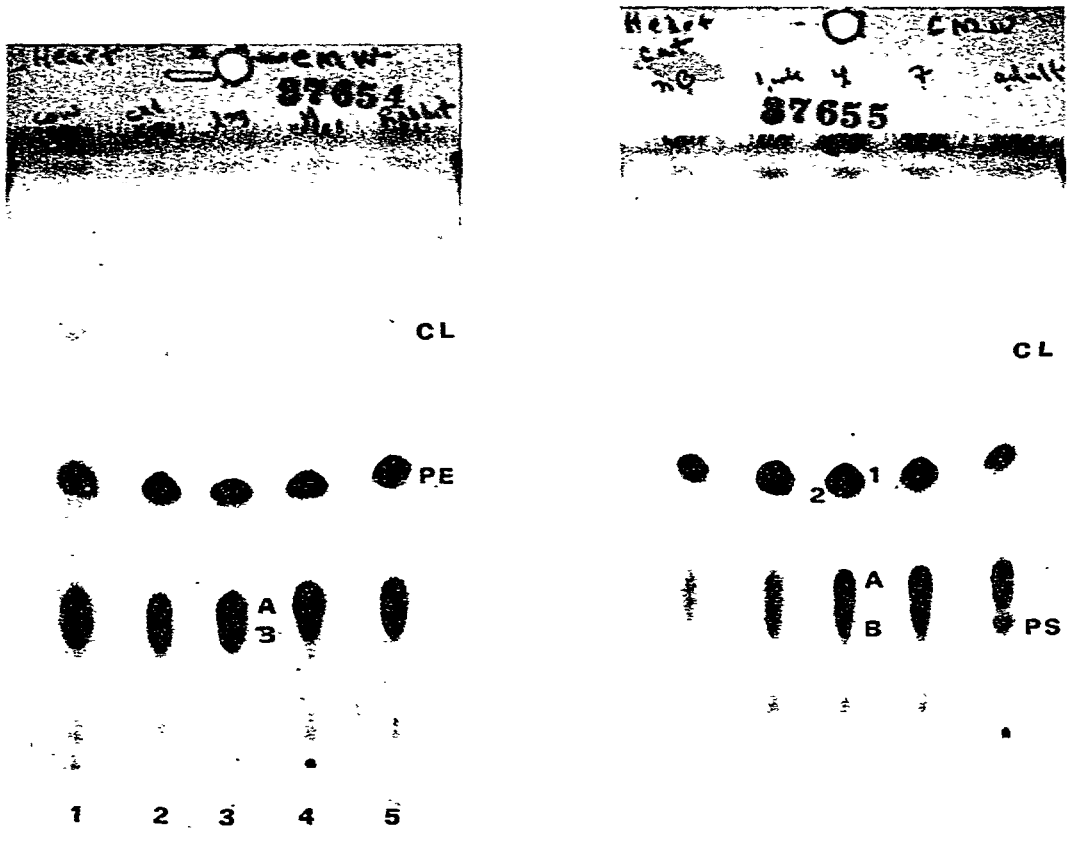

Fig. 2. CMW chromatogram, plasmal and Rhodamine 6G staining of heart extract; from left to right: cow, cat, dog, man, rabbit. Two PC plasmalogens are clearly evident (A, B) representing PC-1 and PC-2 respectively as indicated in the text with varying ratios of the two among the different samples. The resolution of the cow and cat heart PE is essentially undetectable.

Fig. 3. This CMW chromatogram shows the increasing PC plasmalogen content of maturing cat heart (left to right: new-born, 1 week, 4 weeks, 7 weeks and adult). Plasmalogen is absent from the B spot (PC-2) of new-born but it rapidley becomes a dominant component of both PC spots as growth proceeds as does the serine plasmalogen (PS).

DISCUSSION

Fatty acid analysis of isolated phosphatide preparations continues to provide data supporting the general thesis that they exist in nature as molecular species varying in their explicit fatty acid content (*cf.* refs. 4–6). This is no longer generally explainable as a random distribution of the fatty acid population unrelated to specific metabolic control. The physical resolution of molecular species of such phosphatides as PE and PC has generally been difficult to achieve without resorting to chemical binding procedures such as argentation TLC[4]. Our observations on the PE-2 plasmalogen of myelin and here on the PC-1 and PC-2 plasmalogens of developing and mature cardiac muscle seems to provide some evidence that specific plasmalogen molecular species

(*i.e.* specific with respect to (a) the base group, (b) the 1-alk-1′-enyl portion and (c) the 2-acyl group) are required for certain metabolic functions of (1) myelin and therefore of the nerve impulse and (2) mature cardiac muscle.

We have focused here essentially on the plasmalogen (*i.e.* alk-1-enyl) analogs of PC and PE but fully expect that further examination will reveal some similar specific relationship, although perhaps with other tissues, of the alkyl and diacyl analogs as well. From this should come additional data bearing on the precursor relationship of the alkyl glyceryl ethers to the plasmalogens.

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