The use of chromatographic procedures in the synthesis of ^{14}C-labelled phosphodiesters

L-Serine ethanolamine phosphodiester (L-SEP) and L-threonine ethanolamine phosphodiester (L-TEP) are natural phosphates which have been recently crystallized from the tissues of many animal species[1–7]. Much attention has been paid to L-SEP and L-TEP, since it is thought they may be involved in some biosynthetic reactions of phospholipid metabolism[2,4–6], and have in addition a clear evolutionary role.

A method of organic synthesis for ^{14}C-labelled L-SEP and L-TEP has been described earlier by us[8,9] and we now wish to report briefly on the use of the ion-exchange chromatography procedures and thin-layer chromatography techniques which have enabled us to obtain pure crystalline material and to control easily and quickly the process of separation and isolation of the labelled phosphodiesters.

Experimental and results

The synthesis of crude ^{14}C-L-serine ethanolamine phosphate and ^{14}C-L-threonine ethanolamine phosphate has been reported elsewhere[8,9]. The hydrogenolysis of the neutral phosphate ester mixture was carried out as previously described[8] and subjected to separation and purification as follows.

After removal of the catalyst and addition of suitable amounts of 10% NaOH to give a pH of 1.8–2.0, the mixture was concentrated and analyzed at this stage by thin-layer chromatography and radioautography where the single clearly defined components of the phosphate ester mixture were checked. Desalting was then carried out through a column (2.05 × 9.2 cm) of Dowex 50 W × 4 (H^+ form, 200–400 mesh), until the conductivity of the eluate had almost disappeared; the adsorbed material was then eluted with 2 *N* aqueous ammonia, which was removed by evaporation in a small rotary evaporator. The concentrated eluate was again tested by thin-layer chromatography and radioautography.

The eluate was now transferred to a column (2.25 cm × 123 cm) of finely graded[10] Dowex 50 W resin (15–40 μ particle size diameter), NH_4^+ form, and allowed to pass through at a rate of about 30 ml/h, with water as eluant. A complete separation of the various labelled and non-labelled compounds was achieved with only one treatment (Fig. 1). Rapid screening of alternate fractions by thin-layer techniques revealed the chromatographic purity of each peak. With regard to the synthesis of L-TEP, a satisfactory but incomplete separation of the mixture was obtained by this procedure (Fig. 2), although this phosphodiester was well separated from the other components.

The final steps of the purification process were the evaporation nearly to dryness of the relevant fractions, the crystallization of the dry residue and the further purification of the crystalline material, as described elsewhere[4,5,8,9,11].

At the end of the process the labelled crystalline L-SEP and L-TEP showed the same physical and chemical properties (melting point, optical rotation, infrared spectra, hydrolysis products, R_F values and chemical composition) as the unlabelled corresponding products synthesized previously[3–5,11]. In addition, other labelled compounds have been isolated, purified and characterized by this procedure, *e.g.* L-serine-L-serine phosphodiester (SSP) (Fig. 1), which is a by-product in the synthesis of L-SEP, and what is also most probably L-threonine-L-threonine phosphodiester (TTP) (Fig. 2),

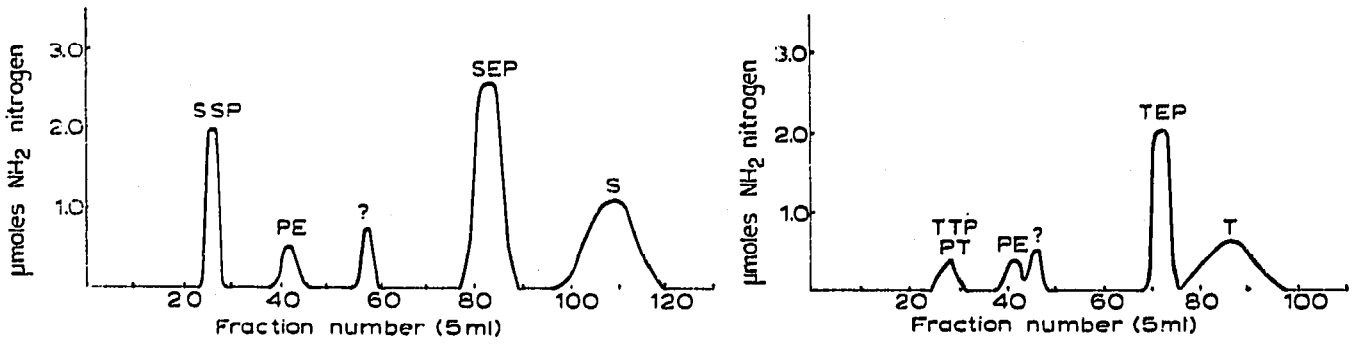

Fig. 1. Synthesis of L-SEP. Ion-exchange chromatogram on a Dowex 50 W resin column, NH_4^+ form, of the reaction mixture after desalting. SSP = L-serine-L-serine phosphodiester; PE = phosphorylethanolamine; SEP = L-serine ethanolamine phosphodiester; S = serine.

Fig. 2. Synthesis of L-TEP. Ion-exchange chromatogram on a Dowex 50 W resin column, NH_4^+ form, of the reaction mixture after desalting. TTP = L-threonine-L-threonine phosphodiester; PT = phosphorylthreonine; PE = phosphorylethanolamine; TEP = L-threonine ethanolamine phosphodiester; T = threonine.

which is presumably a by-product in the synthesis of L-TEP. SSP was obtained as a dry powdery product after numerous evaporations of the homogeneous chromatographic fractions, but TTP was contaminated with some traces of phosphorylthreonine even after the final purification steps.

The chromatographic procedures briefly outlined in this note provide a simple and rapid way of purifying labelled synthetic L-SEP, L-TEP, L-SSP and L-TTP from their reaction mixtures. The yields of these phosphodiesters were found to be satisfactory.

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