A New Method for the Synthesis of Serine Ethanolamine Phosphate

Serine ethanolamine phosphate (SEP) is a naturally occurring phosphodiester. The L-stereoisomer is found in the tissues of fish, amphibians, reptiles and birds[1,2]; the D-form is found only in earthworms[3]. Although the biosynthesis[4] and degradation[5] of L-SEP have been described in detail, its biological function is still unknown. Since metabolic studies of this compound are likely to involve labelled material, a method for the preparation of small quantities (about 0.1 mmoles) of carbon- or phosphorus-labelled SEP was developed, and the details are described in this communication.

The only previously described method is that of JONES and LIPKIN[6], which involves the sequential reaction of *N*-carbobenzoxyserine benzyl ester and *N*-carbobenzoxyethanolamine with phenylphosphorodichloridate, followed by the removal of the protecting groups. A scaled down modification of this was used by PORCELLATI and SIMONCINI[7] for the preparation of [^{14}C]SEP and related compounds. Generally applicable methods for the synthesis of phosphodiesters have been described by CRAMER et al.[8], and by KAMPE[9].

The simple procedure described in this communication involves the reaction in aqueous solution of ethyleneimine with serine phosphate to produce serine ethanolamine phosphate. The synthesis is similar to that of CDP-ethanolamine from CDP and ethyleneimine[10].

Apart from its simplicity, this method has the advantage that it is well suited to the small scale necessary in the preparation of radioactive compounds; a reasonable yield is obtained without recourse to protective groups, and most of the unreacted labelled serine phosphate can be recovered.

Labelled L-serine phosphate was prepared by the method of NEUHAUS and BYRNE[11] using either [^{32}P]$POCl_3$ or L-[3-^{14}C]serine and the appropriate unlabelled compound. The yield in terms of serine was 60%. In a typical experiment 0.05 ml (1000 μmoles) of ethyleneimine was added to an aqueous solution containing 30 μmoles of labelled L-serine phosphate, the pH was adjusted to 7.0 with acetic acid and the volume adjusted to 10 ml. The reaction mixture was incubated at 45 °C for 17 h and then concentrated under reduced pressure on a rotary evaporator. The complete mixture was applied to a 45 cm wide sheet of Whatman 3 MM paper and electrophoresis was carried out at pH 2.0 in a cooled plate apparatus[12] for 45 min under a voltage gradient of 100 V/cm. The buffer used contained 12.4 ml of 98% formic acid and 43.5 ml of glacial acetic acid/l. The paper was dried in an air stream and then autoradiographed. The separation of SEP from serine phosphate and various by-products was complete. In this system SEP moved towards the cathode with an $R_{alanine}$ of 0.38 (where alanine = 1.0), whereas serine phosphate moved in the direction of the anode. SEP and serine phosphate were eluted from the paper, and the recoveries determined by measuring radioactivity. The yield of L-SEP was 17% from serine phosphate, and the recovery of serine phosphate was 35%. The identity of L-SEP was established firstly by means of co-chromatography in a number of solvents with natural L-SEP; and secondly by the chromatographic identification of serine and ethanolamine phosphate produced by the action of a specific SEP-diesterase[5] on the synthetic compound.

Related phosphodiesters were formed by the reaction of ethyleneimine with alanine phosphonate (the phosphonic analogue of serine phosphate), threonine phosphate (threonine ethanolamine phosphate occurs in fish[13]), and the dipeptide phosphoserylvaline.

Apart from the usefulness of this procedure for the production of labelled phosphodiesters, the reaction between serine phosphate and ethyleneimine may be of importance in the field of protein chemistry. RAFTERY and COLE[14], in a modification of LINDLEY's procedure[15], showed that ethyleneimine was capable of converting the cysteine residues of proteins to S-β-aminoethylcysteine. The resulting amino acid is a lysine analogue and bonds adjacent to it are cleaved by trypsin. It was also shown that cysteine was the only amino acid normally found in proteins which reacted with ethyleneimine and from this the authors concluded that the reaction was specific for cysteine residues of proteins. In this communication I have shown that serine phosphate, either as a free amino acid or combined in a dipeptide, reacts with ethyleneimine, it is therefore probable that there will be some reaction between ethyleneimine and serine phosphate residues in proteins (perhaps particularly in the 'active centre' of enzymes). I found that aminoethylated phosphoserylvaline was not cleaved by trypsin, so it is likely that such bonds in proteins would be similarly resistant. Therefore the treatment of phosphoproteins with ethyleneimine if followed by tryptic digestion, is likely to cause changes in electrophoretic and chromatographic properties of the resultant peptides. This would be due not only to the expected cleavage following aminoethylation of cysteine residues, but also to a considerable change in charge brought about by the conversion of highly acidic serine phosphate residues to neutral SEP-residues[16].

Zusammenfassung. Eine einfache neue Methode für die Herstellung von Serine-Äthanolamin-Phosphat, einem natürlichen Phosphorsäurediester, wird entwickelt. Sie benützt die Einwirkung von Äthelinimin auf Serinephosphat und eignet sich besonders für die Herstellung von kleineren Mengen radioaktiver Verbindungen. Auf eine mögliche ähnliche Reaktion zwischen Äthelinimin und der Serinephosphatgruppe von Proteinen wird hingewiesen.

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