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Reaction of a nucleoside 2,4-dinitrophenyl phosphate with fluoride; A convenient method for the preparation of the nucleoside phosphorfluoridate*

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ABSTRACT

Examination of the reaction of 2,4-dinitrofluorobenzene with thymidine-5' phosphate in detail reveals that the initial product is the 2,4-dinitrophenyl ester. This reacts with fluoride to produce thymidine-5' phosphorfluoridate. This second reaction provides the basis for the conversion of preformed thymidine-5' 2,4-dinitrophenyl phosphate to thymidine-5' phosphorofluoridate.

INTRODUCTION

The phosphorofluoridate mono-esters form stable salts which are of potential utility in several areas of biochemistry. Thus, they are efficient intermediates in the base-catalyzed chemical synthesis of phosphodiesters (1,2). In addition, a nucleoside phosphorofluoridate has been used as a very effective nuclease inhibitor (3).

The available route to a phosphorofluoridate mono-ester involves the reaction of a phosphomonoester with 2,4-dinitrofluorobenzene. The latter is a powerful and relatively non-specific reagent (4). We have examined the mechanism of this reaction and, as a result, developed a mild and specific two-step procedure for preparation of phosphorfluoridate mono-esters. This procedure, which involves the synthesis of the 2,4-dinitrophenyl ester from the monoester followed by displacement of 2,4-dinitrophenoxide by fluoride, is the subject of this communication. Some of these results have been published in preliminary form (5).

MATERIALS AND METHODS

DEAE-cellulose was obtained from Schleicher and Schuell. The cation-exchanger AG50 x 2 (used in the interconversion of

nucleotide salts) was obtained from Bio-Rad. Descending chromatography on Whatman 40 paper was performed using the following solvent systems: 1,isopropanol:conc. NH_4OH:water (7:1:2); 2, isobutyric acid:M NH_4OH (5:3); 3, 0.1 M sodium phosphate, pH 6.8:solid $(NH_4)_2SO_4$:n-propanol (100:60:2) using appropriate reference compounds.

The 2,4-dinitrophenyl ester of thymidine-5' phosphate was prepared by esterification of the nucleotide with 2,4-dinitrophenol using dicyclohexylcarbodiimide by the method of von Tigerstrom and Smith (6). Organic solvents were redistilled and dried over CaH_2. Thymidine-5' phosphorofluoridate and thymidine-3' phosphorofluoridate were prepared by the method of Wittmann (7). These two nucleotides were also synthesized using the isolation procedure described below for thymidine-3' phosphorofluoridate.

Thymidine-3' Phosphorofluoridate. The triethylammonium salt of thymidine-3' phosphate (3.5 mmoles) (8) was reacted with 2,4-dinitrofluorobenzene (7 mmoles) in dimethylformamide (14 ml) for 3 days at 20°. Thymidine-3' phosphorofluoridate (2.35 mmoles) was isolated as its freeze-dried ammonium salt by ion-exchange chromatography on a DEAE-cellulose column in the carbonate form (25 mm x 700 mm) eluted with a linear gradient of water (2 litres) and 0.1 M NH_4HCO_3 (2 litres). The product was identical to authentic thymidine-3' phosphorofluoridate in solvents 1 and 2.

Reaction of Thymidine-5' Phosphate with 2,4-Dinitrofluorobenzene to Produce Thymidine-5' 2,4-Dinitrophenyl Phosphate. The triethylammonium salt of thymidine-5' phosphate (0.08 mmole) was allowed to react with 2,4-dinitrofluorobenzene (1.0 mmole) in dimethylformamide (1.0 ml) for 1 hour at 25°. Diethyl ether (50 ml) and water (50 ml) were added and the mixture shaken. The aqueous layer was passed into a column of DEAE-cellulose in the carbonate form (10 mm x 400 mm) and the products eluted with a gradient of water (1 litre) and 0.075 M NH_4HCO_3 (1 litre). The only nucleotide-containing peak was homogeneous and identical with thymidine-5' 2,4-dinitrophenyl phosphate on chromatography in solvents 2 and 3.

Reaction of Thymidine-5' 2,4-Dinitrophenyl Phosphate with Fluoride. The ammonium salt of the nucleotide (0.05 mmoles) in

water (5 ml) was allowed to react with sodium fluoride (5 mmoles) at 25° for 7 days. The solution was diluted with water to a volume of 500 ml and passed onto a column of DEAE-cellulose in the carbonate form (12 mm x 500 mm) and the product was eluted using a gradient of water (2 litres) and 0.05 M NH_4HCO_3 (2 litres). The only nucleotide product was identical with thymidine-5' phosphorofluoridate in solvents 1 and 2. It is important to note that careful chromatography is required to remove contaminating fluoride from a nucleoside phosphorofluoridate; however, this would not be a problem with larger molecules.

Kinetic Studies on the Reaction of Thymidine-5' 2,4-Dinitrophenyl Phosphate with Fluoride. The reactants, in appropriate concentrations, were mixed in water (1 ml) at 50° and the rate of release of 2,4-dinitrophenoxide was determined spectrophotometrically at 360 mm (6). The results are recorded in Table 1.

TABLE I

Reaction of Thymidine-5' 2,4-Dinitrophenyl Phosphate with Fluoride at 50° (see text for experimental details).

Concentration of Reactants (M)				Rate of release 2,4-dinitrophenoxide (nmoles/min)
Thymidine-5' 2,4-dinitro-	Sodium fluoride	Triethylamine	Sodium hydroxide	
10^{-4}	-	-	-	0.00
10^{-3}	-	-	-	0.07
10^{-4}	5×10^{-1}	-	-	0.20
10^{-4}	1.0	-	-	0.50
10^{-3}	5×10^{-1}	-	-	1.70
10^{-3}	1.0	-	-	4.00
10^{-3}	-	10^{-1}	-	0.30
10^{-3}	5×10^{-1}	10^{-1}	-	2.00
10^{-3}	-	-	10^{-2}	0.40
10^{-3}	-	-	10^{-1}	5.00

DISCUSSION

The reaction of a nucleoside phosphate with 2,4-dinitrofluorobenzene provides a convenient route to nucleoside phosphorofluoridates (7). However, 2,4-dinitrofluorobenzene reacts with a variety of nucleophiles (4) and results in at least one

class of unwanted product in reaction with nucleotides (8). Earlier studies suggested that a nucleoside 2,4-dinitrophenyl phosphate was an intermediate in the production of the phosphorofluoridate (8) although it also has been suggested that the reaction proceeds by a concerted mechanism (7). Additional evidence for a two stage mechanism is provided by kinetic studies on the reaction of simple 2,4-dinitrophenyl phosphate esters with fluoride ion (9). Taking all these points into consideration, an investigation of the reaction of fluoride with a nucleoside-5' 2,4-dinitrophenyl phosphate was required. Kinetic studies on the displacement of 2,4-dinitrophenoxide from thymidine-5' 2,4-dinitrophenyl phosphate by fluoride ion in water confirmed that this general type of reaction is quite facile (9) although fluroide is only about one tenth as effective as hydroxide as a nucleophile (Table 1). It has been suggested that a trialkylamine can be a catalyst of the displacement of 2,4-dinitrophenoxide from phosphate by fluoride (9). However, the present kinetic study shows that the effect of triethylamine and fluoride in displacing 2,4-dintrophenoxide from thymidine-5' 2,4-dinitrophenyl phosphate is only additive (Table I). There is no detectable catalytic effect by triethylamine on the reaction of fluroide in our experiments and the release of 2,4-dinitrophenoxide which is produced by triethylamine alone must be a consequence of the great susceptibility of the ester to base-catalysed hydrolysis (6,10). It should be noted that in our earlier work (5) we interpreted our results in terms of trialkylamine catalysis of the reaction of fluoride. It is clear, from the results in Table I, that the determining factor in phosphorofluoridate formation is fluoride concentration. As a consequence (see Materials and Methods), a short exposure of a nucleoside phosphate to 2,4-dinitrofluorobenzene provides an efficient preparative route to the 2,4-dinitrophenyl ester of the nucleotide.

In summary, the present experiments define a mild, specific, two-step route to phosphorofluoridates of mono-esters. First, the 2,4-dinitrophenyl ester is prepared by the method of von Tigerstrom and Smith (6) or by brief reaction with 2,4-dinitrofluorobenzene. Then the 2,4-dinitrophenoxide anion is displaced

by fluoride in aqueous solution. This procedure is exemplified by the preparation of thymidine-5' phosphorofluoridate, but the method should be applicable to other phosphomonoesters. It should be noted that there is an alternate route to nucleoside phosphorofluoridates involving the condensation of nucleotide and fluoride in the presence of dicyclohexyl carbodiimide (11,12).

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