OLIGONUCLEOTIDIC COMPOUNDS. XLVI.*

SYNTHESIS OF ADENYLYL-(5'→3')-URIDYLYL-(5'→5')-URIDYLYL-(3'→5')-ADENOSINE

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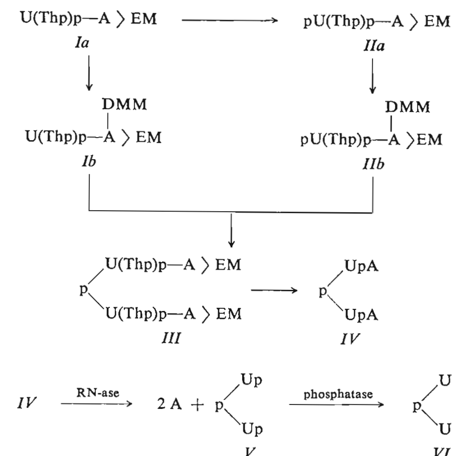
Received May 24th, 1973

2'-O-Tetrahydropyranyluridylyl-(3' → 5')-N^6-dimethylaminomethylene-2',3'-O-ethoxymethylene-adenosine (*Ib*) reacts with 5'-O-phosphoryl-2'-O-tetrahydropyranyluridylyl-(3' → 5')-N^6-dimethyl-aminomethylene-2',3'-O-ethoxymethyleneadenosine (*IIb*) in the presence of N,N'-dicyclohexyl-carbodiimide to give the protected tetranucleoside triphosphate *III*. Removal of protecting groups from compound *III* affords adenylyl-(5' → 3')-uridylyl(5 → 5')-uridylyl-(3' → 5')-adenosine (*IV*). When compared with UpA, compound *IV* shows a doublefold priming activity in the polymerisation of adenosine 5'-diphosphate with polynucleotide phosphorylase (*Micrococcus lysodeicticus*).

The synthesis and priming activity of uridylyl-(5' → 3')-uridylyl-(5' → 5')-uridylyl-(3' → 5')-uridine has been reported in an earlier paper of this series[1]. Further investigations in this direction have now required the synthesis of an analogous tetranucleoside triphosphate composed from two uridylyl-(3' → 5')-adenosines connected at positions $C_{(5')}$ by a phosphodiester bond (compound *IV*). The synthesis was started from 2'-O-tetrahydropyranyluridylyl-(3' → 5')-2',3'-O-ethoxymethyleneadenosine (*Ia*). In the synthesis of this dinucleoside phosphate, an adenosine derivative with an unprotected amino group has been used. This simplification of the synthesis of an internucleotidic bond has been used by Brimacombe and coworkers[2] and, recently, by Narang and coworkers[3]. The $C_{(5')}$-hydroxylic function of compound *Ia* was phosphorylated with an excess of 2-cyanoethyl phosphate after the previous protection of the adenosine amino group on treatment with dimethylformamide dineopentylacetal[4]. The 2-cyanoethyl and dimethylaminomethylene groups were removed by the action of aqueous ammonia and the product *IIa* was isolated by gradient elution on DEAE-cellulose.

By the action of dimethylformamide dineopentylacetal, the mixture of compounds *Ia* and *IIa* was converted into the N^6-dimethylaminomethylene derivatives *Ib* and *IIb* which were then condensed in the form of pyridinium salts by the action of N,N'-dicyclohexylcarbodiimide. The reaction mixture was processed with aqueous am-

* Part XLV: This Journal *38*, 3642 (1973).



monia and then separated on DEAE-Sephadex. The product *III* was eluted along with the unreacted compound *IIa* and purified by preparative paper chromatography. Deblocking of compound *III* with 20% aqueous acetic acid afforded compound *IV*. This product was characterised by pancreatic ribonuclease degradation to give adenosine and 3'-O-phosphoryluridylyl-(5' → 5')-uridine 3'-phosphate (E_{Up} 1·22). By the action of alkaline phosphatase, the latter compound affords uridylyl-(5' → 5')-uridine (E_{Up} 0·53).

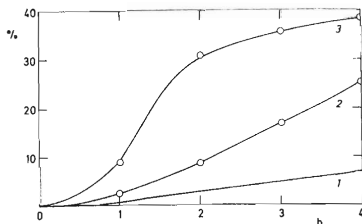


FIG. 1

Polymerisation (%) of Adenosine 5'-Diphosphate with Polynucleotide Phosphorylase

1 Without primer, *2* primed with uridylyl-(3'→ 5')-adenosine, *3* primed with compound *IV*.

The priming activity of compound *IV* in the polymerisation of Adp with polynucleotide phosphorylase was compared with that of UpA by the colorimetric determination of the inorganic phosphate in samples of reaction mixtures. For the results see Fig. 1.

EXPERIMENTAL

Descending chromatography was performed on paper Whatman No 1 in the solvent systems S_1, 2-propanol–conc. aqueous ammonia.water (7 : 1 : 2), and S_2, 1-propanol–conc. aqueous ammonia–water (55 : 5 : 40). The preparative runs were performed on paper Whatman 3 MM. Electrophoresis was carried out on paper immersed in tetrachloromethane; buffer solution E, 0·05M triethylammonium hydrogen carbonate (pH 7·5).

The enzymatical hydrolysis was performed with 1—2 μmol of the substance, volume 0·1 ml. Pancreatic ribonuclease (Sigma, St. Louis, U.S.A.), 25 μg in 0·05M-Tris-HCl, pH 8. Alkaline phosphatase *E. coli* (Sigma), 1 μl of a suspension in ammonium sulfate, 0·05M-TRIS-HCl, pH 9·5. Snake venom phosphodiesterase (C. F. Boehringer & Söhne, Mannheim, Germany), 10 μl of a glycerol solution in 0·05M ammonium hydrogen carbonate. The polynucleotide phosphorylase was purchased from P-L Biochemicals, Milwaukee, U.S.A. The primer-dependent form of the enzyme was prepared according to Klee and Singer[5] and used as an unconcentrated active fraction from a G 200 column.

5′-O-Phosphoryl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-O-ethoxymethyleneadenosine (*IId*)

2′-O-Tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-O-ethoxymethyleneadenosine (*Ia*) triethylammonium salt (2 mmol; prepared from 2′,3′-O-ethoxymethyleneadenosine) is dissolved in dimethylformamide (50 ml), the solution is concentrated to a half of its original volume, and dimethylformamide dineopentylacetal (5 ml) is added. After 20 h, the solution is evaporated under diminished pressure, the residue is dissolved in 30% aqueous pyridine (20 ml), and the solution is passed through a column (10 ml) of pyridinium Dowex 50. The column is eluted with 30% aqueous pyridine and the combined eluates are evaporated under occassional additions of pyridine. 2-Cyanoethyl phosphate pyridinium salt (4 mmol) is added to the residue and the whole is coevaporated with five portions of pyridine. The residue is dissolved in pyridine (10 ml), N,N′-dicyclohexylcarbodiimide (4 g) is added to the solution, and the whole is kept at room temperature for 3 days. Water (5 ml) is then added and, after 1 h, the unreacted diimide is removed by extraction with cyclohexane. The lower layer is filtered, the filtrate diluted with conc. aqueous ammonia (25 ml), and heated in a closed vessel at 60°C for 2 h. The reaction mixture is concentrated to half of its original volume and the concentrate is applied to a column (800 ml) of DEAE-cellulose (HCO_3^-). Linear gradient elution is then performed with the use of 2·5 l of water in the mixing chamber and 2·5 l of 0·25M triethylammonium hydrogen carbonate in the reservoir. The peak eluted at the 0·18—0·22M buffer concentration ($31\,000 A_{260}$) is evaporated and the residue is repeatedly coevaporated with ethanol to afford 1·12 g of the triethylammonium salt of compound *IIa*; R_F, 0·20 (in S_1); E_{Up}, 0·81. By the action of 20% aqueous acetic acid, compound *IIa* is converted to 5′-O-phosphoryluridylyl-(3′ → 5′)-adenosine, the snake venom phosphodiesterase degradation of which affords uridine 5′-phosphate and adenosine 5′-phosphate.

Adenylyl-(5′ → 3′)-uridylyl-(5′ → 5′)-uridylyl-(3′ → 5′)-adenosine (*IV*)

A solution of triethylammonium salts of compound *Ia* (1·45 mmol) and compound *IIa* (0·62 mmol) in dimethylformamide (50 ml) is concentrated to the volume of 10 ml and dimethylformamide dineopentylacetal (6 ml) is added to the concentrate. The whole is kept at room temperature for 2 days, evaporated, the residue coevaporated with two portions of pyridine, and dissolved in 30% aqueous pyridine (10 ml). The solution is passed through a column (10 ml) of pyridinium Dowex 50 ion exchange resin and the column is eluted with 30% aqueous pyridine. The combined eluates are evaporated under occasional additions of pyridine, the residue is coevaporated with five portions of pyridine, and finally dissolved in pyridine (10 ml). N,N′-Dicyclohexylcarbodiimide (3 g) is added and the whole is kept at room temperature for 3 days. Water (2 ml) is then added, the mixture kept for 1 h, and evaporated. The residue is dissolved in a mixture of methanol (30 ml), conc. aqueous ammonia (30 ml), and cyclohexane (60 ml), the solution heated at 50°C for 1 h, and filtered. The lower layer of the filtrate is concentrated in the presence of a few drops of 1-dodecanol, and the concentrate is applied to a column (600 ml) of DEAE-Sephadex (HCO_3^-). The elution is performed with a linear gradient of triethylammonium hydrogen carbonate (4 l of 0·05M buffer solution in the mixing chamber and 4 l of 0·6M buffer solution in the reservoir). The broad peak (0·3—0·55M buffer solution) is evaporated, the residue coevaporated repeatedly with ethanol, and dissolved in water. The aqueous solution is chromatographed on 6 sheets of paper Whatman No 3 MM in the solvent system S_1. The UV-absorbing bands (R_F 0·30) are eluted with water, the eluates evaporated, and the residual compound *III* ($6900A_{260}$) is dissolved in 20% aqueous acetic acid (5 ml). The solution is heated at 50°C for 1 h and then chromatographed for 3 days on 4 sheets of paper Whatman No 3 MM in the solvent system S_1. The UV-absorbing band at the startline is eluted with 0·5% aqueous ammonia ($2400A_{260}$) and the eluate is freeze-dried to afford 86 mg of the ammonium salt of compound *IV*. UV spectrum λ_{max} 257—258 nm, λ_{min} 239 nm; $A_{250/260}$ 0·82, $A_{280/260}$ 0·29. The pancreatic ribonuclease degradation of compound *IV* leads quantitatively to adenosine and compound *V* in the ratio 2·02 : 1 (R_F value 0·02 in S_1 and 0·69 in S_2; E_{Up} 1·22). The alkaline phosphatase degradation of compound *V* affords compound *VI* (R_F value 0·16 in S_1; E_{Up} 0·53).

Priming Activities of Compound *IV* and Uridylyl-(3′ → 5′)-adenosine

Mixtures containing in 0·1 ml of Tris-HCl (pH 9; 10 μmol) sodium ethylenediamine tetraacetate (0·5 μmol), magnesium chloride (1 μmol), sodium adenosine 5′-diphosphate (2 μmol), the primer (0·1 μmol), and a solution of the primer-dependent polynucleotide phosphorylase[5] (50 μl) were incubated at 37°C and the withdrawn samples were frozen down to −70°C. The inorganic phosphate was determined photometrically[6]. For the results see Fig. 1.

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Translated by J. Pliml.