5-CHLORO-8-HYDROXYQUINOLINE (CHLOROXINE) ESTERS OF CARBOXYLIC ACIDS - SELECTIVE REAGENTS FOR ACYLATION OF NUCLEOSIDE AND NUCLEOTIDE AMINOACYL DERIVATIVES

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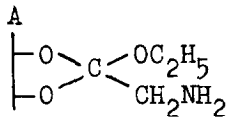
Peptidyl-tRNA is an important intermediate of protein biosynthesis (Nathans and Lipmann, 1961). It has been postulated (Nakamoto and Kolakofsky, 1966) that N-acylaminoacyl-tRNA may serve as an initiator of protein synthesis in E.coli. The chemical synthesis of these compounds may therefore provide very useful tool for detailed investigation of individual steps of protein synthesis. However, it requires an agent which would effect a selective and quantitative acylation of only the amino group in aminoacyl-tRNA and would not react with the aminoacyl--tRNA remaining functional groups in the t-RNA chain. Moreover, the reaction should be performed under conditions that preclude the possibility of cleavage of the aminoacyl residue or of other changes in the structure of t-RNA (e.g., isomerisation of 3′⟶5′diester bonds). Some of the methods used earlier turned out to be nonselective (cf. Lapidot et al., 1967). Other, milder methods for aminoacyl-tRNA acylation have been devised, such as those which make use of activated esters, i.e. of p-nitrophenyl esters (Marcker and Sanger, 1964) and N-hydroxysuccinimide esters (de Groot et al., 1966; Lapidot et al., 1967).

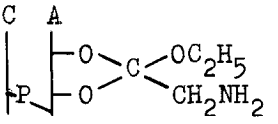
In our investigation the latter method has been extended to

the synthesis of certain 2′(3′)-O-peptidyl- and 2′(3′)-O-(N-acyl) aminoacyl nucleosides and oligonucleotides (Chládek and Žemlička, 1968, 1969) which can be regarded as simple models of peptidyl t-RNA.

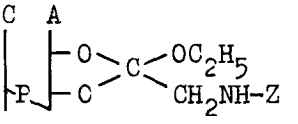
During the acylation of the glycine amino group of the adenosine derivative I by p-nitrophenyl acetate (IVa) and N-hydroxysuccinimide acetate (Va) respectively, we did not observe - in accordance with earlier findings (Lapidot et al., 1967) - simultaneous acylation of the amino group of adenine (Chládek and Žemlička, 1968).



I



II



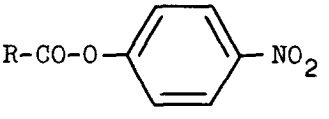
III

Z = $C_6H_5CH_2$-O-CO-

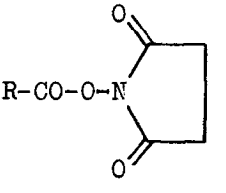
We made the same observation also when we used a series of p-nitrophenyl and N-hydroxysuccinimide esters of protected amino acids (Chládek and Žemlička, 1968). Moreover we have now found that uridine and guanosine (the latter was tested as 2′,3′-O--isopropylidene derivative) do not react with activated esters IVa and Va as well. On the other hand, cytidine reacts readily both with p-nitrophenyl acetate (IVa) and N-hydroxysuccinimide acetate to give N^4-acetylcytidine. A similar reaction, eventhough to a lesser extent, was observed with cytidine-5′-phosphate (Table I). These results are contrary to those of reported studies in which the reaction of cytidine-5′-phosphate with various N-hydroxysuccinimide esters has not been observed. (The conditions of our experiments correspond both to the conditions under which the reaction of cytidine-5′-phosphate with N-hydroxysucci-

nimide esters was tested (Lapidot et al., 1967) and also to the conditions of acetylation of phenylalanyl-tRNA (de Groot et al., 1966)). The cytosine ring obviously reacts with the p-nitrophenyl and N-hydroxysuccinimide esters also in the molecule of ribodinucleoside phosphate CpA. We examined the UV-spectra of products of the reaction of derivatives CpA II and III with esters IVa, IVb, Va and Vb. A partial acylation of the cytosine ring manifests itself by an increase in extinction at 250 nm and thus also by an increased E 250/260 ratio.

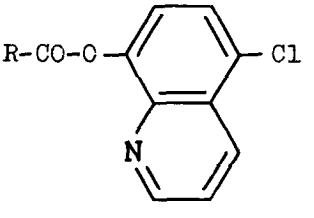
It is obvious that the acylation of the cytosine ring does not represent a drawback to the synthesis of the peptidyl derivatives (Chládek and Žemlička, 1969) using the orthoester intermediate II. The acyl group can be readily removed from the cytosine moiety by ammonolysis since the orthoester group is stable under the conditions of this method. If, however, the orthoester derivative is not accessible and the alkali-labile 2′(3′)--O-aminoacyl derivative (e.g. aminoacyl-tRNA) is used as starting material, these methods are not capable of affording satisfactory results.



IVa,b



Va,b



VIa,b

a = CH_3CO-

b = $ZNH(CH_2)_4CH(NHZ)$-CO-

Z = $C_6H_5CH_2OCO$-

A novel method of activation of the carboxyl group for peptide synthesis using 5-chloro-8-hydroxyquinoline (chloroxine) esters was developed recently (Jakubke and Voigt, 1966). The reactivity of chloroxine esters is comparable with the reactivity of p-nitrophenyl esters in spite of the fact that the acidity of chloroxine is several times lower than that of p-nitrophenol. This fact together with other findings supports the view that the aminolysis of chloroxine esters is governed by a different mechanism, probably involving the participation of the nitrogen hetero atom (Jakubke et al., 1967). We deemed likely that in the case of cytidine the formation of such an intermediate might proceed with difficulty and that the aminolytic reaction might therefore be suppressed. As expected, unlike with esters IVa and Va, cytidine reacts with chloroxine acetate (VIa) only to a negligible extent (Table I).

On the other hand, the reaction of chloroxine acetate (VIa, 0.2M) with aminoorthoester I (0.1M) in dimethylformamide is complete in 4 hours. The analogous reaction of orthoester II with chloroxine esters VIa and VIb is essentially quantitative. In accord with expectation we did not observe in these cases a difference in the UV-spectra of the obtained products and the spectrum of CpA (Table II).

In view of their high selectivity and reactivity, chloroxine esters appear to be well-suited to the acylation of 2′(3′)--O-aminoacyl derivatives of oligonucleotides, especially of those which contain cytidine. In our opinion these esters could be exploited also for the acylation of aminoacyl-tRNA. Experiments following this line of investigation are at present in this Laboratory.

Table I

Reaction of Cytidine and Cytidine-5′-phosphate with Activated Acetic Acid Esters

(in dimethylformamide at room temperature, after the reaction time given an aliquot of the reaction mixture was subjected to paper chromatography or electrophoresis (see footnotes [+] and [x]). The ratio of the starting material and product was determined spectrophotometrically at 260 nm after elution of the corresponding spots with 0.01 N HCl.

Compound	Reagent	Reaction Time (h)	Comp. conc.	Ratio Reagent/ Compound	% of acetylated compound
Cytidine	IVa	4	0.2M	4	48[+]
Cytidine	VIa	4	0.1M	4	0
Cytidine	IVa	24	0.2M	4	48
Cytidine	Va	23	0.2M	4	45
Cytidine	VIa	21	0.1M	4	5
Cytidine-5′-phosphate[++]	Va	24	0.1M	2	9[x]
Cytidine-5′-phosphate[++]	Va	48	0.1M	2	18
Cytidine-5′-phosphate[xx]	Va	24	0.004M	100	36

[+] N^4-Acetylcytidine (van Montagu et al., 1968) was identified by comparison with an authentic sample (a gift of Dr. van Montagu) by paper chromatography in the system 1-butanol--acetic acid-water (5:2:3; R_F 0.43) and by UV-spectrum λ_{max} 244 nm, 299 nm, λ_{min} 270 nm; pH 2.0)

[++] Monotributylammonium salt, suspension.

[x] N^4-Acetylcytidine-5′-phosphate was identified by electrophoresis at pH 3.4 (electrophoretic mobility 3.6 of that of cytidine-5′-phosphate), paper chromatography in the system ethanol-1M ammonium acetate (5:2; R_F 0.67) and by UV-spectrum identical with that of N^4-acetylcytidine.

[xx] Reaction in a mixture of 0.1M sodium acetate (pH 5.4) and dimethylformamide (1:4).

Table II

UV-Spectroscopic Characterisation (in 96% ethanol) of the Products of the Reaction of CpA Derivatives (II and III) with Active Esters IV-VI

(in dimethylformamide at room temperature, the concentration of III was 0.2M and that of II 0.05M, after the reaction time given, the excess of absolute ether was added and the precipitated product was separated by centrifugation and examined by UV-spectroscopy)

Compound	Reagent	Reaction Time (h)	Ratio Reagent/ Compound	λ_{max} nm	250/260	280/260	290/260
III	-	-	-	260	0.82	0.46	0.16
III	Va	24	4	257	0.97	0.41	0.24
III	IVa	24	4	258	0.95	0.50	0.22
II	VIa	24	5	261	0.86	0.47	0.17
II	Vb	24	5	257	0.95	0.41	0.21
II	IVb	24	5	256	0.96	0.42	0.22
II	VIb	6	5	260	0.74	0.50	0.22

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