tics suggests that the controlling seasonal fluctuations are not merely local phenomena. In addition, the controlling parameters should display intraseasonal variations capable of accounting for the occasional secondary bands observable in sample 2 (see Fig. 1).

Water temperature (*9*) and available sunlight (*10, 11*) have been the factors most frequently suggested as controlling coral growth rates. A recent study in Hawaii (*11*) has indicated that available light is a dominant factor in determining growth rates of reef corals there. Our results are consistent with this finding; seasonal variations in cloud cover in combination with variations in the length of day occur even in locations where water temperature is almost invariant, and can account for the annual banding mechanism. Year-to-year variations in the degree and dates of occurrence of cloudiness can account for both the secondary bands observed in sample 2 and the modest differences between annual bands.

The fact that there are internal, seasonal growth bands permits the retrieval of information on both the differential and integral long-term growth rates of corals without the necessity for real-time in situ experiments. The response of coral growth rates to imposed stresses such as storms, pollution, and radioactivity inclusions may also be preserved in the density pattern record.

Other possible applications of the coral internal calendar are the following: (i) retrieval of global distribution patterns of ^{90}Sr and ^{14}C in surface ocean waters during the early stages of the nuclear era; (ii) measurement of annual variations in cloudiness from the band dimensions and, concurrently, of water temperature variations determined by isotope thermometry (*12*); (iii) eventual construction of a "coral ring chronology" analogous to the long-term tree-ring chronologies successfully developed by dendrochronologists; and (iv) paleoclimatic studies and investigations of the natural historic variations in oceanic ^{14}C activity, provided that sufficiently old or preserved coral heads can be found and dated.

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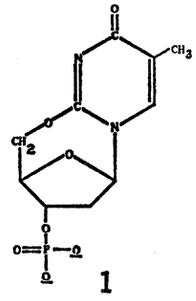
Oligothymidylates: Formation by Thermal Condensation of O²,5′-Cyclothymidine 3′-Phosphate

Abstract. *Thermal activation of* O²,5′-*cyclothymidine 3′-phosphate in solution and in the solid state led to the formation of thymidine oligonucleotides containing up to approximately 12 nucleotide units. Only the 3′,5′ internucleotide diester bonds were formed. This polymerization occurs without the addition of any activating agent or catalyst.*

The chemical synthesis of oligonucleotides (*1*) has been undertaken by the stepwise condensation or polymerization of mononucleotides. Both of these processes involve the activation of the phosphate moiety of the suitably protected nucleotides. Even in the absence of activating agents, 2′-deoxynucleotides could be polymerized at elevated temperatures in anhydrous solution with a variety of mild acidic catalysts. Oligonucleotides containing up to eight or nine nucleotide residues have been obtained (*2*). Another route for the synthesis of the internucleotide linkage involves an intermediate formation of phosphotriesters (*3*). Besides these methods of phosphate activation, a different principle is based on the use of cyclonucleosides (*4*). Various pyrimidine and purine cyclonucleosides have been used for this purpose, but the products obtained were no larger than the dinucleoside monophosphate or the trinucleoside diphosphate.

We report our results on the self-condensation of *O*², 5′-cyclothymidine 3′-phosphate (**1**), which led to the formation of a mixture of oligonucleotides. This molecule (**1**) contains both the nucleophile and the leaving group, and consequently is able to polymerize. The polymerization is not dependent on the addition of an activating agent or a catalyst, and it can be performed in solution as well as in the solid state.



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*O*²,5′-cyclothymidine 3′-phosphate was obtained after a series of reactions starting from 5′-*O*-tosylthymidine (*5*), which was phosphorylated with 2-cyanoethyl phosphate by the method of Tener (*6*). The cyanoethyl group was removed by alkali, and the 5′-*O*-tosylthymidine 3′-phosphate was purified by column chromatography on Dowex-1 formate with 0.05*M* ammonium formate in 50 percent alcohol (*p*H 4). This was converted into 5′-*O*-tosylthymidine 3′-phosphoromorpholidate by activating the phosphate moiety with diphenyl phosphorochloridate (*7*) in a mixture of dioxane and hexamethylphosphorotriamide, followed by the addition of morpholine. The product was purified on a silica gel column with *n*-butanol : water (86 : 14) as the eluant. Treatment of this with four equivalents of *N*,*N*′-dicyclohexyl-4-morpholinocarboxamidine (*8*) in a mixture of dimethylformamide and dioxane at 90°C for 18 hours led to the formation of *O*²,5′-cyclothymidine 3′-phosphoromorpholidate in 55 to 60 percent yield. The phosphoromorpholidate was hydrolyzed in 0.5*N* HCl at 0°C for 5 minutes, and the *p*H of the solution was quickly adjusted to 4. The solution was placed on Dowex-1 chloride resin (10 ml of

resin per 4000 adsorbance$_{250}$ units of product) and eluted with an HCl gradient from *p*H 3 to 1.5. The first peak contained the product followed by a small amount of thymidine 3′-phosphate. O^2,5′-Cyclothymidine 3′-phosphate was obtained as the triethylammonium salt after the eluate was neutralized with triethylamine and lyophilized as rapidly as possible. It was freed of salt by precipitation from ethanol with ether. The structure of these compounds was established by elemental analysis (*9*) and spectral analysis. The cyclonucleotide and its morpholino precursor exhibited the characteristic absorption maxima at 250 to 252 nm (*5*) and a negative Cotton effect (*10*).

The polymerization in solution was performed as follows. The triethylammonium salt of the cyclonucleotide (0.07 mmole) was dissolved in a minimum amount of anhydrous hexamethylphosphorotriamide in a tube, tri-*n*-butylamine (0.007 mmole) was added, and the solution was concentrated to a thick gum under vacuum. The reaction tube was sealed under vacuum and heated at 85° to 88°C for 96 hours. The slightly yellowish product was put on a Sephadex G-25 column (2.5 by 120 cm). The material in the last peak in this separation, which constituted about 5 percent of the original material absorbing ultraviolet light, was identified as thymine and discarded. The remaining fractions were pooled, evaporated, and rechromatographed on a Sephadex G-50 column (2.5 by 120 cm), with 0.1*M* triethylammonium bicarbonate as the eluant. The separation of the oligomers larger than the tetramer was not satisfactory (Fig. 1a). However, the material in fractions 240 to 330 (55 to 60 percent of the material on the column) was resolved into four peaks (Table 1a). Each peak was contaminated with the lower and the higher homolog, and all were found to be about 75 to 80 percent pure, as judged by paper chromatography in two solvent systems and electrophoresis in acidic and neutral buffer. Thus peak 1 was mainly thymidine 3′-phosphate (Tp) mixed with small amounts of thymidine 3′,5′-cyclic phosphate and thymidine. Peak 2 was $(Tp)_2$, peak 3 was $(Tp)_3$, and peak 4 was largely $(Tp)_4$. The chain length of the products was determined by the standard procedure of removing the terminal phosphate with phosphatase and subjecting the enzymatic mixture to paper chromatographic separation (*1*). The purified products $(Tp)_nT$ thus obtained were treated with snake venom phosphodiesterase, and the ratio of thymidine 5′-phosphate to thymidine was determined.

Table 1. Product distribution after chromatographies in Fig. 1. Values of R_F are relative to Tp in ethanol : 1*M* ammonium acetate, *p*H 7.5 (7 : 3); electrophoretic mobilities (EM) are relative to Tp in 0.05*M* ammonium acetate, *p*H 4.5 (P, peak; TM, total material).

P	Fraction	TM (%)	Compound	R_F	EM
		a. Sephadex G-50 (Fig. 1a)			
1	303–322	18	Tp	1.00	1.00
2	278–292	13	$(Tp)_2$	0.67	1.28
3	257–272	14	$(Tp)_3$	0.46	1.62
4	240–252	13	$(Tp)_4$	0.22	1.80
		b. DEAE cellulose (bicarbonate) (Fig. 1b)			
1	10–12	5	TpT	1.76	
2	13–17	25	$(Tp)_2T$	1.21	
3	18–21	12	$(Tp)_3T$	0.84	
4	22–28	15	$(Tp)_4T$	0.55	
5	29–35	12	$(Tp)_5T$	0.38	
6	36–44	11	$(Tp)_6T$	0.26	
7	45–49	5.5	$(Tp)_7T$	0.21	
8	50–55	4.4	Not identified	0.09	

Fractions 140 to 235 were pooled and evaporated, and the residue, after treatment with bacterial phosphatase, was rechromatographed on a diethylaminoethyl (DEAE) cellulose (bicarbonate) column (Fig. 1b and Table 1b). The appropriate fractions were pooled, checked for purity, and treated with snake venom phosphodiesterase for chain length determination.

The solid-state polymerization of O^2,5′-cyclothymidine 3′-phosphate was performed by heating the triethylamine salt (100 A_{250} units) of the nucleotide in a drying pistol over P_2O_5 at 110° to 114°C for 120 hours. The reaction product was analyzed for chain length distribution on a Sephadex G-25 column by the method of Hohn and Schaller (*11*). Chain lengths from 1 to 12 nucleotides were observed. Of the total material, 10 percent had a K_d of 0.01 to 0.05 and was irresolvable from the void volume, 23 percent (K_d, 0.06 to 0.20) had chain lengths of 8 to 12 nucleotides, and 44 percent (K_d, 0.21 to 0.50) had chain lengths of 4 to 8 nucleotides.

Although the preparation of the starting material is time-consuming and is not presently suitable for production of larger quantities, this unique polymerization reaction has several attractive features. The efficiency of previous chemical polymerizations in solution was limited by the competing cyclization process and the formation of pyrophosphates (*2*, *12*). An inherent advantage of the cyclonucleotide method of condensation is that no activating agent is required; and cyclonucleotides can be in high concentration or the solvent can be omitted. Accordingly, no significant amounts of cyclic oligothymidylates and pyrophosphates were detected. Some cleavage of glycosidic linkages in the starting material was observed, but this undesirable side reaction could be decreased by lowering the temperature. The major limitation to attaining higher chain length has been the hydrolysis of the cyclic isourea ether group by traces of moisture. This effect is expected to be less important when the experiments can be scaled up to the quantities (> 1 mmole) that are optimal in other methods of polymerization. The chain length distribution of experiments described here is promising

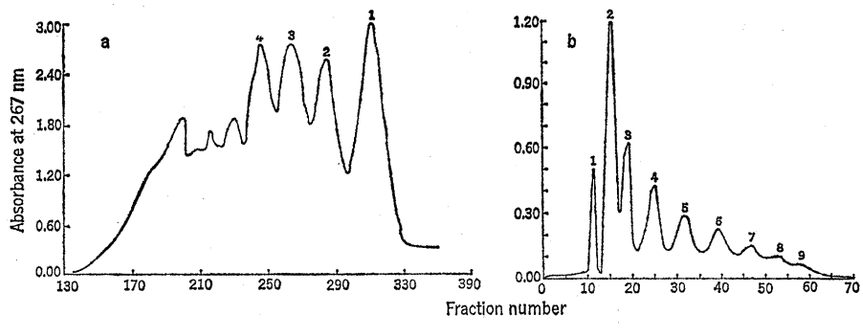

Fig. 1. Chromatography of the thymidine oligonucleotides. The polymerization of O^2,5′-cyclothymidine 3′-phosphate was done in solution (85°C, 96 hours). (a) Reaction products were separated on a Sephadex G-50 column with 0.1*M* triethylammonium bicarbonate (*p*H 7.5). Fractions of 2 ml were collected every 15 minutes. The product distribution is given in Table 1a. (b) Fractions 140 to 235 in (a) were treated with bacterial phosphatase and chromatographed on a DEAE cellulose (bicarbonate) column (1.8 by 30.0 cm) with a gradient of triethylammonium bicarbonate (0.04 to 0.05*M*). Fractions of 18 ml were collected every 15 minutes. The distribution of products is given in Table 1b.

in view of the small amounts of starting material (0.01 to 0.07 mmole).

The polymerization of O^2,5′-cyclothymidine 3′-phosphate is of immediate interest because of the novelty of the approach and its theoretical aspects. In all polymerizations of deoxyribonucleotides, the underlying principle has been phosphate activation to phosphoric anhydrides. We have introduced a different principle of polymerization, that of displacement on carbon of an intramolecular leaving group by a phosphomonoester as a nucleophile. A related approach that led to the formation of unnatural polynucleotides has been described (*13, 14*). We have suggested (*14*) that this principle might have relevance to the prebiotic formation of polynucleotides, although the nature of the prebiotic leaving groups is an enigma. Research on the behavior of cyclonucleotides should include such practical aspects as catalysis by acid or metal ions or irradiation as possible means of providing activation energy.

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Anemic Stress as a Trigger of Myelogenous Leukemia in the Unirradiated RF Mouse

Abstract. *Ninety-six percent of mice that were bled of 50 percent of their blood volume when they were 9 weeks old succumbed to myelogenous leukemia by 15 months after phlebotomy, the majority of them dying between 7 and 10 months after this treatment. These results suggest that (i) anemia is an effective stress for triggering myelogenous leukemia in animals that are predisposed to the disease, (ii) the RF mouse is "naturally" prone to the development of myelogenous leukemia, and (iii) the concept of two-step de novo induction of myelogenous leukemia appears to be applicable in this animal.*

Myelogenous leukemia (ML) can be induced in rats that apparently are not predisposed to spontaneous development of the disease (*1*). The induction process requires that the animal be first rendered prone to leukemia by x-irradiation and, second, that the leukemia be triggered by an acute anemia such as that induced by phlebotomy. If this two-step mechanism of leukemogenesis is generally valid, then (i) any particular population showing a tendency for the development of ML is bound to have more members prone to leukemia than members manifesting the overt disease; (ii) the tendency could be artificially induced (for example, by x-irradiation in the rat) or it could be spontaneously or "naturally" occurring (in other words, not dependent on any prior experimental conditioning); and (iii) the actual incidence of frank myelogenous leukemia in a population that is prone to leukemia would then be a function of the probability that individual animals would develop an anemia of sufficient severity to trigger the disease. Because of the relatively high incidence of spontaneous ML in the RF mouse, up to 4 percent (*2*), it was thought that this strain might be naturally prone and would thus be a suitable subject for testing these hypotheses. We report here that essentially all RF mice developed ML after half of their blood volume was removed. Since no other treatment was used, it would appear that this strain was indeed spontaneously prone to the development of ML.

Thirty female RF mice (*3*), 9 weeks old, were bled of 50 percent of their total blood volume (6.6 percent of body weight) from the posterior orbital sinus. Ten animals were nonbled controls. The two groups were kept in separate plastic cages lined with wood shavings but were otherwise treated in identical fashion; they were given free access to water and Purina chow and were weighed and examined daily. Hematocrit values and counts of total white blood cells and of percentages of different cell types were made at various intervals in the majority of the mice. All animals survived the acute effects of the phlebotomy; the first death occurred 17 weeks after bleeding. By 65 weeks after this treatment, all bled mice and three of the controls had died. (Three of the bled animals could not be analyzed.) The average survival time of the experimental mice was 39 weeks after venesection (50 percent had died after 37 weeks) (*4*). In all

Table 1. Summary of blood and marrow values found in animals with myelogenous leukemia. Blood films obtained before death from only 16 mice and marrow smears obtained after death from 23 animals were available for examination. Blood or marrow samples, or both, were obtained from each of 26 bled mice ($\bar{X} \pm$ S.E.M., mean $\pm$ standard error of the mean; *N*, number of animals; WBC, white blood cells; RBC, red blood cells; Neut., neutrophilic).

Test	$\bar{X} \pm$ S.E.M.
Blood values (N = *16*)	
WBC per cubic millimeter	32,000 ± 1,400
Differential count (%)	
Myeloblasts*	7.4 ± 1.1
Basophils*	4.1 ± 0.6
Eosinophils	2.7 ± 0.5
Neutrophils	62.4 ± 4.3
Monocytes	4.0 ± 0.5
Lymphocytes	21.0 ± 0.6
Marrow values (N = *23*)	
WBC : nucleated RBC (modal)	20 : 1
Differential count (%)	
Myeloblasts*	8.8 ± 0.8
Neut. promyelocytes and myelocytes	20.0 ± 1.1
Neut. metamyelocytes	31.8 ± 1.5
Neut. stab cells	13.1 ± 0.5
Mature neutrophils	6.6 ± 1.1
Basophils (all ages)*	4.8 ± 0.5
Eosinophils (all ages)	5.2 ± 0.5
Nucleated RBC	5.8 ± 0.4
Lymphocytes	6.5 ± 0.3

* Myeloblast values in seven nonleukemic (nonbled) mice were 0.4 ± 0.2 percent in the blood and 1.0 ± 0.4 percent in the marrow; basophil values in these mice were 0.4 ± 0.1 percent in the blood and 0.5 ± 0.1 percent in the marrow. Ranges of values in the blood and marrow samples of the leukemic mice were 2.5 to 12.0 percent basophils and 3.0 to 18.5 percent myeloblasts; and in the nonleukemic animals, 0.0 to 1.0 percent basophils and 0.0 to 1.5 percent myeloblasts.