THE ACTION OF PANCREATIC DESOXYRIBONUCLEASE*

I. ISOLATION OF MONO- AND DINUCLEOTIDES

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(Received for publication, November 30, 1953)

The nature of the products of the action of pancreatic desoxyribonuclease (DNase) upon highly polymerized desoxyribonucleic acid (DNA) has been the subject of numerous investigations. Titrimetric studies (1–5) have revealed that, during the digestion, approximately one secondary phosphate group becomes titratable per 4 phosphorus atoms, which may be interpreted to mean that the products are, on the average, of the magnitude of tetranucleotides.[1] More recently, ionophoretic (9), diffusion (5), and dialysis (10, 11) studies of the digest have suggested that it contains a complex mixture of polynucleotides of a range of magnitudes. Direct chromatographic fractionation of the digestion products has led to the demonstration of mono- (12, 13) and dinucleotides (14, 15).

Refinement of our ion exchange chromatographic procedures has now led to the isolation from the enzymatic digest of all possible mononucleotides and most of the possible dinucleotides. If both of the possible sequential isomers of a mixed dinucleotide are present, they are obtained as a mixture. The methods developed make it highly probable that any dinucleotide not detected is simply not present. The techniques have been applied to DNA from calf thymus and from wheat germ.

Materials and Methods

Materials—The calf thymus DNA was prepared from fresh thymus gland. Nucleoprotein was isolated according to the method of Mirsky and Pollister (16) and deproteinization was carried out by using the dodecyl sulfate denaturation described by Marko and Butler (17). The yield of DNA was 1.9 gm. from 150 gm. of thymus. The DNA, after drying over

* The work described in this paper was accomplished with the aid of grants from the Rockefeller Foundation and the United States Public Health Service.

[1] In addition, groups titratable in the pH range of the enolic hydroxyls are freed during digestion (4). However, these latter groups may be rendered titratable by simple acid or base treatment of the high polymer (6–8) without extensive degradation; hence their release during digestion is probably a secondary result of the enzyme action.

$CaCl_2$, had a P content of 7.52 per cent and an ϵ(P) (18) of 7500. The ribonucleic acid content was less than 1 per cent according to the method of von Euler and Hahn (19). Application of the method of protein denaturation of Sevag *et al.* (20) showed no trace of protein.

The wheat germ DNA was prepared from type B wheat germ, generously supplied by the B. A. Eckhart Milling Company, Chicago, Illinois. The germ was first defatted by extraction with hexane (from which all ultraviolet-absorbing impurities had been removed by passage through a column of activated silica gel) for 24 hours. Isolation of the nucleoprotein and deproteinization were carried out in the same manner as for the thymus DNA, save that the nucleoprotein was precipitated by 1:10 dilution of the 1 M NaCl solution as recommended by Daly *et al.* (21). After deproteinization with dodecyl sulfate, the nucleate was treated twice with the octyl alcohol-chloroform method of Sevag *et al.* to remove the last traces of protein. The yield of DNA was 395 mg. from 1 kilo of germ. The DNA had a P content of 7.38 per cent and an ϵ(P) of 7500. The ribonucleic acid content was less than 1 per cent.

Pancreatic DNase, twice crystallized, was obtained from the Worthington Biochemical Sales Company.

The ion exchange resins employed have been Dowex 1-8× (8 per cent cross-linked), 250 to 500 mesh, and Dowex 1-2× (2 per cent cross-linked), 200 to 400 mesh. Before use the resins are alternately washed with a 1 M HCl-1 M acetic acid mixture and with 1 M NaOH, until both acid and alkaline effluents have an optical density less than 0.010 at 260 mμ. Once thus cleaned, the stock resin is kept clean by dripping acetic acid through it, occasionally alternating with alkali. Before use for fractionation, the resin, in the amount to be used, is first equilibrated with 0.1 M ammonium acetate buffer at the pH of the first eluent to be used, and then poured into a column. Dilute NH_4OH is run through immediately prior to addition of the (alkaline) material to be fractionated. The columns are operated under about 12 feet of hydrostatic pressure. Fractions of eluate are collected at regularly timed intervals.

All ultraviolet absorption measurements were made with a Beckman ultraviolet spectrophotometer. Each absorption cell is individually calibrated for all measurements at wave-lengths below 260 mμ.

Digestion—The enzyme digestions were carried out at the pH meter so that the secondary phosphate released could be titrated almost continually to insure that the reaction reached completion. In addition, measurements of the increase in ultraviolet absorption (3) were made during digestion.

200 mg. of the thymus DNA (containing 48.8×10^{-5} mole of P) were digested with 700 γ of DNase in a volume of 30 ml. in the presence of

0.015 M magnesium acetate. The secondary phosphate released amounted to 11.0×10^{-5} equivalent or 1/4.43 atom of P. The increase in ultraviolet absorption at 260 mμ was 27 per cent.[2] The addition of more DNase and Mg^{++} caused no further release of acid.

200 mg. of the wheat germ DNA (containing 47.8×10^{-5} mole of P) were similarly digested with 700 γ of DNase in a volume of 30 ml. in the presence of 0.015 M magnesium acetate. The secondary phosphate released amounted to 11.6×10^{-5} equivalent or 1/4.12 atom of P. The increase in ultraviolet absorption at 260 mμ was 23 per cent. Again, the addition of more DNase and Mg^{++} caused no further release of acid.

With both nucleic acids, the products of the digestion were brought to pH 10.0 to 10.5 by addition of 1 M NH_4OH, and then were added to a column of Dowex 1-2×, in acetate form, 9 cm. long and 1 cm. in diameter.

Elutions—It has not been possible to establish conditions that would permit the separation of all of the nineteen components to be described in one column run. Instead, a procedure has been developed which permits separation of the mononucleotides into one group and of the dinucleotides into three groups. Each of these groups is then refractionated under a new set of conditions. One pair of dinucleotides is still unresolved after this and must be separated by an additional fractionation.

The first elution of the digest is carried out with acetate buffer at pH 5.5 (Figs. 1 and 6). The objective of this fractionation is to separate the mononucleotides and the dinucleotides in distinct groups from the remainder of the digest. The separation of the dinucleotides is not perfect, as a portion of the most retarded dinucleotide, didesoxyguanylic acid, overlaps the elution of the most advanced trinucleotides. This overlap is greater in the case of the wheat germ DNA because of the presence of significant amounts of trinucleotides containing the 5-methyldesoxycytidylic acid.

The points of division of the dinucleotides from this elution are selected for convenience in the refractionations. The division point between the first and second groups (D-1, D-2) is not critical and is best selected by observation of the D_{260}/D_{280} ratio of the effluent. Starting from a value of 0.9, this ratio rises to a flat maximum of about 2.20, which is maintained for 8 to 10 hours; division is made after about one-third of the volume of material of this ratio has been eluted. This division point is chosen in order to separate the dinucleotides[3] TT and CG and to coincide with the

[2] The ε(P) of the DNase digestion products is generally in the range 9200 to 9600 regardless of the ε(P) of the initial DNA.

[3] Throughout this paper the following abbreviations will be used: M = 5-methyldesoxycytidylic acid, C = desoxycytidylic acid, T = thymidylic acid, A = desoxyadenylic acid, G = desoxyguanylic acid. For dinucleotides, the letters representing the component nucleotides will be used in arbitrary sequence. Dinucleotide frac-

time when only CA is coming off the column so that it is the only fraction to be split.

The point of division between the second and third groups (D-2, D-3) divides the bulk of the dinucleotides from the early trinucleotides plus trailing GG. The advent of these early trinucleotides (which are tripyrimidine nucleotides) in the eluate is signaled by an increase of the D_{270}/D_{250} ratio, and the separation is made at the beginning of this increase.

The termination of group D-3 is made at the time when the D_{270}/D_{250} ratio of the effluent levels off at a value of about 1.4. This plateau indicates the completion of elution of the GG.

All subsequent fractionations are carried out on columns of Dowex 1-8×. Columns 10 cm. long × 1 cm. in diameter are satisfactory for the mononucleotide separations (12), while columns of 20 cm. length are used for the dinucleotides. For readsorption to the column, the solutions are made alkaline with NH_4OH and flowed onto the column by gravity. Conditions of pH and ionic strength of the eluents are chosen for optimal separation of the individual components. The presence of numerous components containing the 5-methyldesoxycytidylic acid in the wheat germ DNA digests complicates the refractionation of the corresponding groups D-1 and D-3. (Compare Figs. 2 and 5 with Figs. 7 and 8.)

The solutions are added to the resin while it is in acetate form, as this condition facilitates adsorption of the nucleotides. Elutions are frequently carried out with solutions containing a mixture of acetate and chloride anions, in which the acetate serves to maintain the pH, while the chloride provides most of the eluting power. The replacement of acetate by chloride permits the use of solutions with lower salt content and lower ultraviolet absorption at wave-lengths below 260 mμ. When the elution is carried out with these mixed salts, the resin will be converted largely to the chloride form because of the much greater affinity of chloride for the resin. When the chloride-containing solution is first added to the resin, a sharp boundary between the chloride and acetate forms moves down the column. If the strength of the eluent used is sufficient to move some of the nucleotides down the column faster than the chloride-acetate boundary can move, these substances will be crowded up against this boundary and will be eluted without resolution when the boundary reaches the bottom of the column.[4] For this reason it is essential to use, for the conversion of the column to the chloride form, a solution of low chloride concentration,

tions composed of unlike nucleotides will in general consist of a mixture of the two possible sequential isomers, and no implication should be derived from the sequence of letters used to describe the fraction. Studies of the ratios of sequential isomers in these fractions are in progress.

[4] A similar circumstance has been described by Tompkins *et al.* (22).

which will not appreciably affect the adsorbed nucleotides. The completion of conversion of the resin to the chloride form, which is marked by the appearance of chloride in the effluent, is indicated on each chromatograph.

Identification of Components—The components of a di- or trinucleotide may be determined either by an analysis of its ultraviolet absorption spectrum or by quantitative degradation to mononucleotides (23) and chromatographic analysis of the mononucleotide mixture (12). The best criterion of the homogeneity of a fraction is that the molar ratios of the mononucleotides thus obtained shall be simple integral numbers.

TABLE I

*Ultraviolet Absorption Spectra of Desoxyribonucleotides**

Wave-length	$\epsilon \times 10^{-3}$							
	5-Methylcytidylic		Cytidylic		Thymidylic	Adenylic		Guanylic
	pH 4.3	pH 5.5–7.0	pH 4.3	pH 5.5–7.0	pH 4.3–7.0	pH 4.3	pH 5.5–7.0	pH 4.3–7.0
mμ								
240	3.47	6.84	4.14	7.38	3.06	5.98	6.61	10.1
250	2.89	5.24	4.48	6.54	6.00	12.1	12.5	14.5
253	3.08	5.00	5.06	6.68	7.07	13.7	14.1	14.9
260	4.25	5.45	7.30	8.10	9.13	15.3	15.7	12.8
270	7.37	7.74	11.0	10.1	10.0	10.5	10.4	10.4
280	10.1	8.55	11.5	7.95	6.60	2.48	2.21	8.55
290	9.49	5.64	6.90	2.42	1.99	0.18	0.11	3.43
300	5.27	1.39	1.59	0.20	0.16	0	0	0.36

* Calculated from organic phosphorus contents of solutions judged to be homogeneous by chromatographic analysis.

These methods can determine only the ratios of the component nucleotides and cannot indicate the molecular magnitude of the fraction. This latter may be determined either by removal of the terminal phosphate and measurement of the ratio of P released to total P of the fraction (14), or by removal of the terminal phosphate, treatment of the residue with phosphodiesterase, and ion exchange fractionation of the resultant nucleosides and nucleotides. The molar ratio of nucleotides to nucleosides will then be a measure of the original magnitude. In addition, if the fraction consists of a mixture of sequentially isomeric dinucleotides, the molar ratio of the two nucleotides obtained in this manner will equal the initial ratio of the two isomers.

After once having established the di- or trinucleotide character of various fractions and the principles underlying the sequence of elution of these

fractions under various conditions, the magnitude of other fractions may then be deduced from their position in the elution pattern.

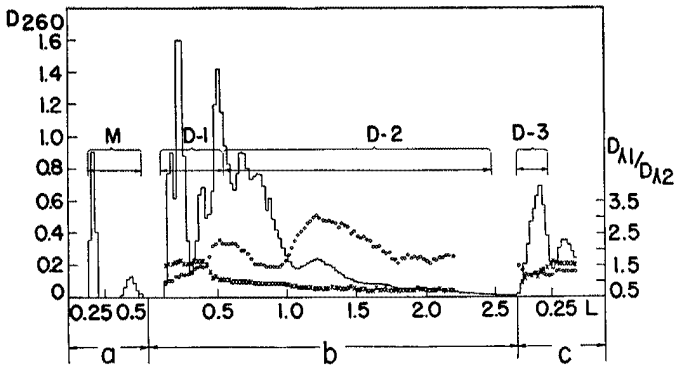

FIG. 1. Fractionation of DNase digest of thymus DNA. Column, Dowex 1-2×, 9 cm. × $\pi/4$ cm.^{2}. Flow rate, 23 ml. per hour. Stepped line, D_{260}; ○, D_{260}/D_{280}; ×, D_{270}/D_{250}. *a*, 0.10 M Ac, pH 5.5; *b*, 0.24 M Ac, pH 5.5; *c*, 0.45 M Ac, pH 5.5. L. = liters.

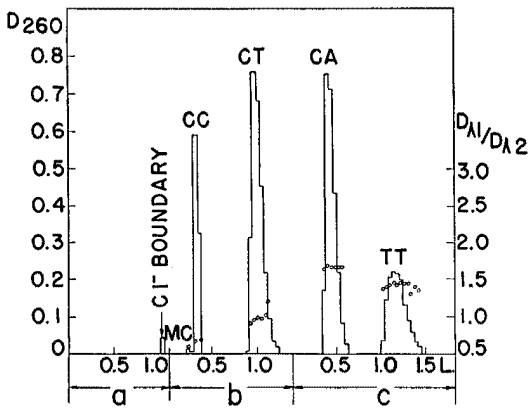

FIG. 2. Refractionation of group D-1 of thymus DNA digest. Column, Dowex 1-8×, 20 cm. × $\pi/4$ cm.^{2}. Flow rate, 21 ml. per hour. Recovery of absorption at 260 mμ = 93 per cent. ○, D_{260}/D_{280}. *a*, 0.05 M Ac, pH 4.3 + 0.015 M Cl^-; *b*, 0.05 M Ac, pH 4.3 + 0.04 M Cl^-; *c*, 0.05 M Ac, pH 4.3 + 0.055 M Cl^-.

For the spectroscopic analysis of polynucleotides, accurate data of the extinction coefficients of the monodesoxyribonucleotides are necessary. Such data are presented in Table I. The absorption spectra of di- and trinucleotides match quite well those computed by a linear summation of the spectra of their component mononucleotides. However, the intensity of the absorption of the dinucleotides is always significantly lower

than would be computed by such a linear summation. This effect is particularly marked with those polynucleotides containing desoxyguanylic acid.

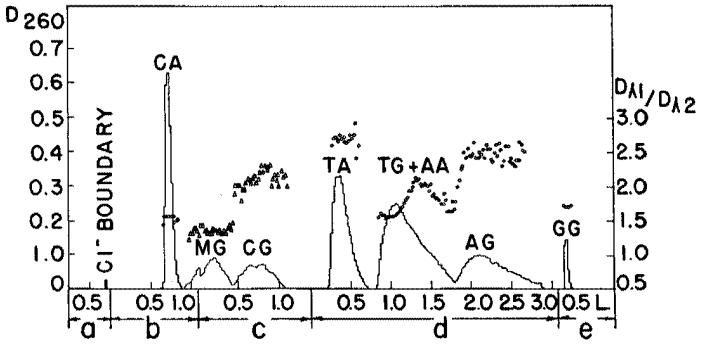

FIG. 3. Refractionation of group D-2 of thymus DNA digest. Column, Dowex 1-8×, 20 cm. × $\pi/4$ cm.^{2}. Flow rate, 21 ml. per hour. Recovery of absorption at 260 mμ = 97 per cent. ○, D_{260}/D_{280}; △, D_{270}/D_{290}. *a*, 0.10 M Ac, pH 4.0 + 0.02 M Cl^-; *b*, 0.10 M Ac, pH 4.0 + 0.045 M Cl^-; *c*, 0.05 M Ac, pH 4.4 + 0.06 M Cl^-; *d*, 0.05 M Ac, pH 4.4 + 0.08 M Cl^-; *e*, 0.05 M Ac, pH 4.4 + 0.20 M Cl^-.

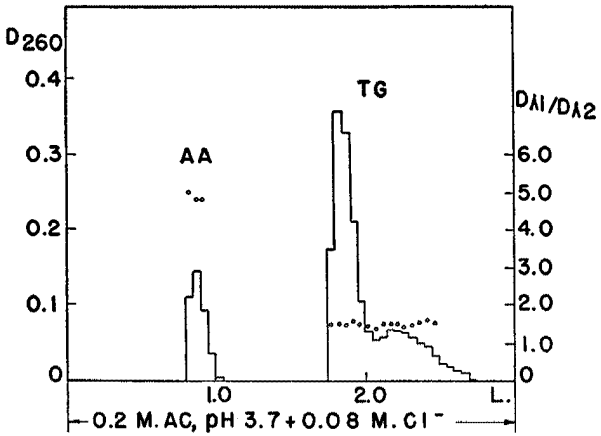

FIG. 4. Refractionation of TG-AA from group D-2 of thymus DNA digest. Column, Dowex 1-8×, 20 cm. × $\pi/4$ cm.^{2}. Flow rate, 24 ml. per hour. Recovery of absorption at 260 mμ = 91 per cent. ○, D_{260}/D_{280}.

Results

The details of the fractionations leading to the isolation of the mono- and dinucleotides from the thymus DNA digest are presented in Figs. 1 to 5; corresponding stages in the fractionation of the wheat germ DNA

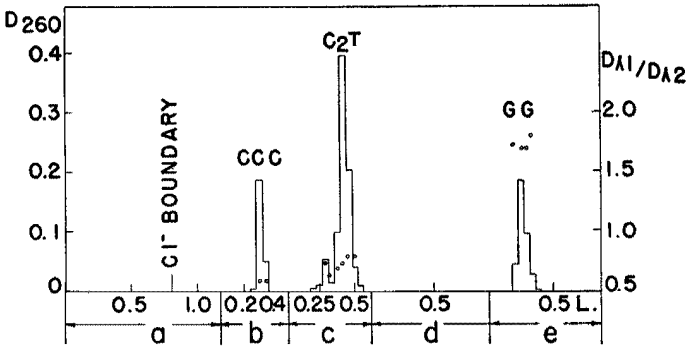

FIG. 5. Refractionation of group D-3 of thymus DNA digest. Column, Dowex 1-8×, 20 cm. × $\pi/4$ cm.^{2}. Flow rate, 24 ml. per hour. Recovery of absorption at 260 mμ = 84 per cent. ○, D_{260}/D_{280}. *a*, 0.10 M Ac, pH 4.0 + 0.02 M Cl^-; *b*, 0.10 M Ac, pH 4.0 + 0.06 M Cl^-; *c*, 0.10 M Ac, pH 4.0 + 0.08 M Cl^-; *d*, 0.10 M Ac, pH 4.0 + 0.13 M Cl^-; *e*, 0.10 M Ac, pH 4.0 + 0.20 M Cl^-.

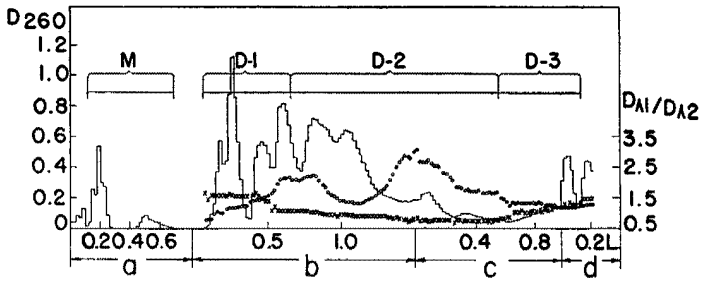

FIG. 6. Fractionation of DNase digest of wheat germ DNA. Column, Dowex 1-2×, 9 cm. × $\pi/4$ cm.^{2}. Flow rate, 20 ml. per hour. Stepped line, D_{260}; ○, D_{260}/D_{280}; ×, D_{270}/D_{250}. *a*, 0.10 M Ac, pH 5.5; *b*, 0.24 M Ac, pH 5.5; *c*, 0.30 M Ac, pH 5.5; *d*, 0.45 M Ac, pH 5.5.

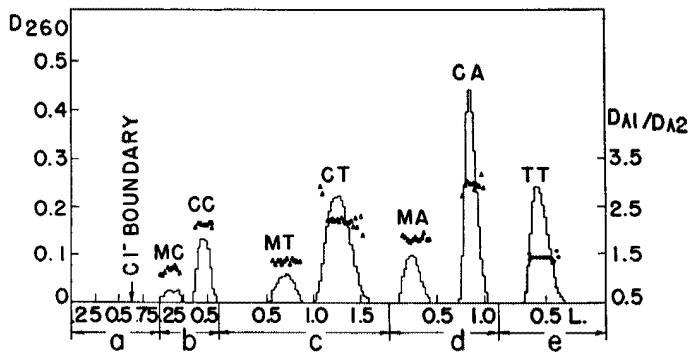

FIG. 7. Refractionation of group D-1 of wheat germ DNA digest. Column, Dowex 1-8×, 20 cm. × $\pi/4$ cm.^{2}. Flow rate, 25 ml. per hour. Recovery of absorption at 260 mμ = 92 per cent. ○, D_{260}/D_{280}; △, D_{270}/D_{290}. *a*, 0.05 M Ac, pH 4.3 + 0.015 M Cl^-; *b*, 0.05 M Ac, pH 4.3 + 0.025 M Cl^-; *c*, 0.05 M Ac, pH 4.3 + 0.03 M Cl^-; *d*, 0.05 M Ac, pH 4.3 + 0.04 M Cl^-; *e*, 0.05 M Ac, pH 4.3 + 0.065 M Cl^-.

digest are presented in Figs. 6 to 8. The fractionations of group D-2 of the wheat germ digest and of the TG-AA fraction from this chromatogram are carried out exactly as for the thymus DNA. The fractionation of the mononucleotides is carried out essentially as previously described (12, 23). The numerical results of these fractionations are summarized in Tables II

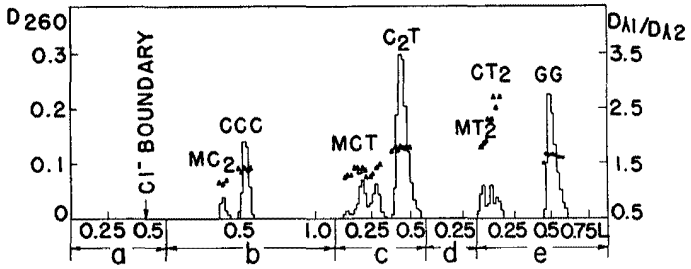

FIG. 8. Refractionation of group D-3 of wheat germ DNA digest. Column, Dowex 1-8×, 20 cm. × $\pi/4$ cm.^{2}. Flow rate, 18 ml. per hour. Recovery of absorption at 260 mμ = 77 per cent. ○, D_{260}/D_{280}; △, D_{270}/D_{290}. *a*, 0.10 M Ac, pH 4.0 + 0.02 M Cl^-; *b*, 0.10 M Ac, pH 4.0 + 0.06 M Cl^-; *c*, 0.10 M Ac, pH 4.0 + 0.08 M Cl^-; *d*, 0.10 M Ac, pH 4.0 + 0.13 M Cl^-; *e*, 0.10 M Ac, pH 4.0 + 0.20 M Cl^-.

TABLE II

Mononucleotide Components of DNase Digests

Nucleotide	Calf thymus DNA		Wheat germ DNA	
	Per cent of absorption at 260 mμ	Per cent of P	Per cent of absorption at 260 mμ	Per cent of P
M	0	0	0.08	0.17
C	0.13	0.17	0.07	0.09
T	0.61	0.63	0.49	0.49
A	0.13	0.08	0.13	0.08
G	0.07	0.06	0.05	0.04
Total	0.94	0.94	0.82	0.87

and III. Analytical data on the fractions isolated, providing direct evidence as to their nature, are presented in Table IV. The molar proportions (per cent of P) listed in Table III are computed by using the numerical ratios of ultraviolet absorption to phosphorus given in Table IV.

Recoveries of ultraviolet absorption during these fractionations generally range from 91 to 94 per cent, with the exception of the initial DNase digest fractionations, for which the recoveries were 98 and 101 per cent, and the group D-3 fractionations, for which the recoveries were only 84

TABLE III

Dinucleotide Components of DNase Digests

Dinucleotide	Calf thymus DNA		Wheat germ DNA	
	Per cent of absorption at 260 mμ	Per cent of P	Per cent of absorption at 260 mμ	Per cent of P
MM	0*	0	0	0
MC	0.02	0.03	0.11	0.17
MT	0	0	0.31	0.43
MA	0	0	0.43	0.41
MG	0.72	1.03	1.52	2.08
CC	0.82	1.11	0.48	0.63
CT	2.42	3.12	1.57	1.97
CA	3.50	3.32	2.43	2.23
CG	0.79	0.87	0.44	0.47
TT	1.28	1.38	1.11	1.16
TA	1.71	1.46	1.68	1.39
TG	2.41	2.77	1.97	2.20
AA	0.53	0.46	0.46	0.39
AG	1.44	1.17	1.15	0.90
GG	0.76	0.82	0.76	0.79
Total	16.40	17.54	14.42	15.22

* 0 = certainly less than 0.02 per cent.

TABLE IV

Analytical Data on Dinucleotide Components

Dinucleotide	$E^{1\,\gamma\ P\ per\ ml.}_{1\ cm.}$	Per cent of P released by phosphomonoesterase	Molar ratio of nucleotides after phosphodiesterase action
MC	0.187*	†	†
MT	0.216*	†	†
MA	0.310	52.0	M:A = 1.02
MG	0.217	50.6	M:G = 1.02
CC	0.226	50.2	C only
CT	0.236	50.3	C:T = 1.04
CA	0.322	49.3	C:A = 1.00
CG	0.276	52.5	C:G = 1.08
TT	0.284	51.5	T only
TA	0.360	51.1	T:A = 1.04
TG	0.265	47.0	T:G = 1.07
AA	0.356	50.6	A only
AG	0.376	51.0	A:G = 1.01
GG	0.303	46.4	G only

$E^{1\,\gamma\ P\ per\ ml.}_{1\ cm.}$ = optical density at 260 mμ of a 1 cm. path of solution containing 1 γ of organic P per ml.

* Computed value.

† Composition and magnitude deduced from ultraviolet absorption spectra and position in the chromatogram.

and 77 per cent.[5] The values given in Tables II and III are uncorrected for these apparently incomplete recoveries, with the exception of the TG and AA fractions, which are corrected for loss in their final separation (Fig. 4) on the assumption that the losses are in proportion to the amounts recovered. In this latter instance, the material to be fractionated was composed of a simple and well defined mixture, and such correction is considered valid. Because of the poor recoveries of the D-3 groups, the values given for GG may be significantly low.

While the absolute accuracy of the proportions of these fractions is uncertain because of the incomplete recoveries, the reproducibility of the proportions given appears to be better than ±5 per cent.

DISCUSSION

Techniques—The utility of the 2 per cent cross-linked resin[6] for the initial separation into groups of nucleotides of similar magnitude is derived from its converse inefficacy for the separation of nucleotides of similar magnitude. Thus, the resolution of mononucleotides on the 2 per cent resin is much poorer than on the 8 per cent resin, for which reason it is not satisfactory for the separation of the various dinucleotide fractions. At pH 5.5 differences in the net charge of nucleotides of the same magnitude are negligible (24); hence the spread of fractions of a given magnitude is presumably caused by variations in affinity for the resin produced by secondary valence forces. These forces appear to be appreciably stronger for purines than for pyrimidines, resulting in the retardation of the elution of the purine-containing fractions. The overlap of nucleotides of different magnitudes, which begins with the dipurine and tripyrimidine nucleotides, consequently becomes greater with increasing magnitude.

Over 95 per cent of the DNase digests may be eluted from the 2 per cent resin at pH 5.5 by the use of 2 M acetate buffer; by extrapolation then from the known affinities of di- and trinucleotides, it may be estimated that the largest fractions to occur in the digests in appreciable amount are of the order of hepta- or octanucleotides.

[5] The low recoveries of the group D-3 fractionations are probably due to the presence of GG. The behavior of diguanylic acid is consistently anomalous; it has been impossible to carry out conventional procedures (concentration, lyophilization, enzyme digestion) without encountering appreciable loss (disappearance) of this substance. This anomalous behavior may possibly be linked to the low solubility which might be expected of diguanylic acid.

[6] The use of the 2 per cent cross-linked resin was initially suggested to us by Dr. Waldo Cohn with the idea that its more open structure might improve the resolution of polynucleotides. In our hands, however, the 2 per cent resin has shown no significant advantage over the 8 per cent in this respect.

The sequence of elution of dinucleotides at pH 4.0 or 4.3 may be predicted accurately from the sequence of elution of their component mononucleotides (12, 24) at these pH values. This fact, taken together with the evidence for the symmetrical form of the elution pattern of individual components, indicates that these resins can adsorb and release di- and trinucleotides as efficiently as mononucleotides.

Results—The action of pancreatic DNase upon highly polymerized DNA evidently results in an extremely complex mixture of mono- and polynucleotides of various magnitudes and compositions. The fractions identified in this paper account for but one-sixth of the total digest. Representatives of almost all of the mononucleotide and dinucleotide fractions are present. The quantities of mononucleotides are so small as to suggest the possible presence of trace amounts of a phosphodiesterase which might occur as an impurity in either the DNA or the DNase. However, it would have to be a diesterase of peculiar specificity to account for the marked predominance of thymidylic acid among the mononucleotides from both thymus and wheat germ DNA.

The most striking outcome of the dinucleotide analysis is the extraordinarily large amount of 5-methyldesoxycytidylic acid associated with desoxyguanylic acid in both digests. The MG fraction of the thymus DNA digest accounts for more than 30 per cent of all the M present. No other single fraction accounts for more than 8 per cent of any nucleotide (C in CA). It is believed that this association of M and G must represent a similar association in the nucleic acid structure. The unusual distribution of M in the wheat germ DNA has also been noted by Smith and Markham (15).

The molar proportions of mononucleotides among the dinucleotide fractions differ significantly from their proportions in the entire nucleic acid (Table V); in particular the proportion of (C + M) is considerably increased, while that of A is markedly decreased. Similar observations of the excess of pyrimidines in the more readily dialyzable portion of the digest have been reported (10, 11).

With the obvious exceptions of the fractions containing 5-methyldesoxycytidylic acid, the similarities between the distribution of dinucleotide fractions from the thymus and wheat germ DNA digests would seem to be more remarkable than the differences. Except for these fractions, an arrangement of the dinucleotide components in order of amount would be nearly identical in both digests. Significant differences, both relative and absolute, do occur between the proportions of various nucleotides in the two digests, but they are not large. It is noteworthy that the total proportion of mono- and dinucleotides is less in the wheat germ DNA digest

than in the thymus DNA digest, although the extent of titratable acid release was slightly greater in the former.

The proportions of the M and C fractions in the wheat germ DNA digest as compared to those in the thymus DNA digest indicate that no simple correlation can be made, such as the direct substitution of C by M. This distribution may, of course, be influenced to some extent by the mode of action of the enzyme.

No conclusive argument can be made from these data as to the specificity of the pancreatic DNase. The presence of appreciable amounts of di- and trinucleotides of varied composition, with only minute amounts of mononucleotides, suggests that the action of the enzyme may be to attack some

TABLE V

Molar Proportions of Nucleotides in Dinucleotide Fractions and in Whole DNA

	M	C	T	A	G
Calf thymus DNA					
Dinucleotides	0.12	1.09	1.15	0.79	0.85
Whole DNA*	0.067	0.83	1.09	1.15	0.87
Wheat germ DNA					
Dinucleotides	0.41	0.80	1.09	0.75	0.95
Whole DNA†	0.25	0.65	1.10	1.11	0.89

* Sinsheimer and Koerner (23).

† Data obtained by the authors using the methods previously described (23).

particular internucleotide linkage except when such linkage is terminal in the chain fragment, in which case the action may proceed but only very slowly.[7] Such a specificity would permit the existence of all possible di- and trinucleotides in the digest, but would limit the possible composition of tetra- and higher nucleotides.

We are indebted to Dr. Gerhard Schmidt for a generous gift of prostatic phosphomonoesterase.

[7] A similar type of specificity has been postulated for malt α-amylase (25, 26) which can split 1,4-glycoside linkages unless they are initial or terminal, in which case the action proceeds, but very slowly. If α-amylase is allowed to digest amylose to an extent comparable with the digestion of DNA by DNase, there is a considerable resemblance among the distributions of products with respect to magnitude in the two digests (27).

SUMMARY

1. Ion exchange chromatographic procedures are described for the isolation of all mono- and dinucleotide fractions from digests of DNA by pancreatic DNase. The dinucleotides are obtained as mixtures of the two sequential isomers, if both are present.

2. Analytical data are presented for the proportions of the individual mono- and dinucleotide fractions of DNase digests of calf thymus DNA and of wheat germ DNA. The total mononucleotide fraction amounts to less than 1/100 and the dinucleotide fraction to approximately one-sixth of both digests.

3. Significant differences exist between the relative proportions of dinucleotide components in the thymus DNA and wheat germ DNA digests, especially among the fractions containing 5-methyldesoxycytidylic acid. The dinucleotide containing 5-methyldesoxycytidylic and desoxyguanylic acids is present in unusually large proportions in both digests. This one fraction contains over 30 per cent of the 5-methyldesoxycytidylic acid present in the case of the thymus DNA digest.

4. The ratio of pyrimidine to purine nucleotides among the components of the dinucleotide fractions is considerably greater than it is in the entire nucleic acid.

5. The significance of these observations is discussed with reference to the possible mode of action of the pancreatic DNase.

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ARTICLE:

THE ACTION OF PANCREATIC DESOXYRIBONUCLEASE: I. ISOLATION OF MONO- AND DINUCLEOTIDES

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J. Biol. Chem. 1954, 208:445-459.