# THE ACTION OF PANCREATIC DEOXYRIBONUCLEASE*

## II. ISOMERIC DINUCLEOTIDES

By ROBERT L. SINSHEIMER

*(From the Department of Physics, Iowa State College, Ames, Iowa)*

(Received for publication, November 15, 1954)



In Paper I (1) methods were described for the isolation of dideoxyribonucleotides from digests of deoxyribonucleic acid (DNA) by pancreatic deoxyribonuclease (DNase). Dinucleotides composed of unlike mononucleotides were obtained as mixtures of the two possible sequential isomers. Methods have now been developed for the analysis of the proportions of the isomers in such mixtures and applied to the dinucleotide fractions obtained by the action of DNase on DNA from calf thymus.

### *Material and Methods*

Dinucleotide fractions were obtained as described previously (1). Purified venom phosphodiesterase was prepared as before (2). Prostatic phosphomonoesterase was generously supplied by Dr. Gerhard Schmidt. All fractionations were carried out on Dowex 1-8X anion exchange resin.

*Principles of Isomer Analysis*—The method consists of the following steps: (*a*) removal of the terminal phosphate groups of the mixed isomeric dinucleotides with prostatic phosphomonoesterase; (*b*) isolation of the resultant mixed isomeric dinucleotide phosphates by ion exchange chromatography; (*c*) splitting of the mixed isomeric dinucleotide phosphates by purified venom phosphodiesterase; (*d*) recovery of the mononucleotide fractions from this latter digest by ion exchange chromatography. From the optical density of each nucleotide fraction and the extinction coefficients (1), the molar ratio ($X:Y$) of the two mononucleotides ($X$ and $Y$) can be calculated. This molar ratio ($X:Y$) is then taken to be the molar ratio of the dinucleotide isomers containing $X$ and $Y$ as their initial nucleotides ($X$-$Y$/$Y$-$X$).

This conclusion is based upon the assumptions that the terminal phosphate of the dinucleotides is attached to a 5′ position and that the venom phosphodiesterase always splits the 5′-3′ phosphate diester link at the 3′ end. The validity of these assumptions is supported by the following observations.

The mononucleotides obtained by venom phosphodiesterase digestion of a DNase digest are completely dephosphorylated by 5-nucleotidase of

* The research described in this paper was supported by a grant from the United States Public Health Service.

snake venom ((3), and personal observation). Since this enzyme will not attack 3′-deoxyribonucleotides (4), the terminal phosphate groups of all the polynucleotide fractions must be attached at the 5′ position, and the phosphodiesterase must always attack at the 3′ end of the 5′-3′ phosphate diester.

TABLE I

*Strength of Acetate Buffer (pH 4.3) Employed to Elute Dinucleotide Phosphates*

| Dinucleotides* | Molarity of buffer of pH 4.3 |
|---|---|
| CT | 0.04 |
| CA | 0.05 |
| MG | 0.16 |
| CG | 0.18 |
| TA | 0.20 |
| TG | 0.35 |
| AG | 0.35 |

* C = deoxycytidylic acid; T = thymidylic acid; A = deoxyadenylic acid; M = 5-methyldeoxycytidylic acid; G = deoxyguanylic acid.

TABLE II

*Molar Ratios of Mononucleotides Obtained by Phosphodiesterase Degradation of Dinucleotide Phosphates*

| Dinucleotide | Molar ratio |
|---|---|
| CT | T:C = 3.0 |
| CA | A:C = ∞ |
| TA | A:T = 13.3 |
| CG | C:G = 5.4 |
| MG | G:M = ∞ |
| TG | G:T = 15.7 |
| AG | A:G = 4.9 |

Similarly, if the pooled dinucleotide fractions (D-1, D-2, and D-3; see (1)) from a DNase digest are treated with prostatic phosphomonoesterase to remove all terminal phosphate groups and then, after inactivation of the prostate enzyme,[1] are treated with rattlesnake venom, which contains phosphodiesterase and 5-nucleotidase (3, 6), the entire digest is converted to nucleosides. The phosphodiesterase must again attack only at the 3′

[1] The prostatic phosphomonoesterase may be inactivated by shaking the solution with ether for 30 minutes at room temperature (5) (this procedure leaves less than 5 per cent of the original activity) and, further, by adjustment of the pH to 9.2 for the snake venom action, which reduces the residual prostate enzyme activity below detectable levels (less than 0.01 per cent of the original activity).

end of the 5′-3′ phosphate diester link in the dinucleotides without terminal phosphate groups.

*Technique of Isomer Analysis*—125 to 250 $\gamma$ of dinucleotide, in a volume of approximately 100 $\mu$l., are added to 1 ml. of 0.2 M acetate buffer, pH 5.5. 50 $\mu$l. of the prostatic phosphatase preparation are added, and the solution is incubated at 37° for 4 hours. The digest is then made alkaline by addition of 1 N $NH_4OH$ and added to a column of resin, 10 cm. long × 3 mm. in diameter. An appropriate strength (Table I) of ammonium acetate buffer, pH 4.3, is then used to elute the desired dinucleotide phosphate from the column. The fractions containing the eluted substance, detected by its ultraviolet absorption, are pooled and lyophilized to remove water and salt.

TABLE III

*Molar Proportions of Dinucleotides in Thymus DNA Digest*

*XY* = X-p-Y-p.*

| x \ y | M | C | T | A | G |
|---|---|---|---|---|---|
| M | 0 | | 0 | 0 | 0 |
| C | | 1.11 | 0.78 | 0 | 0.75 |
| T | 0 | 2.34 | 1.38 | 0.10 | 0.16 |
| A | 0 | 3.22 | 1.36 | 0.46 | 0.97 |
| G | 1.03 | 0.12 | 2.61 | 0.20 | 0.82 |

* *Y* refers to the nucleoside carrying the phosphate monoester group in each case.

The dinucleotide phosphate is then taken up in 1 ml. of 0.2 M $NH_4OH$-$NH_4$ acetate buffer, pH 9.2. To this are added 0.2 ml. of 0.3 M magnesium acetate and 50 to 100 $\mu$l. of purified venom phosphodiesterase. The digest is incubated at 37° for 4 hours and then added to a column of resin, 10 cm. long × 3 mm. in diameter. The mononucleotides are analyzed as described previously (7, 8).

## *Results*

The molar ratios of the nucleotides found in the phosphodiesterase digests of the dinucleotide phosphate fractions, obtained as above from a DNase digest of calf thymus DNA, are presented in Table II. These values are in each case the averages of two or more determinations made upon dinucleotide fractions prepared at different times from different DNase digests. The variations in isomer ratios of fractions from different digests have in all instances been less than ±3 per cent.

From these ratios, knowing the molar fractions corresponding to each of the mixed isomers in the DNase digest (Table III in Sinsheimer (1)),

we have calculated the molar fraction of each dinucleotide isomer in the digest (Table III).[2]

## DISCUSSION

The only previous report of the analysis of a dideoxyribonucleotide relative to its isomeric composition has been the finding of Smith and Markham (9) that the dinucleotide fraction containing deoxyadenylic and deoxycytidylic acids in a DNase digest of calf thymus DNA was entirely of the sequence adenylic-cytidylic. Our results confirm this report.

The most striking result of the complete isomer analysis is to be found among the dinucleotides composed of one purine and one pyrimidine nucleotide. Here, with one exception, the dinucleotides with the sequence purine nucleotide-pyrimidine nucleotide are in great excess over those with the sequence pyrimidine nucleotide-purine nucleotide. In fact, two of the latter type isomers (cytidylic-adenylic and 5-methylcytidylic-guanylic) could not be detected. The single exception is the dinucleotide with cytidylic and guanylic acids, in which the sequence cytidylic-guanylic is over 5 times as abundant as its isomer.

That the dinucleotide of 5-methylcytidylic and guanylic acids should be all of the sequence purine nucleotide-pyrimidine nucleotide, while that of cytidylic and guanylic acids should be largely of the opposite sequence, again emphasizes the special position of 5-methylcytidylic acid in this nucleic acid.

It is a pleasure to acknowledge the technical assistance, at various times, of James F. Koerner, Jostein Vadla, Kenneth Lunan, and Bennie Friesen.

## SUMMARY

The ratios of the sequential isomers of those dinucleotides composed of unlike mononucleotides have been determined in the digest of calf thymus DNA by pancreatic DNase. Among those dinucleotides containing a purine and a pyrimidine nucleotide, there is a great excess of the isomer with the sequence purine nucleotide-pyrimidine nucleotide, with the striking exception of the dinucleotide containing deoxycytidylic and deoxyguanylic acids.

## BIBLIOGRAPHY

1. Sinsheimer, R. L., *J. Biol. Chem.*, **208**, 445 (1954).
2. Sinsheimer, R. L., and Koerner, J. F., *J. Biol. Chem.*, **198**, 293 (1952).
3. Hurst, R. O., and Butler, G. C., *J. Biol. Chem.*, **193**, 91 (1951).
4. Michelson, A. M., and Todd, A. R., *J. Chem. Soc.*, 34 (1954).

[2] The amounts of MC obtained have been too small to permit an analysis for the ratio of sequential isomers.

5. Kutscher, W., and Wörner, A., *Z. physiol. Chem.*, **239,** 109 (1936).
6. Gulland, J. M., and Jackson, E. M., *Biochem. J.*, **32,** 590 (1938).
7. Sinsheimer, R. L., and Koerner, J. F., *Science*, **114,** 42 (1951).
8. Cohn, W. E., *J. Am. Chem. Soc.*, **72,** 1471 (1950).
9. Smith, J. D., and Markham, R., *Nature*, **170,** 120 (1952).

ARTICLE:

# THE ACTION OF PANCREATIC DEOXYRIBONUCLEASE: II. ISOMERIC DINUCLEOTIDES

Robert L. Sinsheimer

*J. Biol. Chem. 1955, 215:579-583.*