water (5 ml.) and pyridine (5 ml.) were added, and the solution was allowed to stand for 3 hr. Paper chromatography[19] showed the presence of only the diacetyl derivative (R_f 0.63). The product was concentrated from aqueous pyridine solution *in vacuo* a number of times to remove acetic acid and finally taken up in water (20 ml.) and the pH adjusted to 7 with sodium hydroxide. The solution was cooled to 0° and 2 *N* sodium hydroxide (20 ml., previously cooled to 0°) was added. After 3 min. at 0° the solution was quickly neutralized by the addition of an excess of Dowex 50 (pyridinium) ion-exchange resin. The mixture was placed on top of a short column of Dowex 50 (pyridinium) and the product was washed through with water. The total eluate was concentrated *in vacuo* in the presence of pyridine. Paper chromatography[19] showed the presence of the N-acetyldeoxyadenosine 5′-phosphate (R_f 0.53) with a trace amount of deoxyadenosine 5′-phosphate (R_f 0.37). The N-acetyl derivative was rendered anhydrous by repeated evaporation *in vacuo* of its pyridine solution and finally was dissolved in 10 ml. of dry pyridine. Some glass beads were added and dicyclohexylcarbodiimide (4 mmoles) and after the mixture was shaken for 5 min. half of the pyridine was removed by evaporation *in vacuo*. The mixture was shaken for 1 week, added to a mixture of dry cellulose (6 g.), dicyclohexylcarbodiimide (2 g.), and pyridine (50 ml.), and then shaken for a further 6 days. The cellulose was isolated by centrifugation and washed with pyridine. It was then mixed with aqueous pyridine and allowed to stand overnight. The dicyclohexylurea was removed by exhaustively washing the product with warm ethanol, chloroform, and then ethanol. Finally, the N-acetyl groups were removed by mixing the product with 7 *N* ammonia and allowing it to stand for 24 hr. After washing with water the cellulose was ready for use. Spectrophotometric analysis of the combined washings as described for the thymidine polynucleotide–cellulose showed that 55% of the original deoxyadenylic acid had been incorporated onto the cellulose.

Separation of Thymidine and Deoxyadenosine Hexanucleotides.—The thymidine polynucleotide–cellulose prepared as above was mixed with 1 *M* sodium chloride–0.01 *M* sodium phosphate solution, pH 7, and packed as a slurry into a water-jacketed column. The final dimensions of the cellulose were 24 × 0.9 cm. and the column was brought to 4° by a circulating coolant. Ten optical density units (260 mμ) each of thymidine and deoxyadenosine hexanucleotides were applied to the thymidine polynucleotide column in 1 *M* sodium chloride–0.01 *M* sodium phosphate solution, pH 7. Elution was carried out at 4° and the thymidine polymer (λ_{max} 266 mμ) emerged quantitatively as a sharp peak at the solvent front. Elution at 24° produced no further material and at 35° all of the deoxyadenosine polymer (λ_{max} 258 mμ) emerged. The products were dialysed to remove salt and submitted to paper electrophoresis in ammonium citrate buffer (0.03 *M*, pH 2.7). The thymidine polymer migrated at a rate 2.6 times that of the deoxyadenosine polymer, and no contamination of one polymer by the other was apparent.

Fractionation of Deoxyadenosine Polymers.—A mixture of deoxyadenosine oligonucleotides was prepared from the reaction of N-acetyldeoxyadenosine 5′-phosphate with dicyclohexylcarbodiimide as described above in the preparation of the deoxyadenosine polynucleotide–cellulose. The product was treated with 7 *N* ammonium hydroxide for 12 hr. and the mixture of oligonucleotides was fractionated on a DEAE–cellulose column.[13] A mixture of approximately 20 optical density units (260 mμ) each of the deoxyadenosine tri-, tetra-, penta-, hexa-, and heptanucleotides in 1 *M* sodium chloride–0.01 *M* sodium phosphate, pH 7 (1 ml.), was applied to the top of the thymidine polynucleotide–cellulose column and elution was carried out at 0° with the salt solution at *ca.* 2 ml. hr.$^{-1}$. The temperature of the column was then raised in a stepwise manner (Fig. 1) during the elution and the peak fractions were combined. The fractions were then dialysed for 5 hr. against water to remove salt, a procedure which caused only small losses of the shorter oligonucleotides. The products were identified by paper chromatography[20] alongside samples of standard oligonucleotides as prepared above (see Table II).

TABLE II

Fraction		R_f
3	Deoxyadenosine trinucleotide	0.33
4	Deoxyadenosine tetranucleotide	0.27
5	Deoxyadenosine pentanucleotide	0.21
6	Deoxyadenosine hexanucleotide	0.15
7	Deoxyadenosine heptanucleotide	0.11
	Deoxyadenosine-5′ phosphate	0.49

Fractionation of Uridine Oligonucleotides.—The deoxyadenosine polynucleotide column was prepared in a similar way to the thymidine column above and a mixture of uridine oligonucleotides [99 optical density units (262 mμ)] was applied in 1 *M* sodium chloride–0.01 *M* sodium phosphate solution, pH 7.0, at −2°. Elution rate was then maintained at 2–4 ml. hr.$^{-1}$ and at appropriate points the temperature was raised in a stepwise fashion. The optical density peaks were collected and dialysed against water and the solutions were concentrated. Identification was effected on Whatman No. 1 paper by partition chromatography,[20] extended over some days, of samples of the products applied in small spots alongside standard samples of uridine oligonucleotides.[15] The R_f values recorded in Table I were obtained from a single descending chromatogram in which the solvent front was not allowed to reach the bottom of the paper.

(19) Solvent system: ethanol–1 *M* ammonium acetate (7:3), pH 7.5.

(20) *Cf.* Table I, ref. *a*.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, PURDUE UNIVERSITY, LAFAYETTE, INDIANA]

The Use of Polynucleotide–Celluloses in Sequence Studies of Nucleic Acids[1]

BY P. T. GILHAM AND W. E. ROBINSON

RECEIVED JUNE 8, 1964

Thymidine polynucleotide–cellulose has been used to fractionate some of the larger polynucleotides derived from the ribonuclease digestion of the ribonucleic acid from the bromegrass mosaic virus. By the use of specific nucleases partial sequence determinations have been carried out on the fractions obtained and thus the relationship between the base sequences of various polynucleotides and their positions of elution from the polynucleotide–cellulose column has been defined. The use of this method of analysis in studies on the structure of nucleic acids is discussed.

As an approach to the determination of the fine structure of nucleic acids we have considered the need for a method which will separate fragments of nucleic acids by making use of the property of their base sequences. Thus, we have described a procedure[2] by which chemically synthesized polynucleotides may be permanently attached to cellulose, and it was shown that mixtures of simple polynucleotides may be fractionated on columns of these substituted celluloses, the fractionation depending on the relative stabilities of the complexes formed between the components of the mixture and the polynucleotides attached to the cellulose. While a series of homologous polynucleotides could be separated in this way it remained to be seen whether

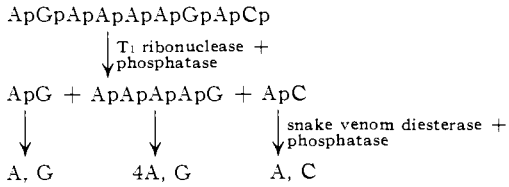
(1) This work has been supported by the National Institutes of Health, U. S. Public Health Service, and the National Science Foundation.

(2) P. T. Gilham, *J. Am. Chem. Soc.*, **86**, 4982 (1964).

mixtures of polynucleotides containing complex sequences would behave in a similar fashion.

The present work describes the isolation of some polynucleotides from the ribonucleic acid of the bromegrass mosaic virus, their fractionation on thymidine polynucleotide–cellulose, and the partial determination of their sequences. The initial study has been made on the polynucleotides formed when the viral ribonucleic acid is degraded by pancreatic ribonuclease and it was considered desirable that these should be first resolved into fractions of fragments of equal chain length. The products from the nuclease digestion of 180 mg. of the ribonucleic acid were separated by means of a DEAE–cellulose column in 7 *M* urea by the technique described by Tomlinson and Tener.[3] The elution pattern (Fig. 1) shows the peaks and the chain length of the polynucleotide fractions obtained. Each of the peaks was purified by readsorption on DEAE-cellulose and recovered by elution with a volatile salt solution. The hepta-, octa-, and nonanucleotide fractions were then separated on a thymidine polynucleotide–cellulose column using a temperature-gradient elution and the resulting elution patterns are shown in Fig. 2, 3, and 4.

For structural analysis the fractions were dialysed, concentrated, and degraded enzymically. Since the oligonucleotides had been produced by pancreatic ribonuclease digestion they consisted of consecutive purine sequences terminated by a pyrimidine. Thus, by further digestion with a mixture of phosphatase and T_1 ribonuclease,[4] the nuclease specific for guanosine linkages, each fraction produced one or more of the following: guanosine, uridine, cytidine, or consecutive adenosine oligonucleotides terminating in one of these three nucleosides. The fragments were separated chromatographically and the oligonucleotides further characterized by spectra, R_f values, and by complete degradation to their component nucleosides with a mixture of snake venom diesterase and phosphatase. The resulting nucleosides were separated chromatographically and their amounts determined spectrophotometrically. This degradative procedure is illustrated with respect to the following polynucleotide



While the information obtained in this way is not sufficient to reconstruct the sequence unambiguously in every case, it does permit the estimation of the type and size of the consecutive adenosine sequences.

In the case of the heptanucleotides, only the largest adenosine-containing fragments, obtained by T_1 ribonuclease and phosphatase digestion, were determined and analysed in this manner. These fragments (Table I, X = cytidine or uridine) were identified by degradation to their component nucleosides and by comparison of their R_f values with similar products obtained from the octa- and nonanucleotides. It is apparent that these results fulfill the expectation that the fractionation of polynucleotides by the above method would depend primarily on the extent of their consecutive adenosine sequences. However, since some of the peaks show the same major adenosine sequence, it is also apparent that other factors are involved and an evaluation of these has come from a closer study of the fractions from the octa- and nonanucleotide mixtures.

(3) R. V. Tomlinson and G. M. Tener, *Biochemistry*, **2**, 697 (1963).
(4) K. Sato and F. Egami, *J. Biochem.* (Tokyo), **44**, 753 (1957).

TABLE I

Heptanucleotides

Peak	Components	Peak	Components
A	ApX ApG	F	ApApApApX
B	ApApC ApApG	H	ApApApApG
C	ApApG	I	ApApApApApX
E	ApApApG	K	ApApApApApX

The analysis of the three peaks of the octanucleotide fractionation is shown in Table II with a list of the components obtained from the T_1 ribonuclease and phosphatase digestion and a list of possible sequences from which these fragments could arise. In Tables II and III the results in parentheses refer to the base ratios obtained by the degradation of the particular fragments to their component nucleosides as outlined above. Again, the results show that the separation of the oligonucleotides was based on their content of consecutive adenosine sequences. In peak C, however, the amount of the tetraadenosine sequences obtained cannot account for all the octanucleotides present. Thus, taking into account the other fragments isolated, it is necessary to consider that half of the oligonucleotides in this peak contain either or both the interrupted adenosine sequences, –ApGpApApAp–, –ApApGpApAp–.

TABLE II

Octanucleotides

Peak	Components	Possible sequences
A	4G, ApU (A/U = 1.2), ApG (A/G = 0.8)	GpApGpGpGpGpApUp
B	3G, ApX, 5ApG (A/G = 0.9), ApApU, ApApC, ApApG (A/G = 1.8)	GpApApGpApGpApXp GpApGpApGpApApCp GpApGpApGpApApUp
C	2C, 6G, ApU (A/U = 1.3), ApG (A/G = 1.2), ApApU (A/U = 2.3), ApApG (A/G = 2.1), ApApApG (A/G = 3.0), 2ApApApApG (A/G = 4.0)	ApApApApGpGpApUp ApApApApGpGpGpCp ApGpApApApGpGpGpCp GpGpApApGpGpApApUp

TABLE III

Nonanucleotides

Peak	Components	Possible sequences
A	4G, 2ApX, 5ApG (A/G = 0.8)	ApGpApGpGpApGpApXp GpApGpGpGpApGpApXp
C	U, 5G, ApX, 5ApG (A/G = 1.0), ApApX, 2ApApG (A/G = 1.8)	ApGpGpGpApGpApApXp GpApGpApGpApApGpUp GpGpApGpApApGpApXp
F	U, G, ApU (A/U = 1.4), ApC (A/C = 0.9), 3ApG (A/G = 1.2), 3ApApApApG (A/G = 4.2)	ApGpApApApApGpApCp ApGpApApApApGpGpUp ApGpApApApApGpApUp
H	G, 2ApApU (A/U = 2.1), ApApApApG (A/G = 4.0), ApApApApApG (A/G = 4.8)	ApApApApApGpApApUp GpApApApApGpApApUp

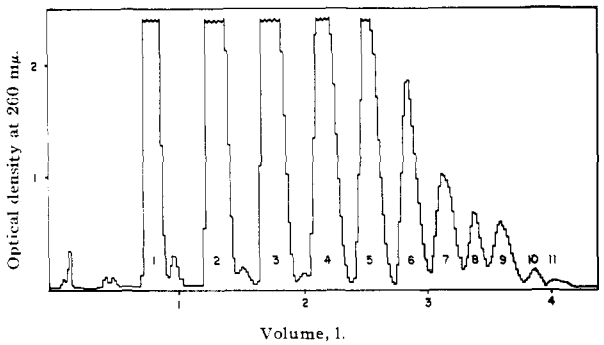


Fig. 1.—Separation of the ribonuclease digest of ribonucleic acid (180 mg.) from the bromegrass mosaic virus on DEAE-cellulose, 23 × 2.5 cm. The elution was carried out with 7 *M* urea–0.01 *M* Tris acetate solution (51.), pH 7.5, containing a linear gradient of sodium acetate from 0 to 0.5 *M* at a flow rate of 36 ml. hr.$^{-1}$

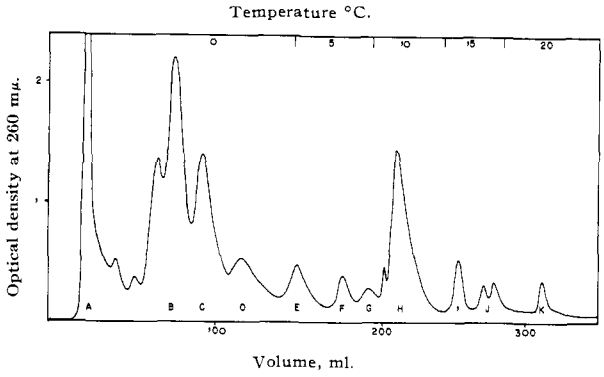


Fig. 2.—Separation of heptanucleotides on the temperature-controlled column of thymidine polynucleotide–cellulose, 40 × 1 cm. Elution was carried out with a 1 *M* sodium chloride–0.01 *M* sodium phosphate solution, pH 7.0, at a flow rate of 1–2 ml. hr.$^{-1}$

A similar situation arises in the analysis of the nonanucleotides (Table III). Although all of the oligonucleotides in peak F contain tetraadenosine sequences, half of peak H must contain similar sequences. The separation of the components in these two peaks can be rationalized by considering that peak F contains nonanucleotides with their tetraadenosine sequence as part of an interrupted pentaadenosine sequence. –ApAp-ApApGpAp–, while half of those in peak H contain tetraadenosine sequences as part of interrupted hexaadenosine sequences, –ApApApApGpApAp–.

While there are probably other subtle structural characteristics which play a part in determining the position at which a particular oligonucleotide will be eluted from the columns the main factor appears to be their content of contiguous adenosine sequences or such sequences which are partially interrupted. If the interactions between the moving and stationary polynucleotide strands described above are similar in structure to the double helix model for double stranded deoxyribonucleic acid, then the presence in the strands of noncomplementary bases which are interrupting an adenosine sequence may be accommodated in the complexes formed by the "looping out" of these bases. The "helix with loops" model has been suggested[5] for the complex formed between polyuridylic acid and a polynucleotide containing both adenosine and uridine bases.

From the above results it is obvious that fractionation of polynucleotide–cellulose results in only partial separation of the oligonucleotide mixtures applied. This is owing in part to the large number of different oligonucleotides produced by the present method of degradation of the nucleic acid. It is expected, however, that further fractionation of the peaks obtained as above will result from the use of finer adjustment of column temperature and salt concentration, or from the subsequent use of other polynucleotide–cellulose columns designed to recognize the presence of sequence characteristics other than the consecutive adenosine sequence. Furthermore, the complexity of the analysis should be reduced by the degradation of the nucleic acid with more specific methods producing fewer fragments of larger size. Thus, in an analysis being conducted at present on the nucleic acid of the MS2 bacteriophage the polynucleotide fragments are being produced by degradation of the nucleic acid with the T_1 ribonuclease.

The analysis of nucleic acids by the use of polynucleotide–cellulose columns should produce some useful information on their structure and function. For example, if one assumes that the whole of the viral nucleic acid contains information for the production of proteins in the form of a triplet code, then the discovery of oligonucleotides containing pentaadenosine sequences implies that, in the present system, the AAA triplet is a meaningful code word, since in these cases the existence of this triplet is independent of how the reading frame is positioned. Further work in progress has shown that the elution patterns from polynucleotide–cellulose separations are reproducible and highly distinctive for the nucleic acid from which they are derived. Although the elution patterns for the DEAE-cellulose separations of the pancreatic ribonuclease digests of the MS2 virus nucleic acid and ribosomal nucleic acid from wheat germ are similar to the one reported here for the bromegrass mosaic virus (Fig. 1), the subsequent fractionation of the various peaks on the polynucleotide–cellulose column gave elution patterns which were markedly different. Thus, these elution patterns represent a distinctive fingerprint for nucleic acids and can be used to compare similarities or differences between their base sequences. It is estimated that with the nucleic acids (mol. wt. *ca.* 10^6) of the viruses mentioned above the sensitivity of the method of analysis could permit the detection of a single base change. The determination of molecular weights of nucleic acids may also be derived from such analyses. These could be computed by comparing the amount of a single oligonucleotide of known chain length with the amount of nucleic acid from which it was derived.

Experimental

Preparation of Ribonucleic Acid from the Bromegrass Mosaic Virus.—Bromegrass mosaic virus was grown and isolated according to the method of Bockstahler and Kaesberg.[6] The purified virus (1.3 g.) in 0.1 *M* sodium acetate, pH 5 (30 ml.), was added to a mixture of sodium dodecyl sulfate (1 g.) and bentonite[7] (1.25 g.) in 0.01 *M* sodium acetate, pH 6 (75 ml.). Liquid phenol (40 ml.) was added and the mixture shaken at room temperature for 15 min. and then centrifuged. The top layer was removed and shaken with a further 20 ml. of phenol for 10 min. After centrifugation, the top layer (80 ml.) was removed and washed

(5) J. R. Fresco and B. M. Alberts, *Proc. Natl. Acad. Sci. U. S.*, **46**, 311 (1960).

(6) L. E. Bockstahler and P. Kaesberg, *Biophys. J.*, **2**, 1 (1962).

(7) H. Fraenkel-Conrat, B. Singer, and A. Tsugita, *Virology*, **14**, 54 (1961).

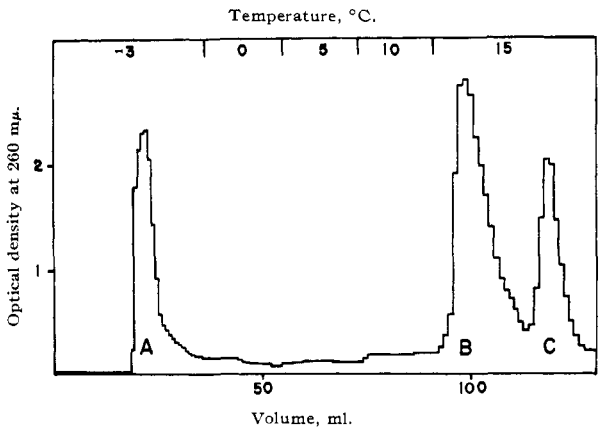


Fig. 3.—Separation of octanucleotides on the temperature-controlled column of thymidine polynucleotide–cellulose, 40 × 1 cm. Elution was carried out with a 1 *M* sodium chloride–0.01 *M* sodium phosphate solution, pH 7.0, at a flow rate of 1–2 ml. hr.$^{-1}$

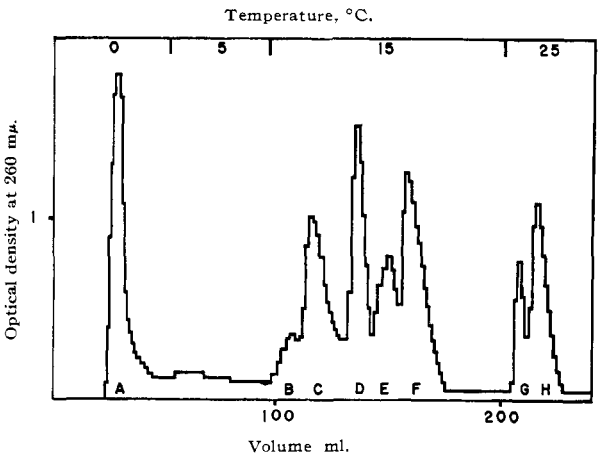


Fig. 4.—Separation of nonanucleotides on the temperature-controlled column of thymidine polynucleotide–cellulose, 40 × 1 cm. Elution was carried out with a 1 *M* sodium chloride–0.01 *M* sodium phosphate solution, pH 7.0, at a flow rate of 1–2 ml. hr.$^{-1}$

TABLE IV

Peak no.	Polynucleotides	O.D. units at 260 mμ
1	Mononucleotides	1030
2	Dinucleotides	1046
3	Trinucleotides	826
4	Tetranucleotides	616
5	Pentanucleotides	458
6	Hexanucleotides	274
7	Heptanucleotides	171
8	Octanucleotides	85
9	Nonanucleotides	101
10	Decanucleotides	26
11	Undecanucleotides	15

with ether. It was centrifuged at high speed to remove traces of bentonite and mixed with 4 *M* sodium acetate, pH 6 (2 ml.). The aqueous solution was mixed with ethanol (240 ml.) and kept at 0° for a few hours. The precipitated nucleic acid was collected by centrifugation, washed with ethanol, and dried *in vacuo* to a white powder (205 mg.). The RNA was stable to incubation at 25° in water for 24 hr. as judged by a study of its sedimentation in the ultracentrifuge. The product had an absorbancy index at 260 mμ of 21.0 cm.^{2}/mg.

Hydrolysis of the Viral Ribonucleic Acid with Ribonuclease.—The ribonucleic acid (180 mg.) prepared as above was dissolved in water (7 ml.) and treated with pancreatic ribonuclease (9 mg.) at 25°. The pH of the solution was kept at 7.5 by the addition of 2 *N* sodium hydroxide. After 20 hr. the solution was made 7 *M* with respect to urea. A DEAE–cellulose[8] column (23 × 2.5 cm.) was prepared from a slurry of the DEAE–cellulose in 7 *M* urea–0.01 *M* Tris acetate solution, pH 7.5. The enzyme hydrolysis solution was applied and a linear gradient elution from 0 to 0.5 *M* sodium acetate effected with the following solutions: 7 *M* urea–0.01 *M* Tris (2.5 l.) and 7 *M* urea–0.01 *M* Tris–0.5 *M* sodium acetate (2.5 l.), both solutions being finally mixed with sufficient glacial acetic acid to give a reading of 7.5 on the pH meter. Fractions of about 18 ml. were collected at 0.5-hr. intervals and their optical density at 260 mμ was determined (Fig. 1). The quantities of each molecular size of polynucleotide obtained from this separation are listed in Table IV.

In order to remove urea and salt from the fractions, they were individually diluted with an equal volume of water and reabsorbed on the same column used for the separation. The urea and acetate ions were removed by washing the column with 0.05 *M* triethylammonium bicarbonate, pH 7.5 (500 ml.), and the polynucleotides were eluted by washing with 1 *M* triethylammonium bicarbonate, pH 7.5. The fractions containing the polymers were combined, evaporated *in vacuo*, taken up in water, and evaporated *in vacuo* a number of times to remove bicarbonate. The recoveries of the polymers were essentially quantitative.

Fractionation of Oligonucleotides on Thymidine Polynucleotide–Cellulose.—The mixture of oligonucleotides of a particular size, obtained as above, was dissolved in 1 *M* sodium chloride–0.01 *M* sodium phosphate, pH 7.0, and applied to a temperature-controlled column of thymidine polynucleotide–cellulose (40 × 1 cm.). Elution was carried out with the same salt solution at a flow rate of 1–2 ml. hr.$^{-1}$ and the temperature of the column was raised at appropriate points in the fractionation. The optical densities of the fractions were read at 260 mμ and the peak fractions (Fig. 2–4) combined, dialysed against water for 6 hr. to remove salt, and concentrated *in vacuo*. The recoveries of material were essentially quantitative and the number of optical density units (260 mμ) obtained for each peak are listed in Table V.

TABLE V

	Peak										
Oligonucleotides	A	B	C	D	E	F	G	H	I	J	K
Hepta-	26.9	26.0	19.4	11.8	6.0	3.8	2.8	22.2	4.4	6.2	4.2
Octa-	13.7	29.0	13.3								
Nona-	11.8	3.3	11.8	10.7	7.0	14.8	3.5	9.4			

Partial Determination of Sequence of Oligonucleotides.—Each fraction from the thymidine polynucleotide–cellulose separations was dissolved in 0.5 ml. of water and treated with a mixture of ribonuclease T_1 (25 μg.) and alkaline phosphatase (25 μg.) in the presence of 20μl. of 0.1 *M* Tris buffer, pH 8.0, and a drop of chloroform. After incubation at 25° overnight the total product was chromatographed[9] on Whatman No. 1 paper. The R_f values of the various fragments obtained in this way are listed in Table VI.

TABLE VI

Fragments	R_f	Fragments	R_f
U, C	0.63	ApApApG	0.22
G, ApU, ApC	0.49	ApApApApG	0.14
ApG, ApApU	0.43	ApApApApApG	0.09
ApApG	0.32		

The various bands were eluted and their amounts measured spectrophotometrically against appropriate paper blanks. For further identification each polynucleotide fragment was dissolved in about 0.5 ml. of water and treated with snake venom diesterase (25 μg.), alkaline phosphatase (25 μg.), and 20 μl. of 0.1 *M* Tris buffer, pH 8.0. The mixture was incubated overnight at 25°, applied to Whatman No. 1 paper, and chromatographed.[10]

(8) Selectacel, Standard Grade, Schleicher and Schuell Co.

(9) Solvent system: 1-propanol–concentrated ammonia–water (55:10:35).

(10) Solvent system: a mixture of ammonium sulfate (40 g.), water (100 ml.), and sodium phosphate (0.01 mole), pH 7.0.

The R_f values of the nucleosides obtained were: adenosine 0.20; guanosine 0.42; cytidine 0.61; uridine 0.62. The spots of the products together with appropriate blanks were cut out and eluted and the amounts and ratios of the component nucleosides of the fragments were determined spectrophotometrically. These results are recorded in parentheses in Tables II and III.

Acknowledgment.—The authors are indebted to Dr. P. Kaesberg and Dr. L. E. Bockstahler for their help in preparing the bromegrass mosaic virus.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY MEDICAL CENTER, DURHAM, NORTH CAROLINA]

Optical Rotatory Dispersion of L-Tyrosine[1]

BY T. M. HOOKER, JR., AND CHARLES TANFORD

RECEIVED JULY 20, 1964

Aqueous solutions of L-tyrosine display a prominent Cotton effect which is associated with the aromatic absorption band near 280 mμ. The effect is enhanced and shifted to higher wave length when the phenolic group is ionized, but is not greatly affected by changes in the ionization states of the amino or carboxyl groups. On the other hand, the Cotton effect is considerably reduced in amides and esters of tyrosine, and is absent altogether in N-acetyl derivatives. No corresponding Cotton effect is associated with the 260-mμ absorption band of L-phenylalanine.

The most prominent feature of the optical rotatory dispersion of the α-amino acids[2-4] is a Cotton effect which shows its first extremum in the region from 220 to 230 mμ, and which has been associated with the carboxyl group absorption band near 210 mμ. Schellman[2,3] has predicted, however, that the aromatic amino acids should have an additional Cotton effect, associated with the aromatic chromophore, and the purpose of this paper is to show that such a Cotton effect indeed appears prominently in the optical rotatory dispersion of aqueous solutions of L-tyrosine. On the other hand, it has not been observed with L-phenylalanine, and it is either not present at all, or considerably less prominent, in a number of derivatives of L-tyrosine.

Experimental

L-Tyrosine was an A-grade sample from the California Corporation for Biochemical Research. The same sample has been used for solubility studies,[5] and proved free from impurities by the criterion that its apparent solubility remained unchanged when the amount of solid phase added exceeded the saturation amount more than tenfold. L-Phenylalanine was obtained from Mann Research Laboratories. It also has been used for solubility studies,[5] and found free from impurities. The tyrosine derivatives which were used were purchased from Mann Research Laboratories or from Cyclo Chemical Corporation. The purest available grades were obtained. These derivatives were used only for preliminary survey experiments.

Optical rotations were measured with a Cary Model 60 spectropolarimeter, using an Osram 450 watt xenon lamp as light source. Measurements were made at 25°, most of them using cells with a 1-cm. light path. In the data reported for tyrosine the amino acid concentration was between 1.5 and 1.6 × 10^{-3} M. The phenylalanine data represent a composite of two curves, using concentrations of 5.4 × 10^{-5} M and 6.8 × 10^{-3} M, respectively. All results are reported in terms of molecular rotations

$$[\phi] = \frac{3}{n^2 + 2} \frac{M_0}{100} [\alpha]$$

where n is the solvent refractive index, M_0 the molecular weight, and $[\alpha]$ the specific rotation at any wave length. The refractive index values for pure water[6] were used, since the small amounts of acid or base, present in most of the solutions to adjust the pH, should have no appreciable effect on the refractive index.

Since solutions with high absorbance may sometimes lead to artifactual optical rotation data,[7] great care was taken to make certain of the validity of our results. The peak absorbance of our tyrosine solutions (1.6 = 10^{-3} M, 1-cm. light path) was about 2.2, which is well within the permissable range for the Cary spectropolarimeter. Solutions of lower concentration were studied as controls, and data were also obtained for 1.6 × 10^{-3} M solutions in cells with a 0.1-cm. light path. Such data were somewhat less precise, because the observable rotation diminishes with the absorbance, but agreed with all other data within the limits of error.

In one experiment a curve was obtained for a solution of 1.06 × 10^{-3} M tyrosine (peak absorbance 1.5), and a second curve was obtained with two cells in the light path, one containing the same solution, and the other containing an equal concentration of *dl*-tyrosine. The peak absorbance was now 3.0, which is near the limit which the instrument can handle. Nevertheless, no significant change in the optical rotatory dispersion curve was observed.

Results

Figure 1 shows typical data for L-tyrosine at three pH values, and also gives for comparison the optical rotatory dispersion curve for L-phenylalanine, which has no Cotton effect above 225 mμ. The figure shows that the peak and trough of the effect lie at 283 and 260 mμ, respectively, at neutral pH. The midpoint thus lies at 272 mμ, close to the absorption peak which occurs in neutral solution at 275 mμ. At high pH, the peak and trough shift to 304 and 274 mμ, respectively, and the midpoint at 289 mμ now lies closer to the alkaline absorption peak of tyrosine, which occurs at 294 mμ.

The point midway between the trough and peak of the curves of Fig. 1 should not be regarded as the exact center of the Cotton effect because Cotton effects which occur at lower wave lengths evidently contribute to the rotation in the 280-mμ region. To obtain a precise quantitative measure of the 280-mμ Cotton effect would require that the contribution of these other Cotton effects could be estimated accurately and subtracted from the data. If this could be done, then the peak and trough rotations would become equal, but opposite in sign, and the midpoint would correspond to the absorption wave length of the transition responsible for the effect. The required correction cannot be estimated with the data presently available. It is evident, however, that the rotation due to lower wave length Cotton

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