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Infrared Spectra of Deoxyribonucleic Acids with Different Base Compositions in Their D_2O Solutions

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In this note we present the result of our examination of the infrared absorption spectra of six DNA's with different guanine+cytosine (GC) contents in their heavy water solutions in the spectral region of 1750—1480 cm^{-1}. A similar examination was once made by Fritzsche[1] with deuterated DNA *films*, and he found that the relative intensity of the absorption bands at 1485 and 1505 cm^{-1} depends on the guanine+cytosine (GC) content of the deuterated DNA samples. In our study in D_2O *solution*, on the other hand, a number of bands characteristic of the guanine-cytosine (G–C) and adenine–thymine (A–T) base-pairs are found in the 1700—1550 cm^{-1} region as shown below.

TABLE 1. DNA SAMPLES USED IN THE PRESENT WORK

Source	GC content (%)
Tetrahymena pyriformis GL	25[a]
Clostridium perfringens	31[b]
Calf thymus	43[b]
Escherichia coli	51[b]
Pseudomonas aeruginosa	65[c]
Micrococcus lysodeikticus	72[b]

a) Estimated from the melting temperature (61.8 °C in 0.015 M NaCl+0.0015 M Na-citrate) and buoyant density in a CsCl density gradient centrifugation (1.686, as given by R. A. Flavell and I. G. Jones, *Biochem. J.*, **116**, 811 (1970).
b) S. Ulitzur, *Biochim. Biophys. Acta*, **272**, 1 (1972).
c) N. Sueoka and T. Y. Cheng, *J. Mol. Biol.*, **4**, 161 (1962).

The DNA samples used in the present study are listed in Table 1. Preparation of DNA from *Tetrahymena pyriformis* was made according to the method described by Allen and Gibson.[2] The cells were kindly provided by Dr. Takashi Mita, National Cancer Center Research Institute. Lysis was effected by adding 10% sodium lauryl sulfate, and within 1 min phenol was added. After treatments with ribonuclease A (Sigma Chemical Co., 50 μg/ml, at 37 °C, for 30 min), with pronase E (Kaken Chemical Co., 1 mg/ml, at 37 °C, for 1 hr), and with α-amylase (Boehringer-Mannheim 50 μg/ml), DNA was precipitated with 2-propanol. DNA samples from *Clostridium perfringens* and Calf thymus were purchased from Miles Laboratories, Inc. DNA preparation from *Escherichia coli*, *Pseudomonas aeruginosa* (kindly provided by Dr. Toshio Ando, The Institute of Medical Science, University of Tokyo), and *Micrococcus lysodeikticus* was made, according to Marmur,[3] by lysing the micro-organism with sodium lauryl sulfate, followed by a shaking with chloroform–isoamyl alcohol, precipitation with ethanol, treatment with ribonuclease A (Sigma Chemical Co., 50 μg/ml, at 37 °C, 30 min), and again precipitation with ethanol.

The purities of the DNA samples were judged by the ultraviolet absorption curves and the optical melting profiles (*i.e.*, the melting temperatures T_m and the amounts of hyperchromicity). The phenol extraction, protease treatment, and ribonuclease treatment were repeated until a proper set of values of T_m and hyperchromicity was reached. To remove disturbing anions the DNA solution was dialyzed against 10^{-3} M Tris buffer+10^{-3} M NaCl and lyophilized.

For infrared absorption measurement, concentrated D_2O solution (about 1.5%) was prepared. The solvent used was Tris buffer (pD≒7). To promote the rate of dissolving of DNA into D_2O, sonication with UMEDA 20 kcycle/s (150 W) was applied for 10 min. It was confirmed that this procedure does not pracitcally affect the ultraviolet and infrared absorption spectra of the DNA solution. The solution was placed in a cell with CaF_2 windows and optical path 50 μ. A Perkin-Elmer 621 infrared spectrophotometer was

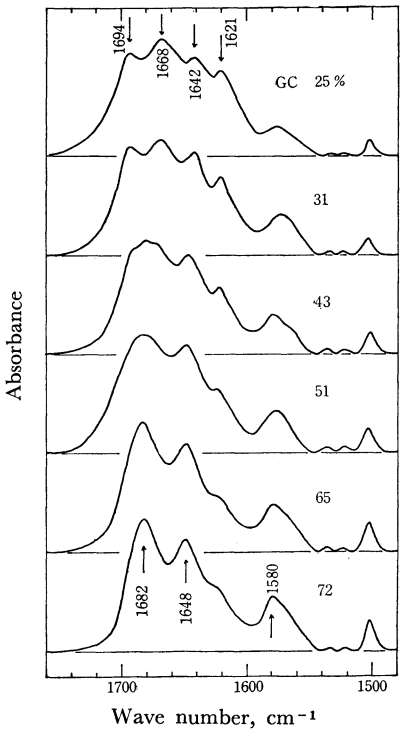

Fig. 1. Infrared absorption curves of DNA's in D_2O solutions at about 40 °C. From the top: DNA's from *Tetrahymena* (GC 25%), *C. perfringens* (31%), Calf thymus (43%), *E. coli* (51%), *P. aeruginosa* (65%), and *M. lysodeikticus* (72%).

1) H. Fritzsche, *Biopolymers*, **5**, 863 (1967).
2) S. L. Allen and I. Gibson, *J. Protozool.*, **18**, 518 (1971).
3) J. Marmur, *J. Mol. Biol.*, **3**, 208 (1961).

used for the absorption measurement.

The observed infrared absorption curves are shown in Fig. 1. As may be seen in the figure, the absorption peaks at 1621, 1642, 1668, and 1694 cm^{-1} become more prominent on lowering the GC content, while those at 1580, 1648, and 1682 cm^{-1} become more prominent as the GC content becomes higher. In fact, after a proper adjustment of the absorbance scale in each absorption curve, the absorbance at each of these frequencies is found to have an almost linear relation with the GC content (see Fig. 2). This fact indicates that the A–T base-pairs (or the G–C base-pairs) in all of these DNA structures are in a similar environment to one another in an average. The vertical stacking interactions between the base-pairs in a double-helical polynucleotide structure should certainly affect the infrared absorptions of the base-pairs,[4,5] but this is not clearly detected in our present examination of DNA's in the range of GC content 25—72%.

The effect of the vertical stacking interaction becomes apparent when the examination is extended to the range of the GC content 0% or 100%. In Fig. 3,

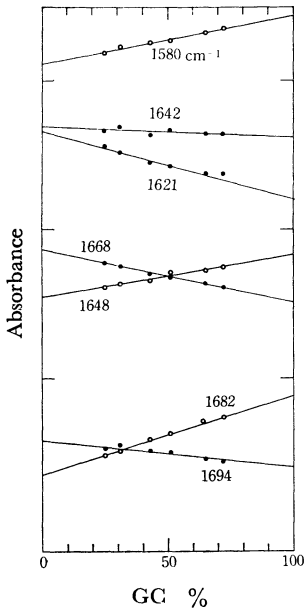

Fig. 2. Plots of absorbance *versus* GC content at several frequencies in the infrared spectra of DNA's.

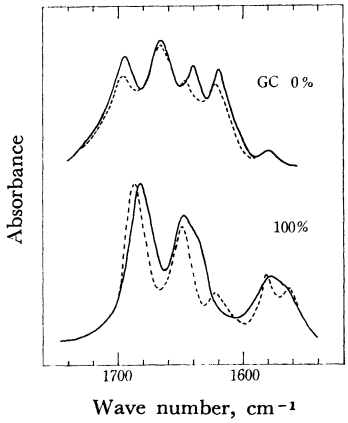

Fig. 3. Full lines: Calculated spectra of A-T and G-C base-pairs obtained by the extrapolation of the absorbance *versus* GC content relations illustrated in Fig. 2. Broken lines: Observed spectra of poly(dA-dT)-poly(dA-dT) (upper) and poly(rG)-poly(rC) (lower)[6] in their D_2O solutions.

infrared absorption curves of a hypothetical A–T base-pair and a hypothetical G–C base-pair are shown which are obtained by an extrapolation of absorbance *versus* GC content relations illustrated in Fig. 2. These are appreciably different from actually observed spectra of A–T and G–C base-pairs, respectively. Thus, the spectrum of the hypothetical A-T pair placed in an average environment of natural DNA's is different from the spectrum actually observed of the A–T pair in poly(dA–dT)–poly(dA–dT), *i. e.*, the double-helical complex containing strands of alternating deoxyriboadenylate and deoxyribothymidylate residues (purchased from Miles Laboratories, Inc). In the latter structure every A–T pair is considered to be always sandwiched by two T–A pairs. The spectrum of the hypothetical G–C pair placed in an average environment of natural DNA's is also different from the spectrum actually observed[6] of the G–C pair in poly(rG)–poly(rC), the double-helical complex of riboguanylate and ribocytidylate homopolymers. This difference should be attributed not only to the difference in the base-sequence but also to the difference between the DNA B structure[7] and the double-helical RNA structure.[8]

4) K. Morikawa, M. Tsuboi, S. Takahashi, Y. Kyogoku, Y. Mitsui, Y. Iitaka, and G. J. Thomas, Jr., *Bipolymers*, **12**, 799 (1973).

5) M. Tsuboi, S. Takahashi, and I. Harada, in "Physico-Chemical Properties of Nucleic Acids," ed. by J. Duchesne, Academic Press, London (1973), p. 91.

6) G. J. Thomas, Jr., *Biopolymers*, **7**, 325 (1969).

7) S. Arnott and D. W. L. Hukins, *Biochem. Biophys. Res. Commun.*, **47**, 1504 (1972).

8) S. Arnott, "Progress in Biophysics and Molecular Biology", Vol. 21, ed. by J. A. V. Butler and D. Noble, Pergamon Press (1970), p. 265.