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Masamichi Tsuboi and Katsuichi Shuto: Infrared Absorptions in the 1300 cm^{-1} Region of Deoxyribonucleic Acid Solutions with Different Base Compositions.

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Deoxyribonucleic acid (DNA) from calf thymus shows two absorption peaks at 1292 and 1277 cm^{-1} in its infrared absorption spectrum.[1] On deuteration, both of these two disappear.[1] The transition moments of these two lie in an almost perpendicular direction to the fiber axis[1] in crystalline states of this DNA. Therefore, these two bands may be assigned to some in-plane vibrations of the bases in the DNA structure. On drying the DNA fiber, both of these two bands completely disappear.[1,2] By a treatment with deoxyribonuclease (for 10 min. at 37°) or by a treatment with formamide, however, such a spectral change takes place only half way, and on heating the DNA solution (*e.g.* to 100° for 10 min.), almost no change takes place in the two absorption bands.[2,3] Therefore, these two bands are considered to reflect a secondary structure of DNA. The secondary structure, here, should be a rather local structure which is retained even if the base pairings and the double-stranded structure are mostly destroyed, and which is broken only when the surrounding water molecules are removed or the primary structure is destroyed.

The purpose of this paper is to report our recent finding that the relative intensities of these two absorption peaks are sensitive to the guanine-cytosine content (GC content) of DNA.

The DNA samples used are listed in Table I.

Table I. DNA Samples used in the Present Work

Source	GC content (%)	Source	GC content (%)
Micrococcus lysodeikticus	72[a]	*Clostridium werchii*	31[b]
Bacille de Calmette-Guérin	65[b]	*Cancer borealis*	3[d]
Calf thymus	44[c]		

a) N. Sueoka, T.Y. Cheng: J. Mol. Biol., **4**, 161 (1962).
b) Determined by Professor T. Tsumita (by hydrolysis).
c) E. Chargaff: "The Nucleic Acids," E. Chargaff and J.N. Davidson, Eds., Vol. 1, pp. 354, Academic Press, Inc., New York, 1955.
d) Reference 7).

DNA from *Micrococcus lysodeikticus* was prepared by ourselves. Mostly, the procedure described by Marmur[4] was followed. For dissociating the nucleic acid from protein, however, not only the Marmur's method but also the method described by Kay, Simmons, and Dounce[5] was adopted. The molecular weight of the product was estimated to be 3.2×10^6 from the sedimentation coefficient. DNA from *Bacille de Calmette-Guérin* (B.C.G.) and DNA from *Clostridium werchii* were prepared by Professor Toru Tsumita in the Institute for Infectious Diseases, University of Tokyo, and kindly

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1) G. B. B. M, Sutherland, M. Tsuboi: Proc. Roy. Soc. (London), **A239**, 446 (1957).
2) M. Tsuboi: Progress of Theoretical Physics, Suppl. No. **17**, 99 (1961).
3) Y. Kyogoku, M. Tsuboi, T. Shimanouchi, I. Watanabe: J. Mol. Biol., **3**, 741 (1961).
4) J. Marmur: J. Mol. Biol., **3**, 208 (1961).
5) E. R. M. Kay, N. S. Simmons, A. L. Dounce: J. Am. Chem. Soc., **74**, 1724 (1952).

placed at our disposal by him. Calf Thymus DNA was purchased from Sigma Chemical Company. The "light DNA" from *Cancer borealis*, which contains 97 moles percent of deoxyadenylate and deoxythymidylate in predominantly alternating sequence[6,7] (dAT), is a gift from Dr. Ts'ai-Ying Cheng, now at the Central Research Department, Experimental Station, E.T. du Pont de Nemours of Company.

All of the DNA samples are in the form of lyophilized, cotton-like, white fibers. Each of these DNA samples was dissolved into distilled water, so that about 5% aqueous solution was obtained. Infrared absorptions were observed by placing the solution between two CaF_2 windows, and then by mounting this on a double-beam infrared spectrophotometer. In the reference beam of the spectrometer, a variable thickness cell with CaF_2 windows and filled with distilled water was placed. On recording the absorption curve, the absorptions due to the solvent (water) were compensated by adjusting the optical path length of the reference cell.

The recorded absorption curves of these DNA's in solutions are reproduced in Fig. 1. As may be seen in the figure, the absorption intensity of the 1277 cm^{-1} band relative to that of 1292 cm^{-1} band is found to increase as the GC content (see Table I) decreases.

What are significant in connection with the present finding may be the following two points: First, we have now a more detailed knowledge of the infrared spectrum of DNA in this spectral region than we have before. The two absorption bands at 1292 cm^{-1} and 1277 cm^{-1} should now be assigned to some vibrations directly related to the base parts in the DNA structure. In addition, it has been established that the adenine-thymine base pair has a contribution to both of these two bands, while the guanine-cytosine base pair only to the 1292 cm^{-1} band. Secondly, the above finding may provide a simple method for the GC content determination of a given DNA sample. At present, about 1 mg. of DNA sample is required for the infrared absorption measurement. The error in the intensity determination of the 1277 cm^{-1} band would be about 5%. However, the amount of the sample required may somewhat be lowered and the accuracy of the intensity measurement may be improved in future.

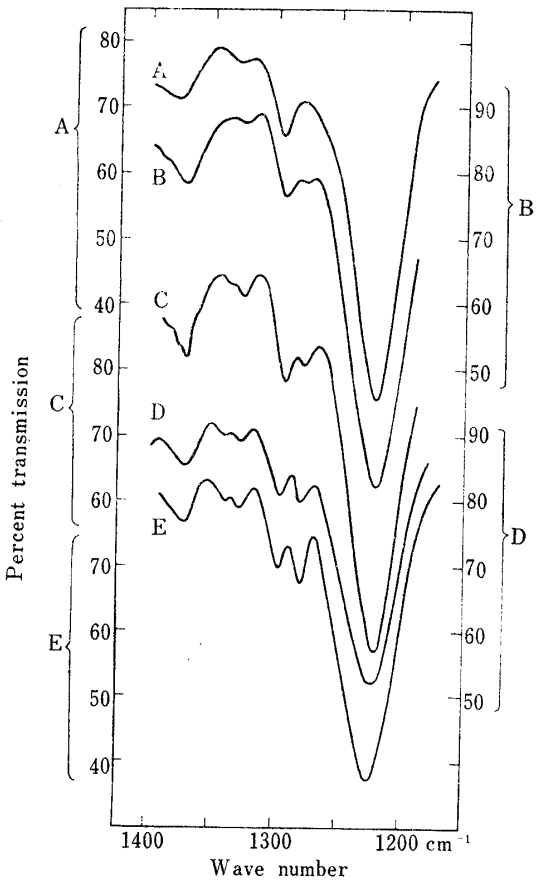

Fig. 1. Infrared absorption spectra of DNA's in H_2O solutions

A: DNA from *M. lysodeikticus*
B: DNA from B.C.G.
C: DNA from calf thymus
D: DNA from *Cl. werchii*
E: "Light DNA" from a marine crab (nearly dAT)

We wish to express our deep appreciation to Professor Toru Tsumita and to Dr. Ts'ai-Ying Cheng for their kindness in providing the DNA samples described above. Our thanks are also due to Dr. Yoshiki Ohba

6) N. Sueoka: J. Mol. Biol., **3**, 31 (1961).
7) N. Sueoka, T. Y. Cheng: Proc. Nat. Acad. Sci. (U. S. A.), **48**, 1851 (1962).

for his help in our preparation of the *M. lysodeikticus* DNA. This work was supported by a grant from the U. S. Public Health Service (GM 10024-02) and by a grant from the Ministry of Education of Japan.

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Hideo Yamada, Teruhisa Ichihashi, Fujiko Kogishi, and Ryuichi Yamamoto : Biopharmaceutical Studies on Factors Affecting Rate of Absorption of Drugs. II.*1 Further Investigation of Absorption of Drugs in Micellar Solution.

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The previous paper*1 has reported the relationship between the intestinal absorption rate of salicylamide in polysorbate-80 solution and the concentration of the surfactant. This relationship is shown in the form of Eq. (1),

$$\frac{k}{k_{Df}}=\frac{f_w}{C_{sm}K+f_w} \qquad (1)$$

where k or k_{Df} is the absorption rate constant of the drug in the micellar solution or in the surfactant-free solution, respectively, under the condition described in the previous paper, C_{sm} is the concentration of the surfactant forming the micelle, K is the partition constant of the drug between in the micellar phase and in the aqueous phase and f_w is the volume fraction of the aqueous phase to the total solution. Eq. (1) indicates that the dependence of the ratio, k/k_{Df}, on the concentration of surfactant is mainly governed by the partition constant. In the case of salicylamide, the experimental results well agreed with the calculated values by Eq. (1).

The present paper describes the further investigation of the relationship, shown in Eq. (1), with methylsalicylate and sulfanilamide which have the respective K-value much different from that of salicylamide in the previous study.

Experimental

Determination of the Partition Constants of Methylsalicylate and Sulfanilamide——The method used in determination of the partition constant was essentially the same as that described in the previous report.*1 The assay methods were as follows : For methylsalicylate, to 4 ml. of the outer solution were added 10 ml. of $M/15$ Na_2HPO_4, 4 drops of 2% 4-aminoantipyrine solution and 2 ml. of 1% $K_3[Fe(CN)_6]$. The volume was made up to 20 ml. with distilled water. For sulfanilamide, the outer solution was diluted 20-fold by distilled water and to 10 ml. of this solution were added 3 ml. of N HCl and 4 drops of 0.2% $NaNO_2$. Ten min. later, 4 drops of 10% $NH_4SO_3NH_2$ was added. Five min. later, 4 drops of 0.2% N-(2-dimethylaminoethyl)-1-naphthylamine solution was added. Then, the volume was made up to 20 ml. with distilled water.

The absorbances of these solutions were read on a spectrophotometer (Hitachi Co., Ltd. model EPU-2) at 510 mμ for methylsalicylate and 544 mμ for sulfanilamide.

Determination of the Rate of Absorption from the Rat Small Intestine——The experimental technique employed was essentially the same as that described in the previous paper.*1 Only few variations in method

*1 Part I : H. Yamada, R. Yamamoto : This Bulletin, **13**, 1279 (1965).

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