Mechanism of mild acid denaturation of deoxyribonucleic acid

It is well known that a saline solution of highly polymerized DNA (deoxyribonucleic acid) titrates about 2 protons per 4 nucleotides in lowering the pH from 7.0 to 3.0. Concomitant changes take place in the macromolecular properties of DNA[2]. Thus at a constant molecular weight, the radius of gyration decreases by a factor of three and the viscosity decreases by a factor of 15. If the temperature is low and if the solution is quickly brought back to neutrality the original macromolecular configuration of DNA is recovered. Since it has been shown that the molecular weight did not change during this process, it is evident that the changes were the result of drastic alterations of the intramolecular structure, the transition from helix to random coil being induced by the co-operative breakdown of hydrogen bonds as a result of the constituent groups taking up protons. A similar phenomenon has been observed by R. F. BEERS AND R. F. STEINER[3] for a synthetic complex containing equal amounts of polyadenylic and polyuridylic acids.

Previous authors have appeared to assume that the protons add to the 6-amino groups of adenine and the 6-amino group of cytosine in the acid denaturation of DNA and the double-chain polynucleotides[1,3] (Fig. 1). But it does not seem to have been appreciated that this does not lead directly to an explanation of the hydrogen-bond breakage. Indeed both the groups in question act as hydrogen donors in their respective hydrogen bonds and the addition of a proton to the amine group does not generally detract from its ability to form a hydrogen bond[4–6]. Furthermore, the fact that adding protons to DNA decreases the interchain repulsion resulting from the negatively charged phosphates makes the breakage doubly difficult to comprehend.

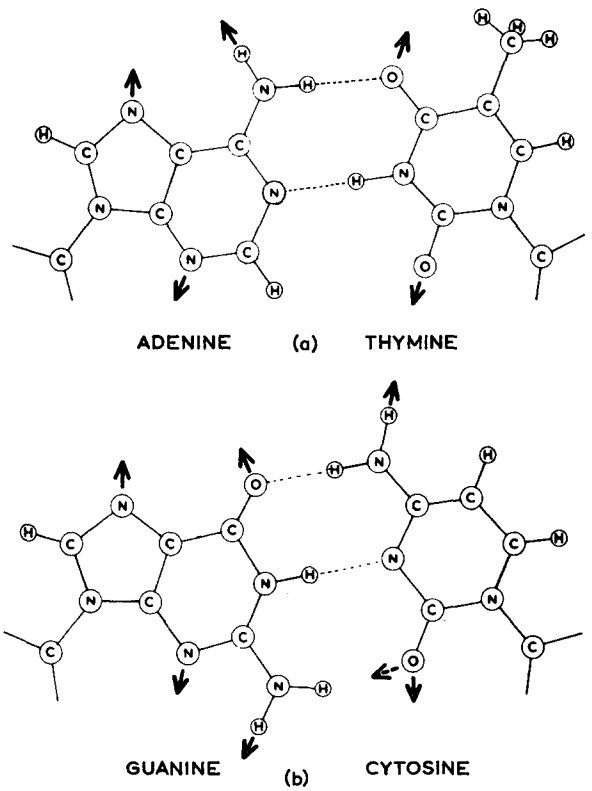

Fig. 1. Purine-pyrimidine base pairs as they exist in DNA
(a) adenine-thymine, (b) guanine-cytosine.

It appears that this dilemma can be resolved by assuming that the proton adds to the N_1 position rather than to the amino nitrogen group. The former nitrogen acts as a hydrogen acceptor in the intact hydrogen-bonded DNA structure and its affinity for hydrogen would obviously be decreased by the addition of a proton. Direct evidence on the position of the extra hydrogen in the heterocyclic six-membered ring of adenine has been given in the crystal-structure determination

of adenine hydrochloride by W. COCHRAN[7]. In COCHRAN's structure the extra hydrogen is placed on the N_1 position in agreement with the assignment suggested here for protonated DNA. Of course, one does not "see" a proton with X-rays. The X-ray F_0—F_c transform indicates a maximum in electron density at a position 0.89 Å from the center of the N nitrogen, demonstrating the presence of an N_1–H bond in the vicinity. The positive charge added by the proton appears to be shared between the N_{10}, N_1, and N_9 positions on adenine via resonance between four different structures. Although the N_9 of adenine has its N_9–H bond replaced by an N_9–C bond in DNA it is still possible to draw the same four resonating structures. Whether it is justifiable to say that cytosine also protonates on its N_1 position in DNA is less certain. However, it is possible to draw three reasonable resonating structures of cytosine with the extra positive charge shared by the N_1, N_4 and N_7 nitrogens, respectively, and on this basis the comparison is believed to be a good one. Thus it appears most likely that mild acid treatment protonates the N_1 positions of adenine and cytosine in DNA and also that this gives a better explanation of the hydrogen-bond breakage by acid.

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1 D. O. JORDAN, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. I, Academic Press, Inc. New York, 1955 p. 478.
2 C. A. THOMAS AND PAUL DOTY, *J. Am. Chem. Soc.*, 78 (1956) 1854.
3 R. F. BEERS AND R. F. STEINER, *Nature*, 181 (1958) 30.
4 C. H. BAMFORD, A. ELLIOT AND W. E. HANBY, *Synthetic Polypeptides*, Academic Press, Inc., New York, 1956, p. 109.
5 J. MONTEATH ROBERTSON, *Organic Crystals and Molecules*, Cornell University Press, Ithaca, N. Y., 1953, p. 237.
6 J. DONOHUE, *J. Phys. Chem.*, 56 (1952) 502.
7 W. COCHRAN, *Acta Cryst.*, 4 (1951) 81.

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Studies of ribose metabolism
IV. The metabolism of D-glucuronolactone in normal and pentosuric human subjects

A pathway for ribose biosynthesis from D-glucuronic acid in the mammalian organism has been postulated[1,2] as a result of the demonstration in animal tissues of the following reactions:

1. D-glucuronic acid ⟶ L-gulonic acid[3]
2. L-gulonic acid ⟶ L-xylulose[2]
3. L-xylulose ⟶ xylitol[4]
4. xylitol ⟶ D-xylulose[4]
5. D-xylulose ⟶ D-xylulose-5-phosphate[5]
6. D-xylulose-5-phosphate ⇌ D-ribulose-5-phosphate[6]
7. D-ribulose-5-phosphate ⇌ D-ribose-5-phosphate[7]

A block in this pathway has been suggested[8] to explain the urinary excretion of L-xylulose by persons with the genetic disturbance, essential pentosuria[9].

We have recently obtained evidence in man[10] for ribose synthesis from glucose *via* the oxidative and the nonoxidative reactions of the pentose phosphate pathway[11]. Our studies were carried out with a ribose "trapping" technic, suggested by the observation of TABOR AND HAYAISHI[12] that imidazoleacetic acid (IAA) riboside appears in the urine of rats given IAA. Using this technic, we have now demonstrated ribose biosynthesis from D-glucuronolactone, uniformly labeled with ^{14}C, in a human subject with normal carbohydrate metabolism. Evidence is also provided for an impairment of this pathway in a subject with pentosuria.

A 67-year-old man with coronary artery disease but with normal carbohydrate metabolism and a 52-year-old man with pentosuria were each given IAA hydrochloride[13] by mouth, and 30 min later, intravenous D-glucuronolactone, uniformly labeled with ^{14}C. (This compound, which was obtained through the generosity of Dr. N. E. ARTZ of the Corn Products Refining Co., Argo, Ill., was dissolved in 0.9% saline and sterilized by passage through a bacteriological filter.) Urine was collected during the ensuing 10 h, and a portion was passed over Dowex-1 acetate, which

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