THE REACTION OF NITROUS ACID WITH DEOXYRIBONUCLEIC ACID

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If infective ribonucleic acid (RNA) from tobacco mosaic virus (TMV) is treated with nitrous acid (HNO_2) the inactivation and mutation rates both follow a one-hit curve, showing that the deamination of only one of the amino-bases A,[1] G or C in the RNA is lethal or mutagenic, respectively (Schuster and Schramm, 1958; Gierer and Mundry, 1958). At present it cannot be determined, which of the three types of chemical alteration caused by HNO_2 (A → HX, G → X, C → U) are mutagenic or lethal, because the amino-bases in the RNA all react with nearly the same velocity.

Several investigators have studied the lethal and mutagenic effect of HNO_2 on biological active material containing deoxyribonucleic acid (DNA).[2] The kinetics of both inactivation and mutation were studied, but not the kinetics of the chemical alterations.

In this paper the reaction of DNA with HNO_2 was chemically investigated, using DNA's from different sources. The reaction rates of the amino-bases in DNA were studied under different conditions.

Experimental and Results

Calf thymus DNA was treated at pH 4.2 (20°C) with HNO_2; the concentrations per volume of reaction solution being 0.1% DNA, 0.25 M acetate buffer, 1 M $NaNO_2$. Samples were withdrawn from the solution at various times. After neutralization and dialysis, the base composition of the DNA was determined spectrophotometrically after formic acid hydrolysis (0.5 hours, 175°C; Wyatt, 1953), and separation of the bases by paper chromography (Schuster and Schramm, 1958). The reaction velocities of the

[1] The following abbreviations are used: A = adenine, G = guanine, C = cytosine, HX = hypoxanthine, X = xanthine, U = uracil, T = thymine.

[2] For a survey of the literature see Vielmetter and Schuster (1960).

amino-bases in the DNA were determined from the decrease of the bases during the reaction time relative to thymine, assuming the latter to be constant throughout the treatment. The decrease of the amino-bases A and C equaled the increase of the keto-bases HX and U, respectively. But less X was found than would be expected from the decrease of G. The deficiency of X was found because the glycosidic bond between X and deoxyribose is more labile than the corresponding bonds of A and G.

The reaction velocities, α_m of the bases in DNA are summarized in Table 1. The α_m values of the bases in native thymus DNA vary considerably (column 2) in contrast to the similar α_m values of the bases in TMV RNA (column 1), treated with HNO_2 under identical conditions. The bases containing an amino-group in the 6-position of either a purine or pyrimidine ring (A and C) react much slower than does the guanine, whose amino-group is in the 2-position. The former bases reacted more rapidly when the thymus DNA was denaturated by the heat treatment of Rice and Doty (1957) prior to HNO_2 treatment (column 3).

The reaction rates of the bases are not influenced, if the native thymus DNA is treated with phenol (1 hour, 20°C) prior to HNO_2 treatment (column 4). It should be mentioned that the activity of a transforming factor (DNA of H. influenzae) is not destroyed by incubation in phenol (0.65 M phenol, 50°C, 1 hour; Zamenhof et al., 1953).

Table 1

The reaction velocities α_m+ of the amino-bases in nucleic acids treating the nucleic acid with HNO_2 at pH 4.2

		Thymus DNA			DNA	DNA
Base	TMV-RNA	native	heat-denaturated	phenol treated	Salmon Sperm	in E. coli
A	265	70	160	80	150	60
G	250	355	330	335	320	305
C	190	160	255	165	210	185

+ α_m = μM deaminated base/1 M base x minute

The reaction velocities of the bases in DNA from salmon sperm, isolated by the procedure of Emanuel and Chaikoff (1953), are very similar to those of a heat-denaturated DNA (column 5). This similarity might have been caused by keeping the DNA several months in the dried state.

When DNA, isolated from E. coli by a rigorous purification procedure, was treated with HNO_2 the reaction velocities of the amino-bases gave non-reproducible values. Therefore, the intact bacterial cells (10^{10} - 10^{11} cells/ml) were treated with HNO_2. The treated cells were harvested by centrifugation and the DNA was isolated thereafter using alkaline solutions to remove all the contaminating RNA. The base determinations were then carried out as usual. The reaction rates of the bases in this E. coli DNA are about the same as those of native thymus DNA (column 6).

In order to determine the influence of the H-ion concentration on the reaction rates of the bases, thymus DNA was incubated at different pH values with a constant amount of 1 M $NaNO_2$. The reaction velocities increase with increasing H-ion concentration, since more molecules of HNO_2 are produced from the $NaNO_2$. Therefore, the ratios of the reaction velocities $\alpha_m A/\alpha_m G$ and $\alpha_m C/\alpha_m G$ were determined at different pH values and compared. The results of the treatment of thymus DNA at two pH values, 3.4 and 4.2 are summarized in Table 2. At pH 3.4 the ratios of $\alpha_m A/\alpha_m G$ and $\alpha_m C/\alpha_m G$ are greater than at pH 4.2. Therefore, at a higher H-ion concentration more A and C relative to G undergo reaction with HNO_2.

Table 2

Comparison of the reaction velocities α_m of the thymus DNA bases by deamination at pH 3.4 and 4.2

Ratios of α_m	pH 3.4	pH 4.2
A/G	0.66	0.20
C/G	0.74	0.45

Discussion

In native DNA the different amino-bases react with HNO_2 with considerably different reaction velocities. After denaturation of the molecule these differences are less. From these results it is concluded that the H-bonds between the amino-groups of A and C and the corresponding keto-groups of T and G inhibit the reaction of the former groups. The results also suggest that no H-bond exists between the amino-group of G and the 2-keto-group of C, as was also suggested by earlier titration studies (Gulland et al., 1947). The reason for the difference in the reaction rates of A and C in native DNA is not

known, nor is the reason for the greater reactivity of G in DNA in comparison to the G in RNA.

The similar reaction velocities of the bases of DNA within bacterial cells and those of thymus DNA in solution indicate that the HNO_2 molecules can react with the DNA within intact cells without an inhibitory influence by the surrounding cell constituents.

The pH dependence of the relative reaction rates of the bases $\alpha_m A / \alpha_m G$ and $\alpha_m C / \alpha_m G$ suggest that differentiation between the three possible types of base deaminations for their ability to induce mutagenic or lethal changes may be possible. This is discussed in the following paper.

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