THE BASE SPECIFICITY OF MUTATION INDUCED BY NITROUS ACID IN PHAGE T2

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Nitrous acid has been shown to be an effective inactivating and mutagenic agent for the RNA of tobacco mosaic virus (Schuster and Schramm, 1958; Mundry and Gierer, 1958), for the phages T2 (Vielmetter and Wieder, 1959), T4 (Freese, 1959), ϕ x 174 (Tessman, 1959) and for transforming DNA (Litman and Ephrussi-Taylor, 1959). In all cases analysed, both inactivation and mutation follow first order kinetics with respect to the time of treatment. Therefore lethal or mutagenic changes are caused by single chemical alterations in the nucleic acid. The chemical nature of these alterations is known to be the deamination of either G,[1] A or C, which is thereby converted into X, HX or U, respectively (Zamenhof, 1953; Schuster and Schramm, 1958; Schuster, 1960).

In DNA, the relative deamination rate ratios, $\alpha A/\alpha G$ and $\alpha C/\alpha G$, are different for different pH values (Schuster, 1960). Therefore, in this paper the deamination rates of G, A and C in phage T2 treated with HNO_2 at different pH values are compared with the corresponding rates of mutation and inactivation. This reveals which types of base deaminations can lead to either mutagenic or lethal changes.

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[1] G = guanine, A = adenine, C = cytosine, or in phage T2 hydroxy-methyl-cytosine (HMC), X = xanthine, HX = hypoxanthine, U = uracil, or in phage T2 hydroxy-methyl-uracil (HMU).

EXPERIMENTAL

T2 wild type phage were incubated at 20°C in a mixture containing ~2 mg/ml phage, 1 M $NaNO_2$, 0.25 M acetate buffer and 0.08 M NaCl. One experiment was performed at pH 4.20 for 60 hrs and another at pH 5.00 for 550 hrs. At regular time intervals samples were withdrawn and after dialysis the DNA was extracted and purified using the methods of Wyatt and Cohen (1953). After formic acid hydrolysis the bases were chromatographed as previously described (Schuster, 1960). The extent of deamination for each sample was measured as the decrease in G, A and HMC and the increase in HMC, assuming T to be constant. Under the same conditions, but at various pH values, the rate of induction of r^- mutation and the inactivation rate were measured as described previously (Vielmetter and Wieder, 1959). The number of plates for each sample was chosen so that at least 50 r^- plaques were observed. The fraction of rII^- mutants was determined by assaying the purified progeny of randomly picked r^- plaques on strain E. coli K (Benzer, 1957).

RESULTS AND CONCLUSIONS

In Fig.1 the rate of the induction of r^- mutation and the inactivation rate are plotted against the pH. It is found that the log of the mutation rate decreases linearly with increasing pH. Linear regression was used to obtain accurate values for the rate constants at pH 4.20 and pH 5.00. These values can be compared with the deamination rates of the three bases (Table I). The deamination rate of each base has been found to follow first order kinetics for a long period of time. Therefore, it is likely that the same applies also for the beginning of the reaction, at which time mutation and inactivation have been measured.

The results lead to the following conclusions.

(1) The deamination rate, α, of both A and C decreases by a factor of about 90 from pH 4.20 to pH 5.00, whereas the deamination rate of G decreases by a factor of only 35. The corresponding factor for

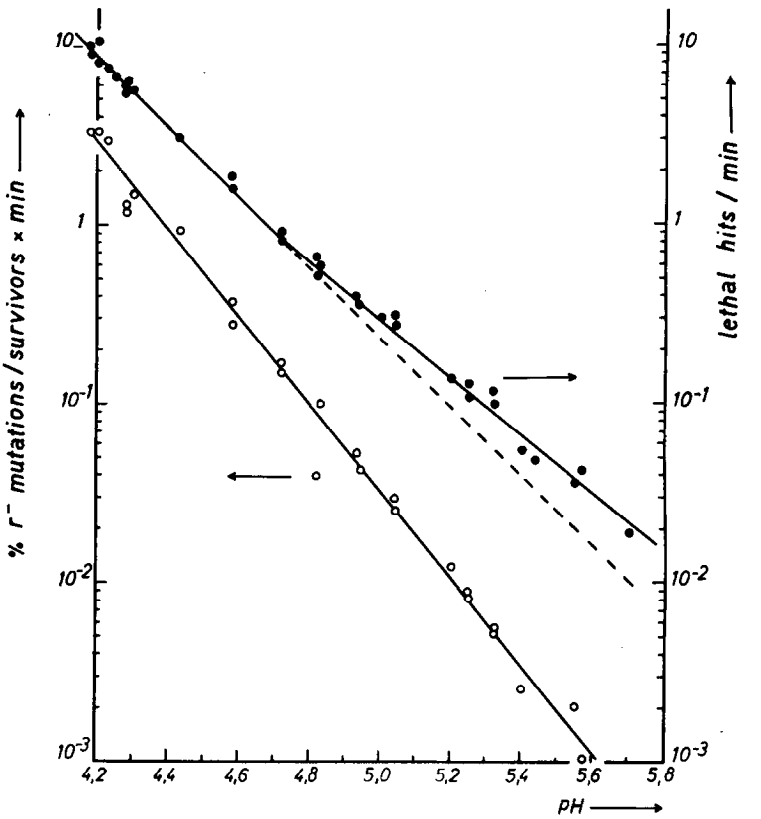

Figure 1.

Dependence of the rate of r^- mutation and the inactivation rate on the pH for phage T2 treated with 1 M $NaNO_2$ at 20°C

Table I

	Percent of Total DNA	α (Deaminations/min x 10^6) pH 4.20	pH 5.00	$\frac{\alpha\ 4.2}{\alpha\ 5.0}$
C	17	116	1.3	89
A	33	18.5	0.2	90
G	17	297	8.55	35
Average deamination per Total DNA		76.3	1.74	44
$\frac{\%\ r^-\text{mutants/survivors}}{\text{min}}$		2.9	3.3×10^{-2}	88
phage lethal hits/min		9	0.3	30

the decrease of the rate of r^- mutation has been found to be 88. Therefore it is concluded that the deamination of mainly A, C or both, but not of G, is responsible for the induction of mutations.

(2) This result is expected from the base pairing scheme proposed by Watson and Crick (1953). In this scheme A pairs specifically by hydrogen bonding with T and C pairs with G. The pairing specificity is primarily determined by the 6' keto-group in G and T and the 6' amino-group in A and C. The amino-group in G is 2', therefore its removal does not affect the pairing specificity. However, in both A and C deamination leads to analogues (HX and U) which pair like G and T, respectively. Therefore two types of conversions of base pairs (so called "transitions", Freese, 1959), should result from deamination and subsequent replication:

$$CG \longrightarrow TA \text{ (type 1) and } AT \longrightarrow GC \text{ (type 2)}$$

For T2 treated at pH 4.2 it can be calculated, that mutants resulting from conversions of type 1 should be 3-times more frequent as those derived from conversions of type 2.

(3) At either pH 4.20 or pH 5.00 the conversion of any one of about 570 base pairs/phage genome gives rise to a r mutation. Since we have found that the proportion of rII mutants/total induced r^- mutants is 65 %, the conversion of any one of about 370 base pairs should lead to a visible rII mutation. This is roughly 1/2 to 1/3 of the total base pairs/rII region, as estimated from data of Benzer (1957) for the closely related phage T4.

(4) The inactivation rate decreases from pH 4.20 to pH 5.00 by a factor of only 30. This indicates that in contrast to the mutagenic inducibility, inactivation can also be caused by the deamination of G (Table I). This conclusion however, remains tentative, because deaminations in the protein might also contribute to the inactivation.

(5) If in T2 the rate of the unspecific inactivation at a low pH of

treatment does not exceed 20% of the total inactivation [as indicated from experiments with phage PLT22 (Vielmetter, unpublished) and T4 (Freese, personal communication)] the target size for inactivation due to deamination of A, C and G can be calculated to be about 5×10^4 nucleotide pairs or 25% of the total T2 DNA.

A detailed account and discussion of the results will be published in the Zeitschrift für Naturforschung.

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