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THE MECHANISM OF THE REACTION OF HYDROXYLAMINE AND O–METHYLHYDROXYLAMINE WITH CYTIDINE

N.K. Kochetkov, E.I. Budowsky, E.D. Sverdlov, R.P. Shibaeva, V.N. Shibaev, G.S. Monastirskaya

Institute of Chemistry of Natural Compounds Academy of Sciences of USSR, Moscow, USSR

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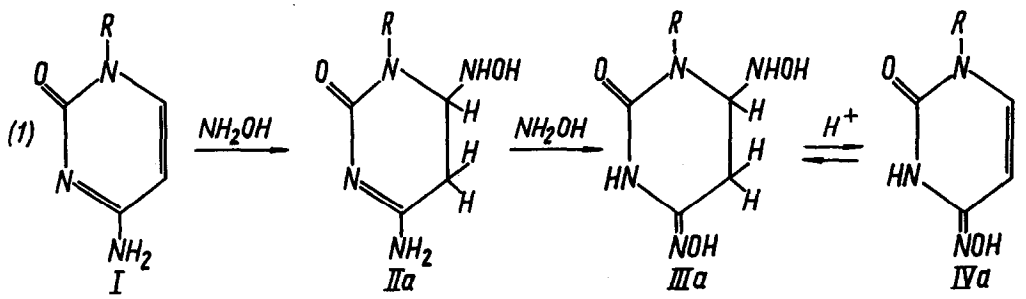
The mutagenic action of hydroxylamine (HA) and O–methylhydroxylamine (OMHA) appears mainly to be due to their reactions with cytosine residues in nucleic acids (1–6). The mechanism of these interactions is not fully understood; the interpretations of the genetic data from the chemical point of view are rather contradictious (7 – 9).

The present communication deals with the preliminary results of the investigation of the kinetics and the mechanism of the reactions of cytidine with HA and OMHA under the conditions usually used in genetic studies (the concentration of HA or OMHA 0.1 – 3.0 M; pH 4.0 – 6.5; 28–30°; the concentration of cytidine is 10^{-2} – 10^{-4} M).

The following mechanism of the interaction between cytidine and HA is now commonly accepted (see Scheme 1):

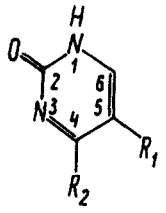


Uridine 4–oxime (IVa) and 6–hydroxyamine–5,6–dihydrouridine 4–oxime (IIIa) are the main reaction products with HA (1, 3, 10, 11). OMHA gives the similar products (IVb and IIIb) (11–13). The direct formation of IVa from cytidine and HA is considered by previous authors as hardly probable, although in the case of 5–alkylcytosines occurs only direct substitution of the aminogroup at C_4 (14).

We have calculated the values of the nucleophylic localization energy for the addition to the 5,6–double bond and substitution on C_4 for several derivatives of 2–oxopyrimidine with the use of MO method (15) (see Table 1). The results obtained show close similarity of the latter energies for cytosine and 5–methylcytosine; these data suggest the opportunity of direct conversion I → IV in the reactions of cytidine with HA or OMHA.

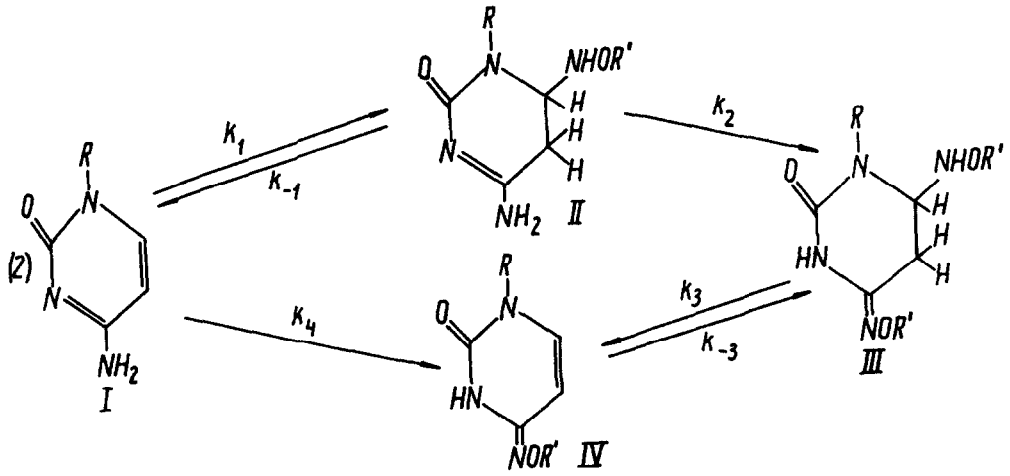
TABLE I

The Nucleophylic Localization Energies (in β – Units) for Some Derivatives of 2–Oxopyrimidine



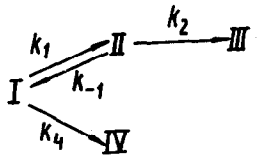
R_1	R_2	C_4	$C_5 = C_6$
H	OH	2.7911	2.9240
H	NH_2	4.5938	2.9912
CH_3	NH_2	4.6216	6.8300

If this possibility really takes place then the mechanism of the cytidine reactions should be represented by Scheme 2*.



The rate constants of the conversions III → IV (k_3) and IV — III (k_{-3}) were directly determined by the time dependence of the optical densities of the solutions III (pH 4.5 – 6.5) and the solutions IV containing 0.5 M HA or OMHA in the same pH–range (17) (Table 2). These reactions were proved to be slow as compared to the reactions leading to decrease of cytidine and Scheme 3 could be used instead of Scheme 2 for describing of reaction kinetics.

*This scheme was given in Brown's paper (16). However, based on the experimental data he rejected the formation of intermediate IV.



(3)

The direct formation of IV from I is proved by the following facts. Firstly, as it has been mentioned above (see also Table 2) the formation of IV from III may be neglected under the reaction conditions. Secondly, the accumulation of IVa in the reaction mixture (determined by the change of the optical density at 310 mμ (at this wavelength the absorption of IVa much more than these of I, IIa, or IIIa (17)) takes place without any noticable lag period (Fig. 1).

According to the Scheme 3 the accumulation of IV in the reaction mixture may be described by the equation (4):

$$(4) \qquad \frac{d[IV]}{dt} = k_4 [I]$$

If the concentrations of IV (Calculated from optical density at 310 mμ) and I* are known for any moment the rate constant k_4 can be easily calculated (Table 2). The calculated value of k_4 does not vary with time, this fact further confirms the direct conversion I – IV.

Thus the constants of pseudo–first order k_3, k_{-3} and k_4 were determined directly. The rate constants of other stages (k_1, k_{-1}, k_2) were determined by more detailed analysis of the changes in the spectra of the reaction mixtures.

TABLE 2

The Rate Constants of Different Stages in the Reaction between Cytidine and HA or OMHA (15)

	k_1^{0} [a] 1/mole.hr	k_{-1}^{0}/k_2^{0} [a] mole/l	k_3 hr^{-1}	k_{-3}^{0} [a] 1/mole.hr	k_4^{0} [a] 1/mole.hr
HA [b]	0.72	2.4	<0.001	0.014	0.094
OMHA [c]	0.6	11	<0.001	< 0.001	0.042

[a] calculated from the corresponding pseudo–first order constants. [b] 30°, pH 6.0 μ = 1.0.

[c] 28°, pH 4.9, μ = 3.0.

*Knowing the concentration of IVa, the concentration of I can be easily calculated from optical density at 275 mμ, where the absorptions of IIa and IIIa are negligible.

FIG 1

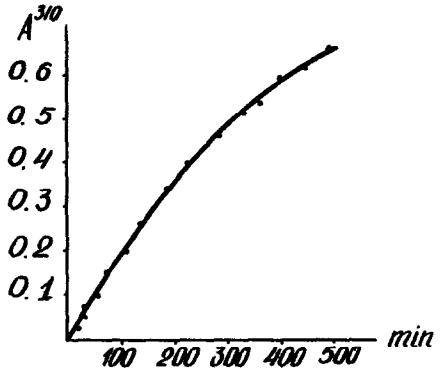


FIG. 2

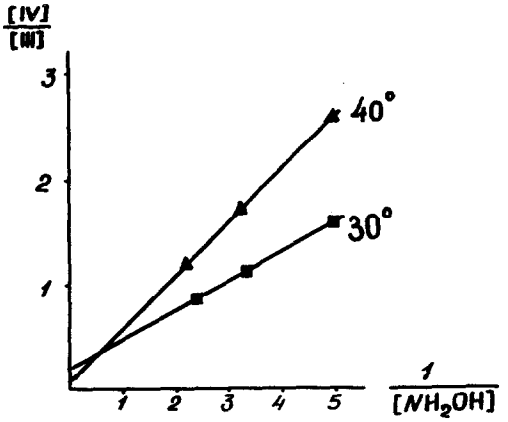


Fig. 1. The change of the optical density at 310 mμ in the course of the reaction between cytidine and hydroxylamine. Cytidine concentration is about 10^{-2} M, optical path is 1.0 cm, hydroxylamine concentration 1.0 M, pH 5.5, 30°, μ = 1.

Fig. 2. The relationship of the IV/III at the end of the reaction and reciprocal concentration of hydroxylamine at pH 6.

The analysis of differential equations system corresponding to the Scheme 3 enables to draw the following important conclusions.

The ratio of the concentrations of the products III and IV at the moment of disappearing I and II from the reaction mixture is described by the equation:

$$(5)\quad \frac{[IV]}{[III]} = \frac{k_4^\circ}{k_1^\bullet}\,\frac{k_{-1}}{k_2^\circ \cdot [NH_2OH]} + \frac{k_4^\circ}{k_1^\circ}$$

This ratio is dependent on the concentration of HA and OMHA; in full agreement with the equation (5) a linear relationship is really observed between the ratio IV/III and reciprocal concentration of HA (Fig. 2).

In addition the investigation of the dependence of the course of the reaction on pH-value shows that the ratio IV/III is increased with a decrease of pH from 6.5 to 4.5. In the case of HA this ratio is increased with an increase in the temperature (Fig. 2).

A complicated character of the reaction of HA and OMHA with cytidine permits to explain the shift of optimal pH-value for the rate of cytidine decrease to alkaline region as compared with that calculated for the simple reaction of protonated I with HA base (5) by the presence of the reversible reaction II → I the rate of which is increased with a decrease of pH.

Our experimental data show that interaction of cytidine with HA and OMHA is a complex parallel-consequent reaction which results in the formation of the compounds IV directly from I already at the first moment of the

reaction*. The ratio of the products vary widely depending on the reaction conditions (particularly on pH and the concentrations of hydroxylamine derivatives). These results are to be taken into consideration studying the changes of the template activity of polynucleotides by the action of HA or OMHA.

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*When this paper was ready for publication, the communication of Lawley (18) has appeared where the possibility of the direct formation of deoxyuridine 4–oxime from deoxycytidine was also demonstrated.