REACTION OF THE CYTOSINE NUCLEUS WITH HYDRAZINE IN THE PRESENCE OF BISULPHITE

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1. Introduction

The presence of a reactive hydrazine group in N^4-amino cytosine provides ample opportunities for the specific introduction of labels (fluorescent or radioactive), combinations with carbonyl-containing carriers (cellulose or Sephadex) and for other specific secondary reactions. Also, N^4-amino cytidine is an effective mutagen [1], this effect being evidently caused by the double functional specificity of this compound in the systems of matrix biosynthesis similar to N^4-hydroxy- and N^4-methoxy-derivatives of cytidine and adenosine [2].

By hydrazine action in aqueous neutral solutions the conversion of cytosine into the N^4-amino cytosine is slow even in rather vigorous conditions [3]. As was shown earlier [4–8], the nucleophilic substitution of the exocyclic amino group of cytosine is greatly facilitated by the presence of bisulphite.

In this paper it is shown that N^4-amino cytosine derivatives can be obtained in mild conditions when cytidine is treated with a bisulphite–hydrazine mixture and some kinetic characteristics of this reaction are adduced.

2. Materials and methods

Sodium metabisulphite $Na_2S_2O_5$ was prepared as described earlier [8].

Hydrazine hydrate was an analytically pure preparation.

The reaction was followed by the changes of u.v.-spectra in 100-fold water dilutions of portions of the reaction mixture.

7 mg of 2′-desoxycytidine-5′-phosphate were dissolved in 2 ml of 1 M sodium metabisulphite solution containing hydrazine (hydrazine concentration, pH values and temperature are given in table 1). Immediately after nucleotide dissolution and then after fixed intervals of 0.1 ml portions were taken and diluted in 10 ml of water. The spectra were measured 10–15 minutes after dilution.

To study the velocity of the reverse reaction, the mixtures were incubated until no further spectral changes occurred and then portions were diluted 50-fold with 0.5 M buffer solutions (pH 5.0 – sodium acetate, pH 6.0 and 7.0 – sodium phosphate, pH 7.9 – Tris·HCl, pH 9.0 – glycine–NaOH). The diluted mixtures were incubated at the required temperature and their spectra measured periodically. The constants of reaction velocity were calculated according to the formula:

$$K = \frac{2.3}{t} \lg \frac{D_0 - D_\infty}{D_t - D_\infty}$$

Table 1
Half-time of dpC conversion (min) (calculated according to the changes of A_{270} of diluted reaction mixture) at 1 M bisulphite and hydrazine

Temp	2.5 M NH_2NH_2		5 M NH_2NH_2	
pH	30°C	40°C	30°C	40°C
5.0	30	21	–	13
5.5	22	20	16	12
6.0	25	22	15	7
6.5	34	27	18	14
7.0	59	50	45	23
7.5	–	60	60	44

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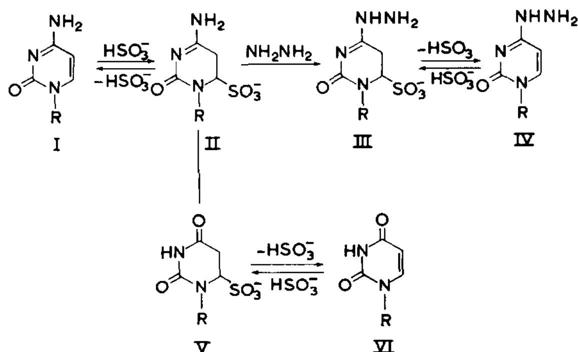
where t is the incubation time, min.; D_0, D_t and D_∞ are the optical densities at 270 nm initially, after t minutes, and the maximum reached during the reaction, respectively.

3. Results and discussion

Bisulphite adds easily to the C^5–C^6 double bond of the cytosine nucleus [4–8] thus contributing to the substitution of the exocyclic amino group even by weak nucleophiles under mild conditions [7,8]. The general pattern of cytosine nucleus transformations in the presence of bisulphite–hydrazine mixture can be represented as in Scheme 1.



The reduction in bisulphite concentration by 50–100-fold dilution of the reaction mixture (pH ⩾ 5) is associated with rapid and almost quantitative transformation of II → I [4,5] and correspondingly by increase of the absorption in the long-wavelength part of the spectrum. The rate of V → VI transformation at pH ⩽ 7 is low and the new equilibrium after dilution is established slowly, thus the decrease in optical density at 270 nm of dpC mixture with bisulphite measured after dilution reflects the degree of I → V transformation, i.e. deamination of the cytosine nucleus. As is shown below the III → IV transformation is also rather slow. Thus the changes in optical density at 270 nm measured after diluting the mixture containing dpC, bisulphite and hydrazine reflects the total transformation of the cytosine nucleus into compounds III and V.

Incubation of dpC with 2.5 and 5 M hydrazine at pH from 5.0 to 7.5 (5 h, 30–40°C) does not cause any spectrum changes. On incubation of dpC with 1 M bisulphite deamination of the cytosine nucleus occurs. However, by the action of bisulphite–hydrazine mixture on dpC the decrease of optical density at 270 nm is much faster than with bisulphite alone (table 1). In the time taken for the reaction with hydrazine and bisulphite to be practically complete only 20–30% deamination occurs in the presence of bisulphite. Thus by the action of 1 M bisulphite mixture with 2.5 or 5 M hydrazine on dpC the basic reaction is substitution of the exocyclic amino group of the cytosine nucleus with formation of compound III. This is supported by the spectral changes seen in diluted portions of the reaction mixture – after incubation at 20–30°C the absorption in the long-wavelength region increased and the final spectrum is characteristic of N^4-amino cytidine (λ_{max} = 275 nm) rather than of uridine.

The rate of the splitting off of water, bisulphite and hydroxylamine from the subsequent adducts with restoration of the C^5–C^6 double bond of the pyrimidine nucleus largely depends on the nature of the C^4 substituent. The rate decreases from cytidine to uridine and to N^4-oxy and N^4-methoxycytidine derivatives [4–11].

The rate of bisulphite splitting from compound III (table 2) is comparable with the rate of V → VI conversion [4,5] as may be judged by the rate of spectral changes of diluted reaction mixture. The increase in optical density at pH 7–9 is well described by the first-order reaction equation while at pH 5 and 6 noticeable deviations from it are observed and the reaction does not come to an end. This is apparently related to the rather high concentration of product III in the equilibrium mixture after dilution at pH 5 and 6.

Thus the action of bisulphite–hydrazine mixture under mild conditions is associated with transformation of the cytosine nucleus into that of N^4-amino-6-

Table 2
Rate constants of sulphogroup splitting from N^4-amino-5,6-dihydro-6-sulphocytidine versus pH and temperature (K, min^{-1} × 10^2)

Temp. pH	20°C	30°C
7	0.60	1.1
8	0.46	0.86
9	0.37	–

sulphonate-5,6-dihydrocytosine, that is rather easily transformed into N^4-amino cytosine after removal of excess reagents. In other words, the proposed reaction allows transformation of the monomer or macromolecule cytosine nucleus into N^4-amino cytosine under mild conditions.

A detailed exploration of this reaction and the possibilities of its application to the study of the structure and functions of nucleic acids and nucleoproteins is being conducted.

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