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Catalysis of Diethylenetriamine for Bisulfite-Induced Deamination of Cytosine in Oligodeoxyribonucleotides

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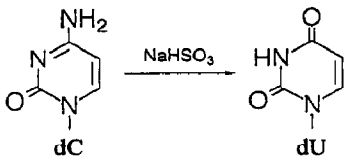
Abstract: Diethylenetriamine catalyzes bisulfite ion-induced deamination of 2'-deoxycytidine in oligodeoxyribonucleotides to 2'-deoxyuridine at pH 5, much more efficiently than ammonia, ethylenediamine, 3,3'-diaminodipropylamine, and spermine.

Transformation of nucleic acid bases to others has been attracting increasing interest. More than 20 years ago, Shapiro *et al* [1] and Hayatsu *et al* [2] independently reported that sodium bisulfite deaminates cytosine to uracil and that the reaction is applicable to artificial mutagenesis.[3] However, information on the catalysts of the deamination, required for the further applications of this unique reaction, has been scarce.[4] Here we show that diethylenetriamine effectively catalyzes bisulfite-induced deamination of 2'-deoxycytidine (dC) in oligodeoxyribonucleotides to 2'-deoxyuridine (dU) (eq. 1).



dC dU (eq. 1)

The deamination reactions were achieved at pH 5 (unless otherwise noted) where bisulfite ion satisfactorily functioned as a buffer agent. The mixtures were prepared by use of $NaHSO_3$ and Na_2SO_3. The initial concentration of the oligodeoxyribonucleotide substrate was 10^{-4} *M*. After the reactions, the mixtures were first subjected to ultrafiltration to remove bisulfite ion and then were incubated at pH 9 to decompose the deoxycytidine-bisulfite and the deoxyuridine-bisulfite adducts. The resultant oligodeoxyribonucleotides were hydrolyzed to nucleosides by a phosphodiesterase and by an alkaline phosphatase, and were analyzed by a reversed-phase HPLC (Merck LiChrosphere RP-18(e) column; water/acetonitrile = 97.5/2.5). Assignments of HPLC signals were made by coinjection of authentic samples. The dC→dU transformations in the presence and the absence of amines satisfactorily showed first-order kinetics. The results presented here are the averages of at least duplicated runs.

Figure 1 depicts typical reversed-phase HPLC patterns for the enzymatic digests for the products of the bisulfite ion (1.0 *M*)-induced reactions of a 7-mer oligodeoxyribonucleotide (TGTCTAG) at pH 5 and 35°C . Diethylenetriamine greatly promotes the deamination of dC to dU (compare (a) with (b)). Conversion of the dC→dU transformation at 4 h is 73 mole% when $[\text{diethylenetriamine}]_0 = 1.0$ *M*, whereas the value in the absence of the amine is only 9.4 mole% (13 fold acceleration). All the nucleo-

sides other than dC remained intact, and no other by-products were perceived. The rate constant for the transformation linearly increased with increasing concentration of diethylenetriamine, as depicted in Fig. 2. The reaction proceeds as a bimolecular one, rather than via the complex between the oligodeoxyribonucleotide and the amine. The transformation is the most effective around pH 5.[5]

The rates of the dC→dU transformation in the oligodeoxyribonucleotides TGACAAG and TGACTAG by diethylenetriamine were almost identical with that for TGTCTAG. The kinds of the adjacent nucleotides cause only minimal effects on the catalysis. The dC→dU transformation in a 39-mer oligodeoxyribonucleotide (CTGAAGATCTGGAGGTCCTGTGTTCGATCCACAGAATTC), in which nine dC residues are distributed rather randomly, was also catalyzed by diethylenetriamine in a similar magnitude.

The activity of diethylenetriamine is overwhelmingly larger than those of any other amines investigated (Fig. 3). No measurable catalysis was observed for ammonia, 3,3'-diaminodipropylamine, and spermine. All these amines are virtually completely protonated at pH 5, whereas diethylenetriamine mostly (85 %) exists as a dication with the two terminal amino residues protonated (the pK_a values are 9.84, 9.02, and 4.23).[6] Apparently the neutral amino residue at the center of diethylenetriamine is essential for the catalysis of the dC→dU transformation. Although ethylenediamine showed some catalysis, the activity was around 10 fold smaller than that of diethylenetriamine. Highly substituted diamines, N,N,N',N'-tetramethylethylenediamine, piperazine, and 1,4-diazabicyclo[2.2.2]octane, were not active at all.

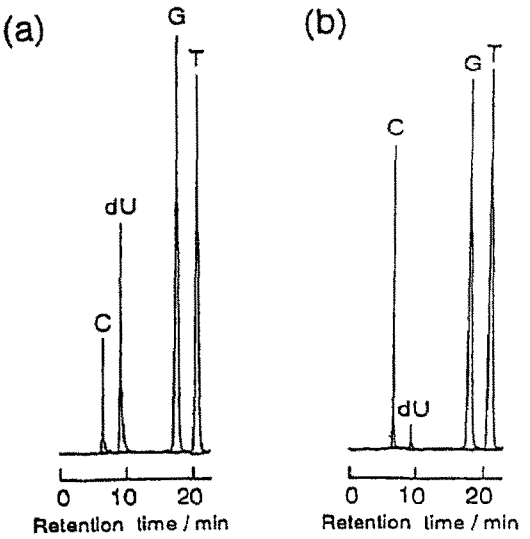


Fig. 1. Reversed-phase HPLC patterns for the enzymatic digests of the products of the reactions of a 7-mer oligodeoxyribonucleotide (TGTCTAG) with bisulfite ion (1.0 *M*) at pH 5 and 35°C for 4 h: (a) in the presence of diethylenetriamine (1.0 *M*); (b) in the absence of diethylenetriamine. The peaks for adenosine are at the retention time 48 min.

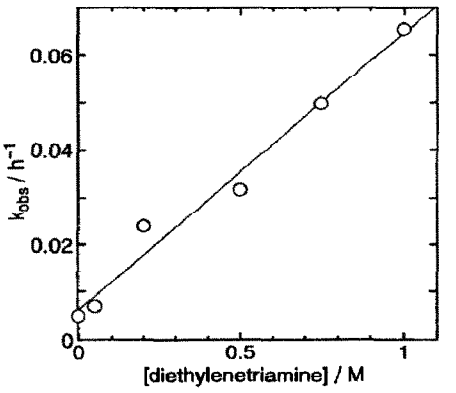


Fig. 2. Plot of the pseudo first-order rate constant for the bisulfite-induced dC→dU transformation in a 7-mer oligodeoxyribonucleotide (TCTCTAG) vs. the concentration of diethylenetriamine at pH 5 and 35°C: $[bisulfite]_0 = 1.0\ M$.

$H_2N(CH_2)_2NH(CH_2)_2NH_2$ >> $H_2N(CH_2)_2NH_2$
>> NH_3 , $H_2N(CH_2)_3NH(CH_2)_3NH_2$, $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$,
$(CH_3)_2N(CH_2)_2N(CH_3)_2$, $HN(C_2H_4)_2NH$, $N(C_2H_4)_3N$ = 0.

Fig. 3. Catalytic activities of various amines for the dC→dU transformation.

The most plausible mechanism for the diethylenetriamine catalysis is schematically depicted in Fig. 4. The central unprotonated amino residue as a general base catalyst promotes the nucleophilic attack of water toward the C4 carbon in the cytosine–bisulfite adduct (the direct nucleophilic attack by the amine toward the C4 is ruled out, since the corresponding modified nucleoside is not detected by HPLC).[7] Consistently, the small acceleration of the transformation by bisulfite ion was previously ascribed to a similar general base catalysis.[4] In addition, one of the two terminal amino residues of diethylenetriamine, which are protonated under the conditions, can cooperatively function as an acid catalyst. Assumedly, the other cationic amino residue of the diethylenetriamine electrostatically interacts with the sulfonate residue at the C6 position of the adduct, promoting the acid–base catalysis described above.

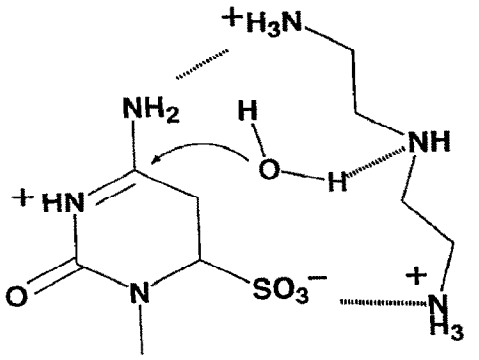


Fig. 4. A proposed mechanism of the diethylenetriamine catalysis for the bisulfite–induced dC→dU transformation.

The small catalysis by ethylenediamine is ascribed to the monocationic species. Although its molar fraction at pH 5 is only 0.82 % (pK_a 9.89 and 7.08),[6] the catalytic rate constant should be larger than that of diethylenetriamine due to the larger basicity of the neutral amino residue. When the α value of the Brønsted plot for the general base catalysis is tentatively taken as 0.4, the activity (the product of the concentration of the catalytic species and its catalytic rate constant) is estimated to be 8 times as small as that of diethylenetriamine (the α value for the general base catalysis is, in most of the cases, in the range of 0.2–0.6).[8,9]

The cooperative acid–base catalysis proposed in Fig. 4 is furthermore supported by the significant steric effect on the catalysis. As described above, highly hindered diamines such as N,N,N',N'–tetramethylethylenediamine, piperazine, 1,4–diazabicyclo[2.2.2]octane showed no catalysis, although ethylenediamine was somewhat active. All the amines have similar pK_a values, and are in virtually the same ionization states. If only the general base catalysis by the neutral amino residue were operative, the steric effect should not cause such a drastic effect (it is well–known that the general base catalysis is not significantly affected by steric hindrance).[8] In the cooperative acid–base catalysis, however, the mutual conformation of the two amino residues is of dominant importance, and the steric factor in the catalyst should give rise to a crucial effect.

In conclusion, diethylenetriamine efficiently catalyzes the bisulfite-induced deamination of dC in oligodeoxyribonucleotides. The number of the methylene residues between the amino residues must be two for the effective catalysis. Attachment of the amine to sequence-recognizing moieties for the site-selective dC→dU transformation in oligodeoxyribonucleotides is currently under way in our laboratory.

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References and Notes

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5. The pH-rate constant in the absence of the amine shows a maximum around pH 5 (ref. 1).
6. The pK_a values of 3,3'-diaminodipropylamine were determined to be 10.1, 9.1, and 7.6 by a potentiometric titration. All other pK_a values are from "Kagaku Binran Kiso Hen", **1984**, ed by Chemical Society of Japan, Maruzen, pp. II-339 - 341.
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8. M. L. Bender, R. J. Bergeron, and M. Komiyama, "The Bioorganic Chemistry of Enzymatic Catalysis", **1984**, Wiley-Interscience, New York, Chap. 5.
9. The efficiency of the acid catalysis by the adjacent ammonium cation must be also considered for a more detailed analysis.

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