X-Ray Crystallographic Identification of Bisulfite-Uracil Adduct as Sodium 5,6-Dihydouracil 6-Sulfonate

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ABSTRACT

DNA methylation at position 5 of cytosine residues plays an important role in the gene function control. The analytical method for determining the sites of 5-methylcytosine residues utilizes bisulfite treatment of genomes. Cytosines in DNA are converted into uracils by this treatment, while 5-methylcytosines remain unaltered. The bisulfite treatment followed by amplification by polymerase chain reaction and by sequencing the resulting DNA allows determination of the 5-methylcytosine sites in the original. In this chemical modification, key intermediates are those formed by addition of bisulfite across the 5,6-double bond of pyrimidine ring. Their structures were proposed in 1970 as 5,6-dihydropyrimidine 6-sulfonates, but not its 6-sulfurous acid ester, on the basis of spectral data. X-ray analysis has now been performed for a single crystal of sodium bisulfite-uracil adduct and the results showed its structure as sodium 5,6-dihydouracil 6-sulfonate monohydrate, thus providing definite evidence for the C(6)-sulfonate structure.

INTRODUCTION

The functions of genomes are often affected by methylation of cytosine residues in the DNA. The methylation at position 5 of the pyrimidine ring occurs by post-replicational enzymatic actions, and the resulting methylated part of the genome may control the transcriptional activity of a gene. For example, silencing of genes in this way is involved in cancer cell proliferation. The DNA methylation is also associated with many other biological phenomena and thus is a central issue in the currently emerging science of ‘epigenetics’(1). The principal method used in DNA methylation analysis is ‘bisulfite genomic sequencing’ (2). In this method, a given sample of DNA is treated with bisulfite to convert its cytosines into uracil residues. 5-Methylcytosine is known to be resistant to this treatment, and would stay unchanged during the treatment. Amplification by polymerase chain reaction (PCR) of thus modified DNA produces a group of DNA molecules in which the original cytosines are replaced by thymines. Cloning into a vector followed by its proliferation and DNA sequence determination would give sequences in which all cytosines in the original have been changed into thymines. In these processes, 5-methylcytosines in the original DNA molecules stay unchanged and the descendant DNA molecules submitted to the sequencing possess cytosines at those positions. In this way, one can determine the sites of 5-methylcytosines. A large amount of data is now available with respect to the 5-methylcytosine sites in DNA, and the database is growing rapidly. These data are useful in diagnosing cancer in humans, and their use in basic biological sciences is expanding.

Fig. 1. Bisulfite-mediated conversion of cytosine to uracil

The bisulfite genomic sequencing, devised in 1992 by Frommer et al. (2), was based on the chemistry of bisulfite reactions with DNA discovered by Shapiro et al. (3) and by Hayatsu et al. (4) in 1970. The key intermediates in this cytosine-to-uracil conversion are their bisulfite adducts, the 5,6-dihydropyrimidine 6-sulfonates (Fig. 1). The chemical structures of these adducts were determined on the basis of elemental analysis and spectral properties (uv, ir, Raman, and nmr). Detailed studies were performed for an easily obtainable, stable compound, the crystalline monohydrate of sodium 5,6-dihydouracil 6-sulfonate. One basic issue
was whether the addition of sulfite at position 6 of the pyrimidine ring was by the attack of the sulfur atom or by the oxygen atom of sulfite ion. In case the attack was by the sulfur, the product would be a sulfonate (\(-\text{C-S(O)}_2\text{-OH}\)), whereas in case by the oxygen, the product would be a sulfuric acid ester (\(-\text{C-O-S(O)}\text{-OH}\)). The former was assigned to the adduct due to the presence of strong IR (3,4) and Raman (4) bands at around 740 cm\(^{-1}\) for a C-S bond. Consequently, the cysteine-bisulfite adduct, from which the uracil-bisulfite being derived, was also given the sulfonate structure.

In view of the present-day widespread use of bisulfite modification of DNA, it is desirable to establish the structures of the bisulfite adducts of pyrimidine bases with absolute certainty. There are precedents in which sulfites add across a carbon-carbon double bond and/or a carbonyl group forming a C-O-S rather than a C-S-O linkage (5-7). We have now performed X-ray crystallographic analysis of sodium 5,6-dihydouracil 6-sulfonate monohydrate and wish here to report the results, that have definitely established the C(6)-sulfonate structure for the adduct.

RESULTS AND DISCUSSION

Sodium 5,6-dihydouracil 6-sulfonate monohydrate was prepared as described previously (4). Single crystals suitable for X-ray analysis were obtained by recrystallization in 0.4 M acetic acid (50 mg sample in 1.25 ml solvent). A colorless prismatic crystal having approximate dimensions of 0.25 x 0.25 x 0.15 mm was mounted on a glass fiber. All measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu-Kα radiation (\(\lambda = 1.54187\) Å).

Crystal data: C\(_9\)H\(_6\)O\(_9\)N\(_2\)NaS, \(M_r = 234.16\), monoclinic, space group \(P2_1/c\) (\#14), \(a = 5.6523\) (6), \(b = 10.9949\) (12), \(c = 13.2528\) (16) Å, \(\beta = 94.701\) (8)\(^\circ\), \(V = 820.84(16)\)Å\(^3\), \(Z = 4\), \(D_r = 1.895\) Mg m\(^{-3}\), \(F(000) = 480.00\), \(\mu (\text{CuKα}) = 42.12\) cm\(^{-1}\). Of the 8489 reflections that were collected, 1473 were unique.

![Fig. 2. A perspective drawing of 5,6-dihydouracil 6-sulfonate together with a sodium ion and a molecule of water.](https://example.com)

The structure was solved by direct methods (SIR92) (8) and expanded using Fourier techniques (DIRDIF99) (9). The anisotropic and isotropic temperature factors were applied to the non-hydrogen atoms and the hydrogen atoms, respectively. The final cycle of full-matrix least-squares refinement on \(F^2\) converged with unweighted and weighted agreement factors of \(R = 0.0265\) and \(R_w = 0.07481\), respectively. All calculations were performed using the CrystalStructure crystallographic software package (10) except for refinement, which was performed using SHELXL-97 (11). Thus, the molecular structure of sodium 5,6-dihydouracil 6-sulfonate has been unambiguously established by the X-ray analysis as shown in Fig. 2. One oxygen (O3) of the sulfonate and the oxygen (O6) of the water of crystallization are connected to the sodium ion by electric force. This sodium ion is bonded, in total, to five oxygens of the surrounding molecules of the sulfonate and the water, and seems to contribute to packing of the molecules in the crystal.

REFERENCES


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