Molecular Biology Reports **26:** 231–238, 1999.

Some unusual nucleic acid bases are products of hydroxyl radical oxidation of DNA and RNA

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Accepted 10 June 1999

Key words: DNA, hydroxylation, hydroxyl radicals, nucleic acids modified bases, random modification, RNA

Abstract

There are over 100 modified bases and their derivatives found in RNA and DNA. For some of them, data concerning their properties, synthesis and roles in cellular metabolism are available, but for others the knowledge of their functions and biosynthetic pathways is rather limited. We have analysed the chemical structure of modified nucleosides of DNA and RNA considering mainly their putative synthetic routes. On this basis we suggest, that in addition to enzymatic biosynthetic pathways well established for some odd bases, many rare nucleosides can be recognised as products of random chemical reactions. We identify them as primary or secondary products of the reaction of nucleic acids with hydroxyl radicals, the most active oxidising agent in the cell.

Introduction

In addition to the five major bases: adenine, guanine, thymine, cytosine and uracil occurring in ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) there is a large number of chemically modified bases which are also found naturally. In tRNAs almost 20% of the base composition is that of rare nucleosides [1, 2]. Similarly, mRNAs, rRNAs as well as DNAs also contain a variety of unusual components. However, it is not clear what the presence of an odd base in nucleic acids implies in terms of their biological properties, although in several cases, their finding has been correlated with certain molecular functions. For example, some modified bases (e.g., nucleoside Q) are essential for tRNA functions [1, 2]. Recently considerable interest in the origin of rare bases has developed studies of various modification to DNA caused by oxidation, methylation, depurination and deamination reactions, and their implications in the pathobiology of cancer and several other diseases, such as Parkinson's disease and amyothropic lateral sclerosis [3, 4]. Furthermore, it has also been suggested that the accumulation of oxidatively modified bases is involved in the aging process [3–5].

The mechanisms of formation of rare bases and their biochemical properties are poorly understood. It is generally thought that modifications of bases and nucleosides occur enzymicaly and post-transcriptionally [6–11]. It is important to differentiate modified components from damage products of DNA and RNA. All non-major nucleotides or bases found in nucleic acids are modified and useful. Only after a negative function to the cell is established they should be classified as 'damage' products, which are harmful. In this review, we suggest that a large number of modified nucleic acid components can be formed by random chemical reactions, particularly by reacting with hydroxyl radicals. Their structural analysis has led us to reappraise their role in terms of whether these modified bases are biologically relevant or they are indicators of the extent of stochastic damage of nucleic acids.

Oxidative modification

It is well known that reactive oxygen species (ROS) are generated in living cells during normal metabolism and by exogenous sources, such as ionising radiation and various chemical oxidants [12, 13]. Many toxic chemicals and drugs also generate intracellular oxygen radicals. The radicals formation is a common phenomenon in biochemical processes. These radicals are not accidents of aerobic metabolism or the agents of environmental toxins. They are formed in different ways in biological systems and can react avidly with all macromolecules [14, 15]. For example, oxygen readily accepts electrons from other molecules, which result in the formation of oxygen derived free radicals. Many intracellular reactions including respiration, reduce oxygen to superoxide ($O_2^-\cdot$) or to hydrogen peroxide. Oxidative modification (damage) to cellular DNA may result from the action of ROS, such as peroxides, superperoxide and hydroxyl radicals, all of which are produced during normal cellular oxidative metabolism. Therefore oxidising by-products of normal metabolism can cause significant damage to DNA (as well as to proteins and lipids), because existing cellular antioxidant defence mechanisms are not perfect. The number of oxidative 'hits' per cell in humans has been estimated at about 100,000 per day [16].

Out of a wide variety of ROS generated the hydroxyl radical (·OH) is highly reactive and could well be directly responsible for most of the oxidative damage (oxidation) of biological macromolecules. The most common sources of ·OH in cell are the Fenton and Harber–Weiss reactions [15]. Hydroxyl radicals, produced in the vicinity of the nucleic acids, can easily modify DNA and RNA because they are highly reactive and can not diffuse from their sites of formation. Therefore, hydroxyl radical-induced nucleic acid modifications constitute the most varied class of DNA and RNA damage or multiple modifications even at times for the same nucleoside.

Identification of hydroxyl radical modified bases

Based on the chemical structures of all modified DNA and RNA nucleosides known to date, we divided them into four classes according to the following properties:

(1) A size of the modified group: small or bulky, e.g., methyl- versus isopentenyl- groups;
(2) A nature of the base substituent: simple or derivatives of other natural components as amino acids and sugars, e.g., thio- versus threonyl-derivatives;
(3) A type of the modification: primary or secondary, e.g., isopentenylation of adenine versus side chain substitution of Q or Y nucleosides;
(4) A synthetic pathway: enzymatic (e.g., isopentenylation and methylation), or random (e.g., hydroxylation or peroxidation).

The above classification of unusual nucleic acid constituents has enabled us to select a group of DNA and RNA components, which are formed as a result of spontaneous modification by ·OH. The products of random reaction with hydroxyl radicals with nucleic acids we divided furthermore into four groups:
Group I. Oxidised (damaged) DNA bases (Figure 1).
Group II. Oxidised pyrimidine nucleosides of tRNA (Figure 2).
Group III. Hypermodified bases of tRNA (Figure 3).
Group IV. Sugar derivatives of nucleosides (Figure 4).

Group I: Oxidised DNA bases

Hydroxyl radicals produce a broad spectrum of modified derivatives of the purine and pyrimidine bases in DNA, which in many cases are unstable and undergo further chemical rearrangements (Figure 1). Generally, pyrimidine residues can be modified with hydroxyl radicals by two mechanisms: (i) hydroxylation of 5, 6 double bonds yielding glycols and 5 (or 6)-hydroxy-5,6 dihydropyrimidines; and (ii) reaction with the substituent at the position 5 of the ring. Thymine glycol, which is a ring-hydroxylation product (mechanism i), undergoes furthermore alkali-catalysed decomposition into various products, producing ultimately urea derivatives, which are N-linked to deoxyribose. Thymine is also a target for oxidation at the exocyclic methyl group (mechanism ii) yielding hydroxymethyluracil.

Recently 5-formyluridine (f^5U) has been identified as a novel type of thymine lesion produced by oxidation [17–25]. It is one of the most abundant products formed by ionising radiation and it is produced in quantities similar to those of 8-oxodeoxyguanosine [17]. It has also been shown that f^5U is produced by the Fenton reaction [17–25]. The major mechanism of the formation of f^5U *in vivo* involves ·OH mediated abstraction of hydrogen from methyl group of thymine, followed by the addition of oxygen. If so, this base can be formed in DNA under physiological conditions even without exposure to ionising radia-

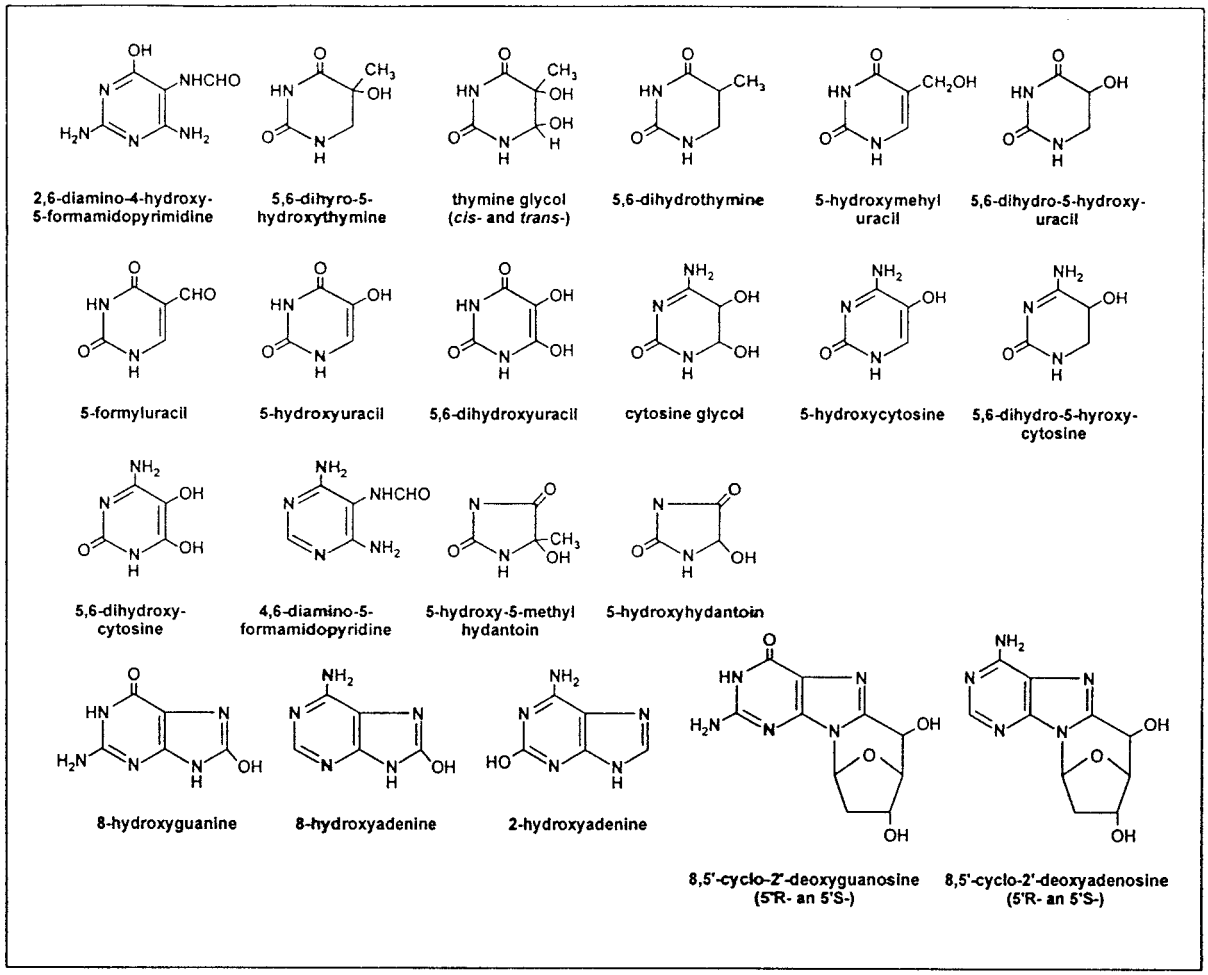

Figure 1. The molecular structures of 22 modified DNA components, the products of hydroxyl radical oxidation (damage) to DNA, for which hydroxy radical oxidation pathway has been proved.

tion. The analysis of the molecular structure of the reaction products clearly suggest that the methylated pyrimidine can be oxidised in two different ways: substitution at heterocyclic ring leads to thymine glycol but side group modification yields the reactive compounds, such as 5-formyluracil and 5-carboxyuridine (Figure 5).

Cytosine glycol on the other hand, is unstable at normal conditions and undergoes decomposition via dehydration and/or deamination to 5-hydroxycytosine, 5-hydroxyuracil or uracil glycol. An acid catalysed hydrolytic deamination of cytosine is accelerated upon saturation of the 5,6-bond and produces the uracil derivatives that can form base pairs preferentially with adenine instead of guanine [15].

The hypermodified base is ß-D-glucosyl-hydroxymethyluracil (J), has been found in *Trypanosoma brucei* DNA telomeric GGGTTA repeats [26–30]. Those authors speculate that an intermediate product in the biosynthetic pathway of this nucleoside is hydroxymethyluracil (Figures 1 and 5), therefore the nucleoside J can be recognised also as the secondary product of ·OH oxidation.

Purine nucleosides can be oxidised at the ring atoms to form two kinds of products. The first one consists of 8-hydroxypurines or their derivatives with opened imidazole ring [31] and the second one containing 2-hydroxydeoxyadenosine [32], (Figure 1). 8-oxo-deoxyguanine (8-oxo-G) is able to form Watson–Crick base pairs with both cytosine and adenine with almost equal feasibility and can thus give rise to transversions from G:C to T:A. Also 8-oxo-deoxyguanosine triphosphate is continually being formed from active oxygen species and is liable to

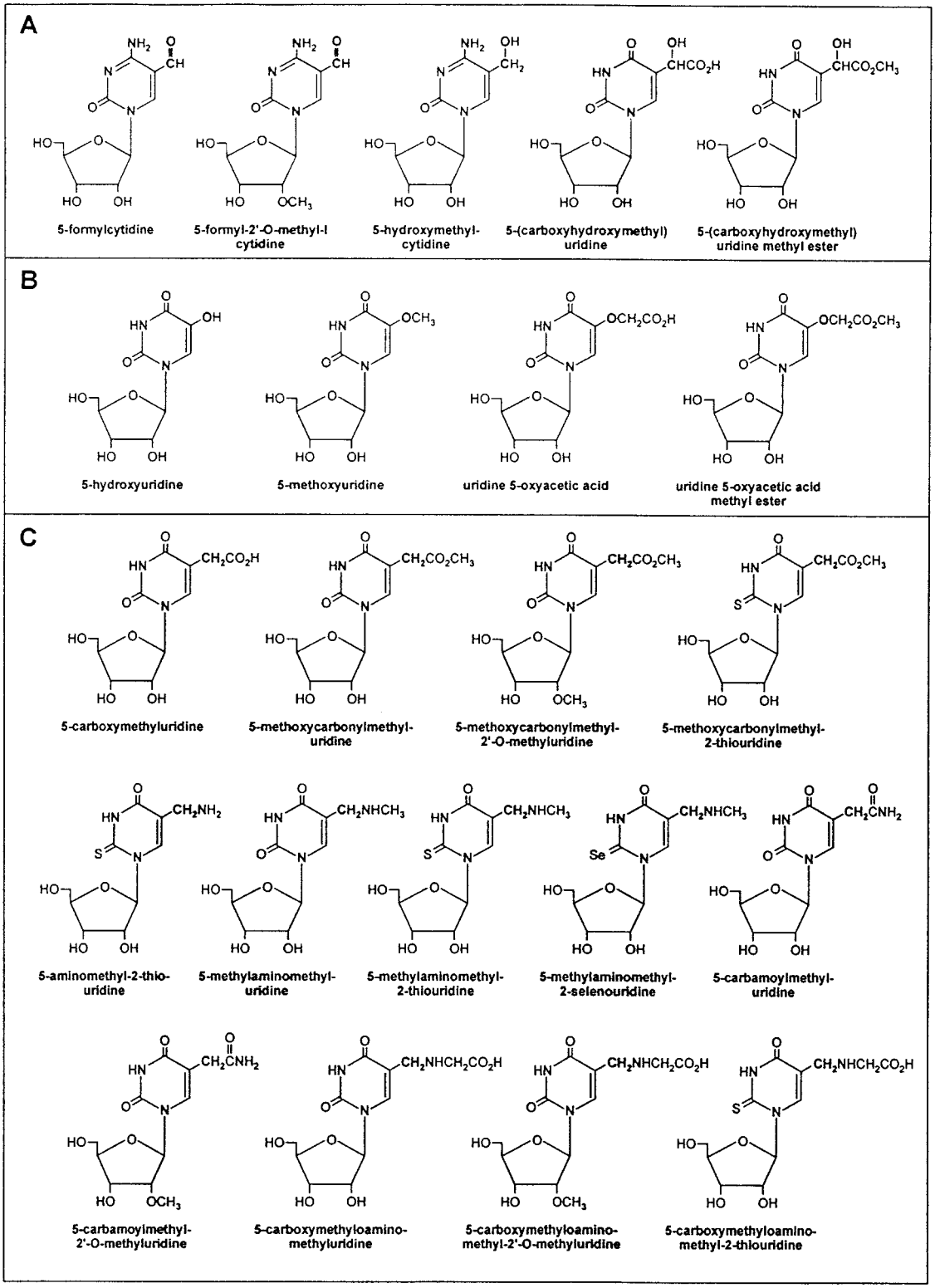

Figure 2. The modified pyrimidine nucleosides found in tRNA. They are divided into three subgroups according to a possible secondary reactions with formylcytosine (A), hydroxyluridine (B) and carboxyuridine (C).

be incorporated into DNA as the complement of adenine producing transversion mutations. Recently, it has been shown that oxidative damage to the RNA precursor pool is also significant and may have important consequences for transcription. The major oxidative product 8-oxo-rGTP is incorporated into RNA opposite adenine on poly(dA-dT) template by *E. coli* RNA polymerase at one-tenth the rate of rGTP incorporation [33, 34].

Group II: Oxidised pyrimidine nucleosides of tRNA

The modified bases and nucleosides categorised in the group II found only in RNA show the following characteristics:

(1) occur at position 34 ("wobble position") of many tRNA;
(2) are pyrimidine residues, mostly uridine derivatives;
(3) are modified at exocyclic position 5 with different substituents,
(4) are(in many cases) 2-derivatives of uridine (Figure 2).

These RNA modified nucleosides can be further divided into three sub-groups according to their putative secondary reactions with: (i) formylcytosine (Figure 2A); (ii) hydroxyluridine (Figure 2B); and (iii) carboxyuridine (Figure 2C).

As one can see ll uracil derivatives can be recognised as products of its reactions with ·OH. Among these modified RNA bases one can find derivatives of formyluracil, hydroxyuracil and carboxyuracil (Figure 2). Although ·OH oxidation reaction seems to be a rather random process, the occurrence of most of the oxidation products in the 'wobble' position of tRNA raises concern about their specific localisation. One reason for that may be that since the anticodon loop is rather an exposed part of the tRNA molecule, it is a good target for oxygen radicals. A high reactivity of the position 5 can be due to the aromatic character of the C5 position in pyrimidines. The fact that mostly uracil derivatives in tRNA are known, could be due to the reason that the 5-hydroxylated derivatives of cytosine are more difficult to detect, mainly because of their deamination reaction.

As formyluracil has been found in DNA (Figure 1), 5-formylcytosine (f^5C) and 5-formyl-2′-0-methylcytosine (f^5Cm) have been identified in the first position of the anticodon of bovine mitochondrial $tRNA^{Met}$ [20, 35] and in the nuclear $tRNA^{Leu}$ [36], respectively. Preliminary quantitation of the f^5C content in mt $tRNA^{Met}$ suggested that there is about 1 mol of f^5C per 1 mol of $tRNA^{Met}$ [20, 35]. Two isoacceptor cytoplasmic $tRNAs^{Leu}$ of bovine liver differ only by one nucleoside in the extra arm, contain that modified base in the same anticodon f^5CmAA [36]. The function of f^5C in the decoding process is yet to be clarified. Interestingly, tRNAs in mitochondria and in *Mycoplasma* have not contain hydroxyl radicals modified nucleosides. Up to now there is no clear data available suggesting their random or enzymatic formation [1, 2].

Group III: Hypermodified bases of tRNA

The group contains hypermodified derivatives of adenine and guanine, such as the i^6A family and the W base family, respectively (Figure 3A, B). Those nucleosides always occur in the anticodon loop of tRNA, next to the 3′ end of the anticodon triplet. It is well known that the side chain of adenine derivatives originates from isopentenyl-pyrophosphate substitution at position N6, but for the W base, the methionine moiety is the precursor of the side chain [1, 2]. However, almost nothing is known about the origin of the hydroxyl substituent on the side chains in these modified bases. There are no specific hydroxylases identified up to now, although it is clear that zeatin and hydroxy-W are the products of hydroxylation reactions. In the case of zeatin it has been shown that the isopentenyl group is added enzymaticaly to the adenine moiety [1, 2], but hydroxylation of the isopentenyl side chain and zeatin formation does not take place in the absence of oxygen [32]. There are also suggestions that enzymes are involved in this reaction [38]. One can therefore suggest that hydroxylation of the methyl group of isopentenyl side chain is result of ·OH reaction [37]. Similarly, the biosynthetic route of the W base involves the binding of methionine to guanine followed by extensive chemical transformation [7, 9], and finally by hydroxylation.

Group IV. Sugar derivatives of nucleosides

To that family of hydroxyl derivatives of nucleic acid bases we have encounted two hypermodified nucleosides: queuosine (Q) and N^6-furfuryladenine or kinetin (K). In both cases the side chain of the modified base consists different derivatives of sugar residues: ribose in the case of Q, and deoxyribose in the case of K. The ribosyl moiety of S-adenosylmethionine is the precursor of the cyclopentanediol residue of Q [39]. The oxidised derivative

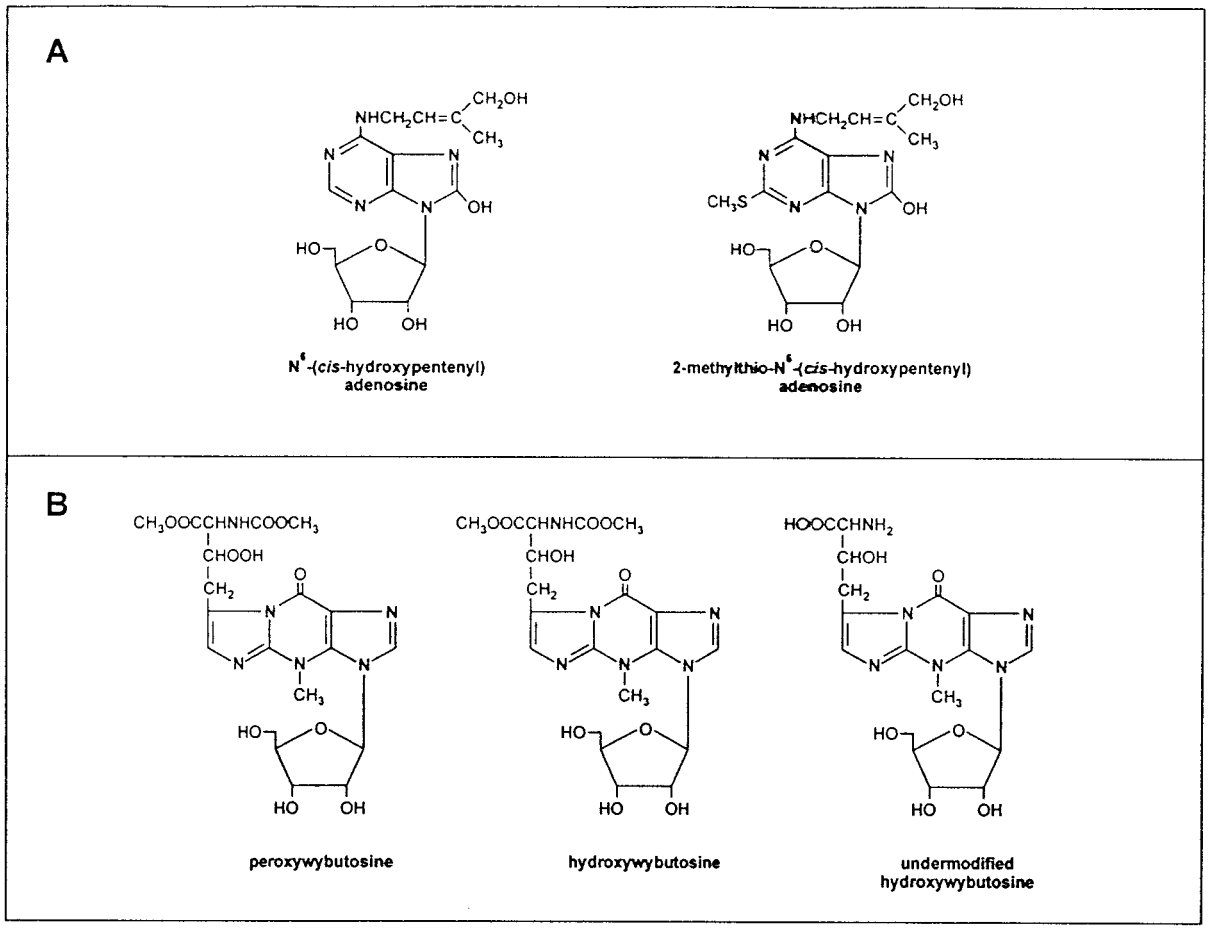

Figure 3. The hypermodified (hydroxylated) nucleosides occurring at position 37 of tRNA (3′ end to the anticodon). A – zeatin and its 2-methylthioderivative, B – the W nucleoside family.

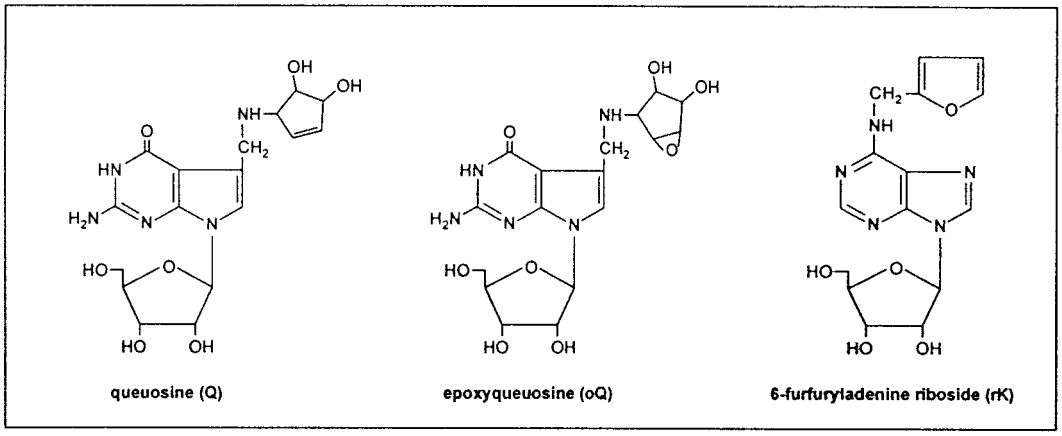

Figure 4. Rare tRNA nucleosides containing sugar derivatives. oQ – is an oxidised form of quosine (Q). Its cyclopentanediol ring arises from the ribose moiety of AdoMet. It is oxidised with peroxide to form oQ. 6-furfuryladenine (kinetin, K) is a product of the reaction of adenine with furfural which on the other hand originates from deoxyribose of DNA after damage at its C5′ with hydroxyl radical.

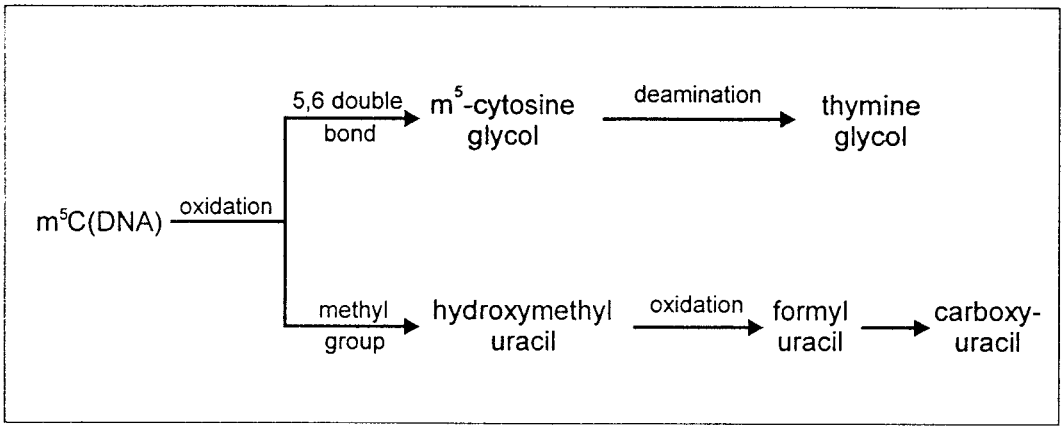

Figure 5. Two pathways of oxidation of the cytosine residue in DNA: the first one shows a substitution reaction at the pyrimidine double bond and the other involves the substitution at the side chain. The end products of these reactions can react furthermore to produce new derivatives (Figure 2).

of Q base (oQ) has been also found in the wobble position of some tRNAs [1, 2]. This oxidation of cyclopentanediol ring with peroxide has been postulated previously [1, 2]. Kinetin, on the other hand, was considered to be a product of thermal rearrangement of DNA [40]. However, by mass spectrometric analysis it has been shown that K is a product of reaction of adenosine of DNA with furfural [41–44] which results from ·OH reaction at the C5′ of a DNA deoxyribose residue, the major radical oxidation product of deoxyribose moiety [45–46]. Therefore, one can classify kinetin as a secondary oxidatively modified base of DNA formed *in vivo*.

Perspectives

From the above discussion it is clear that there is a substantial group of known nucleic acid derivatives, which may be considered as a randomly hydroxylated. They are products of random reactions of ROS, mainly ·OH radicals with nucleic acids. At the same time there is no strong evidence up to now that these modified bases are formed by any enzymatic reactions.

Not too much is known about the function of hydroxylated modified rare bases. Although there are some speculations about their role in tRNA function [1, 2], in the case of DNA it is generally believed that hydroxyl radical modified bases are a sign of damage, which are potentially mutagenic [3, 4]. For example, 8-oxodeoxyguanosine can induce transition mutations by base pairing with adenosine [12]. However in some cases modified nucleosides or the so-called damage products can have beneficial effects on the cell. Two derivatives of adenine, zeatin and kinetin, are very useful well known plant hormones [40, 47]. In addition kinetin has been shown to delay the onset of many age-related characteristics that appeared in normal human skin fibroblasts undergoing aging *in vitro* [48], to slow down development and aging in insects, to reduce their fecundity, to increase the activity of catalase and to prolong the life span of the fruit fly [48–50]. Kinetin also forms a complex *in vitro* with Cu(II) thus having properties of superoxide dismutase, and catalyses efficiently O_2-dismutation [51, 52].

Therefore, the presence of modified nucleosides in the cell may not be only a measure of nucleic acid damage, but it can also have other biological meanings. For example, the formation of modified bases may induce the synthesis of various repair enzymes, which increase the overall protection of the cell. Such phenomenon where small amounts of apparently harmful agents have potentially useful effects on the survival and functioning of cells and organisms is known as 'hormesis' [53]. Some of the modified bases, especially zeatin and kinetin, appear to belong to this category. It will be interesting to find out whether other hydroxylated modified nucleosides also have biologically useful properties. From the impressive amount of energy (and genes) that cells invest in chemical modification of RNA it must be concluded that they are important for cellular metabolism and function [54].

Acknowledgements

This work was supported within the grant from Polish Committee for Scientific Research (KBN) and Senetech PLC.

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