A mechanism for the in vivo formation of N^6-furfuryladenine, kinetin, as a secondary oxidative damage product of DNA

Jan Barciszewski[c], Gunhild E. Siboska[a], Bent O. Pedersen[b], Brian F.C. Clark[a], Suresh I.S. Rattan[a,*]

[a] *Laboratory of Cellular Ageing, Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus-C, Denmark*
[b] *Department of Chemistry, University of Aarhus, DK-8000 Aarhus-C, Denmark*
[c] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, 61704 Poznan, Poland*

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Abstract Recently, we have reported the presence of kinetin (N^6-furfuryladenine) in commercially available DNA, in freshly extracted cellular DNA and in plant cell extracts. We have also found that kinetin has electrochemical properties which can be used for monitoring the level of this modified base in DNA. Here, for the first time, we propose a mechanism for the formation of kinetin in DNA in vivo, based on the analyses of its mass spectra. Since hydroxy radical oxidation at the carbon 5′ of the deoxyribose residue yields furfural, we propose that this aldehyde reacts with the amino group of adenine and, after intramolecular rearrangement, kinetin is formed in vivo. Thus kinetin is the first stable secondary DNA damage product known to date with very well defined cytokinin and anti-aging properties, linked to oxidative processes in the cell. These results also indicate that N^6-furfuryladenine or kinetin is an important component of a new salvage pathway of hydroxy radicals constituting a 'free radical sink'. In this way, the cells can neutralize the harmful properties of hydroxyl radical reaction products, such as furfural, and respond to oxidative stress by inducing defence mechanisms of maintenance and repair.

Key words: Kinetin; 6-furfuryladenine; Oxidation; DNA damage; Base propenal

1. Introduction

Cytokinins are plant hormones which promote cell growth, development and division [1–3]. Kinetin (N^6-furfuryladenine) was the first compound discovered with cytokinin activity and was isolated in 1955 from autoclaved herring sperm DNA, and has been thought to be an artificial product of decomposition of the DNA [4,5]. The first naturally occurring cytokinin, zeatin, was isolated much later [6]. Both zeatin and kinetin are 6-substituted adenine derivatives. The isopentenyl group of zeatin is derived from mevalonic acid which is a precursor of more than a dozen products through its conversion to, for example, isopentenyl-pyrophosphate used in tRNA modification. Such enzymically formed isopentenyladenosine (i^6A) can be further hydroxylated to zeatin (io^6A). There are also suggestions that formation of io^6A is catalysed by a specific hydroxylase [7]. In the case of kinetin however, almost nothing is known about its natural occurrence and its mechanism of synthesis.

Since its discovery, kinetin has been widely used as a cytokinin in various aspects of plant biochemistry and cell biology [1–3]. Kinetin, like other cytokinins, can delay senescence in plants [8,9]. Recently, it has been reported that kinetin delays the onset of many age-related characteristics that appear in normal human skin fibroblasts undergoing aging in vitro [10], slows down development and aging in insects, reduces their fecundity and increases the activity of catalase [11,12]. Because of the intriguing effects of kinetin on plants, animals and human cells, we decided to reinvestigate the problem of kinetin origin and its biological properties. Using high performance liquid chromatography (HPLC) combined with an electrochemical (EC) detector we and others have detected kinetin (6-furfuryladenine) as a naturally occurring component of DNA as well as in plant cell extracts [13–15]. Here, we analyse mass spectra of DNA for the presence of 6-furfuryladenine and propose a mechanism for its formation as a secondary oxidative damage product of DNA in vivo.

2. Materials and methods

2.1. Extraction of kinetin from DNA

Calf thymus DNA (130 mg; Sigma, type 1) was dissolved in 6 ml 0.2 HCl and incubated for 2 h at 50°C. Extraction with 4×50 ml water-saturated ethyl acetate was carried out from neutral solution. In addition, freshly isolated DNA (11 mg) from human cells [15,16] was treated with 0.5 ml 0.2 N HCl and incubated 2 h at 50°C. Under these conditions DNA is totally soluble. The hydrolysate was used for analysis on HPLC and mass-spectrometer without further purification or extraction.

2.2. Mass-spectrometric analysis

MS analyses were performed by direct inlet of the samples to VG Trio-2 machine (UK). Ionisation was achieved by electron impact (70 EV). The ion source was at 200°C and heating of the sample was carried out up to 300°C.

3. Results and discussion

We have measured and collected the mass spectra of DNA by direct inlet in the temperature range 20û to 300û C. We looked particularly for mass signals of adenine (m/e 135) and 6-furfuryladenine (m/e 215). One of these spectra (no. 453) was analysed in detail and showed the molecular ion of 215 m/e (Fig. 1). Its fragmentation pattern is identical to that of a commercial kinetin sample (Fig. 1), as well as to the one deposited in the data library [16]. The lower spectrum (Fig. 1) contains the characteristic signals of 81 or 135 m/e, which correspond to an adenine residue, a basic constituent of DNA and also of kinetin. The identities of both spectra led us to conclude that kinetin (6-furfuryladenine), so far recognized only as a synthetic compound, occurs naturally in DNA mol-

*Corresponding author. Fax: (45) 86 19 61 99.
E-mail: rattan@imsb.au.dk

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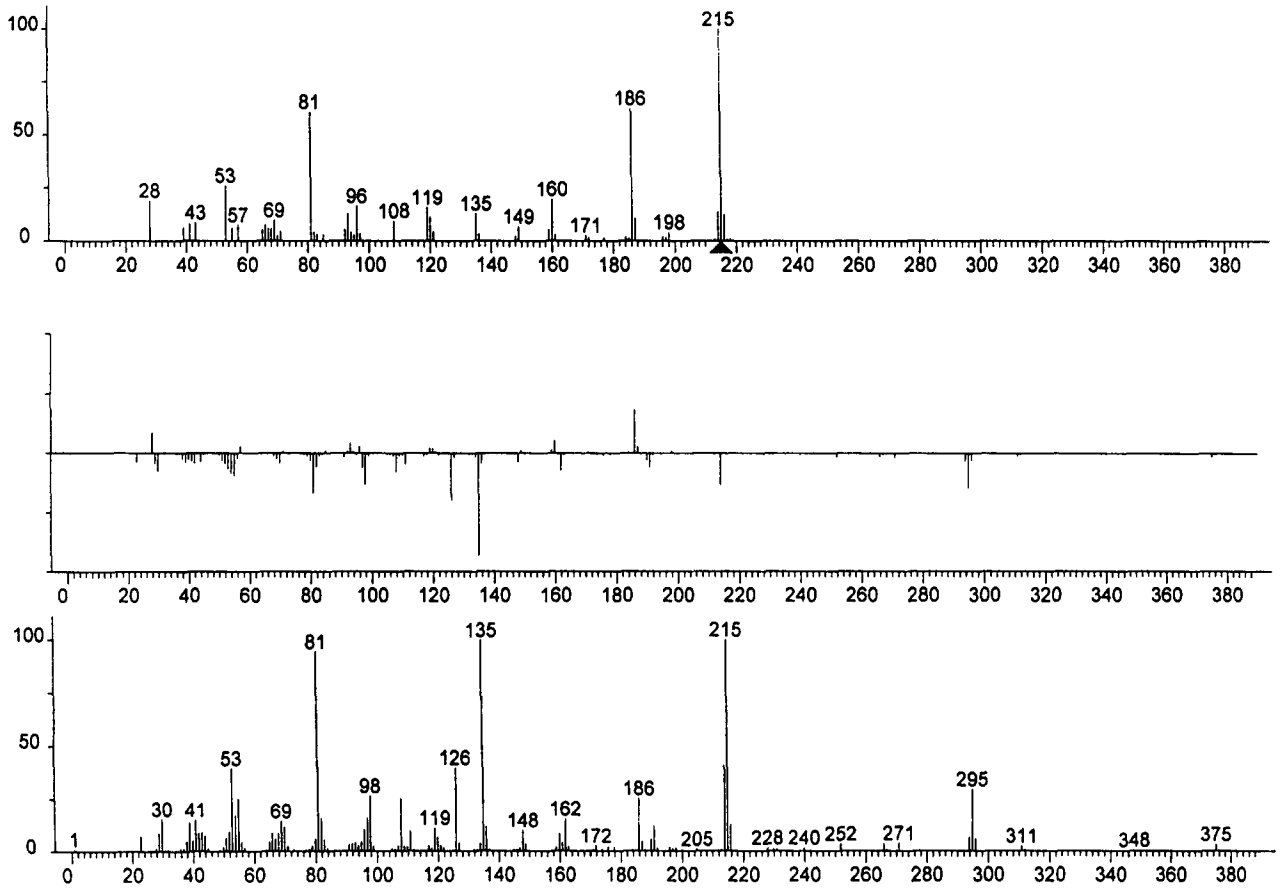

Fig. 1. Mass spectra of commercially available kinetin (upper spectrum) and DNA (lower spectrum). The diagram in the middle shows the result of subtraction of the two spectra. The lower spectrum clearly identifies the presence of kinetin in DNA. The mass spectra of calf thymus DNA (Serva), were recorded on Trio-2-Vg machine: ion source: 200 mA, 700 eV; direct inlet 20°C for 1 min and temperature range 20° to 300°C (10°C/min).

ecule. This raises the question concerning the mechanism of its formation at the nucleic acid level.

The chemical structure of N^6-furfuryladenine clearly suggests the reaction of an adenine residue of DNA with furfural as the synthetic pathway. However, the question how this aldehyde could be formed in the cell has not been clear. It is known that furfural is a dietary mutagen and is a known constituent of various food products, beverages, coffee and white bread and is formed when sugars are heated [17–22]. Furfural is widely used as a solvent in the petrochemical industry and is also present in the vapour phase of cigarette smoke in rather large quantities, ranging from 45 to 110 mg per cigarette [17–22]. Furfural is extensively metabolized in rats prior to excretion with urine [23]. Furfural has been found in normal human plasma, urine and heart homogenate, by gas chromatography-mass spectrometric analysis [24]. Using mass-spectrometry only, we also detected furfural (oxime derivative) in cell-free extracts prepared from cultured human osteoblasts (manuscript in preparation).

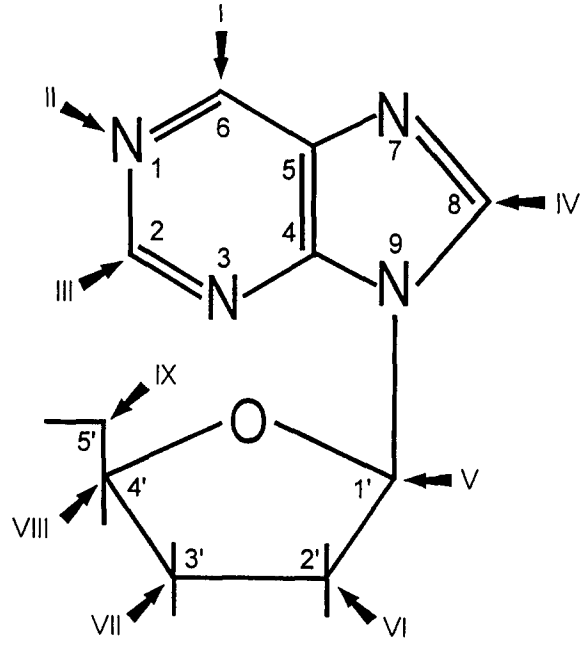

Fig. 2. The possible sites of hydroxyl radicals attack on purine nucleosides in DNA and damage products which can be formed in these reactions. I: 2′-deoxyinosine, II: N1 oxide of adenosine (non radical product), III: 2-hydroxyadenosine, IV: 8-hydroxydeoxyguanosine or 8-hydroxydeoxyadenosine, V: hydroxylation at C1′ leads to 5-methylene-2-furanone (5MF), VI: formation of ribonucleotides (2′ hydroxylation; 2′-oxidation leads to the release of the base), VII: abstraction of hydrogen at the carbon 3′ (the C3′ chemistry might be involved in DNA cleavage), VIII: hydroxylation at the carbon C4′, after cleavage it gives 5« phosphorylated terminus and a 3′ terminus bearing the sugar residue, and IX: abstraction of hydrogen at C5′ leads to the formation of furfural.

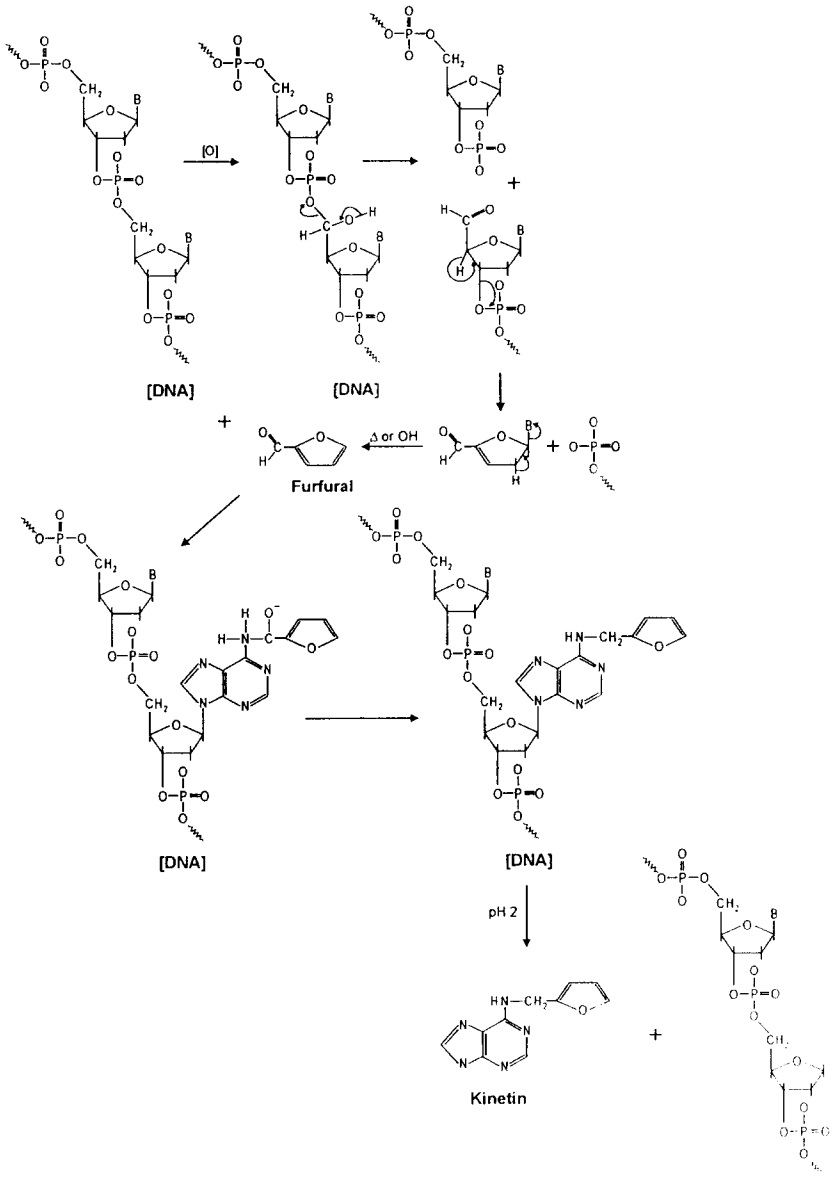

Fig. 3. Proposed mechanism of kinetin formation in DNA. There are two main targets of oxidative damage in DNA-bases and deoxyribose residues. The latter can be hydroxylated at three sites C1′, C4′ and C5′ [12]. The C5′ hydrogen abstraction pathway ends up with the formation of furfural which easily reacts with adenosine to form kinetin.

Recently, it has been reported that furfural, the oxidized sugar residue, is formed during the oxidative damage of DNA in vitro [17–22]. This pathway has also been considered in an analysis of the products of the action of bleomycin with DNA [25]. Although the main target of deoxyribose oxidation is the anomeric carbon (C1′ hydroxylation) which yields 5-methylene-2-furanone [26,27], furfural has also been found among many of the reaction products of metalloporphyrins with DNA [25]. The mechanisms of these reactions indicate that furfural results from apparent hydroxylation at the carbon 5′ of deoxyriboses in DNA [20–25]. The calculated amount of furfural (C5′ hydroxylation) relative to 5-methylene-2-furanone (C1′ hydroxylation) is 15% [17–22]. These results suggest that furfural is one of the primary products of hydroxy radical damage of DNA, which is shown in Fig. 2.

We suggest that furfural forms an adduct with an adenine residue in DNA which is further dehydrated under acidic conditions to form kinetin (Fig. 3). This is consistent with previous observations that reaction of furfural with DNA and AT rich oligonucleotides destabilizes DNA secondary structure through the modification of DNA bases and phosphates [17–22,25]. The presence of 6-furfuryladenine in DNA, forms a direct link between oxidation of deoxyribose and a new type of base modification. Therefore, kinetin is suggested to be a secondary oxidation product, and is formed by the reaction of an adenine moiety with furfural, the very first such radical oxidation product of DNA.

Analysis of our data and those from the literature suggests that kinetin can influence the cell in two distinct ways. First, it can induce the synthesis of repair enzymes which either remove modified bases from DNA and/or are involved in protection against oxygen stress [28–32]. Second, its complex with copper(II) initiates superoxide dismutase, in that the kinetin-Cu(II) complex catalyses O^{2-}. dismutation very efficiently at physiological pH, with a turnover of 2.7×10^{9} mol per second [33–35]. These properties help to understand why exoge-

nously added kinetin to various cells has several positive effects, including anti-aging effects on them. It seems that cells have invented yet another mechanism of free radical sink to neutralize harmful hydroxyl radicals and their products by forming non-toxic secondary reaction compounds, with new properties. It will be interesting to find out whether the levels of kinetin change during aging, cancer and other pathological situations, and which enzyme systems are involved in the removal of kinetin from the DNA.

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