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Furfural, a Precursor of the Cytokinin Hormone Kinetin, and Base Propenals Are Formed by Hydroxyl Radical Damage of DNA

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Recently, we have detected kinetin (N^6-furfuryladenine), a well known cytokinin plant hormone, in commercially available DNA, in freshly extracted cellular DNA and in plant cell extracts. We had suggested that the furfuryl moiety of kinetin originates from furfural which is one of the primary oxidation products of deoxyribose in DNA. Here we show that the human cell extracts treated with O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) give rise to oxime derivatives of various aldehydes present in the cell. Mass spectrometric analysis of silylated oximes showed several mass signals of different species, one of which was identified as furfural. Furthermore, detailed inspection of the mass spectra of DNA showed the mass signals of 165, 180, 189 and 206 m/z which correspond to cytosine-propenal, thymine-propenal, adenine-propenal and guanine-propenal, respectively. The presence of furfural, along with four base-propenals in the cell extract, as the primary oxidation products of deoxyribose, suggests that degradation of sugar residues in DNA is one of the major routes of cellular damage in addition to the modification of nucleic acid bases.

Kinetin, (N^6-furfuryladenine), is a well-known cytokinin hormone and has anti-aging effects on plants (1–3), insects (4, 5), and human cells (6). It also stimulates ribosomal RNA transcription in *Arabidopsis thaliana* (7), which suggests that cytokinins may act as general regulators of protein synthetic capacity. Furthermore, in *Spirodela polyrrhiza*, kinetin represses the accumulation of abscisic acid-induced TUR2 transcripts, an analog of the yeast PDR5 gene which encodes for the ATP-binding cassette (ABC) transporter (8). Thus, although a wide variety of biological properties of kinetin have been reported, very little is known so far about the mechanism of its formation *in vivo*.

Since kinetin was originally isolated from autoclaved herring sperm DNA, it was thought to be an artificial product of decomposition of the DNA (9, 10). Recently, we have detected kinetin as a naturally occurring component of DNA and in plant cell extracts (11). Kinetin has been also detected in the root nodules of *Casuaria equisetifolia* (12, 13). We have proposed a mechanism in which kinetin is formed by the intramolecular rearrangement of oxidatively modified deoxyribose, furfural, with adenine (14). Here, we, for the first time, provide direct evidence for the occurrence of furfural in human cells. This was achieved by treating cell extracts with O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA), in order to obtain oxime derivatives of aldehydes present in the sample, and then by converting the reaction mixture to silylcompounds suitable for analysis with mass spectrometry. In addition, we have also detected other primary products of DNA hydroxylation, particularly the base-propenals (15– 17). These results are discussed in a wider context of the role of reactive oxygen species in the formation of modified bases with significantly altered biological properties.

MATERIALS AND METHODS

Normal human skin fibroblasts were grown in culture by standard procedures, as described previously (11). A cell extract from about 1 million cells (ca. 250 μg protein) was prepared by brief homogenization in 500 μl of triple-distilled water containing 0.4 mM of EDTA. To this homogenate, 200 μl of 50 mM solution of O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA-HCl; Sigma) was added, and the mixture was incubated for 30 min at room tem-

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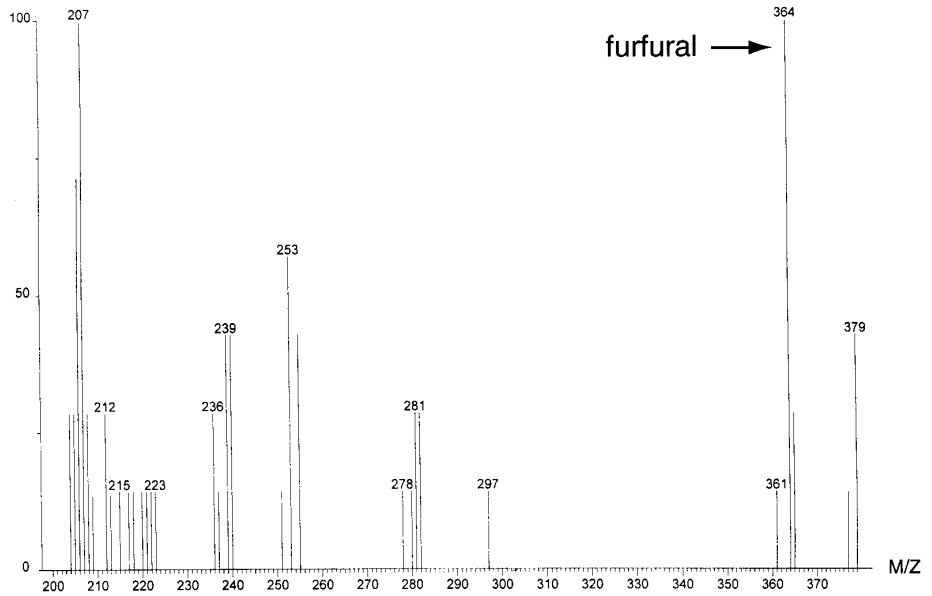

FIG. 1. The mass spectrum of PFB-oxime-TMS derivative of furfural isolated from human cells extracts. The m/z 364 corresponds to furfural.

perature. Next, 500 μl methanol, 2 ml hexane and 180 μl sulfuric acid were added to the mixture which was then vortexed for 1 min followed by centrifugation at 3000 rpm for 2 min. The hexane upper layer was collected, dried on sodium sulfate for 30 min and the dried organic phase was evaporated. Finally, 50 μl of N, O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA; Sigma) was added and incubated for 5 min at 80°C (18). Furfural was purchased from Sigma; whereas calf thymus DNA was from Serva.

The mass spectra were recorded on Trio-2-Vg machine: ion source: 200 mA, 700 EV; direct inlet 20° C for 1 min and temperature range 20-300° C at 10° C/min (11).

RESULTS AND DISCUSSION

We have detected furfural in human cell extracts by mass-spectrometric analysis of its PFB-oxime-TMS derivative. PFBHA is a very sensitive derivatizing agent and has been used previously for analysis of ketosteroids (19,20). Its high potential for reacting with ketocompounds has been already confirmed in an analysis of aldehydes and other lipid peroxidation products in human urine and plasma (18). In our approach, we registered 840 scans using the direct inlet mode of the mass analysis. It is calculated that the molecular signal for furfural-PFB-oxime-TMS derivative is 364 m/z. An analysis of the spectral data gave us several spectra which showed m/z 364, identified as furfural derivative (Fig. 1). The control reaction performed with commercially obtained furfural gave identical spectra (*picture not shown*). Therefore, we can conclude that furfural is present in human cell extracts, due to oxidation reaction of DNA. Once the aldehyde is formed within the cell, its direct reaction with amine-group-containg compounds, among them adenine, is obvious. However, there is also a possibility that aldehydes can be converted to acids and their derivatives by the action of dehydrogenases (21).

Previously, it has been claimed that in addition to furfural, other products of DNA hydroxylation are also formed, among them base-propenals (16). Formation of the base-propenals has been confirmed in reaction of nucleosides with peroxynitrite, a strong oxidant (22). Therefore, it was interesting to check the mass spectra of the DNA for the presence of these compounds. We have measured and collected the mass spectra of DNA by direct inlet in the temperature range 20-300° C. Detailed inspection of these spectra showed that the scan no. 453 contained the mass signals of 165, 180 and 206 m/z, which correspond to cytosine-propenal, thymine-propenal and guanine-propenal, respectively (Fig. 2). The absence of a mass signal 189 m/z for adenine-propenal may be due to the fact that adenine is mostly converted to kinetin with m/z 215 (14).

The presence of furfural, along with base-propenals, as the primary oxidation products of deoxyribose suggests that degradation of sugar residues is one of the major routes of DNA damage in addition to the modification of nucleic acid bases. Formation of furfural as one of the first steps of DNA damage products in the cell and then its transformation into kinetin, a very potent cytokinin, is an example of the salvage pathway of hydroxy radicals constituting a free radical sink (14). Furthermore, these observations are very much in line with a novel approach to therapeutic potentiation of the immune system by Schiff-base-forming drugs (23). According to this, Schiff-base-forming molecules may substitute for the physiological donor of carbonyl groups, be constitu-

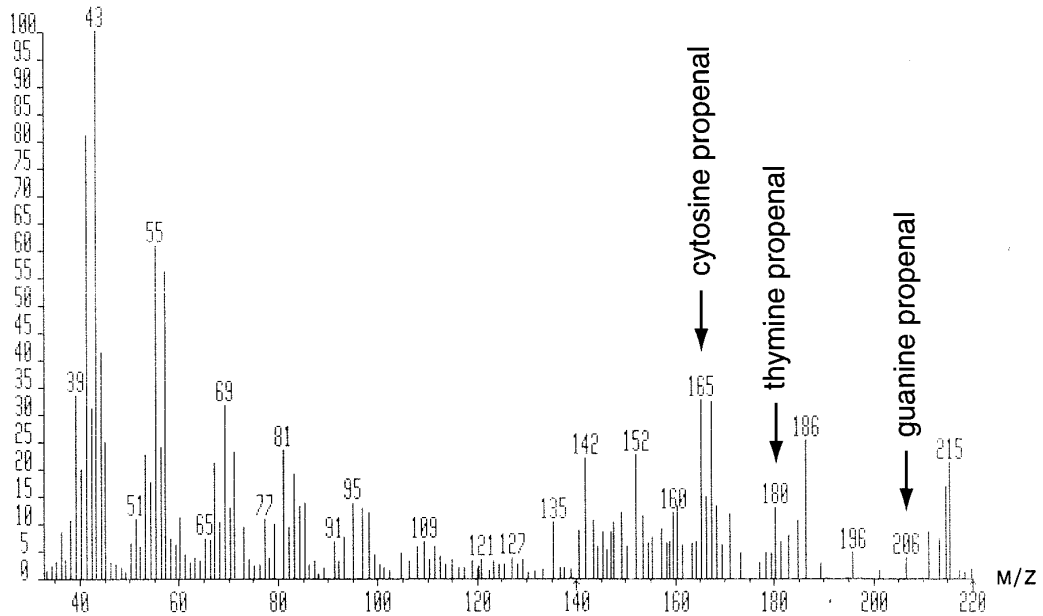

FIG. 2. Mass spectra of commercially available calf thymus DNA (Serva). The m/z 165, 180, 206 correspond to cytosine-, thymine- and guanine-propenal, respectively.

tively expressed on antigen-presenting cells, and thus interact with amines on T-cell receptors, providing thereby a costimulatory signal to T lymphocytes and for the phosphorylation of proteins (23). It will be interesting to find out if furfural, which is also a Schiff-base-forming molecule, has similar effects on the immune system. It will help to understand the diverse biological effects of kinetin, such as its anti-aging antioxidative and life-prolonging properties (4–6).

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REFERENCES

1. Mok, D. W. S., and Mok, M. C. (Eds.) (1994) Cytokinins: Chemistry, Activity, and Function, CRC Press, Boca Raton, FL.
2. Kaminek, M. (1992) *Biotechnology* **10,** 159–164.
3. Gan, S., and Amasino, R. M. (1996) *BioEssays* **18,** 557–565.
4. Sharma, S. P., Kaur, P., and Rattan, S. I. S. (1995) *Biochem. Biophys. Res. Commun.* **211,** 1067–1071.
5. Sharma, S. P., Kaur, P., and Rattan, S. I. S. (1997) *Biochem. Mol. Biol. Int.* **41,** 869–875.
6. Rattan, S. I. S., and Clark, B. F. C. (1994) *Biochem. Biophys. Res. Commun.* **201,** 665–672.
7. Gaudino, R. J., and Pikaard, C. S. (1997) *J. Biol. Chem.* **272,** 6799–6804.
8. Smart, C. C., and Fleming, A. J. (1996) *J. Biol. Chem.* **271,** 19351–19357.
9. Miller, C. O., Skoog, F., von Saltza, M. H., and Strong, F. M. (1955) *J. Amer. Chem. Soc.* **77,** 1392.
10. Miller, C. O., Skoog, F., Okumura, F. S., von Saltza, M. H., and Strong, F. M. (1956) *J. Amer. Chem. Soc.* **78,** 1375–1380.
11. Barciszewski, J., Siboska, G., Pedersen, B. O., Clark, B. F. C., and Rattan, S. I. S. (1996) *FEBS Lett.* **393,** 197–200.
12. Raman, N., and Elumalai, S. (1996) *Indian J. Exp. Biol.* **34,** 577–579.
13. Ratti, N., and Janardhanan, K. K. (1996) *Indian J. Exp. Biol.* **34,** 1126–1128.
14. Barciszewski, J., Siboska, G., Pedersen, B. O., Clark, B. F. C., and Rattan, S. I. S. (1997), submitted.
15. Pratviel, G., Pitie, M., Bernadou, J., and Meunier, B. (1991) *Angew. Chem. Int. Ed. Engl.* **30,** 702–704.
16. Pratviel, G., Pitie, M., Bernadou, J., and Meunier, B. (1991). *Nucl. Acids Res.* **19,** 6283–6288.
17. Pratviel, G., Pitie, M., Bernadou, J., and Meunier, B. (1995) *Angew. Chem. Int. Ed. Engl.* **34,** 746–769.
18. Luo, X. P., Yazdanpana, M., Bhooi, N., and Lehotay, D. C. (1995) *Anal. Biochem.* **228,** 294–298.
19. Koshy, E. T., Kaiser, D. G., and VanDerSlik, A. L. (1975) *J. Chromatogr. Sci.* **13,** 97–104.
20. Nambara, T., Kigasawa, K., Iwata, T., and Ibuki, M. (1975) *J. Chromatogr.* **114,** 81–86.
21. Raner, G. M., Chiang, E. W., Vaz, A. D. D., and Coon, M. J. (1997) *Biochem.* **36,** 4895–4902.
22. Yermilov, V., Yoshie, Y., Rubio, J., and Ohshima, H. (1996) *FEBS Letters* **399,** 67–70.
23. Rhodes, J., Chen, H., Hall, S. R., Beesley, J. E., Jenkins, D. C., Collins, P., and Zhenk, B. (1995) *Nature* **377,** 7175.