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Review - Food Science Section

ADDUCTS OF DEOXYRIBONUCLEIC ACID WITH NITROSAMINES

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Abstract

Chemical and physico-chemical investigations initiated during the 6th decade of the 20th century on the compounds resulted from the interaction between the DNA macromolecule and small molecules led to the idea of appearance of some bioincompatible structures that, at the beginning were named "molecular complexes", an inadequate denomination because such compounds exist in chemistry and inorganic biochemistry. It was also used the term "molecular associations", but it does not explain the existence of such compounds. During years of physical-chemical investigations and mechanic-quantum calculations over the electronic density in the donor-acceptor relationship between DNA and small molecules, the term "adducts" was preferred. This term is used in the present paper following the biogenesis of adducts of DNA with nitrosamines. Nitrosamines are compounds that can exert oncogenic effect due to their ability to form nucleophilic metabolites that binds especially to DNA nucleobases.
***Keywords**: DNA-Nitrosamines adducts*

1. Structural Peculiarities of the DNA Double Helix

Discovery of double chain structure of DNA is of major importance in the development of molecular biology, allowing the interpretation of data from classical genetics and the initiation of important studies in molecular biology, genetics, biochemistry, biochemical pathology, etc.

In order to understand the biogenesis of DNA adducts we must first review the structure of the deoxyribonucleic acid (DNA).

Deoxyribonucleic acid is a polymer or a polyheteronucleotidic chain. The monomer units of DNA are nucleotides. Each nucleotide consists of a 5-carbon sugar (deoxyribose), a nitrogen containing base attached to the sugar, and a phosphate group. There are four different

types of nucleotides found in DNA, differing only in the nitrogenous base. The four nucleotides are given one letter abbreviations as shorthand for the four bases: A for adenine, G for guanine, C is for cytosine, T for thymine (Gârban, 2005).

DNA is a normally double stranded macromolecule. The chemical structure of double stranded DNA is given in figure 1.

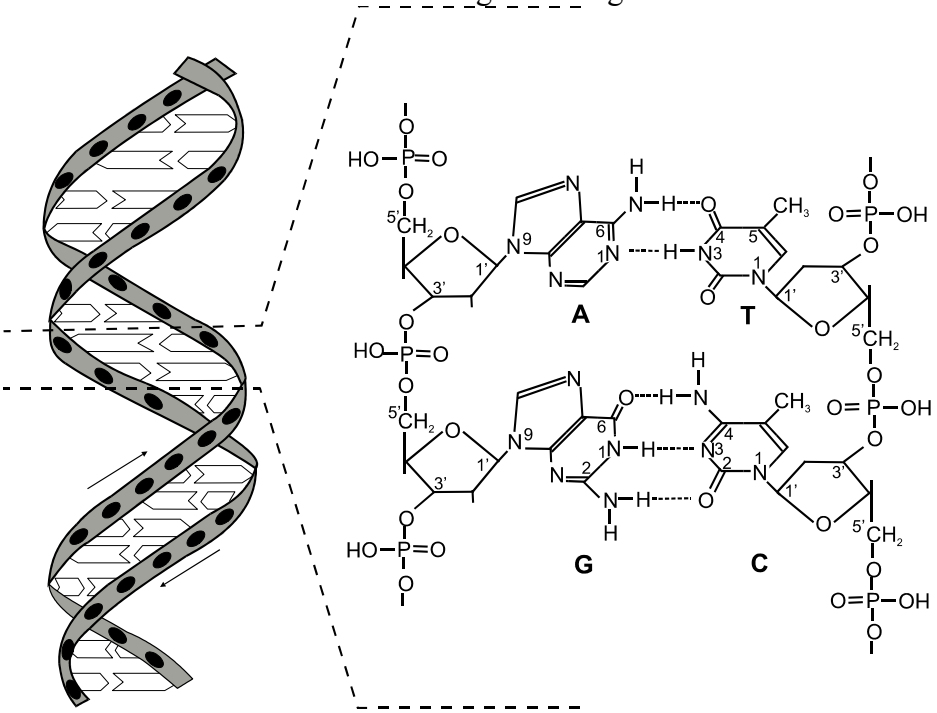


Fig. 1. Double-stranded DNA – structural formula

In figure 1 it is shown a double stranded molecule of DNA, pointing out the way that nucleobases bind according to the Watson - Crick model. So within the double helical DNA, adenine forms 2 hydrogen bonds with thymine on the opposite strand, and guanine forms 3 hydrogen bonds with cytosine on the opposite strand.

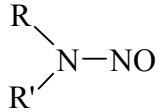
2. Peculiarities of nitrosamines structure–activity relationship

Experimental investigations using different physical and physico-chemical methods over some mutagenic and carcinogenic agents established that they form with DNA characteristic molecular associations, named adduct. A carcinogenic agent can affect DNA directly without a previous activation, or throughout its metabolites.

Humans are exposed to N-nitrosamines from a wide variety of sources, including foods, beverages, tobacco, and cosmetics. Over 300 N-nitrosamines have been demonstrated to be carcinogenic in experimental animals (Preussmann and Stewart, 1984). Like majority of the chemical carcinogens, these chemically inert compounds are metabolized to reactive electrophiles before binding to DNA macromolecules (Lawley, 1984; Gârban et al., 1985).

2.1. Chemical structure of nitrosamines

Nitrosamines are chemical compounds having the general structure as presented below:



where R and R' may be alkyl, hydroxyalkyl, ester, amide or aryl groups. Cyclic nitrosamines are also known.

Generally, nitrosamines are chemically stable, neutral compounds. The lower dialkyl nitrosamines are volatile liquids, soluble both in water and organic solvents, whereas the higher members are solids of low melting point and low water solubility (Ghibu, 2005).

The only nitrosamine known to occur in nature is 4-methyl-nitros-aminobenzaldehyde, a product of metabolism in the edible fungi Clityocybe Suavolens, although it has been claimed that dimethylnitrosamine is present in herring-meal when preserved with sodium nitrite.

Some of the most important nitrosamines with a non-cyclic structure are: N-nitrosodimethylamine (I); N-nitrosodiethylamine (II); N-nitrosodiprophylamine (III); N-diisopropylnitrosamine (IV); N-nitrosomethylethylamine (V); N-nitrosopropilbuthyl (VI); N-nitroso-ethylvinylamine (VII); N- nitrosomethylphenylamine (VIII); N-nitro-sodiphenylamine (IX); N- nitrosophenylbenzylamine (X); N-nitroso-

dibenzylamine (XI). Their chemical structures are shown in figure 2 – compounds I – XI.

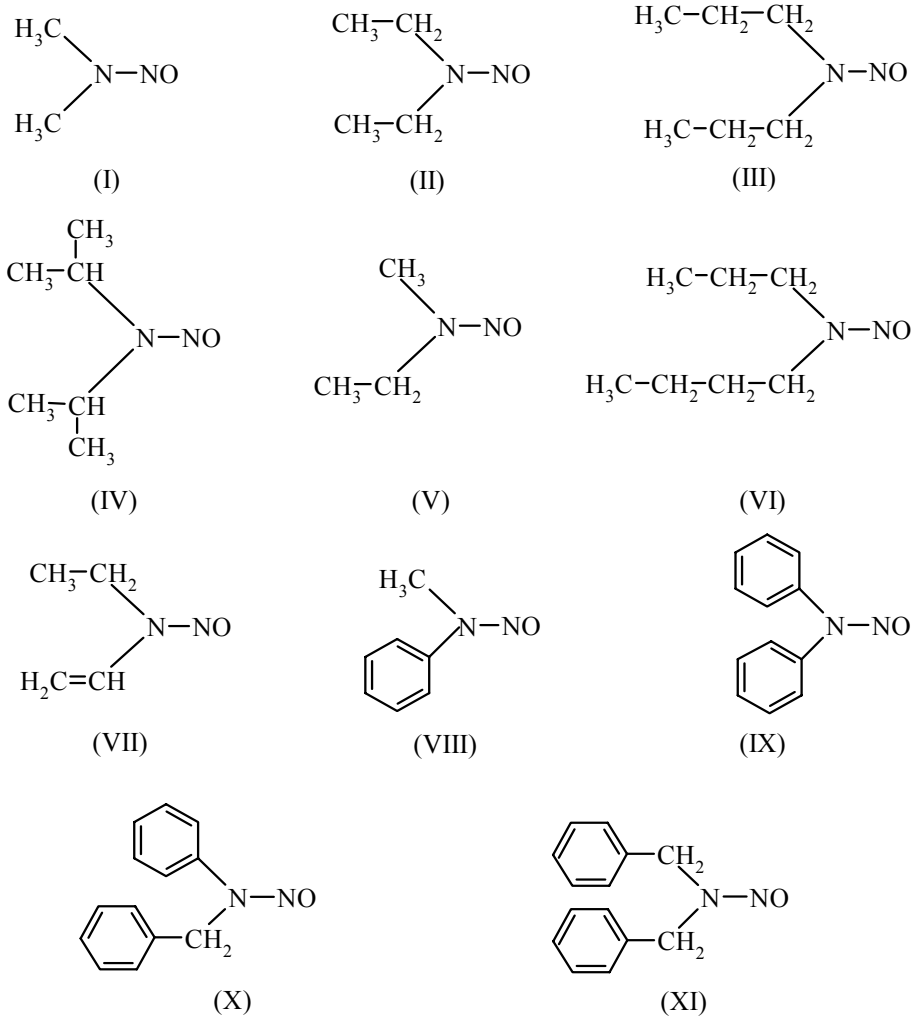
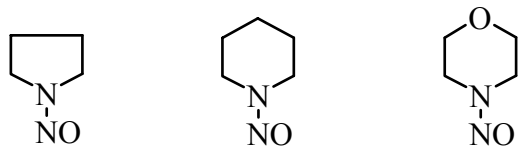


Fig. 2. Non-cyclic nitrosamines – structural formula

As we already mentioned above there are also a small number of cyclic nitrosamines: N- nitrosopyrolidine (XII), N-nitrosopiperidine (XIII) and N-nitrosomorpholine (XIV). Their chemical structures are presented in figure 3 - compounds XII – XIV.

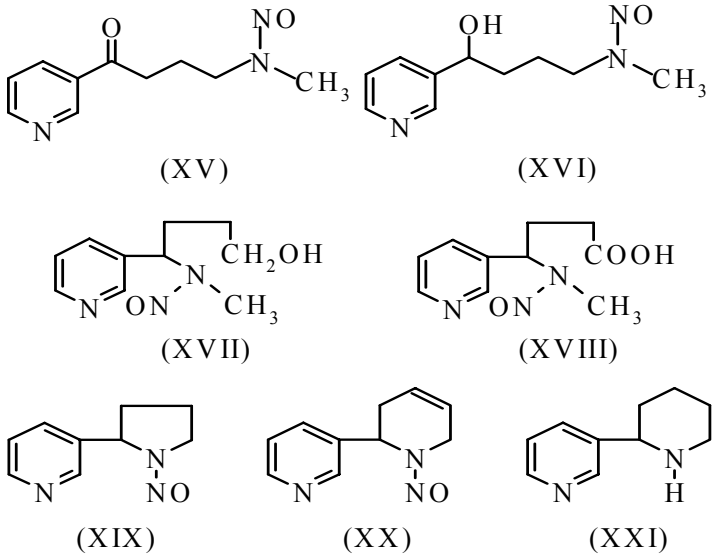


(XII) (XIII) (XIV)

Fig. 3. Cyclic nitrosamines – structural formula

One class of nitrosamines that, by the magnitude of exposure and impact on human health, can be considered of crucial importance for investigation and further research are the tobacco-specific nitrosamines (Belinsky, 1987; Hecht, 2003).

The most important tobacco-specific nitrosamines are: 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (XV); 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanol (XVI); 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (XVII); acid 4-(methylnitrosamino)-1-(3-pyridyl) butiric (XVIII); N-nitrosonornicotine (XIX); N-nitrosoanatabine (XX) and N-nitrosoanbasine (XXI). For details see figure 4 – compounds XV – XXI.



(XV) (XVI)

(XVII) (XVIII)

(XIX) (XX) (XXI)

Fig. 4. Tobacco-specific nitrosamines – structural formula

During the curing and maturation of tobacco the present nitrates are reduced to nitrites (Wiernik, 1995). Nitrite acts as a powerful nitrosant agent towards the secondary and tertiary amines leading to the formation of the tobacco-specific nitrosamines (Hoffmann, 1994).

2.2. Biological activity of nitrosamines

Nitrosamines are produced from nitrites and amines. Their formation can only occur under certain conditions, including strongly acidic conditions such as that of the human stomach.

The nitrite forms nitrous acid, which splits into the nitrosyl cation ($N{=}O^+$) and the hydroxide (OH^-) anion. The nitrosyl cation then reacts with an amine to produce nitrosamine (Gârban, 2003).

Nitrosamines are found in many foodstuffs especially beer, fish, fish byproducts, and in meat and cheese products preserved with nitrite pickling salt. They are formed when the food protein reacts with nitrite salts in the stomach. They can also be formed by frying or smoking. Nitrosamine-content is lower if the food was processed less, less preservatives were used, and if natural production techniques were used. Tobacco-specific nitrosamines are found especially in the cigarettes smoke.

A general reaction of a nitrosamine (nitrosodialkylamine) biotransformation process is given in figure 5 (Gârban, 2004).

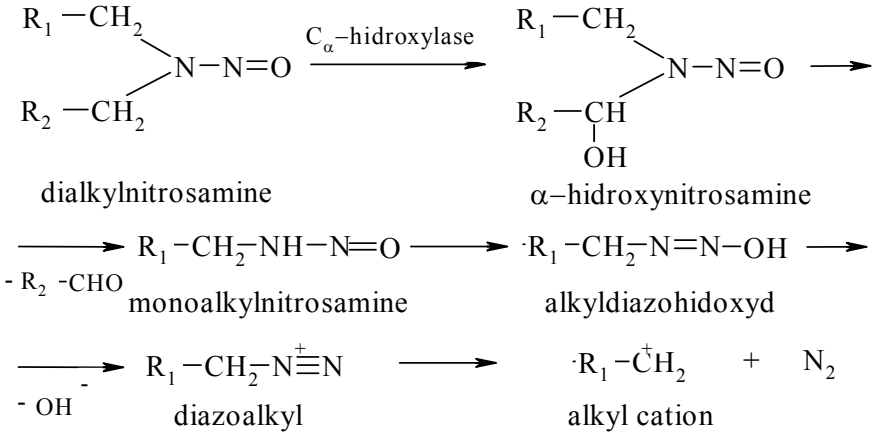


Fig. 5. The mechanism of biotransformation for a nitrosodialkylamine (Gârban, 2004)

The process of metabolisation typically involves the oxidation of the carbon adjacent to the amine nitrogen (α-hydroxylation), as it was shown in the laboratory experiments with the ubiquitously distributed N-nitrosodimethylamine, and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.The resultant α-hydroxy-N-nitrosoalkylamines are unstable, and rapidly decompose to produce aldehydes and alkyl diazohydroxides, which have the ability to alkylate DNA (Hecht, 1986; 2003).

N-Nitrosodimethylamine is a symmetrical N-nitrosamine; thus, α-hydroxylation of either carbon will yield the same methylating agent, while 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone which is an asymmetrical nitrosamine, depending of the carbon that undergoes α-hydroxylation, can methylate or pyridyloxobutylate DNA. To date, the N-nitrosamine DNA adducts that have been identified and studied most rigorously result from either methylation or ethylation of DNA. Formation of such biological incompatible structures may lead to mutagenic and carcinogenic processes; this is why over 300 nitrosamines were found to be carcinogenic in experimental animals (Gârban, 1985; Djordjevic, 1989; Hoffman, 1994).

3. Biogenesis of DNA Adducts

Adducts biogenesis has as origin the formation of some bindings between DNA nucleobases, prevailing guanine (G) and cytosine (C) in regions with critical electron density.

As noted earlier, N-nitrosamines do not react directly with DNA; therefore, to characterize the methylated and ethylated DNA adducts resulting from exposure to these compounds, experiments have been conducted with direct-acting compounds that yield the same electrophilic intermediates. A general rule regarding these alkylating agents is that the potential exists to form adducts with all exocyclic oxygen and ring nitrogen, with the exception of N_1 site of guanine. Except for N-ethylnitrosourea, the primary site of substitution is N_7 of guanine. Ethylating agents bind to a greater extent than methylating agents with phosphodiesters, and exocyclic oxygen are preferentially modified by N-alkylnitrosoureas as compared with dialklysulphates and alkyl methanesulphonates (Singer, 1983).

One of the most discussed and studied nitrosamine is N-nitrosodimethylamine (NDMA). N-nitrosodimethylamine has been a potently carcinogenic in all experimental species examined. Since exposure to NDMA occurs principally through its presence as a contaminant in media to which the general population is exposed, this end-point is expected to be limiting; hence, the focus of testing and, as a result, assessment has been carcinogenicity.

There is strong evidence that the toxicological effects of NDMA are directly correlated with CYP2E1-dependent metabolic conversion of this nitrosamine to highly reactive species. The hepatotoxicity of NDMA was attributed, according to Lee et al. (1996), to the methyldiazonium ion formed via the α-hydroxylation pathway; denitrosation was considered to make little contribution to the overall hepatotoxic effect of this nitrosamine in rats. The principal DNA adduct formed following exposure to NDMA is N_7-methylguanine (representing about 65% of all adducts formed initially upon exposure); O_6-methylguanine is a secondary adduct (representing about 7% of all adducts formed initially). Other DNA adducts formed in smaller amounts include N_3-methyladenine and O_4-methylthymine. Their structures are given in figure 6.

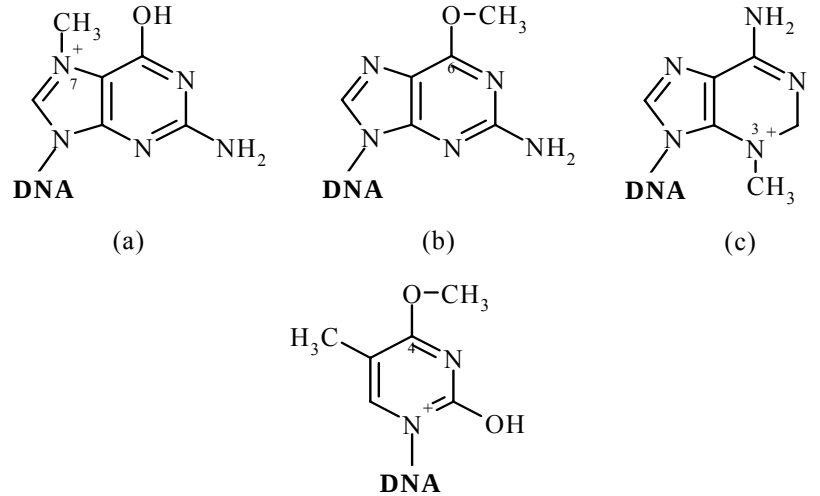


Fig. 6. Chemical structure of some DNA-NDMA adducts: a) N_7dG-NDMA; b) O_6dG- NDMA; c) N_3dA- NDMA; d) O_4dT- NDMA

Another class of nitrosamines that can generate DNA adducts is the cyclic nitrosamines. Carcinogenic cyclic nitrosamines such as N-nitrosopyrolidine (NPYR) and N-nitrosopiperidine (NPIP) are found in the diet and in tobacco smoke, and are probably formed endogenously in humans by nitrosation of the corresponding cyclic amines. NPYR and NPIP, while structurally related, have remarkably different carcinogenic activities in rats. NPYR is a liver carcinogen which never induces tumors of the esophagus while NPIP causes esophageal and liver tumors. The likely reason for this difference is selective metabolic α-hydroxylation of NPIP by cytochrome P450 enzymes in the rat esophagus, a reaction that is barely observable for NPYR (Wong, 2003). Some chemical structures of the DNA adduct with N-nitrosopyrolidine (NPYR), found in laboratory investigations, and are shown in figure 7.

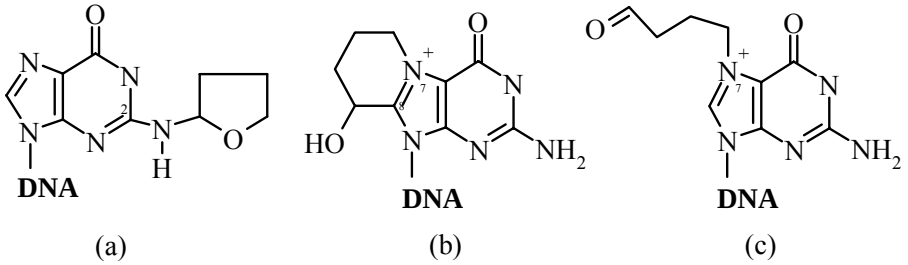


Fig.7. Chemical structure of some DNA adducts formed following exposure to NPYR: a) N_2dG-NPYR; b) N_7C_8dG-NPYR; c) N_7dG-NPYR

The last class of nitrosamines discussed in this paper is the tobacco-specific nitrosamines, a group of carcinogens derived from tobacco alkaloids by nitrosation. The most studied compound from this group of nitrosamines is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). To elicit its carcinogenic effects, NNK requires metabolic activation by cytochrome P-450 - CYP-mediated α-hydroxylation (Adams, 1985). Using mass spectrometric analysis, structures of resulting DNA adducts have been determined (Sturla, 2005). Their structures are given in figure 8.

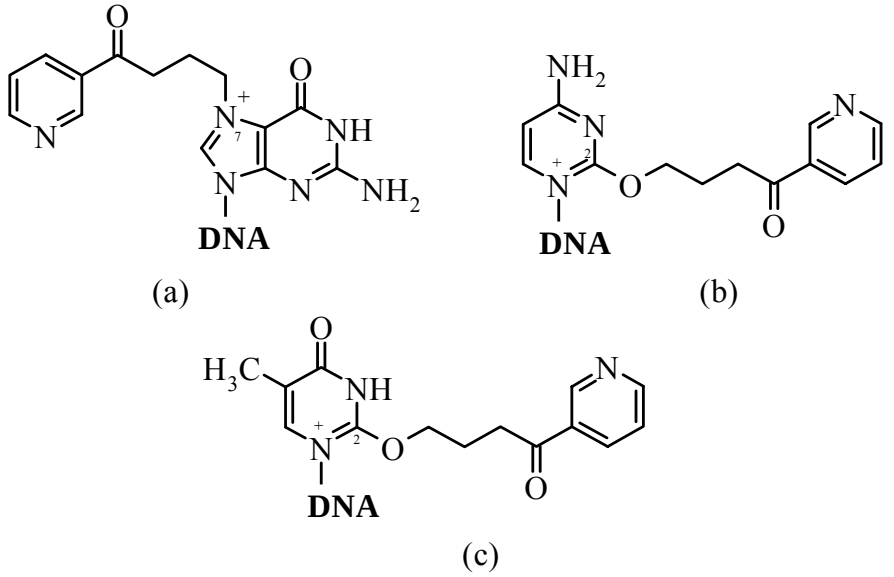


Fig. 8. Chemical structure of some adducts between DNA and NNK:
a) N_7dG-NNK; b) N_2dC-NNK; c) N_2dT-NNK

The resulting adducts have been detected in cells and tissues susceptible to NNK carcinogenesis in rodents. The methylation and pyridyloxobutylation pathways are both important in NNK carcinogenic effect. NNK also induces single strand breaks and increases levels of 8-oxodeoxyguanosine in DNA of treated animals (Hecht, 1999).

Conclusions

Nitrosamines are produced from nitrites and amines. Their formation can only occur under certain conditions, including strongly acidic conditions such as that of the human stomach.

Before binding to DNA macromolecules, the chemically inert nitrosamines are metabolized to reactive electrophiles. The process of activation typically involves the oxidation of the carbon adjacent to the amine nitrogen (α-hydroxylation).

Interactions between DNA and nitrosaminic metabolites are at the origin of biogenesis of some bioincompatible compounds that can be implied in processes of teratogenesis, mutagenesis and/or oncogenesis.

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