

**ANALYTICAL INVESTIGATION OF ADDUCTS FORMED BY
DEOXYRIBONUCLEIC ACID WITH CHEMICAL XENOBIOTICS
OF FOOD INTEREST**

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Abstract

Investigation of chemical xenobiotics of food interest is of major importance for food safety and for public health. In this context classes of substances, specific reactions of biotransformation (with the xenobiodegradation and xenobiosynthesis phases) and the harmful effects of xenobiotics on the organism are presented.

With reference to the analytical methods applied in the investigation of the deoxyribonucleic acid (DNA)-food xenobiotics adducts, the physico-chemical methods used, the facilities offered by them and the applicability in the genomic technologies are discussed succinctly. Also, the predictive character of data for pathological risk assessment is highlighted.

Keywords: chemical xenobiotics of food interest, DNA-xenobiotics adducts

Introduction

Chemical xenobiotics of food interest reach the organism together with nutrients and undergo biotransformation reactions. Among those, a particular interest show the reactions of xenobiosynthesis which generate adducts of type DNA-xenobiotic

To emphasize the importance of the above mentioned issue there are discussed the physico-chemical methods used in the investigation of adducts with applications in genomic technologies and the importance for nutrition and prophylactic medicine.

1. Specificity of the chemical xenobiotics biotransformation.

In food science it is important to know the specific aspects related to metabolization of nutrients - the field of biochemistry and the biotransformation of chemical xenobiotics - a field of xenobiochemistry.

A general assessment of chemical xenobiotics of food interest leads to the observation that they can be grouped in two classes: a) compounds of deliberate provenance (additives), e.g.: food colouring matters (synthetic), preservatives, sweeteners, antioxidants, etc.;

b) compounds of accidental/illicit provenance (pollutants), e.g.: pesticides, polycyclic aromatic hydrocarbons, mycotoxins, steroid hormones, nitrates, nitrites, metals of toxicogenic potential.

It is known that in xenobiochemistry there are specific biotransformation reactions in the phase of xenobiodegradation (i.e. oxidation-reduction reactions; hydrolysis reaction) and in the phase of xenobiosynthesis (i.e. various conjugation reactions and adduction reactions). Following the adduction reactions various adducts may form, among which those of DNA are of interest in pathobiochemistry with possible consequences in genomics and proteomics.

Biomarkers which are formed following the biotransformation processes are suitable for use in monitoring the physiological / physiopathological status of the organism (at certain time intervals), which can be determined and applied individually or within a population group.

Between biomarkers, various metabolites, particularly proteins, such as enzymes, metalloproteins, nucleoproteins, are noted. Among nucleoproteins deoxyribonucleic acid (DNA) is of particular interest because its adducts with various xenobiotics can be accurately investigated by physico-chemical means.

Among the possibilities to approach the problems of xenobiochemistry in relation to food safety / security, the biomarkers keep the attention due to the fact that they offer a real guide to the measures that are required. The use of biomarkers allows risk prediction and predictive assessment of possible pathobiochemical effects due to xenobiotics (Schulte, 1989; Schugart et al., 1992; Ehrenberg et al., 2000; Gil and Pla, 2001; Gârban, 2016).

Monitoring of biomarkers in biochemistry/xenobiochemistry is also important for the reason that it provides preliminary information on the occurrence of some clinical manifestations. It is, in fact, the stage of the occurrence and expansion of the „molecular injuries”.

The used biomarkers according to their attributes can be classified as: a) exposure biomarkers; b) effect biomarkers; c) susceptibility biomarkers.

2. Biogenesis of DNA-xenobiotics adducts and the pathological risk assessment

Once the xenobiotics and xenobioderivatives entered in the organism they interact with the bioconstituents and / or metabolites. Among them, from the point of view of pathobiochemistry, a particular importance are the xenobiosynthesis reactions with the formation of DNA adducts.

The more known adducts of DNA are formed with polycyclic aromatic hydrocarbons, mycotoxins (e.g. aflatoxins), steroid hormones, some pesticides, nitrosamines (derived from nitrates and nitrites), ions of potential toxicogenic metals (M^{n+}). Those macromolecular adducts are noted by abbreviations of type such as: DNA-HPA; DNA-AFB; DNA- M^{n+}

The mechanisms incriminated in the pathobiochemistry of chemical xenobiotics are mainly their interaction with cellular bioconstituents or cellular metabolites. The manifestation of pathobiochemical effects follows the passage of xenobiotics through biochemical barriers.

Chemical xenobiotics of food interest as well as the xenobioderivatives originated from them are at the origin of „molecular injuries”, specific to pathobiochemistry. Afterwards those evolve to „cellular lesions”, important in the pathophysiology.

In xenobiochemistry but also in the field of food chemistry, clinical chemistry, pharmacology, toxicology, etc. a correlation can be made between the nature of the xenobiotics and the appropriate biomarker. Under these circumstances, the possible changes in

protein, lipid, enzyme metabolites, etc., are taken as a reference. The biomarker-xenobiotic relationship has been studied by Walker et al. (1996).

Among biomarkers of major interest for molecular biology-specific investigations are also adducts of deoxyribonucleic acid (DNA) with various xenobiotics (Alford and Caskey, 1994; Dipple, 1995; Gârban and Drăgan, 2004).

3. Analytical detection of DNA-xenobiotic adducts

Biogenesis of DNA adducts is of particular importance for the consideration that these compounds can be analytically detected to provide information on the consequences of „molecular injury”. Detection of DNA adducts is important especially in the diagnosis of neoplastic disease, but also in the biomonitoring of evolution during oncotherapy.

For the detection of DNA adducts there are currently various physico-chemical methods (Perera et al., 1995; Phillips and Arlt, 2009 a.o.). Between these are mentioned: ³²P postlabelling; high-performance liquid chromatography (HPLC); enzyme-linked immunosorbent assay (ELISA); chemoluminescence immunoassay (CIA); immunohistochemistry (IHC); synchronous fluorescence spectroscopy (SFS), mass spectrometry (MS); accelerator mass spectrometry (AMS) a.o. Systematized data on the main detection methods of DNA adducts are presented in Table 1.

Table 1. Detection methods for DNA adducts applicable for biomonitoring in humans (according to Phillips and Arlt, 2009)

Methods	Variations	Amount of DNA required (µg)	Approximate detection limit
³² P-postlabelling	Nuclease P21 digestion, Butanol extraction, HPLC	1-10	1 adduct per 10 ⁹ -10 ¹⁰ nucleotides
Immunoassay	ELISA, DELFIA, CIA, IHC	20	1.5 adducts per 10 ⁹ nucleotides
Fluorescence	HPLC fluorescence; SFS	100-1000	1 adduct per 10 ⁹ nucleotides
Mass spectrometry	MS	Up to 100	1 adduct per 10 ⁸ nucleotides
Accelerator mass spectrometry	AMS	Up to 100	1 adduct per 10 ¹¹ -10 ¹² nucleotides

For the purpose of applying methods for detection of DNA adducts in human exposure are considered important: 1) the sensitivity of the method for detecting low levels of adducts; 2) the need for quantification only in micrograms of DNA; 3) providing quantifiable quantitative results for exposure; 4) the ability to detect, quantify and identify adducts.

The methods used for the determination of DNA adducts can be classified into two categories: detection methods applicable to the identification of most DNA adduct types and detection methods with limited applicability to certain types of DNA adducts (Hemminiki, 1998). With reference to these methods, the important advantages and disadvantages are presented both bio-analytically and cost-effectively (Table 2).

Table 2. Avantages and disadvantages of the main detection methods of DNA adducts used as exposure biomarkers (according to Hemminiki - 1998)

General methods	Advantages and	Disadvantages
A. For most adducts		
³² P post-labelling	Very sensitive; Small amounts of DNA	Laborious, radiation
Immunoassay	Sensitive, easy to prepare columns	Raising antibodies, specificity
GC-MS	Specific, quantitative	Cost, derivatization, volatile adducts
Accelerator MS	Very sensitive, quantitative	Cost, radioactivity
B. For certain adducts		
Electrochemical	Easy, sensitive, cheap	Specificity, contaminants
Fluorescence	Easy, specific	Large amounts of DNA, contaminants
Alkyltransferase	Specific class of adducts, cheap, easy	Specific O6-alkylguanines?
Atomic absorption	Specific, sensitive	Specific metals, platinum

Determination of exposure biomarkers is the basis of the biomonitoring concept, a concept in which DNA adducts are increasingly applicable with the advancement of their detection techniques.

In biomonitoring, DNA adducts can generally be used to evaluate exposure to certain chemical xenobiotics of food, pharmaceutical or toxicological origin. Thus, in food chemistry - viewed from the point of view of xenobiochemistry - biomonitoring based on DNA adducts can highlight exposure to certain xenobiotics, such as carcinogenic substances occurring in food after processing (e.g. polycyclic aromatic hydrocarbons, heterocyclic amines a.o). In pharmacology, DNA adducts can be used to determine the efficacy of chemotherapeutic cytostatics (e.g. cisplatin, tamoxifen, etc.). From the point of view of xenobiochemistry, the use of DNA biomarkers in biomonitoring is important for determining the exposure especially to some environmental xenobiotics (heavy metals, pesticides, nitrates etc.).

4. Contribution of genomics to the investigation of DNA-chemical xenobiotic adducts

In molecular biology, the issue of chemical xenobiotics - of food, pharmaceuticals, cosmetics, and biocides - is of application interest of „molecular injury” at the level of genes and also its effects on genomic level (Irendale and Longley, 1997; Heijne et al., 2005).

Genomics is a branch of biotechnology in which molecular biology and genetics methods for genome studies are applied to all genes in an organism. In humans, for example, the genome includes about 30,000 genes.

With the help of genomics one can study the structure of an individualized gene, the interactions between genes and interactions of the genes with the environment etc. The appearance and evolution of genomics was favored by the possibilities of sequencing the genome with the development of knowledge about DNA fragmentation and computer applications in the analysis of multiple variables.

In genomics, it is mainly intended: i) knowledge of the molecular mechanisms of adverse biological processes (caused by xenobiotics); ii) identifying changes in homeostasis status; iii) the use of screening means in order to detect harmful effects.

Interestingly, the comparison between genetics and genomics. Classical genetics aimed to locate a gene's locus on the chromosome, then cloning the gene and sequencing DNA from it. Genomics follows DNA sequencing of chromosomes, then identifying all genes. This is followed by the mapping of the genes (going up to the definition of places) of the chromosomes.

Applications of genomics also involve the evaluation of some biomarkers. In relation to xenobiochemistry, exposure biomarkers are of particular importance. They can provide predictive information (Groten et al., 2000; Paustenbach and Galbraith, 2006; Gârban, 2016).

In the genomics investigation, two important groups of so-called „genomic technologies” are distinguished from the applicative point of view: structural genomics and functional genomics. In fig.1 there are presented, after Karahalil (2010), the interrelations between genomic technologies.

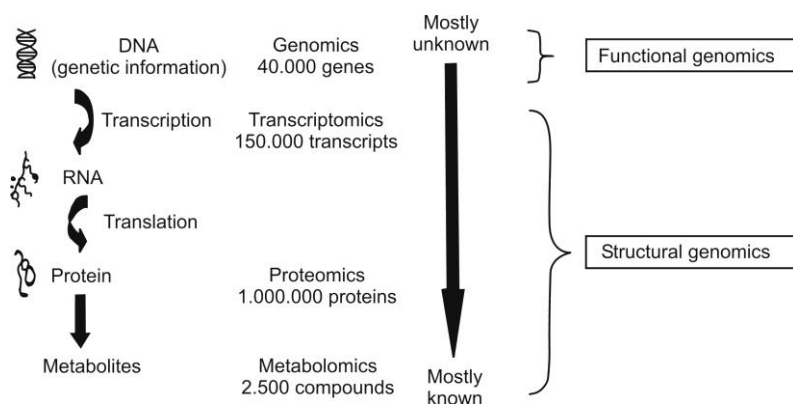


Fig.1. Interrelation between structural and functional genomics (according to Karahalil, 2010)

Structural genomics include genomic mapping, sequencing, genome and genome organization, manipulation of the genome, three-dimensional determination of structures of all proteins, study of networks (of genes, proteins). In this way, following nutrigenomics, one can study the effects of food xenobiotics starting from the DNA macromolecule.

Functional genomics include three distinct investigation directions: a) transcriptomics - evaluating a number of 150.000 transcripts; b) proteomics – considering that there are about one million proteins; c) metabolomics – appreciating that there are around 2600 compounds (Heijne et al., 2005; Fratamico and Luchansky, 2007).

Investigation of the effects of nutrients / chemical xenobiotics in food on organism is important in nutrigenomics. The study of adducts DNA-chemical xenobiotics of food interest are of particular significance because such compounds could be at the origin of teratogenic, mutagenic and oncogenic processes. Detection of the presence of adducts DNA-xenobiotics has a predictive, therapeutic and metaphylactic importance.

Concluding remarks

Approaching the general problems of adducts and of the specific adducts of DNA-xenobiotics was influenced by the progresses in molecular biology. Thus, some issues of

pathobiochemistry based on the applications of genomics were investigated with modern physico-chemical methods.

The information obtained is suitable for use in food xenobiochemistry with applications in nutriviigilance.

References (selective)

1. Alford, R.L., Caskey, C.T. - DNA analysis in forensics, disease and animal/plant identification, *Curr. Opin. Biotechnol.*, 1994, 5(1), 29-33.
2. Dipple A. - DNA adducts of chemical carcinogens, *Carcinogenesis*, 1995, 16, 437-441.
3. Gârban Z., Drăgan P. (Eds.) - *Metal Elements in Environment, Medicine and Biology, Tome VI*, Publishing House Eurobit, Timișoara, 2004
4. Gârban Z. - *Quo vadis food xenobiochemistry* (in romanian) ediția 2-a Editura Academiei Române, București și Editura Politehnica, Timișoara, 2016
5. Gil F., Pla A. - Biomarkers as Biological Indicators of Xenobiotic Exposure, *Journal of Applied Toxicology*, 2001, 21, 245-255.
6. Groten J.P., Butler W., Feron V.J., Kozianowski G., Renwick A.G., Walke R. - An analysis of the possibility for health implications of joint actions and interactions between food additives, *Regulat. Toxicol. Pharmacol.*, 2000, 31, 77-91.
7. Hemminiki K., Byhov V., Yang K., Rajaniemi H. - Use of DNA adducts in Biomonitoring, pp. 133-135, în *Biomarkers: Medical and Workplace Applications* (Mendelson L., Mohr L.C., Peeters J.P., Eds.), Joseph Henry Press, Washington DC, 1998.
8. Karahalil B. - Pharmacogenomics and toxicogenomics in food chemicals, pp.477-496, în *Advances in food biochemistry* (Fatih Yldiz, Ed.), CRC Press, Taylor and Francis Group, Boca Raton – London - New York, 2010.
9. Phillips D.H., Arlt M.V. - Genotoxicity: damage to DNA and its consequences, pp. 87-110, in *Molecular, Clinical and Environmental Toxicology, Vol. 1, Molecular Toxicology* (Luch A., Ed.), Birkhäuser Verlag AG Basel, Switzerland, 2009.
10. Walker C.H., Hopkin S.P., Sibly R.M., Peakall D.B. (Eds) - *Principles of Ecotoxicology*, Taylor and Francis, London, 1996.