Marine Environmental Research 125 (2017) 49–62

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

DNA adducts in marine fish as biological marker of genotoxicity in environmental monitoring: The way forward

Daniela M. Pampanin [a, e, *], Steven J. Brooks [b], Bjørn Einar Grøsvik [c], Jérémie Le Goff [d], Sonnich Meier [c], Magne O. Sydnes [e]

[a] International Research Institute of Stavanger, Mekjarvik 12, NO-4070 Randaberg, Norway
[b] Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, NO-0349 Oslo, Norway
[c] Institute of Marine Research, Box 1870, Nordnes, NO-5817 Bergen, Norway
[d] ADn'tox, Bâtiment Recherche, Centre François Baclesse 3, Avenue du Général Harris, 14076 Caen Cedex 5, France
[e] Faculty of Science and Technology, Department of Mathematics and Natural Science, University of Stavanger, NO-4036 Stavanger, Norway

ARTICLE INFO

Article history:
Received 15 September 2016
Received in revised form
17 January 2017
Accepted 19 January 2017
Available online 21 January 2017

Keywords:
DNA adducts
Genotoxicity
Environmental monitoring
Fish
Polycyclic aromatic hydrocarbons
^{32}P-postlabeling
Mass spectrometry

ABSTRACT

DNA adducts in fish represent a very important genotoxicity endpoint in environmental monitoring, being a pre-mutagenic lesion that plays an essential role in the initiation of carcinogenesis. The analysis of DNA adducts is a challenging task due to the low concentration of the analyte. Methods are available to determine the presence of DNA adducts, although further knowledge is required to fully understand the nature of the adducts and responsible xenobiotics (i.e. position of adduct in DNA, most active xenobiotic and metabolite forms, structural information). At present, ^{32}P-postlabeling is the most used method that has the required sensitivity for DNA adduct analyses in both human health and environmental monitoring. Development of new mass spectrometry based methods for identifying DNA adducts in complex matrixes is now considered as a necessary mission in toxicology in order to gain the necessary information regarding adduct formation and facilitate tracking sources of contamination. Mass spectrometry therefore represents the future of DNA adduct detection, bringing along a series of challenges that the scientific community is facing at present.

Contents

* Corresponding author. International Research Institute of Stavanger, Mekjarvik 12, NO-4070 Randaberg, Norway.
E-mail address: dmp@iris.no (D.M. Pampanin).

http://dx.doi.org/10.1016/j.marenvres.2017.01.002
0141-1136/

1. Introduction

A large variety of contaminants are present in the aquatic environment to which organisms including fish become exposed. In general, fish are able to take up contaminants present in their living environments, metabolise them to more water soluble substances and excrete them *via* the bile (Varanasi et al., 1989a). Throughout this process, some compounds are also metabolically activated to electrophilic metabolites that can bind to nucleic acids forming covalent adducts. It is these covalently-bound compounds that are often referred to as DNA adducts (Dunn, 1991). Measurement of DNA adducts (i.e. a DNA segment that is bound to a cancer-causing chemical, La and Swenberg, 1996), represents one possibility to quantify the biologically effective dose of a contaminant reaching a critical cellular target (i.e. the genetic material). Since the main aim of environmental monitoring is the detection of possible adverse effects on living organisms, and more in general on the ecosystem, due to the presence of contaminants and possibly being able to track sources of contamination, the present review focuses on the study of these type of DNA adducts (chemical entities attached to DNA). However, since the DNA can be attacked by both physical (primarily radiation sources, including UV) and chemical mutagen, a series of damages can be originated. Some chemical compounds can attach alkyl groups covalently to DNA bases (i.e. alkylating agents). DNA damages can also be formed by reactive oxygen species (ROS) directly (e.g. 8-oxo-deoxy guanosine, referred to by some as an adduct, an oxidized base usually very rapidly repaired) or indirectly (e.g. a study from Bar-Ilan et al. (2013) reports the photoactivation of titanium dioxide nanoparticles triggered the production of ROS, which were then responsible for an increase of DNA adducts in zebrafish). DNA adducts can also be the result of endogenous metabolic and biochemical reactions (see section 6). A DNA adduct, once formed, can be repaired, resulting in a return to the original DNA structure or be mis-repaired, resulting in a mutation.

Therefore, DNA adduct levels are useful epidemiological biomarker for detecting exposure to environmental genotoxicants (Shugart, 2000). It is important to notice that the evaluation of adducts, while important, represents the analysis of one subset of types of DNA damage.

The presence and formation of DNA adducts have been extensively studied in many fish species (van der Oost et al., 2003; Pampanin et al., 2013; Pampanin and Sydnes, 2013). Their detection in aquatic organisms is commonly used as exposure indicators since the 90s (Kurelec and Gupta, 1993). Stein et al. (1993) published some of the first data on the kinetics of adduct formation and removal in fish liver, where the persistency of DNA adducts following exposure to benzo[*a*]pyrene (B[*a*]P) and 7*H*-dibenzo[*c*,*g*]carbazole (DBC) were investigated (Fig. 1).

The presence of DNA adducts also represents an evaluation of the mutagenic/carcinogenic risk to the aquatic organisms themselves. At present, hundreds of different DNA adducts are produced from around 20 different classes of carcinogenic and mutagenic compounds, either directly or after activation (Kleihues, 1994).

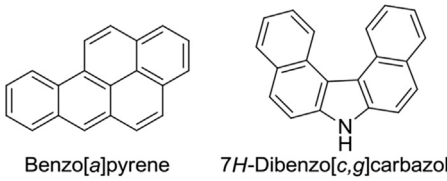

Fig. 1. Structure of benzo[*a*]pyrene (B[*a*]P) and 7*H*-dibenzo[*c*,*g*]carbazole (DBC). Compounds known to increase DNA adduct formation in fish hepatocytes.

Being a pre-mutagenic lesion, DNA adducts play an essential role in the initiation stage of carcinogenesis (Zhou et al., 2011). Their qualitative and quantitative evaluation is therefore of great importance for assessing the health of entire organisms, and consequently the ecosystem (Pfau, 1997). DNA adducts are also very important in providing valuable information for risk analysis. They are in fact considered to be an ideal target for human screening, biomarker discovery, and measurement of exposure levels (see mini review by Klaene et al., 2013), by revealing bioavailability of genotoxicants and reflecting biological effective dose. It is important to note that this type of DNA damage might lead to other effects other than cancer, e.g. reproduction toxicity and neurodegeneration (Shugart, 1995; Sram et al., 1999; Horak et al., 2003).

Analysis of DNA adducts is recognised to be one of the most sensitive genotoxicity test for fish, and it is one of the most applied tests in biomonitoring studies (Pampanin and Sydnes, 2013). In fish, DNA adducts are most often measured in liver, since this is the key organ for biotransformation of xenobiotics, but other tissues can also been analysed (Ericson et al., 1999). Recent findings of relatively elevated DNA adduct levels in fish collected in the Norwegian sector of the North Sea have raised the interest within the scientific community, amongst the oil and gas operators, and the Norwegian regulatory authorities (e.g. Miljødirektoratet) (Grøsvik et al., 2009; Balk et al., 2011). There are several reports showing that exposure of crude oil and produced water can induce DNA adducts in marine fish, both from laboratory studies (Lyons et al., 1997; Aas et al., 2000; Holth et al., 2009; Sundt et al., 2012) and field studies after major oil spills (Harvey et al., 1999; Amat et al., 2006). Likewise, *in vitro* studies showed that oils and oil fractions contain genotoxic compounds that induce DNA adducts (Ingram et al., 2000; Akkineni et al., 2001; Nagy et al., 2004).

2. DNA adducts and environmental monitoring

The effectiveness of using biological effect methods (biomarkers) as assessment tools to evaluate the health of the marine ecosystem, due to anthropogenic activities, has been clearly demonstrated during recent decades (e.g. De Zwart, 1995; Martinez-Gomez et al., 2010). Regarding the North Sea, major environmental concerns have been raised due to the intensive oil exploration activity in the region (Gray, 2002; Hylland et al., 2006; Brooks et al., 2011). A recent study from Hylland et al. (2016) used DNA adducts as a biomarker to monitoring coastal and offshore areas in the Northeast Atlantic, affected by varying pollution inputs. Biomarkers provide valuable early warning information that can be used to improve the process of hazard assessment for aquatic organisms (Moore et al., 2006; Hagger et al., 2006; Martinez-Gomez et al., 2010). In this context, biomarkers of genotoxicity, e.g. DNA adducts, represent an important role in providing information for ecological risk assessment.

This review takes a particular focus towards the effects of polycyclic aromatic hydrocarbons (PAH), which are a group of compounds abundant in oil and gas related mixtures and known to cause adverse effects in aquatic organisms. With the widespread production of oil and gas in the world's oceans, as well as the continued exploration in polar regions, it is essential to understand what effects oil and gas (and PAH) may be having on marine life. The impact of PAHs on genotoxicity through adduct formation will therefore be considered.

Other method commonly used for detecting genotoxicity in marine fish include the alkaline comet assay measuring DNA strand breakage in erythrocytes (Martins and Costa, 2015) and micronucleus in blood and kidney cells (Barsiene et al., 2006). The Comet