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Review Research Paper

Genomic Biomarker Approaches in Environmental Monitoring Processes - A Review

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ABSTRACT

Sufficient knowledge on the effects of pollutants at different levels of biological organization in an aquatic environment is needed for the reliable environmental risk assessment of pollutants, or hazard identification of environmental contaminants/pollutants. Biochemical biomarkers have been considered as the most promising tool for monitoring the early damages caused by pollutants to aquatic organisms, either at acute or sub-acute levels. These biomarkers of early chemical exposure can aid in avoiding further chemical exposure and those specific biomarkers may help to minimize further damage to the environment. In addition to assessing the presence or absence of an exposure or effect, ability to quantify the exposure and dose-response in some way would be useful for risk assessment. New approaches are needed for environmental risk assessment to catch up with the backlog of contaminants and keep pace with the increasing surge of new potential risks. These biomarkers provide us the confidence of accurate prediction to employ suitable prevention processes. Thus, it can be considered as an ounce of pollution prevention that can be worth a pound of waste treatment. An attempt has been made in this review to describe the importance of biomarker research and exploring the possibility of employing suitable new molecular approaches to protect and preserve the health of the environment.

INTRODUCTION

Aquatic pollutants have received attention from environmental scientists, and regulators due to their introduction into the environment, unforeseen effects associated with these pollutants, or enhanced analytical techniques presently capable of detecting them even at a low concentration. Sufficient knowledge on the effect of these pollutants at different levels of biological organization is needed for reliable environmental risk assessment of pollutants, or hazard identification of environmental contaminants/pollutants. Although the effect of pollutants on aquatic organisms encompassing both, whole organism and sub-lethal responses, the term 'biomarker' refers most commonly to the latter. Biochemical biomarkers have been considered as the most promising tool for monitoring the early damages caused by pollutants to aquatic organisms, either at acute or sub-acute levels. Initially, the biomarker concept was applied in medical diagnostics as an indicator of a particular state or disease in humans (Paone et al. 1980). In the early 1990s, it became very appealing in environmental studies, as happened for various other kinds of applications (McCarthy & Shugart 1990, Walker 1992, Depledge & Fossi 1994, Peakall 1994). At the beginning of biomarker research, there were high expectations for the successful utilization of biochemical biomarkers in environmental studies and much was learned about their use during the last two decades. Biomarker has been defined by Walker (1999) as a biologic response to an environmental chemical at the individual level or below which demonstrates a departure from normal status. They were expected to provide information on the qualitative and quantitative relationships among chemical exposure, biological response and adverse effects and between biomarker responses and population and community level responses (McCarthy & Shugart 1990). Hence, environmental biomarkers can be generally classified as measurable indicators or signalling changes in biological systems or samples of measurable changes at the molecular, biochemical, cellular, physiological, pathological, or behavioural levels in response to environmental pollutants. The Biomarkers Definition Working Group of the National Institutes of Health (NIH), USA has defined the biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes or pharmacological responses to a therapeutic agent."

BIOMARKERS

One of the important criteria for the application of biochemical biomarkers in environmental risk assessment research is that they should exhibit a strong link to adverse effects at the organism level, including, metabolic processes such as growth, reproduction and mortality (Depledge & Fossi 1994).

As a consequence of their ability to identify causal mechanisms potentially responsible for effects at higher levels of organization, biochemical biomarkers used to be considered the most promising tools for ecotoxicological applications during the early days of biomarker research in environmental pollution studies (Peakall & Walker 1994, Adams 2002). Many applications of this biomarker approach in environmental studies have included the following aspects:

- Investigation of a pollutant's mechanism of action.
- Fast screening of different pollutants in the environment (i.e. stressor identification).
- Risk assessment
- Improved analysis of the effects of mixtures of pollutants (Snell et al. 2003).

However, background knowledge in biochemistry, test organism physiology and toxicology are required for accurate interpretation of data derived from biomarker studies. Lack of suitable biomarkers for every available pollutants and possible misinterpretation of the data are considered to be a major constraint of biomarker research. For example, possible misinterpretation of enzyme activities, due to chemical exposure, using proteins as a reference was pointed out (Jemec et al. 2007b, 2008a) and has also been reported by others (Knowles & McKee 1987, Radenac et al. 1998, Brown et al. 2004). Moreover, at the present time, biomarker assays are not available for many different types of chemicals. Risk assessment is the process used by toxicologists to evaluate the potential for adverse biological effects from exposure to the chemicals which can be found in aquatic environments.

ENVIRONMENTAL RISK ASSESSMENT

Environmental risk assessment (ERA) includes several distinct steps with different purposes: Hazard identification step, effect assessment, exposure assessment and risk characterization. In hazard identification and risk characterization, it is crucial to have as much information as possible on the effects at different levels of biological organization (Van der Oost et al. 2003). Therefore, a combination of a battery of biomarkers from different levels of biological complexity and also an array of biomarkers within a single level could identify hazard adequately. The use of a range of biochemical biomarkers involved in different metabolic processes could reduce either false positive or false negative hazard assessments. While biomarkers help to provide a framework to attribute possible risk factors to environmental deterioration, more understanding is needed to achieve identification of environmental or genetic factors which generate potential adverse environmental health effects. Currently, few biomarkers can be considered as validated and mature for use in risk assessment.

NEW MOLECULAR APPROACHES

Two decades ago, biochemical biomarkers were considered to be a 'new powerful approach' (Depledge et al. 1995), a 'diagnostic tool for individual health', a 'predictive tool for changes at population level' (Lagadic 1999) and a 'logical approach to ERA which has already proven its worth' (Walker 1999). Similar expectations have been recently expressed for '-omics' approaches (Benninghoff 2007, Mi et al. 2007, Chora et al. 2008), although some limitations and a need for further validation have been discussed (Neumann & Galvez 2002). In the beginning of the 1980s, a significant increase in the number of scientific publications containing the keyword 'biomarker' has also been observed. Between 1990 and 2010, the total number of scientific publications concerning biomarkers has increased by a factor of 200 and currently approaches 3,000 publications annually. At the present time, the number of publications reporting the use of novel techniques, such as genomic and proteomic biomarkers in environmental studies, is also gradually increasing. By recent estimations, the number of publications (with search terms of 'genomic and pollution' and 'proteomic and pollution') published on the use of these novel biomarkers in environmental studies is even increasing. Biomarkers may have applications to all kinds of toxic compounds, especially in the field of pesticides, metals, mycotoxins, and petroleum hydrocarbons. Integration of novel and existing biomarkers with a multidisciplinary approach appears fruitful for the quest of developing the most sensitive and reliable biomarkers. Furthermore, a multibiomarker approach may be able to provide more information and accuracy than a single biomarker approach.

Overall, biomarkers have been useful for evaluating exposure, early indicators of toxicity or environmental related processes. It should be noted that even commonly used biomarkers are far from ideal, but that the combinations of biomarkers for the same compound may give complementary information.

OMICS APPROACHES

"Omics" is a recent development in biomarker methodology with the use of high-throughput techniques that employ highly sophisticated robotic and instrumental techniques, image analysis, and bioinformatics to process the enormous amount of information generated by these technologies. In order to facilitate the application of 'omic' biomarkers in environmental studies, the substantial body of experience obtained with biochemical biomarkers should be exploited in the development of new generation biomarkers. In the future, the application of biomarkers in

environmental studies will require a combination of both traditional, e.g. biochemical, and new-generation 'omic' biomarkers. For example, precise investigation of background variation expression profile unrelated to the contaminants is necessary. For research purposes, complete ecotoxicity information should include contributions from the molecular fingerprint revealed by the use of 'omic' techniques to the whole organism responses. However, in routine use, the group of biomarkers applied will probably depend on their reproducibility, ease of use, robustness and affordability of the methodology as well as the type of chemicals, organisms and ecosystem of interest. With the use of data obtained by transcriptomic/proteomic tools, it is possible to identify entire groups of genes and proteins involved in stress response and in such a way acquire new knowledge which might encourage again the development and use of traditional types of biomarkers (e.g. biochemical, cellular, histological, physiological, etc.).

ECOTOXICOLOGY

Ecotoxicology has in recent years embraced the genomic technologies to create the rapidly growing field of ecotoxicogenomics (Snape et al. 2004). Genomic tools target the molecular responses, the organism experiences in reaction to the pollutant, and provide an illustrative picture suggestive of the toxic effects experienced by the organisms and the compensatory mechanisms the microorganism has mobilized in its defence or degradation. Currently, ecotoxicogenomics and ecotoxicoproteomics are viewed as the next generation steps in the evolution of environmental biomarkers, and great expectations are associated with such 'omic' techniques (Bishop et al. 2001, Moore 2002, Neumann & Galvez 2002, Calzolai et al. 2007, Scholz et al. 2008). These novel approaches are based on measurements of gene or protein expression following exposure to a pollutant and result in an "exposure fingerprint", which provides information concerning the response of cells and organisms to changes in the ambient environment (Calzolai et al. 2007). Among invertebrates, much attention was given to the use of these novel techniques in aquatic crustacean *Daphnia magna*, and it has already been suggested as a leading model invertebrate in ecotoxicogenomics (Poynton et al. 2007, Heckmann et al. 2008, Shaw et al. 2008) of heavy metals (Connon et al. 2008), anti-inflammatory drugs (Heckmann et al. 2008), pentachlorophenol and β-naphthoflavone (Watanabe et al. 2007). These studies focused mainly on providing mechanistic insight into the mode of action of stressors, but the use of these biomarkers in other environmental pollution studies, such as risk assessment or monitoring, is still at an early stage of application and require extensive validation (Neumann & Galvez 2002, Snell et al. 2003, Poynton et al. 2007).

GENOMIC APPROACHES

Genomic technologies have been employed in many areas of biology to study disease states and the interaction of chemicals and nutrients with organisms. Signature gene expression profiles offer the potential to uncover novel biomarkers of exposure and predict the presence and fate of these contaminants in aquatic environment. The No Observed Transcriptional Effect Level (NOTEL) may play a role in determining if a predicted environmental concentration poses a risk to a sensitive species within an ecosystem. Additionally, molecular approaches may add a complementary approach to Toxicity Identification Evaluations (TIE) and help to characterize causal agents in complex effluents.

MICROORGANISMS

Of the aquatic organisms, microorganisms could be considered as suitable candidates to study the environmental perturbation because they are the only group of organisms which can act or react or both. Hence, the microorganisms react to the pollutant at multiple levels which includes altering the expression of genes, protein levels, or metabolite concentrations. The particular set of genes (or proteins or metabolites) which are expressed will be dependent on and specific for the pollutant's mechanism of action. The particular pattern of response therefore can represent a fingerprint for a specific mode of action and pollutant. Expression profiles, and other genome wide approaches have helped generate testable hypotheses of the mode of action (Hamadeh et al. 2002a & 2002b, Waring et al. 2001) of toxicants and enabled classification of chemicals based on their mode of action. Signature gene expression profiles offer the potential to uncover novel biomarkers of exposure and predict the presence of these contaminants in microorganisms. Since emerging and existing contaminants are a complex and pressing concern in environmental health, new approaches are needed to evaluate their environmental risks.

Additionally, gene expression signatures could aid in identifying the causal agents responsible for an observed toxicity (Nuwaysir et al. 1999, Miracle & Ankley 2005). In addition to identifying biomarkers, many have suggested that the gene expression profile may be used to predict exposure to pollutants in the environment (Ankley et al. 2006). Field studies have demonstrated the capability to distinguish between reference sites and contaminated sites using gene expression profiling (Williams et al. 2003, Denslow et al. 2001, Maples & Bain 2004, Roling et al. 2004). Ecotoxicogenomics, in its present form, typically identifies genes that are differentially expressed in a largely descriptive manner and devoid of hypotheses and assumptions.

Another promising approach is the inter-species comparison of gene expression profiles, which will reveal evolutionary conserved molecular events in response to toxicosis. Ecotoxicogenomics is therefore not limited to identifying novel candidate genes as biomarkers but also aims to pinpoint stressor specific expression signatures and reveal stress modulated molecular pathways.

New approaches are needed for environmental risk assessment to catch up with the backlog of contaminants and keep pace with the increasing surge of new potential risks. Genomic approaches including DNA microarrays will continue to help us understand the effects of conventional pollutants. Ankley et al. (2006) discussed the utility of these types of molecular methods (gene-genomics, protein-proteomics or small molecule-metabolomics), especially toxicogenomic approaches for regulatory ecotoxicology. Exposure of cells or organisms to toxic substances (or any stressor) results in changes in their normal gene expression pattern. Specific patterns of gene expression can reflect different mechanisms of action of toxicity or the responses of microorganisms. The use of toxicogenomics in regulatory toxicology is complicated by many issues, including the fact that other stressors can contribute to changes in gene expression patterns; the vast amount of data generated by the experiments; and the lack of experience and know how in translating the genomic data into regulatory decisions. The major benefits identified for investing in toxicogenomics for ecotoxicology decision making are its potential to reduce uncertainty (better science for decision making) and to optimize testing resources (more samples tested faster and cheaper) (Ankley et al. 2006)

The future of microbial biomarkers lies in a combination of traditional biochemical and new-generation biomarkers. The latter are not only a potential replacement for existing biomarkers but will also provide new knowledge which might encourage renewed research and development of traditional biomarkers. For research purposes, complete ecotoxicity information should include contributions from molecular fingerprint of an organism, as well as whole organism, population and ecosystem responses. Still, the type of biomarkers used for routine purposes will depend on their reproducibility, their ease of use, robustness, affordability of the methodology and the type of chemicals, organisms and ecosystem of interest.

CONCLUSION

With the development of toxicogenomic approaches, the use of microorganisms for environmental monitoring purposes is expected to become even more extensive because of better knowledge about potential analogies in toxicity mechanisms between higher organisms and microbes. Advancing the application of a new generation of 'omic' biomarkers and identifying possible links between these two groups of biomarkers are important in future research. The process is anticipated to reach a better understanding of the molecular mechanism of carcinogenesis to improve environmental health risk assessment, and support decision making in supplementing successful environmental health policy. In conclusion, the past experiences gained on biochemical biomarkers in environmental pollution studies should be exploited to new-generation '-omics' biomarkers. The future of biomarker research lies in combining the knowledge of both traditional and new generations of biomarkers.

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REFERENCES

Adams, S.M. 2002. Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland, USA.

Ankley, G.T., Daston, G.P. and Degitz, S.J. 2006. Toxicogenomics in regulatory ecotoxicology. Environ. Sci. Technol., 40: 4055-4065.

Benninghoff, A.D. 2007. Toxicoproteomics-the next step in the evolution of environmental biomarkers. Toxicol. Sci., 95: 1-4.

Bishop, W.E., Clarke, D.P. and Travis, C.C. 2001. The genomic revolution: what does it mean for risk assessment? Risk. Anal., 21: 983-987.

Brown, R.J., Galloway, T.S., Lowe, D., Browne, M.A., Dissanayake, A., Jones, M.B. and Depledge, M.H. 2004. Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. Aquat. Toxicol., 66:267-278.

Calzolai, L., Ansorge, W., Calabrese, E., Denslow, N., Part, P. and Lettieri, T. 2007. Transcriptomics and proteomics. Applications to ecotoxicology. Comp. Biochem. Physiol., 2: 245-249.

Chora, S., McDonagh, B., Sheehan, D., Starita-Geribaldi, M., Romeo, M. and Bebianno, M.J. 2008. Ubiquitination and carbonylation as markers of oxidative-stress in Ruditapesdecussatus. Mar. Environ. Res., 66:95-97.

Connon, R., Hooper, H.L., Sibly, R.M., Lim, F.L., Heckmann, L.H., Moore, D.J., Watanabe, H., Soetaert, A., Cook, K., Maund, S.J., Hutchinson, T.H., Moggs, J., De Coen, W., Iguchi, T. and Callaghan, A. 2008. Linking molecular and population stress responses in Daphnia magna exposed to cadmium. Environ. Sci. Technol., 42: 2181-2188.

Denslow, N.D., Lee, H.S., Bowman, C.J., Hemmer, M.J. and Folmar, L.C. 2001. Multiple responses in gene expression in fish treated with estrogen. Comp Biochem. Physiol. B, 129: 277-282.

Depledge, M.H. and Fossi, M.C. 1994. The role of biomarkers in environmental assessment (2). Invertebrates. Ecotoxicology, 3(3): 161-172.

Depledge, M.H., Aagaard, A. and Györkös, P. 1995. Assessment of trace metal toxicity using molecular, physiological and behavioural biomarkers. Mar. Pollut. Bull., 31: 19-27.

Hamadeh, H. K., Bushel, P., Tucker, C. J., Martin, K., Paules, R. and Afshari, C. A. 2002a. Detection of diluted gene expression altera-

tions using cDNA microarrays. Biotechniques, 32: 326-329.
Hamadeh, H. K., Bushel, P. R., Jayadev, S., DiSorbo, O., Bennett, L., Li, L., Tennant, R., Stoll, R., Barrett, J. C. and Paules, R. S. et al. 2002b. Prediction of compound signature using high density gene expression profiling. Toxicol. Sci., 67: 232-240.
Heckmann, L.H., Sibly, R.M., Connon, R., Hooper, H.L., Hutchinson, T.H., Maund, S.J., Hill, C.J., Bouetard, A. and Callaghan, A. 2008. Systems biology meets stress ecology: linking molecular and organismal stress responses in Daphnia magna. Genome Biol., 9: R40.1-R40.14.
Jemec, A., Drobne, D., Tišler, T., Trebše, P., Roš, M. and Sepèiæ, K. 2007a. The applicability of acetylcholinesterase and glutathione S-transferase in Daphnia magna toxicity test. Comp. Biochem. Physiol., 144: 303-309.
Jemec, A., Tišler, T., Drobne, D., Sepèiæ, K., Fournier, D. and Trebše P. 2007b. Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazinon to a non-target arthropod, the microcrustacean *Daphnia magna*. Chemosphere, 68: 1408-1418.
Jemec, A., Tišler, T., Drobne, D., Sepèiæ, K., Jamnik, P. and Roš, M. 2008. Biochemical biomarkers in chronically metal-stressed daphnids. Comp. Biochem. Physiol., 147: 61-68.
Knowles, C.O. and McKee, M.J. 1987. Protein and nucleic acid content in *Daphnia magna* during chronic exposure to cadmium. Ecotoxicol. Environ. Saf., 13: 290-300.
Lagadic, L. 1999. Biomarkers in invertebrates. In: Peakall DB, Colin HW, Migula P (eds) Biomarkers: a pragmatic basis for remediation of severe pollution in Eastern Europe. NATO Science Series, Kluwer Academic, Dordrecht, Netherlands.
Maples, N.L. and Bain, L.J. 2004. Trivalent chromium alters gene expression in the mummichog (*Fundulus heteroclitus*). Environ. Tox. Chem., 23: 626-631.
McCarthy, J.F. and Shugart, L.R. 1990. Biomarkers of Environmental Contamination. Lewis Pub., Chelsea, USA.
Mi, J., Apraiz, I. and Cristobal, S. 2007. Peroxisomal proteomic approach for protein profiling in blue mussels (*Mytilus edulis*) exposed to crude oil. Biomarkers, 12: 47-60.
Miracle, A.L. and Ankley, G.T. 2005. Ecotoxicogenomics: linkages between exposure and effects in assessing risks of aquatic contaminants to fish. Repro. Toxicol., 19: 321-326.
Moore, M.M. 2002. Biocomplexity: the post-genome challenge in ecotoxicology. Aquat. Toxicol., 59: 1-15.
Neumann, N.F. and Galvez, F. 2002. DNA microarrays and toxicogenomics: applications for ecotoxicology? Biotechnol. Adv., 20: 391-419.
Nuwaysir, E.F. and Bittner, M. et al. 1999. Microarrays and toxicology: the advent of toxicogenomics. Molecular Carcinogenesis 24: 153-159.
Paone, J.F., Waalkes, T.P., Baker, R.R. and Shaper, J.H. 1980. Serum UDP galactosyltransferase as a potential biomarker for breastcarcinoma. J. Surg. Oncol., 15: 59-66.
Peakall, D.B. 1994. The role of biomarkers in environmental assessment-introduction. Ecotoxicology, 3: 157-160.
Peakall, D.B. and Walker, C.H. 1994. The role of biomarkers in environmental assessment (3). Vertebrates. Ecotoxicology, 3: 173-179.
Poynton, H.C., Varshavsky, J.R., Chang, B., Cavigiolio, G., Chan, S., Holman, P.S., Loguinov, A.V., Bauer, D.J., Komachi, K., Theil, E.C., Perkins, E.J., Hughes, O. and Vulpe, C.D. 2007. *Daphnia magna* ecotoxicogenomics provides mechanistic insights into metal toxicity. Environ. Sci. Technol., 41: 1044-1050.
Radenac, G., Bocquene, G., Fichet, D. and Miramand, P. 1998. Contamination of a dredged-material disposal site (La Rochelle Bay, France). The use of the acetylcholinesterase activity of *Mytilus edulis* (L.) as a biomarker of pesticides: the need for a critical approach. Biomarkers, 3: 305-315.
Roling, J.A., Bain, L.J. and Baldwin, W.S. 2004. Differential gene expression in mummichog (*Fundulus heteroclitus*) following treatment with pyrene and fluoranthene: comparison to a creosote contaminated site. Mar. Environ. Res., 57: 377-395.
Scholz, S., Fischer, S., Gündel, U., Küster, E., Luckenbach, T. and Voelker, D. 2008. The zebrafish embryo model in environmental risk assessment-applications beyond acute toxicity testing. Environ. Sci. Pollut. Res., 15: 394-404.
Shaw, J.R., Pfrender, M.E., Eads, B.D., Klaper, R., Callaghan, A., Sibly, R.M., Colson, I., Jansen, B., Gilbert, D. and Colbourne, J.K. 2008. Daphnia as an emerging model for toxicological genomics. Adv. Exp. Biol., 2: 327-328.
Snape, J.R., Maund, S.J., Pickford, D.B. and Hutchinson, T.H. 2004. Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology. Aquat. Toxicol., 67: 143-154.
Snell, T.W., Brogdon, S.E. and Morgan, M.B. 2003. Gene expression profiling in ecotoxicology. Ecotoxicology, 12: 475-483.
Van der Oost, R., Beyer, J. and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Phar., 13: 57-74.
Walker, C.H. 1992. Biochemical responses as indicators of toxic effects of chemicals in ecosystems. Toxicol. Lett., 64/65: 527-533.
Walker, C.H. 1999. The use of biomarkers in vertebrates. In: Peakall D.B., Colin H.W., Migula P. (eds.) Biomarkers: a pragmatic basis for remediation of severe pollution in Eastern Europe. NATO Science Series, Kluwer Academic Publishers, Dordrecht, Netherlands.
Waring, J.F., Ciurlionis, R., Jolly, R.A., Heindel, M. and Ulrich, R.G. 2001. Microarray analysis of hepatotoxins in vitro reveals a correlation between gene expression proûles and mechanisms of toxicity. Toxicol. Lett., 120: 359-368.
Watanabe, H., Takahashi, E., Nakamura, Y., Oda, S., Tatarazako, N. and Iguchi, T. 2007. Development of a *Daphnia magna* DNA microarray for evaluating the toxicity of environmental chemicals. Environ. Toxicol. Chem., 26: 669-676.
Williams, T.D., Gensberg, K., Minchin, S.D. and Chipman, J.K.A. 2003. DNA expression array to detect toxic stress response in European flounder (Platichthysflesus). Aquat. Toxicol., 65: 141-157.