Available online at www.sciencedirect.com
SCIENCE DIRECT®
Toxicology 207 (2005) 451–462
TOXICOLOGY
www.elsevier.com/locate/toxicol

Functional assays in marine biotoxin detection

Gian Paolo Rossini*

Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Via Campi 287, I-41100 Modena, Italy

Received 20 September 2004; received in revised form 27 October 2004; accepted 27 October 2004
Available online 8 December 2004

Abstract

The contamination of seafood by algal toxins regularly affects animals living in several areas of the world, and the number of toxic phycotoxins which are being characterized is steadily increasing. The extreme dynamics characterizing the field of algal toxins has stimulated the development of tools to be implemented in the monitoring of contamination of seafood by individual toxin classes. Under these circumstances, functional assays which can encompass the analytical potential of chemical methods and the predictive features of biological tests are sought.

A variety of functional assays for the detection of phycotoxins has been developed in the last 20 years, and the analysis of their features reveals that their specificity is related to the hierarchical level of the biological response to the toxin that has been exploited for its detection. Ideally, analytical methods which could allow accurate estimates of the overall toxicity of multiple classes of toxins in a single procedure would provide the best means for the highest standards in consumer protection and the most rational and economical tools in the management of risks posed by phycotoxins in a wider scale. The achievement of a "systemic functional assay for marine biotoxins" does not appear to be at hand, but its inclusion among the foreseeable events is fully justified by the new research tools and approaches which have become available for the high throughput analysis of entire molecular domains at the cellular level.

Keywords: Harmful algal blooms; In vitro assay; Receptor; Signal transduction; Effector

1. Introduction

Outbreaks of toxic algal populations in coastal waters, the so-called harmful algal blooms (HAB), have been recorded in the past, together with the human and animal intoxications resulting from ingestion of contaminated seafood and/or direct contamination with algal toxins (Hallegraeff, 2004). In most recent years the phenomenon has shown two worrying developments. On the one hand, HABs have been more frequently reported in those areas known for their recurrence, and have been recorded in coastal areas which had not been touched in the past (Hallegraeff, 2004). On the other hand, new toxins are being continuously isolated and characterized, holding a clear indication

* Tel.: +39 059 205 5388; fax: +39 059 205 5410.
E-mail address: rossini.gianpaolo@unimo.it.

0300-483X/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2004.10.012

about an evolving threat to human health (Yasumoto, 2000).

The risk management linked to phycotoxin contamination of bivalve molluscs is based on monitoring of toxins in material destined for human consumption. While several chemical methods are currently used to assess the contamination of seafood by toxic agents, the available panel of methods is far from being both complete and adequate for the efficient risk management of every known toxin (Quilliam, 1999; Hallegraeff, 2004), and monitoring of shellfish contamination due to algal toxin in many cases is still based on mouse bioassays (Japanese Ministry of Health and Welfare, 1981; AOAC, 1990; European Communities, 2002). In spite of the inconsistencies, lack of specificity and questionable ethical justification of mouse bioassays (Quilliam and Wright, 1995), their use in risk assessment has been maintained over the years (Fernández et al., 2004), on the basis of:

- the limited availability of standards and reference material for every toxin, to be used for routine monitoring programmes of seafood toxicity;
- the continuously changing patterns of toxins found in molluscs in different areas and years, which demand some firm knowledge about the toxicity of individual analytes before conclusions about the risks associated with consumption of contaminated material can be drawn.

The extreme dynamics characterizing the field of algal toxins is a challenge for pharmacologists, toxicologists and biochemists interested in shedding light onto the molecular mechanisms by which bioactive compounds affect biological systems. Furthermore, the discovery of new toxins poses the need for the development of additional tools to be implemented in the monitoring of contamination of seafood by individual toxins.

Under these circumstances, functional assays which can encompass the analytical potential of chemical methods and the predictive features of biological tests are sought.

In this paper we will critically review some well-established functional assays for algal toxin detection and measurement, and analyze the approaches used to develop those methods, in order to discuss future perspectives in this area. Excellent and comprehensive reviews on the different analytical tools available for algal toxin analysis and measurement can be found elsewhere (Hallegraeff et al., 2004).

2. Basic concepts of functional assays

The scope of measurement of chemicals exerting their effects in biological systems has two different meanings for chemists and toxicologists. In the first instance, the total amount of compounds possessing the same type of chemical structure is sought. In the latter case, the overall toxicity of compounds in the tested sample should be determined. Thus the best procedures for chemical analysis possess the highest sensitivity and capacity to discriminate the many compounds which are present in the test sample. In the case of toxicity, instead, the capacity of the system to detect the potency, hence the biological activity of the compounds, must accompany the property to discriminate among chemicals. As a consequence, the application of chemical procedures to measure the toxin content in a sample demands the availability of coefficients of activity for the different compounds, in order to obtain a measurement of the total concentration of toxic agents, which are then expressed as equivalents of the reference toxin in the group (Fig. 1).

A functional assay is based on the capacity of bioactive agents, including phycotoxins, to bind a molecular component which selectively recognizes the structure of the chemical, and then operationally behaves like its receptor. The interaction of the compound with this putative receptor then sets in motion a train of events, leading to a response in sensitive systems.

Since the recognition of chemical structures by the ligand-binding site on the receptor constitutes the molecular basis underlying the effect of toxins, a clear advantage of functional assays is their capacity to discriminate between functionally active and inactive compounds belonging to the same chemical group, but differing according to the substituents modifying the reference “productive” structure.

Any biological entity expressing the receptor and the components of its signal transduction apparatus may constitute a target for the selected compound.

In this general scheme, three steps can be recognized, comprising

(i) the ligand–receptor interaction;