Chemosphere 174 (2017) 643–654

Contents lists available at ScienceDirect

Chemosphere

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

Effect-directed analysis reveals inhibition of zebrafish uptake transporter Oatp1d1 by caulerpenyne, a major secondary metabolite from the invasive marine alga *Caulerpa taxifolia*

P. Marić [a], M. Ahel [b], I. Senta [b], S. Terzić [b], I. Mikac [b], A. Žuljević [c], T. Smital [a, *]

[a] Laboratory for Molecular Ecotoxicology, Division for Marine and Environmental Research, Ruđer Bošković Institute, 10 000 Zagreb, Croatia
[b] Laboratory for Analytical Chemistry and Biogeochemistry of Organic Compounds, Division for Marine and Environmental Research, Ruđer Bošković Institute, 10 000 Zagreb, Croatia
[c] Laboratory for Benthos, Institute of Oceanography and Fisheries, 21 000 Split, Croatia

HIGHLIGHTS

- A novel mechanism of biological action of *C. taxifolia* was identified.
- Caulerpenyne is a potent inhibitor of zebrafish uptake transporter Oatp1d1.
- EDA approach allows reliable identification of new biologically active substances.

ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form
31 January 2017
Accepted 1 February 2017
Available online 2 February 2017

Handling Editor: David Volz

Keywords:
Caulerpa taxifolia
Caulerpenyne
Oatp1d1
Oct1
Zebrafish
Effect-directed analysis (EDA)

ABSTRACT

Caulerpa taxifolia is a marine alga of tropical and subtropical distribution and a well-known invasive species in several temperate regions. Its invasiveness mainly stems from the production of secondary metabolites, some of which are toxic or repellent substances. In this study we investigated the possible inhibitory effects of *C. taxifolia* secondary metabolites on the activity of two zebrafish (*Danio rerio*) uptake transporters that transport organic anions (Oatp1d1) and cations (Oct1). Both transporters were transiently transfected and overexpressed in human embryonic kidney HEK293T cells. Transport activity assays using lucifer yellow (LY) and 4-(4-(dimethylamino)styryl)-*N*-methylpyridinium iodide (ASP+) as model substrates were applied for the determination of Oatp1d1 and Oct1 interactors. A two-step Effect-Directed Analysis (EDA) procedure was applied for the separation and identification of compounds. We identified caulerpenyne (CYN) as the major metabolite in *C. taxifolia* and reveal its potent inhibitory effect towards zebrafish Oatp1d1 as well as weak effect on zebrafish Oct1 transport. The observed effect was confirmed by testing CYN purified from *C. taxifolia*, resulting in an IC_{50} of 17.97 μM, and a weak CYN interaction was also determined for the zebrafish Oct1 transporter. Finally, using Michaelis-Menten kinetics experiments, we identified CYN as a non-competitive inhibitor of the zebrafish Oatp1d1. In conclusion, this study describes a novel mechanism of biological activity in *C. taxifolia*, shows that CYN

Abbreviations: ASP+, 4-(4-(dimethylamino)styryl)-*N*-methylpyridinium iodide; CYN, caulerpenyne; EDA, effect-directed analysis; ESI, electrospray ionization; HEK293T, human embryonic kidney cells; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; LY, lucifer yellow; OATs, organic anion transporters; OATPs, organic anion transporting polypeptides; OCTs, organic cation transporters; PEI, polyethyleneimine; QTOFMS, quadrupole-time-of-flight/mass spectrometry; TIC, total ion current; UPLC, ultra-performance liquid chromatography.

* Corresponding author. Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia.
E-mail addresses: pmaric@irb.hr (P. Marić), ahel@irb.hr (M. Ahel), isenta@irb.hr (I. Senta), terzic@irb.hr (S. Terzić), Iva.Mikac@irb.hr (I. Mikac), zuljevic@izor.hr (A. Žuljević), smital@irb.hr (T. Smital).

http://dx.doi.org/10.1016/j.chemosphere.2017.02.007

was a potent non-competitive inhibitor of zebrafish Oatp1d1, and demonstrates that EDA can be reliably used for characterization of environmentally relevant complex biological samples.

1. Introduction

Some marine green algae belonging to the genus *Caulerpa* are well-known invasive species. Among them, *Caulerpa taxifolia* (M. Vahl) C. Agardh has been described as one of the most invasive in several temperate regions due to its tendency to spread rapidly and negatively impact different ecosystems (Boudouresque et al., 1992, 1995; Meinesz, 1999). Since it was first discovered outside its native habitat in 1984 near the Oceanographic Museum in Monaco, *C. taxifolia* has been found throughout the Mediterranean, California and southeastern Australia (Meinesz et al., 2001; Schaffelke et al., 2002; Creese et al., 2004). After 2009, a significant decline in the abundance of *C. taxifolia* meadows was recorded at most of the invaded areas, probably due to large annual changes in the temperature of the Mediterranean Sea, as *C. taxifolia* exhibits greater sensitivity to colder temperatures (Tejada and Sureda, 2013; Montefalcone et al., 2015). Nevertheless, this marine species still remains in the focus of research studies due to its unique characteristics and invasive potential.

The complex composition of secondary metabolites is thought to be one of the greatest advantages of the *Caulerpa* species, largely contributing to their invasive behavior. Most have been shown to be toxic or repulsive, and it is mainly believed that they serve the *Caulerpa* species as a chemical defense mechanism against herbivores and in interspecies competition (Klein and Verlaque, 2008). The diverse chemical structure of these metabolites includes mainly bisindole alkaloids, sesquiterpenoids and diterpenoids, with aldehyde and/or enol acetate functional groups (Guerriero et al., 1992; 1993; Smyrniotopoulos et al., 2003). Various biological effects attributed to the functional groups of these compounds have been described, including antimicrobial, insecticidal, antifouling, ichthyotoxic, feeding deterrent, anti-inflammatory, cytotoxic, and growth regulatory properties (Paul and Fenical, 1982; Paul et al., 1987; Fischel et al., 1995; Smyrniotopoulos et al., 2003; de Souza et al., 2009; Alarif et al., 2010; Nagaraj and Osborne, 2014). Caulerpenyne (CYN), a unique acetylenic sesquiterpen, has been identified as a major secondary metabolite in *C. taxifolia* (Amico et al., 1978). Various minor metabolites have also been identified. They are produced during CYN transformation processes such as hydrolysis, degradation, deacetylation or oxidation (Guerriero et al., 1992; Lemée et al., 1993; Guerriero and D'Ambrosio, 1999). Toxic effects of secondary metabolites from the genus *Caulerpa* have been studied in the context of cellular detoxification pathways, especially their interaction with biotransformation processes (Schröder et al., 1998; Uchimura et al., 1999a, 1999b; Sureda et al., 2006, 2009; Felline et al., 2012; Tejada et al., 2013). Nevertheless, a mechanistic understanding of the toxic effects of secondary metabolites from the genus *Caulerpa* on the cellular and/or molecular level is still lacking. It is therefore important to identify the biologically active substances responsible for the observed toxic effects and understand the mechanisms of their toxic action.

Effect-Directed Analysis (EDA) is a powerful multidisciplinary diagnostic tool developed in the field of environmental science. EDA combines the use of advanced chemical and biological methods to identify, characterize and prioritize the toxicants present in complex environmental matrices. Sometimes also called "effect-based" or "effect-oriented", it integrates stepwise fractionation procedures together with biotesting and chemical analyses to reduce sample complexity, detect toxic effects and ultimately identify specific chemicals of concern (Brack, 2003; Brack et al., 2003, 2005). So far the EDA approach has been exclusively utilized for the identification and evaluation of environmental contaminants present in non-biological complex environmental samples (sediment, surface water, wastewater, soil). This study is the first to use the EDA approach for the characterization of a complex biological sample.

One of the key factors of the cellular detoxification system are biological membranes, which play a vital role in the uptake and elimination of various endo- and xenobiotics (Simkiss, 1995). Uptake transporters are responsible for the uptake of these compounds into cells via membranes, contributing to the phase 0 of the cellular detoxification mechanism (Klaassen and Lu, 2008; König et al., 2013). Organic Anion Transporting Polypeptides (OATPs), Organic Cation Transporters (OCTs), and Organic Anion Transporters (OATs) are major groups of polyspecific uptake transporters in the cell (Koepsell and Endou, 2004; König, 2011; Roth et al., 2012; Koepsell, 2013). However, despite their toxicological relevance in mammals, knowledge on the presence of polyspecific uptake transporters in non-mammalian organisms is scarce, and their potential ecotoxicological relevance is still poorly understood. We recently characterized the first uptake transporter of organic anions in zebrafish, named Oatp1d1, and demonstrated its interaction with various environmentally relevant compounds (Popovic et al., 2014). Continuing along this line of research, an uptake transporter of organic cations, Oct1, was also identified in zebrafish, and our first data indicated that Oct1 may function as an integral part of cellular defense against potentially hazardous organic endo- and xenobiotic cationic compounds through biliary and renal excretion (Mihaljevic et al., 2016).

Consequently, as the influence of secondary metabolites from the genus *Caulerpa* on the uptake phase of cellular detoxification has not been investigated, the primary goals of this study were (1) to determine the potential inhibitory effects of *C. taxifolia* secondary metabolites on the activity of two zebrafish uptake transporters: the anionic transporter Oatp1, and the cationic transporter Oct1, and (2) to perform a preliminary identification of the biologically active compounds responsible for the observed interaction by applying the EDA approach. A modified EDA protocol using non-selective and non-target preparation of the samples along with specific biological assays was applied, allowing the identification of both the mechanism of toxic action and the chemical identity of toxic substances present in a highly complex biological material.

2. Material and methods

2.1. Chemicals

Lucifer yellow (LY), 4-(4-(dimethylamino)styryl)-*N*-methylpyridinium iodide (ASP+), Trypsin-EDTA solution and Hepes were purchased from Sigma-Aldrich, St. Louis, MO, USA. Dulbecco's Modified Eagle's Medium (DMEM) (Powder, High Glucose, Pyruvate), Fetal Bovine Serum (FBS) and Phosphate Buffer Saline (PBS) were purchased from Gibco Invitrogen, Life technologies, CA, USA.