Science of the Total Environment 490 (2014) 121–133

Contents lists available at ScienceDirect

Science of the Total Environment

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

Redox proteomics as biomarker for assessing the biological effects of contaminants in crayfish from Doñana National Park

Ricardo Fernández-Cisnal [1], José Alhama [1], Nieves Abril, Carmen Pueyo, Juan López-Barea *

Department of Biochemistry and Molecular Biology, Agrifood Campus of International Excellence (ceiA3), University of Córdoba, Córdoba, Spain

HIGHLIGHTS

- Great differences between the four sites were observed among thiol oxidized proteins.
- Thirty five spots showed intensity differences compared to the reference site.
- Nineteen proteins were identified showing thiol oxidation differences among sites.
- Evidence of reversible oxidation was found for specific Cys residues.
- Redox proteomics is useful as a new pollution biomarker for Doñana National Park.

ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form 14 April 2014
Accepted 28 April 2014
Available online xxxx

Editor: Eddy Y. Zeng

Keywords:
Procambarus clarkii
Pollutant-elicited oxidative stress
Protein thiol redox state
Ferritin
Nucleoside diphosphate kinase

ABSTRACT

Despite its environmental relevance and sensitivity, Doñana National Park (DNP) is under high ecological pressure. In crayfish (*Procambarus clarkii*), the utility of redox proteomics as a novel biomarker was evaluated in the aquatic ecosystems of DNP and its surroundings, where agricultural activity is a serious concern. After fluorescence labeling of reversibly oxidized Cys and 2-DE separation, the total density of proteins with reversibly oxidized thiols was found to be much higher in animals from the *Matochal* (MAT) and *Rocina* (ROC) streams, while no difference was found in crayfish from *Partido* (PAR) stream compared to those from the DNP core at *Lucio del Palacio* (the negative control). The 2-DE analysis revealed 35 spots with significant differences in thiol oxidation, among which 19 proteins were identified via MALDI-TOF/TOF. While 3 spots, identified as ferritin, showed higher oxidation levels in ROC, other identified proteins were more intense at MAT than at ROC (superoxide dismutase, protein disulfide isomerase and actin) or were overoxidized only in MAT (nucleoside diphosphate kinase, fructose-biphosphate aldolase, fatty acid-binding protein, phosphopyruvate hydratase). For most of the identified proteins, spots corresponding to different Cys oxidized forms were detected, and the native forms, without oxidized thiol groups were also found in some of them. Evidence of reversible oxidation was found for specific Cys residues, including Cys13 in ferritin as well as Cys76 and Cys108 in nucleoside diphosphate kinase. The identified thiol-oxidized proteins provide information about the metabolic pathways and/or physiological processes affected by pollutant-elicited oxidative stress.

1. Introduction

Doñana National Park (DNP) is a 50,720 ha wildlife reserve located in Huelva province (Andalusia, SW Spain). Due to the variety of ecosystems, plants and animal species it harbors, DNP was declared a UNESCO Biosphere Reserve in 1981, a Ramsar Site in 1982 and a World Heritage Site in 1994. Despite the environmental relevance and sensitivity of the park, it has been exposed to increasing ecological pressures, as diverse human activities carried out in its surroundings threaten its ecosystems in the following ways:

1) DNP is under the influence of industries (petrochemical, fertilizer, paint) located at Huelva Estuary, 40 km to the west. The estuary is formed by the Odiel and Tinto Rivers, which also carry metals from pyrite mines located north of Huelva (Montes-Nieto et al., 2007; 2010).
2) Early studies showed that wind and the water courses that feed the Doñana marshes carry most of the contaminants (Albaiges et al., 1987). Doñana is watered by the *Rocina*, *Partido* and *Guadiamar*

Abbreviations: AM-labeled, acetamido-labeled; AF-labeled, acetamidofluorescein-labeled; 2-DE, two-dimensional electrophoresis; IAM, iodoacetamide; IAF, 5-iodoacetamidofluorescein; IEF, isoelectric focusing; LP, *Lucio del Palacio*; MAT, *Matochal*; PAR, *Partido* stream; PTMs, post-translational modifications; ROC, *Rocina* stream; ROS, reactive oxygen species; RNS, reactive nitrogen species; SSH, suppression subtractive hybridization; TBP, tributylphosphine.

* Corresponding author at: Department of Biochemistry and Molecular Biology, University of Córdoba, Rabanales Campus, S. Ochoa Building, 2nd floor, 14071 Córdoba, Spain. Tel.: +34 957 218139; fax: +34 957 218082.
E-mail address: bb1lobaj@uco.es (J. López-Barea).

[1] Both authors contributed equally to this work and should be considered first authors.

http://dx.doi.org/10.1016/j.scitotenv.2014.04.117
0048-9697/

streams and the Guadalquivir River which are surrounded by agricultural areas. To the NE, the marshes between the Guadiamar stream and Guadalquivir River are responsible for most Spanish rice production. To the NW, near the *Partido* and *Rocina* streams, strawberry and citrus are cultured using large amounts of agrochemicals.

3) In 1998, the tailings dam of the Aznalcóllar pyrite mine, 60 km to the north, collapsed and released acidic water and mud with high levels of toxic metals into the course of the Guadiamar. Although the spill did not have a major effect in Doñana, due to the early implementation of an emergency plan (Grimalt et al., 1999), it alarmed the authorities and the scientific community, raising concern about the presence of contaminants unrelated to the Aznalcollar spill in DNP (Pueyo et al., 2011). In fact, most of recent studies point to agriculture as the main threat to DNP because of the high level of agrochemical use in nearby areas (Bonilla-Valverde et al., 2004; Gomara et al., 2008; Pueyo et al., 2011; Ruíz-Laguna et al., 2001; Vioque-Fernández et al., 2009). The most serious gap in the knowledge about environmental quality within Doñana is related to agrochemical pollution, as this type of contamination is diffuse and difficult to assess (Gomara et al., 2008; Pueyo et al., 2011).

In environmental assessments, sentinel organisms serve as bioindicators in which biomarkers are measured to determine pollutant exposure, effects or risks (Livingstone, 1993; López-Barea, 1995). Most such studies are concentrated in polluted environments, whereas they are scarce in natural ecosystems, such as national parks (Pueyo et al., 2011). The decapod crustacean *Procambarus clarkii* (red crayfish), native to southeastern USA, was introduced to the Doñana marshes (SW Spain, Fig. 1) in 1973, subsequently generating dense populations and becoming an invasive species. *P. clarkii* can be used in the assessment of fresh water ecosystems, particularly to study the effects of metals and pesticides near DNP (Martin-Diaz et al., 2006; Pueyo et al., 2011; Vioque-Fernández et al., 2007b, 2009). The use of conventional biomarkers (esterases, biotransforming enzymes, oxidative stress) in pollution studies is biased because these markers concentrate in few proteins, thus most of the other proteins that are altered, but whose relationship to pollution is still unknown, would be ignored (López-Barea and Gómez-Ariza, 2006). "Omic" approaches have become powerful tools in environmental studies, as they allow the identification of novel biomarkers as well as the elucidation of toxicity mechanisms or proteins that are involved in toxic responses that have not been described previously (Montes-Nieto et al., 2007; Pueyo et al., 2011; Vioque-Fernández et al., 2009). After the identification of key proteins indicating exposure or effects, omic analyses can be used in risk assessments (Abril et al., 2011; Pueyo et al., 2011).

Several types of pollutants, such as metals and oxidative organic chemicals, generate reactive O and N species (ROS/RNS) that induce oxidative stress (Braconi et al., 2011; López-Barea, 1995). When such oxidative damage is excessive, it ends in cell death and various pathologies (Butterfield and Dalle-Donne, 2012; Dalle-Donne et al., 2006; Eaton, 2006; Roberts et al., 2009). ROS/RNS also play key roles in signaling (Eaton, 2006; Tell, 2006). Proteins are major targets of ROS/RNS, in which many post-translational modifications have been described (Butterfield and Dalle-Donne, 2012; Cabiscol and Ros, 2006; Davies, 2005) with essential roles in cellular localization, protein–protein interactions, and protein structure or biological activity (Cabiscol and Ros, 2006; Hwang et al., 2009; Lee et al., 2009; Yamakura and Kawasaki, 2010).

Cysteine is a key residue involved in protein catalysis, oxidative folding/trafficking, redox signaling and regulation. However, its unique redox properties also render Cys vulnerable to many electrophiles, especially ROS/RNS, leading to redox modifications and alteration of protein structure, function, or redox signaling (Ying et al., 2007). Although Cys residues are rare, representing 1–3% of total residues, protein thiols (10–30 mM) are more abundant than

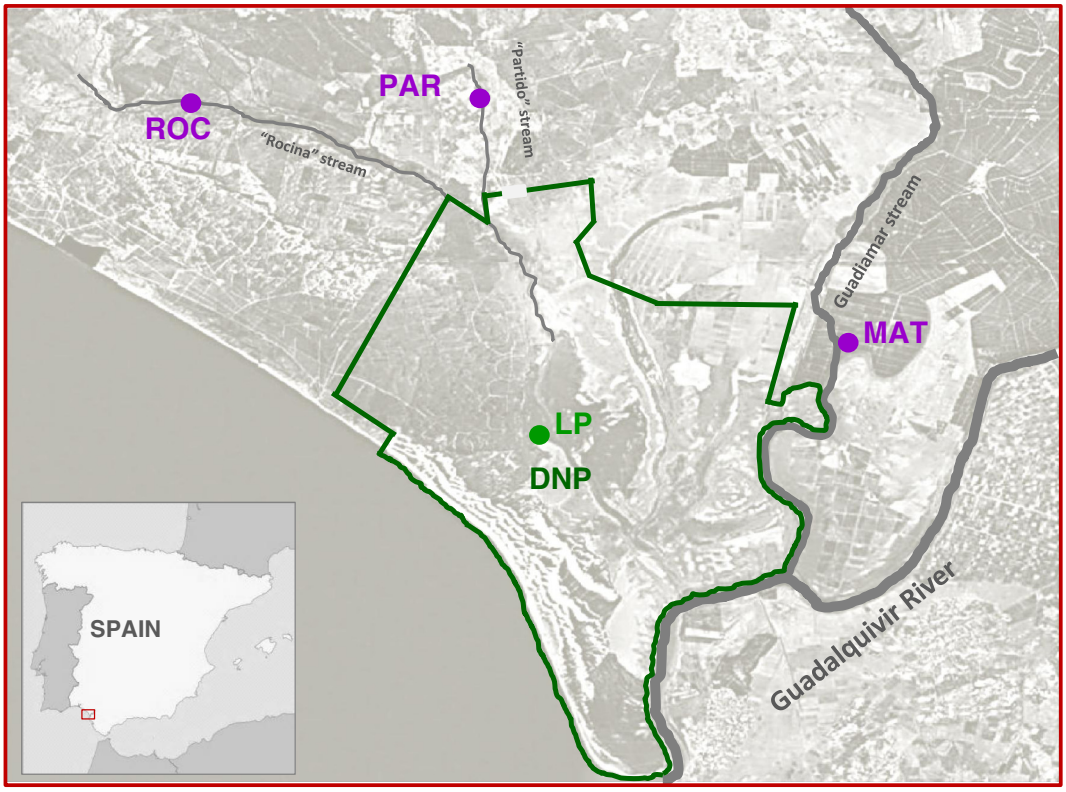

Fig. 1. Map of Doñana National Park (DNP) and its surroundings showing the sites where crayfish (*P. clarkii*) where collected. The locations of the sites (and their UTM coordinates) are indicated: *Lucio del Palacio* (LP, X = 193,800, Y = 4,099,515); *el Matochal* (MAT, X = 208,681, Y = 4,102,207); *Partido* stream (PAR, X = 191,173, Y = 4,124,977) and *Rocina* stream (ROC, X = 178,653, Y = 4,119,937).