Final project report

Project ID	2004/1.5
Title	Low temperature adaptation of osmoregulatory mechanisms in Antarctic teleosts
Principal investigator	Maffia Michele
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Duration	3 years
Assigned funding	60000,00 Euro

Activities and results

Antarctic marine heterotherms live, grow and reproduce in waters at a temperature constantly under 0°C, having developed efficient adaptive answers that permit their survival. Besides the physical problem of ice formation in the body, it must be considered that low temperatures thermodynamically impair all the biochemical processes with the slowing down of all biological activities until a limit beyond which they stop at all. Among these processes, the osmoregulatory system represents a very conspicuous part of the energetic cost for basal metabolism in fish. The physiological osmoregulatory systems represent therefore a crucial issue of the overall study of the adaptive evolution of Antarctic teleosts. Their adaptation allowed the maintenance of efficient biological mechanisms for water, ion, nutrients and catabolites exchange between the organism and its environment also in very unfavourable thermodynamic conditions such us the below zero water of the Antarctic seas. The research activity carried out in the context of this project has allowed to clarify various elements of the adaptation relative to these aspects. The study was focused on gill and intestine of some species of Antarctic teleosts, organs specialized in the control and regulation of the hydro-saline homeostasis. Fresh tissues were used for the analysis of the kinetic parameters of enzymatic and carrier proteins involved in the osmoregulatory processes. Frozen and fixed tissues were used for the studies of cellular localization and characterization of transporters and enzymatic proteins, for RNA and DNA extraction and amplification and sequencing of specific proteins. The cloning allowed us to obtain sufficient amount of protein for bio-structural analysis and the expression of transport and enzymatic protein in heterologous systems. In this regard, the functional and structural characterization of the intestinal peptide transporter of the Antarctic icefish *Chionodraco hamatus* (Ice-PEPT1) is in progress. The cDNA of this transporter has been completely sequenced (Rizzello et al., GenBank accession number AY170828.2) and expressed in the *Xenopus laevis* oocytes. The structural and functional characterization of this transporter has allowed the identification of a particular region localized near the C-terminus of the carrier that appears to be involved in its adaptation to cold. Inserting this specific region of the Ice-PEPT1 in a homologous protein of a mammal (rabbit PEPT1) through mutagenesis experiments, we obtained some interesting results (Rizzello et al., submitted) that could represent the rationale for the development of biotechnological applications in different fields.

Bio-structural analyses were also performed to characterize the carbonic anhydrase (CA), a zinc-protein with a key role in pH regulation and trans-epithelial ion translocation. These studies led to the complete structural characterization of gill CA of the icefish *C. hamatus* [Rizzello et al., Protein Journal, 26(5): 335-348 (2007)]. The comparison of CA from the Antarctic organisms with the correspondents from temperate organisms allowed us to identify the existing correlations between the differences in thermodynamic properties and the primary and three-dimensional structure protein [Marino et al., Biophys. J., 93: 2781-2790 (2007); Chiuri et al., Biophys J. 96(4):1586-96. (2009)].

Products

A – papers in scientific magazines

1. P. Katharios, J. Iliopoulou-Geougudaki, S. Antimisiaris, T. Verri, P. Toma, R. Acierno, Maffia M. (2004). Pharmacokinetics of cephalexin in sea bream, Sparus Aurata (L.), after a single intraperitoneal injection. Journal of applied ichthyology. vol. 20, pp. 1-5 ISSN: 0175-8659.
2. L. Blasi, L. Longo, P.P. Pompa, L. Manna, G. Ciccarella, G. Vasapollo, R. Cingolani, R. Rinaldi, A. Rizzello, R. Acierno, C. Storelli and M. Maffia. Formation and characterization of glutamate dehydrogenase monolayers on silicon supports. Biosensors and Bioelectronics, 21: 30-40 (2005). **IF 4.132**
3. L. Blasi, L. Longo, G.Vasapollo, R. Cingolati, R.Rinaldi, T. Rizzello, R. Acierno, M. Maffia. Characterization of glutamate dehydrogenase immobilization on silica surface by atomic force microscopy and kinetic analyses. Enzyme and Microbial Technology, 36: 818-823 (2005). **IF 1.897**
4. Romano A, Kottra G, Barca A, Tiso N, Maffia M., Argenton F, Daniel H, Storelli C, Verri T. (2006). High-affinity peptide transporter PEPT2 (SLC15A2) of the *zebrafish Danio rerio*: functional properties, genomic organization and expression analysis. PHYSIOLOGICAL GENOMICS. vol. 24, pp. 207-217 ISSN: 1094-8341. 2005 Nov 29 published on line.
5. A. Rizzello, M.A. Ciardiello, R. Acierno, V. Carratore, T. Verri, G. di Prisco, C. Storelli and M. Maffia. Biochemical characterization of a S-glutathionylated carbonic anhydrase isolated from gills of the Antarctic icefish *Chionodraco hamatus*. The Protein Journal, 26(5): 335-348 (2007). **IF 0.962**
6. S. Marino, K. Huyakawa, K. Hatada, M. Benfatto, A. Rizzello, M. Maffia and L. Bubacco. Structural features that govern enzymatic activity in carbonic anhydrase from a low temperature adapted fish *Chionodraco hamatus*. Biophysical Journal, 93: 2781-2790 (2007). **IF 4.757**
7. M.G. Lionetto, Rizzello A., Giordano M.E., Maffia M., De Nuccio F., Nicolardi G., Hoffmann E.K., Schettino T. Molecular and functional expression of high conductance Ca^{2+} activated K^{+} channels in the eel intestinal epithelium. Cellular Physiology and Biochemistry. 21:361-372 (2008). **IF 3.558**
8. R. Chiuri, Maiorano G., Rizzello A., del Mercato L.L., Cingolati R., Rinaldi R., Maffia M., Pompa P.P. Exploring local flexibility/rigidità in psychrophilic and mesophilic carbonic anhydrases. Biophys J. 96(4):1586-96. (2009). **IF 4.757**

B – book chapters

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C - proceedings of international conferences

1. A. Rizzello, R. Acierno, T. Verri, I. Zizzo, C. Storelli and M. Maffia. Partial sequencing and tissutal distribution of a H+/Peptide transporter in the haemoglobinless Antarctic teleost Chionodraco hamatus. Pflügers Archiv - European Journal of Physiology. Vol. 448, pp R55, ISSN: 0031-6768 (2004).
2. T. Verri. G. , A. Romano, N. Tiso, M. Peric, M. Maffia, M. Boll, F. Argenton, H. Daniel, C. Storelli (2004). Molecular/Functional Characterization of the Zebrafish intestinal Peptide Transporter PEPT1". Pflügers Archiv - European Journal of Physiology. Vol. 448, pp R58, ISSN: 0031-6768 (2004).
3. R. Acierno, A. Rizzello, M. Maffia. Transport protein adaptation in Antarctic teleost. Polarnet Technical Report. Proceedings of the Fifth PNRA Meeting on Antarctic Biology, 106-110 (2004).

D – proceedings of national meetings and conferences

1. Rizzello A., Maffia M. "Structure and function of gill carbonic anhydrase in the Antarctic fish" Workshop del settore di Biologia del Progetto Nazionale di ricerche in Antartide, Messina, 29-30 aprile 2004.
2. Maffia M., Rizzello A "Characterization of the plasma-membrane proton-peptide cotransporter in Chionodraco hamatus intestine" Workshop del settore di Biologia del Progetto Nazionale di ricerche in Antartide, Messina, 29-30 aprile 2004.
3. M. Maffia, A. Rizzello, R. Acierno, T. Verri, C. Storelli. Intestinal peptide transporter of the antarctic haemoglobinless teleost Chionodraco hamatus. 55° Congresso Nazionale della Società Italiana di Fisiologia (SIF), Pisa (Italia) 4-7 ottobre 2004.
4. A. Rizzello, M.A. Ciardiello, R. Acierno, V. Carratore, T. Verri, G. di Prisco, C. Storelli and M. Maffia. Structural and functional characterisation of gill carbonic anhydrase of the Antarctic icefish Chionodraco hamatus. 57° Congresso Nazionale della Società Italiana di Fisiologia (SIF), Ravenna (Italia) 25-27 settembre 2006.
5. M. Maffia, A. Rizzello, T. Verri, C. Storelli and R. Acierno. Molecular aspects of the adaptation to low temperature of functional protein in Antarctic poikilotherms. 57° Congresso Nazionale della Società Italiana di Fisiologia (SIF), Ravenna (Italia) 25-27 settembre 2006, Acta Physiologica 2006; Volume 188, Supplement 652.
6. A. Rizzello, A. Romano, G. Kottra, R. Acierno, A. Ferrara, T. Verri, C. Storelli, H. Daniel, M. Maffia, Molecular and functional characterisation of an intestinal peptide transporter in the Antarctic icefish Chionodraco hamatus. 58° Congresso Nazionale della Società Italiana di Fisiologia (SIF), Lecce (Italia) 19-21 settembre 2007, Acta Physiologica 2007; Volume 191, Supplement 657.

E – thematic maps

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F – patents, prototypes and data bases

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G – exhibits, organization of conferences, editing and similar

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H - formation (PhD thesis, research fellowships, etc.)

1. **Antonia Rizzello -** Tesi di dottorato in "Ecologia fondamentale", Titolo: Study of the eco-physiological adaptations of the Antarctic Teleosts", Università del Salento 2002-2005

Research units

Research Unit 1	Research Unit 2	Research Unit 3	Research Unit 4
Michele Maffia	Oscar Moran	Luigi Bubacco	Maria Angela Masini
Carlo Storelli	Franco Conti	Mariano Beltramini	Bianca Maria Uva
Raffaele Acierno	Michael Push	Benedetto Salvato	Mario Pestarino
Tomaso Patarnello	Alessandra Picollo	Elisabetta Bergantino	Grazia Tagliaferro
Antonia Rizzello	Chiara Pincin	Paolo Di Mauro	Maddalena Sturla
Antonio Danieli	Laura Elia	Stefano Vanin	Martina Pastorino
Tiziano Verri	Elena Babini	Adriana Zagari	Sara Ferrando
Giuseppe Alfredo Pede	Loretta Ferrera	Luciana Esposito	Maria Carmela Cerra
Marilena Greco		Rita Berisio	
		Ilaria Bruno	
		Giosue' Sorrentino	

Date: May 20, 2010
