

EFFECTS OF THE SEA WATER OSMOCONCENTRATION CHANGES ON OXIDATIVE PROCESSES IN ISOLATED GILL OF SHORE CRAB *CARCINUS MEDITERRANEUS* CSRN

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The gills of marine organisms play an important physiological role in respiration, osmoregulation and volume and acid-base regulation. Although the significance of the gills in ion transport functions in the Crustacean is evidently recognized (LUCU, 1990), the relationship between their respiratory and ion-regulatory functions has been pointed out only by few studies, not very recent (ENGEL, 1975).

The rate of oxygen consumption was measured in the posterior gills isolated from the shore crabs *Carcinus mediterraneus* Csrn collected at the coastline of the Istrian peninsula (Northern Adriatic). The gills from the intermoulting adult crabs were excised and perfused (LUCU and SIEBERS, 1986) before insertion into the oxygen chamber with an identical diluted solution in which OCR (oxygen consumption rate) was measured. Measurements were performed in a closed experimental system in the perspex chamber. A radiometer PO₂ electrode (E 5046) was protruded through the cover into the chamber and the OCR was measured by digital PHM 72 radiometer analyzer (Copenhagen) with technical details described by LUCU and PAVICIC (in prep.)

The main anaerobic end product in Crustacea lactate (HILL *et al.*, 1991) was affected by the sea water osmoconcentration changes. The mean concentration of lactate in the blood was not changed significantly in the crabs acclimated to the sea water in the salinity range from 11.0 to 38 x 10⁻³.

When gills were isolated from the crabs acclimated to various diluted sea water concentrations, the OCR was salinity-dependent and considerably increased as sea water concentration decreased. Prior to respiration measurement isolated gills were perfused by 2.5 x 10⁻³ mol l⁻¹ ouabain dissolved in 50 per cent DSW (diluted sea water) and incubated in the respiration chamber. OCR was depressed by ouabain by approximately 30 per cent compared to the control solution (table 1). In the gills immersed in the N-methyl glucamine solution containing 250 mmol Cl⁻ l⁻¹, the OCR of the isolated preparation was close to zero, but only by the sodium substitution increased steadily. The OCR in which chloride plays a minor role, is a function of the sodium concentration changes. V_{max} was reached at 465 µl O₂ h⁻¹ per gram gill wet weight and K_m at the sodium concentration of 16.8 mmol l⁻¹. Moreover, in the K-free and Ca-free saline, oxygen consumption of the excised gills was also reduced, supporting the indispensable role of the potassium and calcium ions in the respiration processes (table 1).

Dilute sea water		Physiological saline			
Control	Ouabain	Control	K-free	Ca-free	Mg-free
		(µl O ₂ x h ⁻¹ per gram gill w.w.)			
526.19±26	365.78±36	593.41±43	362.47±33	478.15±44	573±48
	P < 0.01*		P < 0.01	P < 0.01	P > 0.05

Table 1. Effect of ouabain (2 x 10⁻³ mol l⁻¹) on gill respiration measured in the isolated posterior gills from *Carcinus mediterraneus* incubated in the DSW (260 mmol Cl/l). The gill was isolated from the crabs acclimated for 2 weeks in DSW. The mean respiration rate was measured after it reached a steady-rate level in the isolated gills immersed in the control artificial saline (in mmol/l; NaCl, 260; KCl, 5; MgCl₂, 4; HEPES, 5; pH, 8) and compared with the gill respiration rates measured in K-free, Ca-free and Mg-free saline. In the K,Ca and Mg-free solution appropriate ions were substituted by isoosmotic NaCl (N = 6 for each mean ± S.E.; * significantly different from corresponding control group). w.w. = wet weigh.

The results suggest that a portion of the energy liberated by the gill respiration is utilized by the gill Na,K ATPase enzyme complex maintaining Na and K concentration gradients between the extracellular and intracellular compartments.

In the Ca-free saline containing 0.1 mmol/l EGTA the OCR was reduced by about 19 per cent relative to the control Ca containing saline. ATP synthesis could be controlled by the supply of energy to the electron transport chain which is in turn controlled by cytosolic free calcium levels. It is known that in mitochondria Ca is coupled with H pumping providing an electrochemical gradient or proton promotion force which is used to generate synthesis of ATP from ADP and P_i. 50 per cent inhibited OCR by KCN was attained at 1 µmol l⁻¹. We suggest that electron transfer chain was blocked, and consequently oxidative capacity mediated via cytochrome oxidase activity was diminished.

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