Characterization of glucidic metabolism of pacific oyster, *Crassostrea gigas*, as a way to study the reserves management

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INTRODUCTION: The reserves of *Crassostrea gigas*

- The reserves are stored in a specialized tissue:

- **This tissue is constituted by vesicular cells.**

- The vesicular cells are specialized in *storage and mobilization* of glycogen.

- **Glycogen** is the major source of energy supporting general metabolism and particularly during reproduction.
INTRODUCTION: glucose transport and these regulations

1st stage of regulation on transport of glucose

2nd stage of regulation on synthesis or degradation of glycogen
I. Characterization of glucidic metabolism

I.1. Parameters of Glucose uptake: global mechanism

I.2. Systems of glucose transport: Existence of different transporters?

II. Seasonal variations

III. Experimental trophic conditioning
I. Characterization of glucidic metabolism

I.1. Parameters of glucose uptake: global mechanism
Results: Kinetic of glucose uptake and effect of extracellular concentration

- Equilibrium is obtained after 3 hours for the highest concentration of substrate.
- Maximal uptake is ≈ 0.3 nmol/10⁶ cells.

(2 hours and 0.33 nmol/10⁶ cells for *Mytilus edulis*, Lenoir 1989)
Results: determination of two components of glucose uptake

Existence of two components: a passive diffusion and a saturable component.

The Characteristics of saturable component were calculated with Michaelis-Menten transformation. (Km = 1.74 mM and Vmax = 0.1057 nmoles/h/10^6 cells)
Results: determination of two components of glucose uptake

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I. Characterization of glucidic metabolism

I.2. Systems of glucose transport: Existence of different transporters?
Results: Different systems of glucose transport?

- **Cytochalasin B**: an effective inhibitor of saturable diffusion (GLUT) (Keller et al., 1989)
- **Phloridzin**: a specific inhibitor of Na+-glucose transport (SGLT) (Hediger et al., 1987)

\[\text{D-glucose uptake (\%)}\]

- **Phloridzin**: 1mM ~ 60%
- **Cytochalasin B**: 10\(\mu\)M ~ 40%
Conclusion: presence of different systems of glucose transport?

1. Passive Diffusion
   - Glucose transport via GLUT transporter
   - Sodium-potassium Pump

2. Saturable Diffusion (GLUT)
   - Glucose transport via GLUT transporter

3. Na+/glucose transport (SGLT)
   - SGLT Sequence available (AY551089, Huvet et al., 2004)
II. Seasonal Variations

Evolution of uptake parameters during the seasons
Results: seasonal variations of the capacity of glucose transport

Difference on the capacity for glucose transport in February and April.

**Interpretation**: The system of glucose transport was different on the two sampling dates. (variability of the number of glucose transporters? Modification on the affinity?)
Results: variations of saturable component

<table>
<thead>
<tr>
<th></th>
<th>Km (mM)</th>
<th>Diffusion (nM/h/10^6 cells)</th>
<th>Vmax (nmol/h/10^6 cells)</th>
</tr>
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<tbody>
<tr>
<td><strong>February</strong></td>
<td>1.78</td>
<td>0.05</td>
<td>0.033</td>
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<td><strong>April</strong></td>
<td>0.95</td>
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<td>0.117</td>
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The lower the value of Km, the higher the ligand affinity to substrate.
Results: comparisons between the constants of Michaelis and the effect of inhibitors

Results are coherent: stronger effect of cytochalasin B during April (GLUT=high affinity for glucose) than February-March.

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Hypothesis: influence of glucose concentration on the uptake?

1. Passive Diffusion
   - Glucose
   - GLUT transporter
   - Sodium-potassium Pump
   - SGLT (Sodium Glucose transporter)

2. Saturable Diffusion (GLUT)
   - 1st step of regulation on transport of glucose
   - Glucose
   - GLUT transporter

3. Na+/glucose transport (SGLT)
   - SGLT Sequence available (AY551089, Huvet et al., 2004)
Results: influence of the concentration of glucose on his uptake and the expression of SGLT?

Method: Different concentrations of glucose during 2 days of incubation (0 to 10 mM) of vesicular cells

Expression of SGLT

* : significant difference  \( p<0.05 \) (test Student-Newman-Keuls)

Expression of SGLT is regulated by the extracellular concentration of glucose. The maximum of expression is obtained for the cells which were in a low concentration of glucose.

→ The high concentrations of glucose regulate negatively the expression of SGLT transporters

This analyze is in process.
III. Experimental Trophic Conditioning

IFREMER (Brest) – University (Caen) Collaboration, with H. Bacca

Effect of the food on glucose metabolism
Reproductive cycle of *Crassostrea gigas*

- **Gametogenesis**
  - June - July
  - October - November
  - March - April

- **Reserves**
  - Mobilization
  - Storage
  - Mobilization

- **Aim**: Study reserves management during these 3 periods
Materials and Methods

Fast (A-) or Abundant diet (A+)
(Skeletonema costatum: 120 cells/μL or fast)

SYstème Contrôlé d’Ambiance MARine

- Cryopreservation of cells
- Tests of cell’s survey and metabolic tests
- Measurement of quantity of glycogen reserves
- Study of the gonad development
- Expression of SGLT

T = 0
Natural reference = control

T = 30 days
Results: Evolution of glycogen reserves

**R** : no significant differences between T0=“beginning of the experiment” (natural reference) and TF=“End of the experiment”; but for the fast diet the glycogen decreased significantly (A- with p=0.0001)

→ At fast diet: ability to mobilize the reserves of glycogen?

**S** : no differences for each condition in term of glycogen storage
Results: Expression of SGLT by real-Time PCR

- R and S showed no significant differences of SGLT expression.

BUT

- the level of SGLT expression is linked to trophic level:
  higher for fast diets: facilitating the glucose uptake and the storage of glycogen during the autumnal period when the nutritive resources are poor.
CONCLUSION

Characterization of glucidic metabolism:

- Existence of two components: a passive diffusion and a saturable component.
- The characteristics of the saturable component can be obtained by Michaelis-Menten transformation.

Seasonal variations:

Difference of the capacity of glucose transport during the year.
Interpretation: The system of glucose transport is modified. (variability of the number of glucose transporters? Modification of the affinity?)

Experimental trophic conditioning:

R oysters are able to use their reserves during fast period contrary to S oysters. 
R and S have not significant differences of SGLT expression but the SGLT expression is linked to trophic level: higher for fast diet: facilitating the glucose uptake and the storage of glycogen during the autumnal period when the nutritive resources are poor.

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Thanks