

**INCUBATION AND PH-DEPENDENT EFFECTS
OF VANADATE OLIGOMERS AND CADMIUM
WITH *Halobatrachus didactylus* SARCOPLASMATIC RETICULUM
CALCIUM PUMP**

Lília Leonardo
Biochemistry Lab., CMQA, Chemistry Dept., FCT
University of Algarve, Campus de Gambelas, 8000-810 Faro, PORTUGAL
Phone: +351 89 800 900; Fax: +351 89 818 353
E-mail: lilialeonardo@hotmail.com

S.S. Soares¹, N. Joaquim¹, J.M. Coucelo.¹ and M. Aureliano²
¹ Group of Comparative Cardiovascular , CCMar, Faculty of Marine and
Environmental Sciences
² Biochemistry Lab., CMQA, Chemistry Dept., FCT
University of Algarve, Campus de Gambelas, 8000-810 Faro, PORTUGAL

EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The Ca²⁺-ATPase of sarcoplasmic reticulum (SR) membrane is a transmembranar protein that catalyses both the hydrolysis and synthesis of ATP (Chini et al; 1993) playing an essential role in relaxation and contraction of the muscle. The activity of Ca²⁺-ATPase is normally described by a cycle postulating the existence of two protein conformations, E1 and E2, with high and low affinity for Ca²⁺ and ATP, respectively (Inesi, 1985). The alternation between the two distinct conformations of Ca²⁺-ATPase during the transport cycle seems affect the interactions with different oligomeric species of vanadate (e.g., decameric and tetrameric) that affects the activity of the calcium pump (Aureliano and Madeira, 1998). Such as, vanadium, cadmium is well known by its toxic effects in live organisms, being an inhibitor of the calcium pump even at very low concentrations.

In this work we compare the effects of cadmium and vanadium on the ATP hydrolysis by the calcium pump at different pH values and incubation times from skeletal muscle of *Halobatrachus didactylus* (Schneider, 1801).

Material and Methods

Ca²⁺-ATPase isolation and characterization

SR vesicles derived from *H. didactylus* – Lusitanian toadfish – skeletal muscle were prepared as described elsewhere (Aureliano and Madeira, 1994).

Metal stock solutions and vanadate stability

Cadmium stock solution (50 mM) was prepared from cadmium chloride. Vanadate stock solutions (50 mM) – “metavanadate” and “decavanadate” – were prepared from ammonium metavanadate, according to described elsewhere (Aureliano and Madeira, 1994). The stability of vanadate solutions at the different experimental conditions was analysed by measuring the absorbance at 400 nm.

Hydrolysis of ATP by calcium pump

ATP hydrolysis was measured by colorimetry, through inorganic phosphate analysis. Experiments were proceeded at 25°C in a reaction medium containing 0.1 M KCl, 25 mM HEPES, 5 mM MgCl₂, 50 mM CaCl₂, pH 6.0, 7.0 or 8.0, in the absence and presence of 2 µM of cadmium or 2 mM (total vanadium) of “metavanadate” or “decavanadate”, with or without 1 hour incubation with 0.285 mg/ml protein. The reaction was started by the addition of 420 mM Mg-ATP.

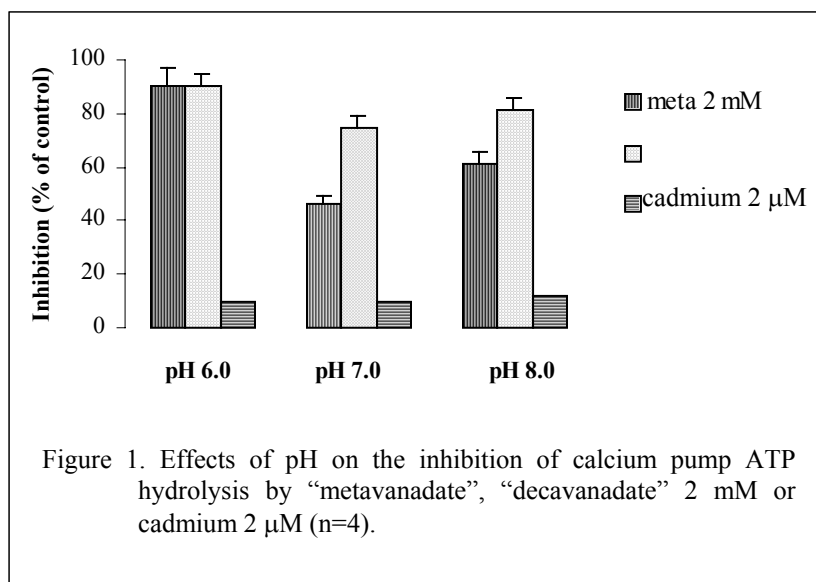
Results

pH effects on the vanadate solutions stability

The “decavanadate” solutions stability decreases with pH (pH 6.0>7.0>8.0) whereas “metavanadate” solutions are stable.

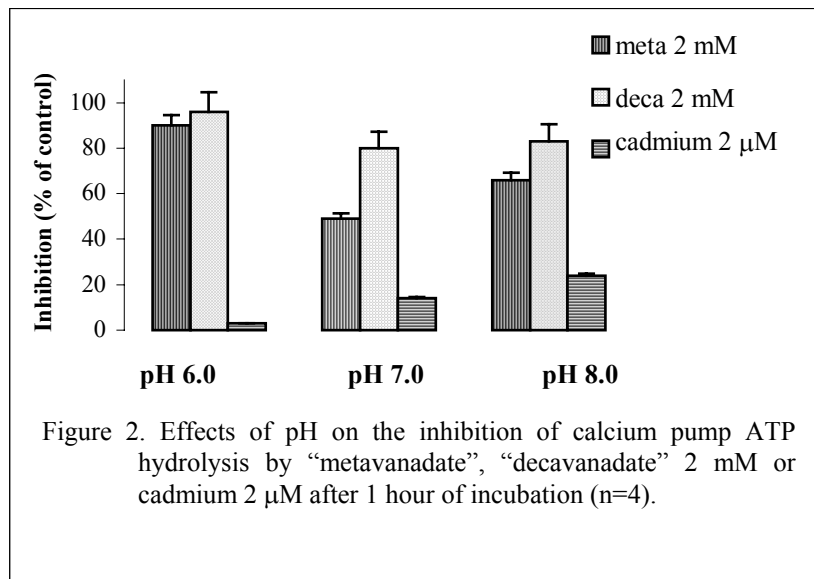
pH effects on Ca²⁺-ATPase activity in vanadium or cadmium inhibition

The ATP hydrolysis by the SR Ca²⁺-ATPase is more strongly affected by “decavanadate” than “metavanadate” and it depends on the pH. The relative inhibitory effects for “metavanadate” and “decavanadate” regarding pH was: pH 6.0>8.0>7.0, whereas for cadmium the ATP hydrolysis was more inhibit at pH 8.0 (pH 8.0>7.0>6.0) (Figure 1).



Effects of Ca²⁺-ATPase incubation with vanadium or cadmium

After 60 minutes of incubation “decavanadate” inhibition on ATP hydrolysis is always higher than “metavanadate”. For both vanadate solutions the relative order of inhibition upon pH is: pH 6.0>8.0>7.0. In fact, the lowest inhibition is observed at pH 7.0 where “metavanadate” and “decavanadate” affect by 49% and 80%, respectively the ATPase activity. Unlike vanadium, the protein incubation with cadmium increases the inhibition of ATP hydrolysis within pH increase from 3% to 24% (Figure 2).



Conclusions

It is concluded that, at pHs 6.0, 7.0 and 8.0, cadmium inhibits strongly calcium pump ATP hydrolysis and the cadmium incubation with the protein favours enzymatic inhibition. “Decavanadate” affects more strongly the ATP hydrolysis by Ca^{2+} -ATPase than “metavanadate”, being the relative order of inhibition affected by pH as followed: pH 6.0>8.0>7.0. It is suggested that oligomeric species of vanadate inhibition of ATP hydrolysis is favoured by the E2 conformation (pH 6.0). Further studies are in course to clarify contribution of vanadate species to vanadium interaction with the calcium pump of *Halobatrachus didactylus*.

Acknowledgements

Sandra Soares thanks to Portuguese Foundation for Science and Technology, FCT and by POCTI program financed through FEDER. Research project 38191/QUI/2001 and University of Algarve.

References

- Aureliano, M. and V.M.C. Madeira. (1994). Interactions of vanadate oligomers with sarcoplasmic reticulum Ca^{2+} -ATPase. *Biochim. Biophys. Acta* 1221: 259-271
- Chini, E.N., F.G.S. de Toledo, M.C. Albuquerque and L. de Meis (1993). The Ca^{2+} -transporting ATPases of rabbit and trout exhibit different pH- and temperature-dependences. *Biochem. J.* 293: 469-473
- Inesi, G. (1985). Mechanism of calcium transport. *Annu. Rev. Physiol.* 47: 573-604
- M. Aureliano and V. M. C. Madeira (1998) Vanadium in the Environment. Part 1: Chemistry and Biochemistry, Wiley & Sons, Inc., New York, pp. 333—357

