Calcium transport and phosphomonohydrolase activity by proteoliposomes harboring Annexin V and Alkaline Phosphatase

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INTRODUCTION

The biomineralization process is initiated inside matrix vesicles (MVs). The initial crystalline hydroxyapatite (HA) generation and its deposition is accomplished by the activities of several proteins, involved in Ca^{2+} and phosphate (Pi) homeostasis, among them Annexins (AnxV), Pi-transporters, PHOSPHO1 and tissue-nonspecific alkaline phosphatase (TNAP). Anx V mediates Ca^{2+} influx and TNAP plays a key role in calcifying bone and cartilage. Dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylserine (DPPS) are found in the MVs membranes and play a crucial role in the biomineralization process, regulating both Ca^{2+} entry into the MVs and formation of HA crystals.

MATERIAL and METHODS

- Rat bone marrow cells and Membrane-bound TNAP was prepared according to Simão et al. [1].
- Liposomes were prepared by the extrusion method as described in Bolean et al. 2010 [2].
- AnxV-containing proteoliposomes (10 µg of protein) were also incubated at 37ºC for 24h in medium (50 mM Tes (N-tris(hydroxymethyl) methyl-2-amino-ethanesulfonic acid), pH 7.65, 85 mM NaCl, 15 mM KCl, 1 mM $MgCl_2$, 30 mM $NaHCO_3$, 1.35 mM $CaCl_2$, 1.97 mM Pi, and 1 mM ATP) containing 25, 50 or 75 µCi.mL^{-1} $^{45}Ca^{2+}$. The $^{45}Ca^{2+}$ influx was determined by scintillation counting.

RESULTS

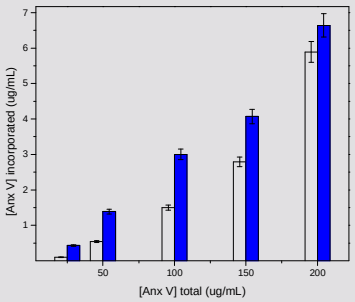


Figure 1: AnxV incorporation into different liposome compositions: [White] DPPC-liposomes; [Blue] DPPC:DPPS 10% - liposomes. The AnxV was able to incorporate into DPPC-proteoliposomes. Moreover, when DPPS was used, we had an significantly increase in protein incorporation.

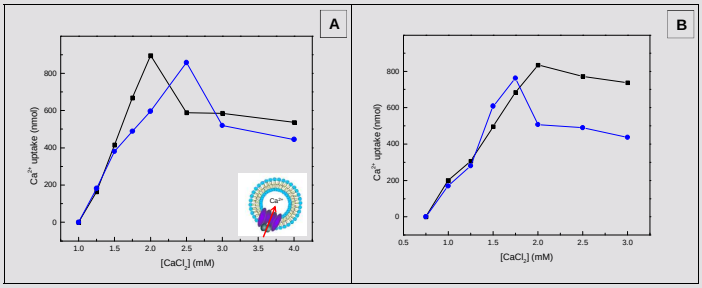
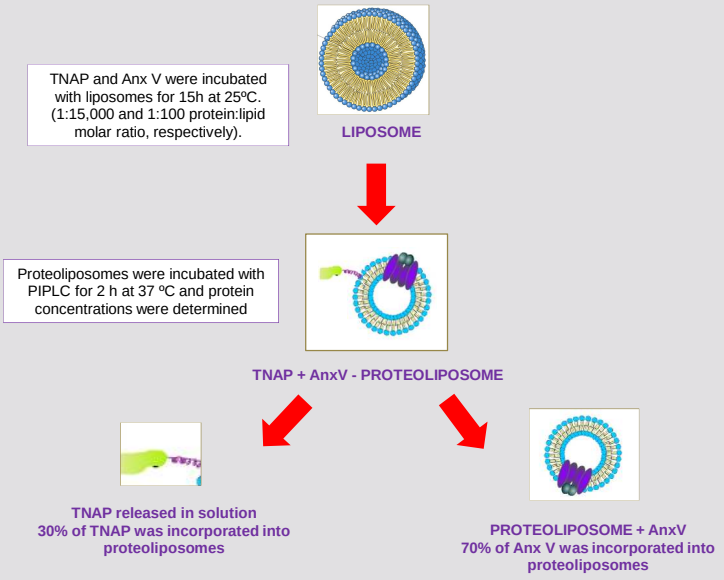


Figure 2. [A] Study of Ca^{2+} uptake mediated by AnxV incorporated into different proteoliposomes compositions: (▬) DPPC - proteoliposomes and (▬) DPPC:DPPS 10% - proteoliposomes. [B] Study of Ca^{2+} uptake mediated by AnxV incorporated into different proteoliposomes compositions in the presence of TNAP: (▬) DPPC-proteoliposomes and (▬) DPPC:DPPS 10%-proteoliposomes. The proteoliposomes (10 µg of protein) were incubated with a fixed concentration of $^{45}Ca^{2+}$ (5.5 µCi.mL^{-1}) and increasing concentrations of Ca^{2+} (from 1 mM to 5 mM), resulting in a linear increased uptake, reaching a maximum with 2 mM Ca^{2+}. Thus, around 0.8 µmol Ca^{2+} was incorporated into the vesicles, with a similar profile for all proteoliposomes curves. The presence of TNAP in the proteoliposomes containing both proteins did not affect significantly AnxV-mediated Ca^{+2} transport.



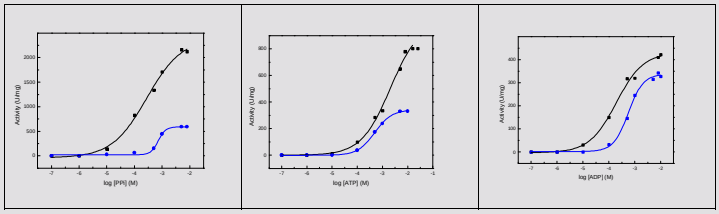


Figure 3. The presence of AnxV affected significantly the hydrolysis of PP_i, ATP and ADP by TNAP. When both proteins are present, the V_m for PP_i hydrolysis decreased by around 19 times and $K_{0.5}$ was not affected significantly. For ATP, V_m decreased around 7 times and $K_{0.5}$ also decreased (9 times).

Table 1. Kinetic parameters for the substrates hydrolysis (pH 7.4) by DPPC-Proteoliposome carrying TNAP or TNAP + AnxV. Influence of the AnxV presence on the kinetic parameters by TNAP hydrolysis.

Substrates	Kinetic Parameters	Proteoliposomes	
		TNAP	TNAP+AnxV
PPi	V_m (U/mg)	2389.60	592.60
	$K_{0.5}$ (mM)	0.279	0.726
	k_{cat} / $K_{0.5}$ ($M^{-1}.s^{-1}$)	17,129.75	1,632.51
ATP	V_m (U/mg)	1,004.55	341.64
	$K_{0.5}$ (mM)	2.042	0.498
	k_{cat} / $K_{0.5}$ ($M^{-1}.s^{-1}$)	983.89	1,372.05
ADP	V_m (U/mg)	430.74	337.02
	$K_{0.5}$ (mM)	0.194	0.565
	k_{cat} / $K_{0.5}$ ($M^{-1}.s^{-1}$)	4,440.62	1,192.99

CONCLUSION

This system can provide more information about the interplay between AnxV and TNAP with other proteins present in MVs, as well as its interactions with the lipid environment in the membrane. Moreover, these studies will help us in the development of mineralization-competent MV biomimetics.

REFERENCES

[1] Simão, A.M. et al. (2010) Proteoliposomes harboring alkaline phosphatase and nucleotide pyrophosphatase as matrix vesicle biomimetics, J. Biol. Chem. 285: 7598–7609.

[2] Bolean, M.et al. (2011) Thermodynamic properties and characterization of proteoliposomes rich in microdomains carrying alkaline phosphatase. Biophys. Chem. 158: 111-8.

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