IN VITRO ASSEMBLY OF PROTEIN NANOTUBES USING A NOVEL TECHNIQUE

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Introduction. Rotavirus VP6 exhibits polymorphic features: it assembles in the form of nanotubes or nanospheres depending on the pH and ionic strength of the media. VP6 nanotubes and nanospheres have applications as vaccines or nanomaterials, and recovery process that are efficient and result in high quality assemblies are needed. The addition of Ca^{2+} above 200mM provokes the disassembly of VP6 nanotubes to monomers, a process that is reverted when Ca^{2+} is removed[1]. Using the insect cell baculovirus system, we express recombinant VP6 nanotubes that are purified from culture supernatants[2]. However, a significant proportion of unassembled protein and aggregates are also obtained.
In this work, we describe a technique for the recovery of unassembled protein using an easy protocol that allowed the formation of VP6 nanotubes in vitro.

Methods. High Five® insect cells were infected with a recombinant baculovirus that contains the gene of rotavirus VP6 (strain SA11). The purification process of VP6 nanotubes was carried out performing anionic exchange and gel permeation liquid chromatography. Unassembled VP6 and aggregates were treated with 300mM $CaCl_2$ to ensure the formation of VP6 monomers. Then, Ca^{2+} was precipitated with the addition of 100mM $NaHCO_3$, which resulted in the formation of VP6 nanotubes.

Results. Fractions collected from the gel permeation chromatography purification of VP6 were observed in a transmission electron microscope (TEM) for the identification of disassembled VP6 (Fig.1). After the addition of $CaCl_2$ and the subsequent precipitation of the Ca^{2+} ions, the sample was also observed by TEM (Fig.2). VP6 nanotubes of several micrometers in length were obtained. This easy method of Ca^{2+} precipitation avoids extensive diafiltration for the elimination of cations and allowed the recovery of VP6 nanotubes from a fraction that is usually discarded.

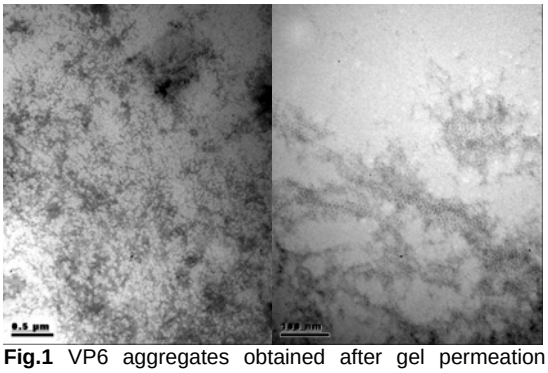

Fig.1 VP6 aggregates obtained after gel permeation chromatography. The protein was stained using uranyl acetate 4% and visualized by TEM. Left: 20 000X. Right: 140 000X.

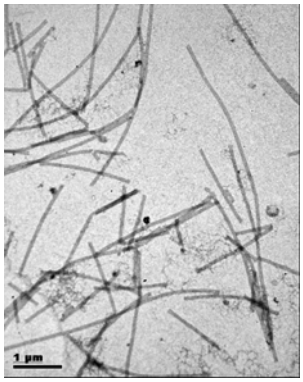

Fig.2 Reassembled VP6 nanotubes. The sample was stained with uranyl acetate 4% and visualized by TEM (12 000X).

Conclusions.
1. Using a fast and easy protocol, VP6 nanotubes were reassembled from monomeric protein and aggregates.
2. Resulting nanotubes had the same size and shape than those purified from culture supernatants.

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