Purification method of FoF1-ATP synthase oligomers for drug delivery target research in diseases caused by mitochondrial dysfunctions

Background

FoF1-ATP synthase localized in mitochondria presents, among others, energy production and membrane Permeability Transition Pore (mPTP) functionalities which, when dysfunctional, can cause mitochondrial or immunometabolic diseases. F-ATP's role in mPTP has also raised interest for conclusive *in vitro* experiments using purified F-ATP synthase in various oligomeric forms. To our knowledge, no protocol for F-ATP synthase oligomers separation has reached the desirable levels of quantity, stability or purity for high-resolution structural and functional analysis required for drug discovery. For these reasons, there is a demand for a purification method answering these research needs. Our purification method for FoF1-ATP synthase has shown excellent ability to produce the desired large amounts of human-derived FoF1-ATP synthase monomers, dimers, tetramers and oligomers, with high purity levels and while steadily retaining high levels of ATP synthase activity. We expect this method to be used for drug discovery purposes, research in drug targets for mitochondrial diseases and other applications. (Fig.1.)

Technical Summary

Our purification method is a column-free purification protocol relying first on a well-established differential centrifugation process to obtain large amounts of pure inner mitochondrial membranes, and second on three sucrose density gradient ultracentrifugation steps, tuned via either LMNG (lauryl maltose neopentyl glycol) or GDN (glyco-diosgenin) to yield respectively monomeric or oligomeric F-ATP synthase. (Fig.2.).

Our innovative approach avoids usual F-ATP synthase partial loss of oligomeric subunits resulting from a chromatography step and achieves greater yields thanks to the density gradient ultracentrifugation process often used in photosynthetic and ribosomal research. Combined with F-ATP synthase's binding properties with IF1 (inhibitor factor 1, a natural inhibitor protein), we overcame the difficulty of the size similarities between its different oligomeric forms and other complexes of the mitochondrial respiratory chain.

The purified FoF1-ATP synthase various oligomeric forms are obtained in satisfying quantity, purity, homogeneity and stability levels for further high-resolution structural and functional analysis. Moreover, we succeeded in maintaining stable activity of F-ATP synthase in liposomes at room temperature, thus opening for further research in drug delivery applications.

Strengths:

- Expertise in F-ATP synthase-IF1 binding and other stabilization mechanisms
- Experimental data available under reasonable request
- Supply potential partners with large amounts of purified human-derived FoF1-ATP synthase oligomers stabilized in liposomes and suitable for structural and functional analysis

Challenges:

- We need partners to help us explore the opportunities for drug discovery and drug target discovery for mitochondrial diseases

Technology Readiness Level

- *2-3*

Potential Applications

- *Drug delivery target*
- *Drug discovery*
- *Mitochondrial diseases*
- *Immunometabolic diseases*
- *In vitro studies*

Possible Collaboration Mode(s)

- R&D collaboration
- Licensing
- Supply of purified F-ATP synthase oligomers

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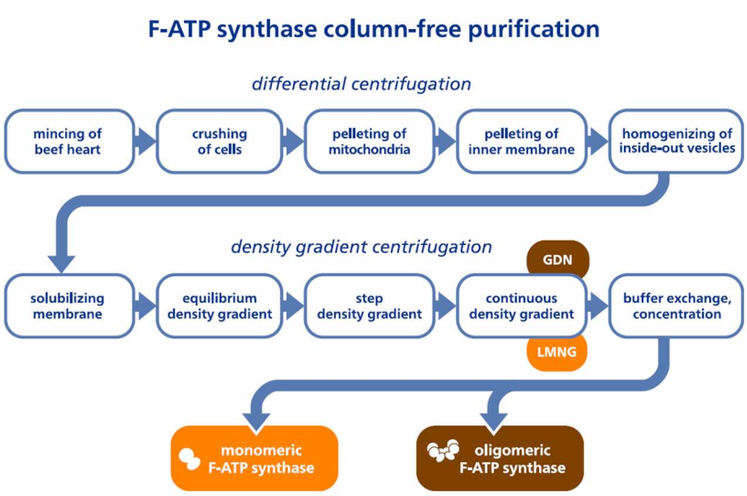

Fig.2. Purification process for FoF1-ATP synthase oligomeric forms

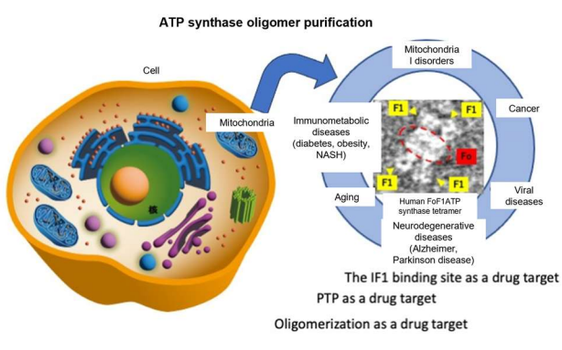

Fig.1. F-ATP synthase oligomer purification applications

INSTITUTIONAL ADVANCEMENT AND COMMUNICATIONS (IAC)
Office of International Expansion

Email: ip-med@saci.kyoto-u.ac.jp
Phone: +81-75-753-5692

International Science Innovation Building
Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto
606-8501 JAPAN