The human mitochondrial ADP/ATP carrier operates by a ping-pong kinetic mechanism

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1. Introduction

The mitochondrial ADP/ATP carrier (AAC) transports ADP into the mitochondrial matrix for ATP synthesis and exports ATP to the cytosol [1]. Historically, the carrier was thought to be a homodimer [2], operating by a simultaneous (sequential) mechanism involving the formation of a ternary complex with the exchanged substrates [3]. Structural, biochemical and biophysical data have shown that ADP/ATP carriers are monomeric with a central translocation pathway [4] and a single substrate binding site [5], which is difficult to reconcile with the mechanism proposed earlier. **Here, we revisit the kinetic properties of AAC by using reconstituted recombinant protein and uptake curves obtained by transport robotics.**

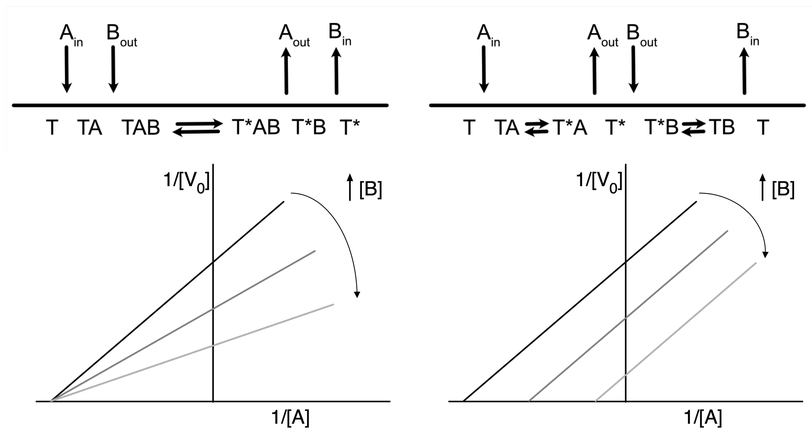

Figure 1. Schematic and experimental differentiation of (left) sequential and (right) ping-pong kinetic mechanisms. A and B are the two exchanged substrates, while T and T* represent the two conformational states of the transporter.

2. The purified human ADP/ATP carrier

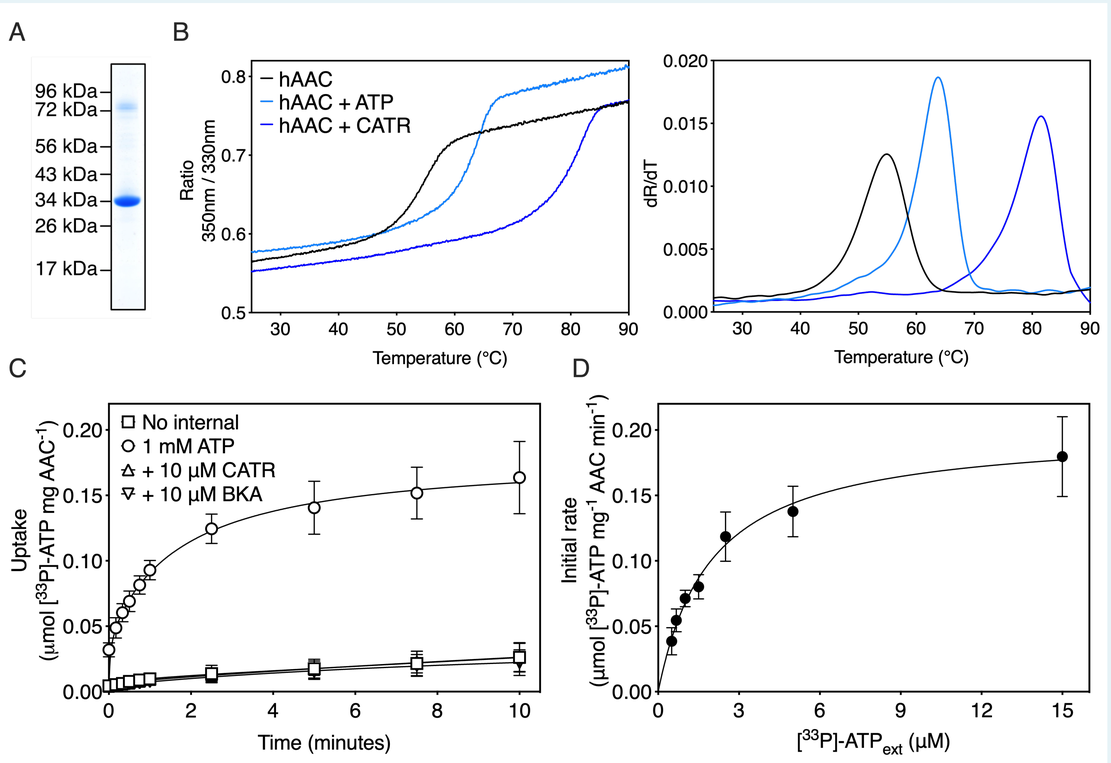

Figure 2. (A) Human AAC was expressed in *S. cerevisiae* and purified by affinity chromatography. The protein is stable, interacts with ATP and CATR as shown by thermostability analysis [5] **(B, C)**, and transports ATP, which is inhibitable by BKA and CATR **(D)**. **(E)** The Km of human AAC is 2 µM. Mean and standard deviation of three biological repeats, each the average of three technical replicates.

3. The kinetics of ADP and ATP homo- and hetero-exchange

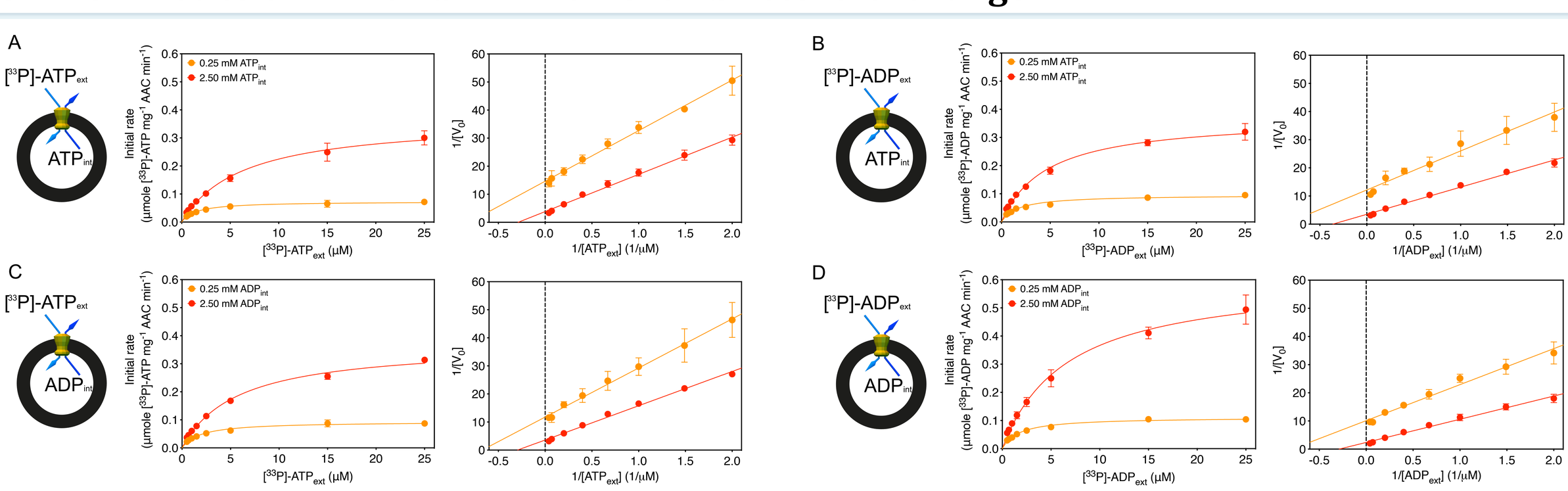

Figure 3. Human AAC was reconstituted into liposomes, and ATP **(A, B)** or ADP **(C, D)** (0.25 mM or 2.50 mM) was internalised by freeze-thaw-extrusion. Transport was initiated by the addition of [^{33}P]-ATP **(A, C)** or [^{33}P]-ADP **(B, D)**, and stopped by filtration and washing. Initial rates were obtained by linear regression analysis. The lines in the Lineweaver-Burke plot are only consistent with a ping-pong reaction mechanism. Mean and standard deviation of three technical replicates.

4. A closer look into the kinetic mechanism

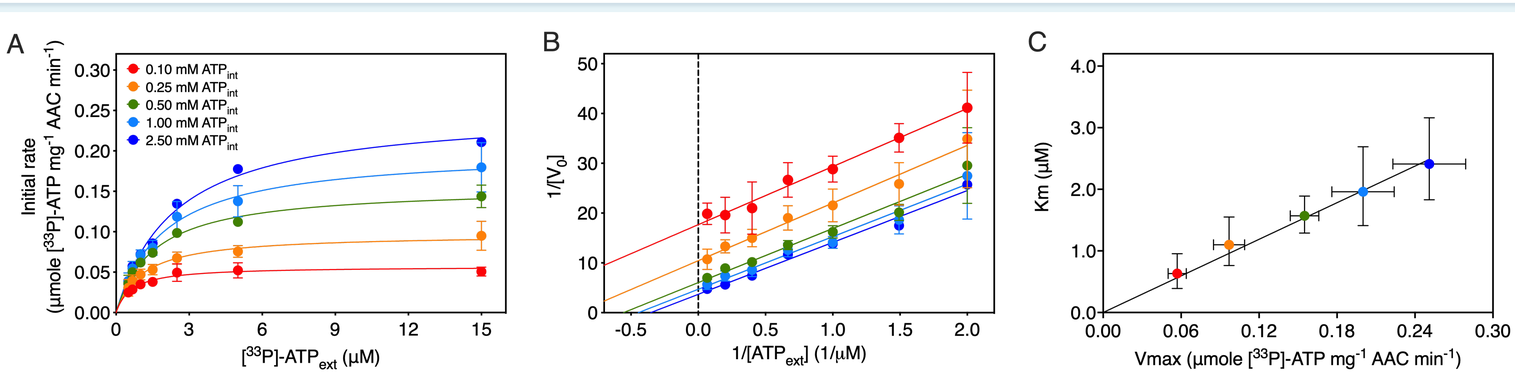

Figure 4. Human AAC was reconstituted into liposomes in the presence of ATP (0, 0.10, 0.25, 0.50, 1.00 or 2.50 mM). Transport was initiated by the addition of [^{33}P]-ATP. Uptake data were fitted using a model in order to obtain the best estimates of the initial rates. **(A)** Michaelis-Menten and **(B)** Lineweaver-Burke plots. **(C)** Km and Vmax for the various substrate gradients were determined by fitting of the Michaelis Menten curves through iteration. The kinetic parameters were plotted against each other, showing the ratio is constant, thus demonstrating a ping-pong kinetic mechanism. Mean and standard deviation of three independent experiments, each the average of three technical repeats.

1. J. J. Ruprecht, E. R. S. Kunji. Structural changes in the transport cycle of the mitochondrial ADP/ATP carrier. Curr. Opin. Struct. Biol. 57, 135-144 (2019).
2. H. Hackenberg, M. Klingenberg. Molecular weight and hydrodynamic parameters of the adenosine 5'-diphosphate-adenosine 5'-triphosphate carrier in Triton X-100. Biochemistry 19, 548-555 (1980).
3. C. Duyckaerts, et al. Kinetic mechanism of the exchanges catalysed by the adenine-nucleotide carrier. Eur. J. Biochem. 106, 1-6 (1980).
4. E. R. S. Kunji, J.J. Ruprecht. The mitochondrial ADP/ATP carrier exists and functions as a monomer. Biochem Soc Trans 48, 1419-1432 (2020)
5. V. Mavridou, et al. Substrate binding in the mitochondrial ADP/ATP carrier is a step-wise process guiding the structural changes in the transport cycle. Nat Commun 13, 3585 (2022).

5. Conclusion

The ADP/ATP carrier operates with a ping-pong kinetic mechanism in which the import and export transport steps occur consecutively. These data are consistent with the carrier being a functional monomer with a single substrate binding site [4, 5].

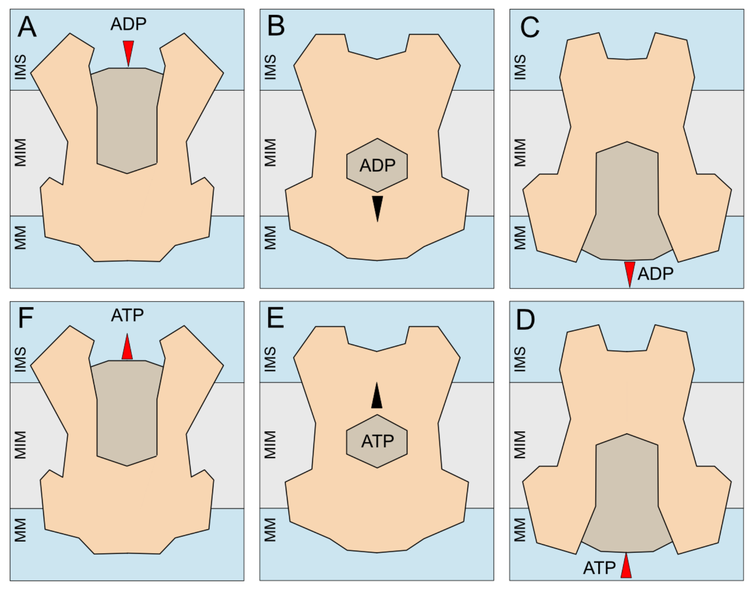

Figure 5. Schematic representation of the transport cycle of the ADP/ATP carrier.