

**P026** The dissociation of the V-ATPase into its  $V_1$  and  $V_o$  complexes is influenced by ATP and ADP

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The reversible dissociation of the  $V_1V_o$  holoenzyme into its  $V_1$  and  $V_o$  complexes is a general regulatory mechanism for V-ATPases, but important aspects are still not understood.

The analysis of endogenous nucleotides tightly bound to the  $V_1V_o$  holoenzyme or to the  $V_1$  complex, revealed that the  $V_1$  complex contained 1.7 molecules of ADP, whereas only 0.3 molecules of ADP were bound to the  $V_1V_o$  holoenzyme. Both complexes contained only negligible amounts of ATP. Since the ATP/ADP ratio reflects the energy content in the cell and could affect the stability of the V-ATPase, we incubated the  $V_1V_o$  holoenzyme with different nucleotides and investigated their effects on its dissociation. Upon treatment with ADP or the non-hydrolysable ATP analog AMP-PNP, no dissociation was observed. On the other hand, already the hydrolysis of a single ATP molecule per copy of V-ATPase and thus the incorporation of ADP into the enzyme without a following ATP binding step was found to be sufficient to induce dissociation. The content of ADP molecules bound to the  $V_1$  complex after its *in vitro* dissociation was equal to its content *in vivo*. Therefore we suggest that ADP plays a similar role in the dissociation process *in vitro* and *in vivo*.