21 Determination of creatine kinase fluxes in heart mitochondria using ^{31}P-saturation transfer NMR

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Mitochondrial creatine kinase (Mi-CK) and extramitochondrial CK systems play a crucial role in the energy transfer of cardiac muscle. Their concerted action provides effective channelling of mitochondrial ATP to the myofibrillae where it is used in the contraction cycle.

Pseudo first order reaction constants of the phosphoryl transfer in the CK-reaction, k_f and k_r, respectively, can be determined by ^{31}P-saturation transfer NMR. In order to study the flux through the Mi-CK reaction *in situ*, we used isolated mitochondria from rat and bovine heart, since it is not possible to determine the Mi-CK activity in the whole tissue. In addition we studied rat liver mitochondria, which do not express Mi-CK, in the presence of soluble MM-CK from rabbit skeletal muscle as a model, where CK is not compartmentalized in the mitochondrial intermembrane space. Mitochondria were oxygenated in the NMR-tube by continuous infusion of low amounts of hydrogen peroxide from which oxygen was liberated by catalase present in the incubation medium.

Mi-CK activity of the mitochondria was estimated from the forward fluxes (→ ATP). The $\text{flux}_{(f)}/\text{V}_{\text{max}(f)}$ ratio was 1.7 for rat heart mitochondria, 0.22 for bovine heart mitochondria and 0.04 for rat liver mitochondria plus MM-CK. Thus the ratio is highest for rat heart mitochondria with a high ATP-ADP-turnover, intermediate with bovine heart and very low for rat liver mitochondria where ATP-ADP-turnover is lowest, since in liver mitochondria ATPase contamination is practically absent. The results also show that compartmentation is not necessary for interaction of the CK-reaction with oxidative phosphorylation, which is probably due to the fact that oxidative phosphorylation has a very low K_M for ADP and competes successfully with CK for this substrate.

On the other hand, we observed a $\text{flux}_{(f)}/\text{V}_{\text{max}(f)}$ ratio greater than 1 for rat heart mitochondria. It is concluded that the $\text{flux}_{(f)}$, which is measured in situ, compared to $\text{V}_{\text{max}(f)}$ which is measured in the diluted detergent extract, shows

the Mi-CK activity in its true *in situ* situation. The localization of the enzyme in the contact sites of mitochondria with ANT and porin [1] yields an environment where the specific activity may exceed the Vmax of the soluble enzyme. In bovine heart mitochondria oxidative phosphorylation is not high enough to cope with the capacity of the Mi-CK, therefore compartmentation will not be as effective as in hearts with high energy turnover. Thus it is the fine tuning of all enzymes and pathways involved in ATP turnover within the cellular compartments which allows optimal rates of energy transfer.

References

1. Wallimann, Th. (1994) Dissecting the role of creatine kinase, *Curr. Biol.* **1**, 42–46.