

Differential Scanning Calorimetry of Glycerinated Muscle Fibres Reveals Distinct Conformational States during the ATP Hydrolysis

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DSC study was performed on glycerol-extracted muscle fibers from rabbit psoas muscle to obtain information about the global conformational changes associated with the different intermediate states of ATP hydrolysis. Using decomposition procedures four single transitions were derived which can be assigned to myosin heads, myosin rods and thin actin filaments at temperatures of 52.9 °C, 57.9 °C, 63.7 °C. The temperature of the fourth transition, which is likely to originate from actin and/or partly from the nucleotide binding domain, depended on the intermediate state of the ATP hydrolysis with transition temperatures varying between 66 °C – 77 °C. Significant difference was found between the strongly and weakly binding states of myosin to actin. Specific binding of ADP to myosin caused only moderate change of the DSC pattern in comparison with rigor, whereas ADP trapped by Vi, AlF₄ or BeF_x to the myosin head induced remarkable stabilization in the globular part of myosin, which was reflected in a 3.0 – 8.0°C upward shift of the transition temperature. A similar effect was observed in the case of the nonhydrolyzable ATP analogue AMP·PNP. Neglecting the changes observed in the line width of transitions at half-height for the myosin rod and the actin filament, the global conformational changes detected by DSC occur mostly in muscle fibers in the globular portion of myosin heads in the vicinity of the nucleotide binding domain and/or in the actin filaments, respectively.