ATP induces a conformational change in the C-terminus domain of the voltage gated skeletal muscle chloride channel (ClC-1)

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Eukaryotic voltage gated chloride channels (ClC) have a distinctive cytoplasmic tail (C-tail) containing two cystathionine β-synthase (CBS) domains and it has been suggested that a nucleotide-binding pocket is produced by the association of the CBS1 and CBS2 regions. Although ClC function is influenced by ATP *via* a cytoplasmic interaction, X-ray crystallography of the purified C-tail of ClC-0, has failed to detect ATP binding. Thus, the exact nucleotide-binding site is not known. In the present study, a distal C-tail fragment, $C_{721\text{-}988}$, containing the second CBS domain, was purified to homogeneity, confirmed by SDS-PAGE analysis and coomassie blue staining. Purified $C_{721\text{-}988}$ tended to form high molecular mass aggregates demonstrated by native, non-reducing and urea-PAGE analysis indicating strong interaction among fragments. An ATP fluorescent analogue, 2′,3′-O-(2,4,6-trinitrophenylcyclohexadienylidene) adenosine 5′-triphosphate (TNP-ATP) has previously been successfully used to study the nucleotide-binding domains of the K_{ATP} and CFTR channel proteins. Significant enhancement of fluorescence was observed during titration of the $C_{721\text{-}988}$ fragment with the TNP-ATP fluorophore indicating a possible nucleotide-binding interaction. However, when the saturated TNP-ATP/$C_{721\text{-}988}$ complex was titrated with ATP fluorescence enhancement also occurred rather than the anticipated displacement of the probe, indicating the possibility of different binding sites. To probe for a possible conformational change in the hydrophobic region of the protein, 8-anilino-1-naphthalenesulfonate (ANS) was used. When $C_{721\text{-}988}$ saturated with ANS was titrated with ATP, the resultant enhanced fluorescence indicated that ATP had induced a conformational change resulting in the formation of additional hydrophobic regions. Such ATP-induced conformational changes could be abolished in the presence of 1M urea. Presence of Mg^{2+} (3 mM) suppressed the allosterism-inducing step at concentrations below 2 mM ATP. Thus, the possible location for nucleotide binding might involve interactions within or between distal parts of C-tail, e.g., $C_{721\text{-}988}$.