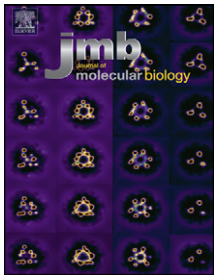

The Protein Kinase CK2 ^{Andante} Holoenzyme Structure Supports Proposed Models of Autoregulation and *Trans*-Autophosphorylation

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Abstract

Eukaryotic protein kinases are typically strictly controlled by second messenger binding, protein/protein interactions, dephosphorylations or similar processes. None of these regulatory mechanisms is known to work for protein kinase CK2 (former name "casein kinase 2"), an acidophilic and constitutively active eukaryotic protein kinase. CK2 predominantly exists as a heterotetrameric holoenzyme composed of two catalytic subunits (CK2α) complexed to a dimer of non-catalytic subunits (CK2β). One model of CK2 regulation was proposed several times independently by theoretical docking of the first CK2 holoenzyme structure. According to this model, the CK2 holoenzyme forms autoinhibitory aggregates correlated with *trans*-autophosphorylation and driven by the down-regulatory affinity between an acidic loop of CK2β and the positively charged substrate binding region of CK2α from a neighboring CK2 heterotetramer. Circular trimeric aggregates in which one-half of the CK2α chains show the predicted inhibitory proximity between those regions were detected within the crystal packing of the human CK2 holoenzyme. Here, we present further *in vitro* support of the "regulation-by-aggregation" model by an alternative crystal form in which CK2 tetramers are arranged as approximately linear aggregates coinciding essentially with the early predictions. In this assembly, the substrate binding region of every CK2α chain is blocked by a CK2β acidic loop from a neighboring tetramer. We found these crystals with CK2 ^{Andante} that contains a CK2β variant mutated in a CK2α-contact helix and described to be responsible for a prolonged circadian rhythm in *Drosophila*. The increased propensity of CK2 ^{Andante} to form aggregates with completely blocked active sites may contribute to this phenotype.

Introduction

CK2, a heterotetrameric protein kinase composed of two catalytic subunits (CK2α) attached to a central dimer of non-catalytic subunits (CK2β) [1] (Fig. 1a), is a pleiotropic enzyme involved in various cellular processes [10–12], among them is the circadian clock [13]. CK2 is therefore supposed to be "in need of control" [12] as is the majority of eukaryotic protein kinases (EPKs) [14,15]. However, regulation of CK2 was referred to as "puzzling" [11] since neither of the typical regulatory mechanisms of EPKs is valid for this enzyme: CK2 is not controlled by second messenger molecules; the catalytic chain CK2α is not phosphorylated at its activation segment and CK2α is active both as a monomer and in complex with CK2β [16], meaning that its interaction partner CK2β is no on/off switch of catalytic activity—in sharp contrast to cyclin proteins in the case of the closest CK2α relatives, the cyclin-dependent protein kinases.

This constitutive activity was corroborated on a structural level by the first CK2α structure [17] (and all subsequent ones) showing the enzyme in a conformation typical for fully active EPKs [15]. In particular, the activation segment is intramolecularly stabilized in an active state by an intimate contact to the N-terminal segment [17]. These findings were confirmed by the crystal structure of a heterotetrameric $CK2\alpha_2\beta_2$ holoenzyme (Fig. 1a) [1], which further revealed that, within the complex, the CK2β binding site is not in direct proximity to CK2α's active site and its activation segment. As a consequence,

protein/protein interactions within the $CK2\alpha_2\beta_2$ holoenzyme did not directly suggest how CK2 regulation might work.

Nevertheless, the butterfly-like architecture of the $CK2\alpha_2\beta_2$ holoenzyme (Fig. 1a) inspired various researchers [3–5] to develop alternative models of CK2 regulation and of regulation-coupled autophosphorylation based on predicted interactions between two (Fig. 1c and d) or more $CK2\alpha_2\beta_2$ tetramers (Fig. 1e) rather than within a single one. The phenomenological basis of these efforts were previous reports about salt- and polycation-dependent inhibitory aggregation phenomena of $CK2\alpha_2\beta_2$ complexes [6,9,18] and of a correlation between autophosphorylation and down-regulation [19] that had provided strong evidence that the regulation of the $CK2\alpha_2\beta_2$ holoenzyme depends on intertetramer rather than intratetramer interactions.

Rekha and Srinivasan were the first to demonstrate that the shape of the $CK2\alpha_2\beta_2$ holoenzyme