doi:10.1016/j.jmb.2009.01.033 *J. Mol. Biol.* (2009) **386**, 1212–1221

JMB

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First Inactive Conformation of CK2α, the Catalytic Subunit of Protein Kinase CK2

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Received 14 November 2008; accepted 20 January 2009
Available online 24 January 2009

Edited by R. Huber

The Ser/Thr kinase casein kinase 2 (CK2) is a heterotetrameric enzyme composed of two catalytic chains (CK2α, catalytic subunit of CK2) attached to a dimer of two noncatalytic subunits (CK2β, noncatalytic subunit of CK2). CK2α belongs to the superfamily of eukaryotic protein kinases (EPKs). To function as regulatory key components, EPKs normally exist in inactive ground states and are activated only upon specific signals. Typically, this activation is accompanied by large conformational changes in helix αC and in the activation segment, leading to a characteristic arrangement of catalytic key elements. For CK2α, however, no strict physiological control of activity is known. Accordingly, CK2α was found so far exclusively in the characteristic conformation of active EPKs, which is, in this case, additionally stabilized by a unique intramolecular contact between the N-terminal segment on one side, and helix αC and the activation segment on the other side. We report here the structure of a C-terminally truncated variant of human CK2α in which the enzyme adopts a decidedly inactive conformation for the first time. In this CK2α structure, those regulatory key regions still are in their active positions. Yet the glycine-rich ATP-binding loop, which is normally part of the canonical anti-parallel β-sheet, has collapsed into the ATP-binding site so that ATP is excluded from binding; specifically, the side chain of Arg47 occupies the ribose region of the ATP site and Tyr50, the space required by the triphospho moiety. We discuss some factors that may support or disfavor this inactive conformation, among them coordination of small molecules at a remote cavity at the CK2α/CK2β interaction region and binding of a CK2β dimer. The latter stabilizes the glycine-rich loop in the extended active conformation known from the majority of CK2α structures. Thus, the novel inactive conformation for the first time provides a structural basis for the stimulatory impact of CK2β on CK2α.

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Keywords: protein kinase CK2; casein kinase 2; CMGC family of eukaryotic protein kinases; inactive conformation of CK2α; regulation of catalytic activity

Eukaryotic protein kinases (EPKs) catalyze the transfer of the γ-phospho group of ATP to the terminal hydroxy groups of specific serine, threonine, and tyrosine residues within substrate proteins. In this way, they act as molecular switches orchestrating almost all fundamental cellular processes as components of signaling pathways and regulatory networks.[1] To ensure the spatial and temporal interplay of molecular processes, EPKs themselves are, in general, strictly regulated,[2] and dysregulations can lead to cell transformation and cancer.

With regard to their structures, EPKs are closely related proteins. They share the same core architecture, consisting of an N-terminal domain based on a central anti-parallel β-sheet and an α-helical C-terminal domain with the active site located in

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Abbreviations used: CK2, casein kinase 2; EPK, eukaryotic protein kinase; CDK, cyclin-dependent kinase; DRB, 5,6-dichlorobenzimidaziole ribofuranoside; AMPPNP, adenylyl imidodiphosphate; PDB, Protein Data Bank.

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between. Typically, EPKs exist in ground states of minimal activity and are activated in response to stimulatory signals.[2] The mechanisms occurring in the change from the "off" state to the "on" state include binding of activator proteins [cyclin-dependent kinases (CDKs)], release of regulatory subunits (cAMP-dependent protein kinase), phosphorylation (mitogen-activated protein kinases), dephosphorylation (glycogen synthase kinase 3), release of pseudosubstrate segments (twitchin kinase), dimerization (RNA-activated protein kinase and epidermal growth factor receptor), or combinations of these.[2–4] In general, these regulatory events are accompanied by large conformational changes—in particular, in the two regulatory key elements, the so-called activation segment at the border of the C-terminal domain and helix αC in the N-terminal domain.[2]

EPKs are conformationally individual and diverse in their inactive states, but they adopt a similar conformation in the "on" state due to the chemical constraints of the phosphotransfer reaction.[2] In this conformation, the activation segment is open to allow substrate binding,[5] whereas it is often nonproductively rearranged in the "off" state. Moreover, in active EPKs, helix αC is oriented such that a conserved glutamate residue can establish a salt bridge to a likewise conserved lysine residue; fixed in this way, the lysine side chain can coordinate the α- and β-phospho groups of ATP in their functional conformations. This lysine/glutamate ion pair is often absent in inactive EPKs and is therefore regarded as a sensitive identification for the active conformation of an EPK.[2]

The importance of activity control for EPK function makes the exploration of regulatory mechanisms and their structural bases a fundamental subject of EPK research. In this respect, the regulation of protein kinase casein kinase 2 (CK2)—a heterotetrameric Ser/Thr kinase composed of two separate catalytic chains (CK2α, catalytic subunit of CK2) attached to a central dimer of two noncatalytic subunits (CK2β, noncatalytic subunit of CK2)[6]—is particularly puzzling, since none of the above-mentioned control mechanisms works in this case. Thus, although CK2 itself is a regulatory factor in apoptosis, proliferation,[7,8] and multiple transitions in the cell cycle,[9] its own regulation is poorly understood.[10] CK2α alone has a significant basal catalytic activity, which is enhanced by CK2β as long as peptides serve as substrates.[11] With complete proteins as substrates, however, the modulatory impact of CK2β on CK2α is more diverse and can sometimes even involve down-regulation (e.g., in the case of calmodulin).[12] At any rate, CK2β is no on/off switch for CK2α and thus not comparable to cyclins in the case of the CDKs.

Various alternative notions of CK2 regulation have been considered so far, for example: (i) formation of inactive filamentous aggregates from heterotetrameric CK2 holoenzyme complexes;[13] (ii) regulation through small molecules such as inositol phosphates;[14] (iii) modulation of specificity through protein–protein interactions;[15,16] (iv) intracellular translocation;[17] and (v) long-term regulation via enzyme concentration through gene expression and protein degradation.[18]

None of these regulatory concepts requires an underlying inactive CK2α conformation. Thus, they are consistent with the fact that, in more than 30 crystal structures, CK2α was found exclusively in the typical conformation of active EPKs, and that this active conformation is, in the case of CK2α, particularly constrained by at least three internal elements: (i) the N-terminal segment that fixes the activation segment and helix αC in a way comparable to cyclins in the case of the CDKs;[19] (ii) an exceptional tryptophan in the magnesium-binding loop[5] that replaces the central Phe residue of the canonical DFG motif (this Trp side chain allows an additional hydrogen bond[19] that disfavors "DFG-out" conformations as known from inactive mitogen-activated protein kinases);[20] and (iii) a structural chloride ion, together with a conserved water cluster, that supports contact between the N-terminal segment and the activation segment.[21]

In accordance with these and further structural features, it was proposed that CK2α was "evolved to be active"[22] and can possibly never occur in an inactive conformation. Therefore, we were surprised to discover a decidedly inactive conformation of human CK2α. Here, we describe this structure. We propose on its basis a concept for the stimulatory impact of CK2β on CK2α and, moreover, discuss its possible consequences for CK2 regulation.

Cocrystallization of *hs*CK2α$^{1–335}$ and glycerol

The inactive CK2α conformation was found by coincidence when we cocrystallized glycerol with *hs*CK2α$^{1–335}$, a C-terminal deletion mutant of human CK2α that is catalytically fully active[23] and capable of associating with CK2β.[24] The motivation for this cocrystallization was based on a recent report that the CK2α/CK2β interface region of human CK2α harbors a relatively unspecific small-molecule binding site[25] to which we refer from hereon as the "remote cavity" in order to distinguish it from the canonical ATP-binding site.

In that study,[24] the remote cavity was occupied by 5,6-dichlorobenzimidaziole ribofuranoside (DRB) in a complex structure with *hs*CK2α$^{1–335}$ and by glycerol in complex with the mutant *hs*CK2α$^{1–335}$-V66A/M163L. In this context, it became evident that the occupation of the remote cavity has a subtle impact on the crystallization behavior of the enzyme enforcing a certain tetragonal crystal packing and salts such as ammonium sulfate or sodium citrate as precipitating agents (rather than polyethylene glycols as in all previous crystallization reports with CK2α[26]). Such an impact should be mediated either by novel possibilities for crystalline contacts through the small molecule, an influence on the enzyme's three-dimensional structure, or a combination of both.

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