Impressions from the Conformational and Configurational Space Captured by Protein Kinase CK2

Jennifer Hochscherf, Alexander Schnitzler, Olaf-Georg Issinger, and Karsten Niefind

Abstract As key components of cellular regulation and signal transduction, eukaryotic protein kinases (EPKs) are strictly regulated. Sophisticated control mechanisms of EPKs are necessary which typically include conformational changes of the enzymes in critical regions. Local structural plasticity is therefore a prerequisite of normal EPK function. Protein kinase CK2, a member of the CMGC family of EPKs, was regarded as an exception from this rule for a long time due to its constitutive activity (lack of an inactive state) and due to its structural rigidity in typical EPK control regions of its catalytic subunit CK2α like the activation segment and the helix αC. Gradually, however, several cases of inherent local plasticity within CK2α were detected, and questions about their crosstalk and their functional significance became an issue. It is very likely now that structural plasticity and dynamics is more important for CK2 function than believed previously. Novel interpretation methods of crystallographic data even confirm an allosteric communication between the ATP site and CK2β-binding site of CK2α which had been only hypothetically postulated before. Similarly, local mobilities of CK2α are subject to modern computational approaches suggesting conformational equilibria in solution as assumed previously. In summary, CK2 structural biology has reached now a mature phase in which sophisticated modern techniques overcome the limitations of classical crystallography so that structural dynamics rather than single "snapshots" is investigated.

Keywords Protein kinase CK2 • Casein kinase 2 • Eukaryotic protein kinases • CMGC kinases • Signal transduction pathways • Structural plasticity • Structural dynamics • Constitutive activity • Hinge region • Allosteric communication

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K. Ahmed et al. (eds.), *Protein Kinase CK2 Cellular Function in Normal and Disease States*, Advances in Biochemistry in Health and Disease 12, DOI 10.1007/978-3-319-14544-0_2

1 CK2: A Protein Kinase of Limited Internal Mobility

"Conformational plasticity" as a basis of regulation and functional control was described as a hallmark of eukaryotic protein kinases (EPKs) more than a decade ago [1]. In recent reviews, this feature received particular attention and emphasis: EPKs were referred to as "dynamic molecular switches" [2] or as "allosteric macromolecular switches" [3] that received plasticity-correlated regulatability during evolution in contrast to similarly folded but more simple small-molecule kinases [4]. In particular, EPKs acquired an activation segment as part of their C-terminal domains (C-lobes) [4]. This activation segment is often the subject of (auto)phosphorylation and adapt able in its conformational state [1, 4], and it is assisted in this regulatorily relevant plasticity by the helix αC, the main control element of the N-terminal domain (N-lobe). Apart from regulation, large conformational changes within EPKs were also discussed to be necessary steps within the catalytic cycle itself [5].

Against this background, protein kinase CK2—a heterotetrameric complex of two catalytic chains (CK2α) attached to an obligatory homodimer of regulatory subunits (CK2β)[6]—was (and essentially still is) regarded as an apparent exception: it is no subject of regulatory phosphorylation at the activation segment of CK2α and does not dispose of a clearly defined inactive state. On a structural level, this "constitutive activity" was underpinned by a remarkable conformational rigidity of the activation segment and the helix αC. Both of them are fixed by the likewise structurally invariant N-terminal segment of CK2α via intramolecular interactions that resemble functionally the intermolecular contacts of the cyclin proteins with their corresponding cyclin-dependent kinases [7].

In the first review on CK2 structural biology published in 2009 [8], the structural conservation of the ensemble of activation segment, helix αC and N-terminal segment was emphasised by systematic structural comparisons including all 46 CK2α structures published at that time. The same analysis, however, illustrated that there are zones of structural variability as well (Fig. 1), some of them are typical loop regions in which flexibility is not unusual; others are with a clear conformational consensus among EPKs so that the plasticity discovered in CK2α (however not in all CK2α orthologues [9]) came as a surprise.

In subsequent years—with increasing numbers of structures—the picture of CK2 as an enzyme being conformationally constrained, where other EPKs are adaptable, but simultaneously disposing of regions of unusual adaptability, intensified and was addressed in a number of original and review publications [10–15], among them a recent metadynamics study to investigate conformational states separated by fairly high energy barriers [16]. Therefore, the local conformational and configurational variability of CK2 was the main subject of our contributions to the 7th International Conference on Protein Kinase CK2 in September 2013 in Lublin, Poland.

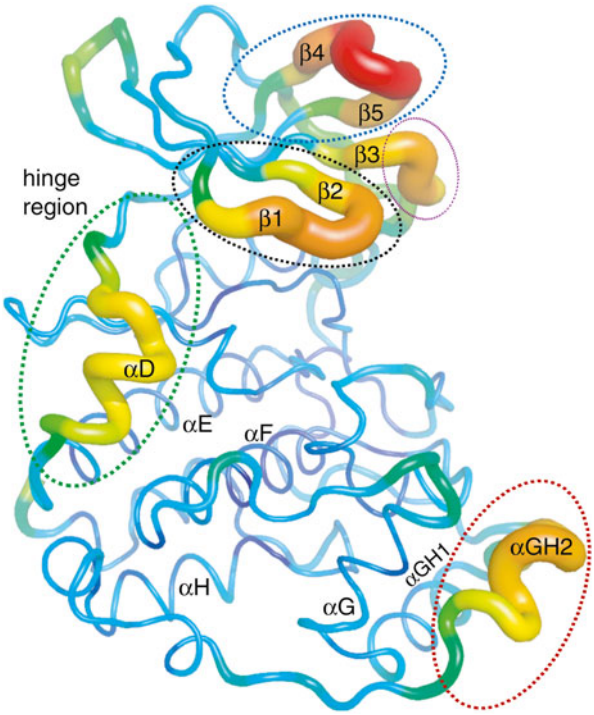

Fig. 1 CK2α structure with regions of increased mobility. The crystallographic B-factors of the atoms are encoded by the colour and the thickness of the tube. Rigid regions are drawn in *blue colour* with thin tubes while high-mobility regions are illustrated as thick and *yellow* to *red tubes*. The picture was created from PDB file 2ZJW using PYMOL [52]

2 Indicators and Study Methods of Structural Plasticity

Basically, X-ray crystallography is of limited use for the investigation of conformational dynamics in proteins because the crystalline state of a protein requires and causes strong restraints on atomic mobility. However, some phenomena and approaches are able to relativise these restrictions:

– B-factors: even under crystalline conditions, the individual atoms retain a certain degree of mobility. In crystallographic refinement, it is taken into account by the so-called B-factor, also known as the atomic temperature factor. The B-factor of an atom is proportional to the square of its mean displacement, i.e. a (hypothetical) ideally frozen atom has a B-factor of 0 while strong mobility correlates with high B-factors. Noteworthy, these B-factors reflect the inherent mobilities of the proteins and are no crystal-packing artefacts [17]. In Fig. 1, regions of human CK2α with high B-factors are illustrated by thick tubes and red colour.

– Polymorphism: many proteins crystallise in different crystal forms so that several snapshots of the conformational space are captured. This phenomenon is even enhanced by a sort of "functional polymorphism", i.e. the fact that the protein can exist and can be crystallised in different functional states, e.g. in complex with various ligands like substrates, inhibitors or other interaction partner. Recent CK2-related examples for a systematic investigation of structural polymorphism are the works of Papinutto et al. [14] and of Klopffleisch et al. [15].
– Starting from one or several experimentally determined conformations, computational methods (molecular dynamics) can be used to explore the conformational space quite efficiently and to expand in this way the structure-based understanding. A valuable example of this approach in the case of CK2 was published by Gouron et al. [16].
– In recent years, sophisticated methods were developed to regard a protein crystal structure even more than just as a "snapshot", but to derive conformational dynamic information directly from the (properly calculated and scaled) electron densities [18, 19]. In this context, room-temperature crystallography might experience a revival [20].

3 In Front of and Behind the β4β5 Loop

The β4β5 loop is a mobile structure element of CK2α with particularly high temperature factors (blue ellipse in Fig. 1). Like other EPKs [21], it harbours a hydrophobic surface cavity at either side: the "N-lobe cap" [21] and the "PIF pocket" [22] (Fig. 2). The plasticity of the β4β5 loop correlates with the occupation states of these two cavities.

3.1 The PIF-Pocket Region of CK2α Is Intramolecularly Plugged by an Absolutely Conserved Trp Side Chain

The intimate hook-up of the N-terminal segment with the helix αC and activation segment was the most conspicuous feature of the first CK2α structure [7]. It inspired Sarno et al. [23] to investigate the importance of the N-terminal segment by a set of point and N-terminal deletion mutants of human CK2α. Remarkably, the mutants CK2α$^{\Delta 2\text{-}24}$ and CK2α$^{\Delta 2\text{-}30}$ were inactive, but catalytic activity was partially rescued by CK2β [23].

Sarno et al.'s [23] results show that an active conformation of the helix αC and the activation segment does not absolutely depend on a network of contacts to the N-terminal segment but can be stabilised by CK2β as well. How CK2β manages this is not understood, in particular, since the CK2 holoenzyme structure (Fig. 3a) [6] revealed that CK2β does not directly touch the helix αC and the activation segment.

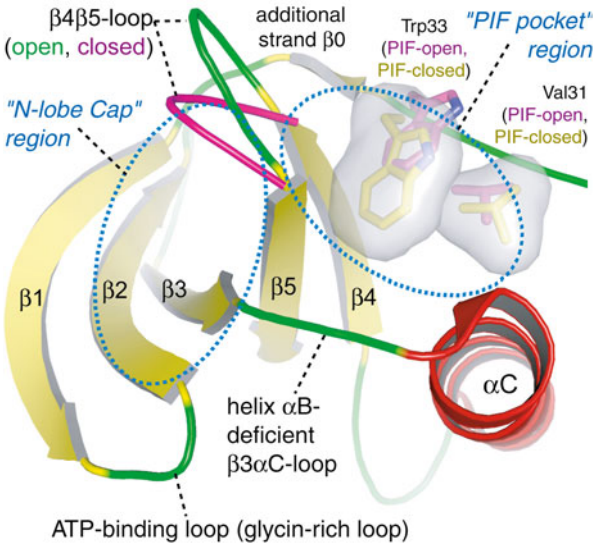

Fig. 2 "PIF pocket" and "N-lobe cap": the two N-lobal cavities flanking the adaptable β4β5 loop The majority of the picture was drawn with the PDB file 3NSZ which shows the PIF pocket filled with the side chains of Trp33 and Val31. The magenta-coloured parts representing on open PIF pocket, but a closed β4β5 loop are extracted from PDB file 2PVR. The figure was generated with PYMOL [52]

Recent observations with CK2α' [24, 25], however, and unpublished mutational data from our group suggest that the rescue effect of CK2β on CK2α$^{\Delta 2\text{-}24}$ and CK2α$^{\Delta 2\text{-}30}$ might have to do with the PIF pocket [22] of CK2α. "PIF" means"PDK1 interacting fragment". PIF is a region within enzymes of a certain AGC kinase subfamily which are phosphorylated and activated by 3-phosphoinositide-dependent protein kinase 1 (PDK1). The PIF fragment of these enzymes binds to PDK1's PIF pocket providing thus the prime example for an external occupation of the PIF pocket. The global analysis of Thompson et al. [21] revealed a more general relevance of the PIF pocket for EPKs: it is a hydrophobic surface cavity that can be used for docking of intra- or intermolecular peptide regions for the purpose of substrate recruitment or activation, the latter being caused by the stabilisation of the adjacent helix αC in its active conformation.

In the case of CK2α, the PIF pocket is mainly occupied by the side chain of a tryptophan residue which is absolutely conserved among all CK2α sequences reported so far (Trp33 of human CK2α, Fig. 2). This Trp side chain can be plugged into the PIF pocket very deeply (PIF closed), as it is the case in the two human CK2α' structures published to date [24, 25], or it can be located a bit more outside as found in most other CK2α structures (PIF open). So far, human CK2α is the only CK2α orthologue to be found with both extreme positions of Trp33 (Fig. 2). It may be possible to replace Trp33 completely from the PIF pocket by suitable small molecules and with consequences for the enzyme that are difficult to predict. Changing

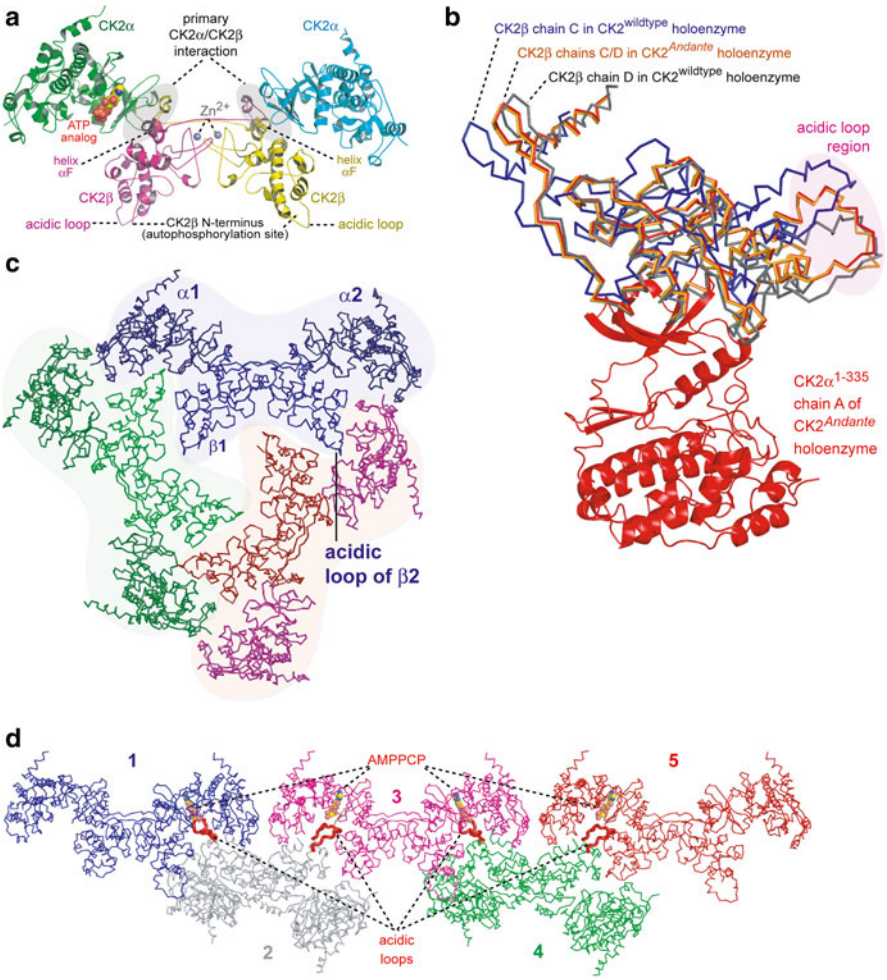

Fig. 3 The primary CK2α/CK2β interaction constituting the CK2 holoenzyme is adaptable which allows the formation of ring-like and linear higher-order aggregates. (**a**) Heterotetrameric $CK2\alpha_2\beta_2$ holoenzyme. (**b**) Superimposition of four CK2α subunits from two different $CK2\alpha_2\beta_2$ holoenzyme structures [6, 33] with attached CK2β subunits to illustrate the adaptability of the primary CK2α/CK2β contact. (**c**) Trimers of $CK2\alpha_2\beta_2$ heterotetramers identified in a hexagonal crystal form [6, 31]. The acidic loop of one CK2β subunit per heterotetramer approaches the positively charged substrate-binding region of CK2α from a neighbouring $CK2\alpha_2\beta_2$ holoenzyme complex. Packing of $CK2\alpha_2\beta_2$ tetramers in this way requires a certain deviation from the twofold symmetry and the adaptability illustrated in part **b** of the figure. (**d**) Linear aggregation of highly symmetrical $CK2\alpha_2\beta_2$ heterotetramers in a monoclinic crystal form [33]. In this arrangement, the acidic loops of all CK2β subunits are embedded in the substrate-binding regions of neighbouring CK2α chains. Part **a**, **b** and **d** of the figure were reprinted from the Journal of Molecular Biology, Vol. 424, Schnitzler, A. et al., "The protein kinase$CK2^{Andante}$ holoenzyme structure supports proposed models of autoregulation and *trans*-autophosphorylation", pp 1871–1882, 2014, with kind permission from Elsevier. Part **c** of the figure was reprinted from Molecular and Cellular Biochemistry, Vol. 274, Niefind, K. and Issinger, O.-G., "Primary and secondary interactions between CK2α and CK2β lead to ring-like structures in the crystals of the CK2 holoenzyme" pp 3–14, 2005, with kind permission from Springer Science+Business Media

Trp33 to alanine in human CK2α has nearly no effect on the K_M value for ATP and the k_{cat} value for the kinase reaction with an artificial peptide substrate; however, the Trp33Ala mutant was significantly more susceptible to activation by CK2β (J. Hochscherf, A. Köhler, unpublished data) which resembles the behaviour of the mutants CK2αΔ2-24 and CK2α$^{\Delta 2\text{-}30}$ observed by Sarno et al. [23].

3.2 *The "N-Lobe Cap" of CK2α Coordinates CK2β and Serves as a Mobile Hinge of the CK2α/CK2β Interaction*

The "N-lobe cap", i.e. the front cavity of the β4β5 loop in EPKs [21], is a prominent functional site of CK2α since it serves for docking of CK2β via the primary CK2α/CK2β interaction [6] (Fig. 3a). CK2β binding to CK2α is an enthalpically driven process [26] that requires the β4β5 loop in an extended ("open") conformation (Fig. 2), whereas in CK2β-unbound form the β4β5 loop can switch to a closed state [27, 28]. The closed β4β5 loop partly occupies the "N-lobe cap" cavity (Fig. 2) and contributes to the coordination of small molecules with a certain CK2β-antagonistic effect [29]. For coordination of peptidic CK2β competitors, however, the β4β5 loop has to be open like in the CK2 holoenzyme as recently shown in the complex structure of human CK2α$^{1\text{-}335}$ with a cyclic peptide (Fig. 4a) [30].

But not only the β4β5 loop itself has conformational plasticity depending on the occupation state of its neighbouring cavities and the crystalline environment. Rather, already the first CK2$\alpha_2\beta_2$ holoenzyme structure (Fig. 3a) [6] revealed that the whole primary CK2α/CK2β interaction is not rigid but adaptable. The structure originated from a hexagonal crystal packing in which the CK2$\alpha_2\beta_2$ tetramers associate to trimeric rings (Fig. 3c) [31]. To fit into this arrangement, the two CK2α chains are attached in significantly different orientations to the CK2β dimer (Fig. 3b). This asymmetry is no inherent property of the CK2$\alpha_2\beta_2$ holoenzyme; rather, in two recent studies with CK2$\alpha_2\beta_2$ tetramers in monoclinic crystalline environments, the twofold symmetry of the CK2$\alpha_2\beta_2$ holoenzyme is well preserved [32, 33]. Thus, three different orientations of the CK2β dimer relative to CK2α are known to date (Fig. 3b) providing the impression that the N-lobe cap cavity acts like a hinge to permit adaptability and mobility to a certain degree.

Whether and how these observations can be functionally interpreted is a subject of debate. While Lolli et al. [32] regarded the higher symmetrical form of the CK2$\alpha_2\beta_2$ tetramer *per se* as the CK2 state of maximum activity, we emphasised the adaptability of the primary CK2α/CK2β contact as a prerequisite of the CK2$\alpha_2\beta_2$ holoenzyme's propensity to integrate into ring-like [31] or linear [33] higher-molecular aggregates (Fig. 3c/d) of reduced activity. The ensembles illustrated in Fig. 3c/d were found in two different crystal forms, but linear and ring-like states of the CK2 holoenzyme exist in solution as well where they are strongly effected by the concentration of salts and of polycationic substances [34]. This downregulatory aggregation propensity is such a prominent feature of the CK2 holoenzyme that it inspired Poole et al. [35] to classify CK2 as a "constitutively inactive" kinase.

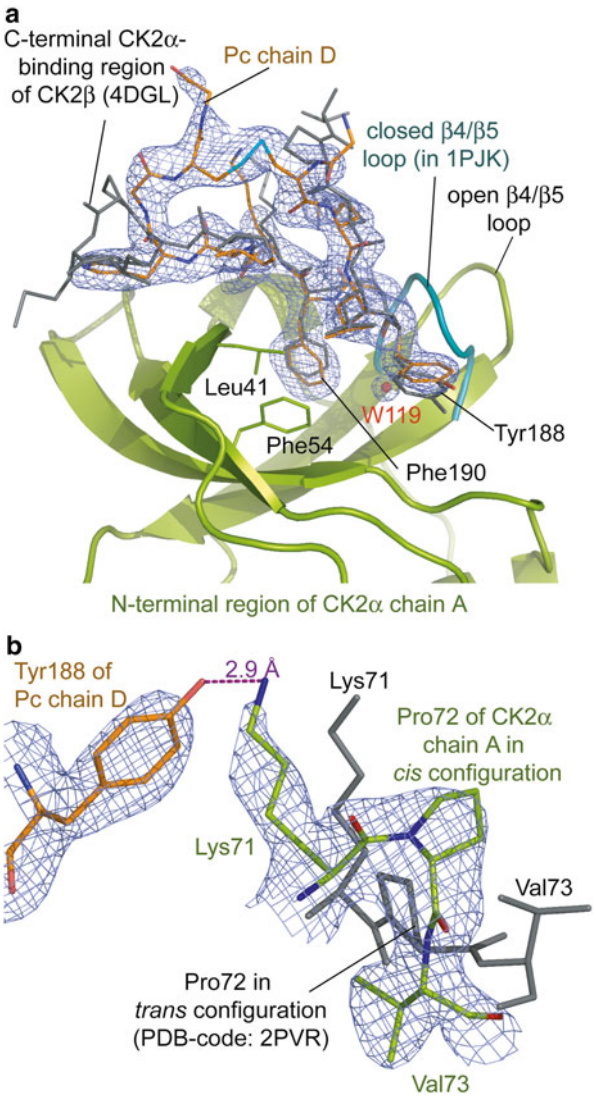

Fig. 4 The structure of CK2α in complex with a CK2β-competitive cyclic peptide revealed that Pro72 within the β3αC loop can switch to the *cis*-configuration (**a**) The CK2β interface of CK2α binds a cyclic peptide [53] which is embedded in its final electron density [30]. For comparison, CK2β in the $CK2\alpha_2\beta_2$ holoenzyme structure 4DGL was overlaid. (**b**) Configurational plasticity of Pro72 that enables a contact of Lys71 to a critical side chain of the cyclic peptide (Tyr188). Both parts of the figure were reprinted from ACS Chemical Biology, Vol. 8, Raaf, J. et al., "First structure of protein kinase CK2 catalytic subunit with an effectiveCK2β-competitive ligand", pp 901–907, 2013, with kind permission from the American Chemical Society

4 Proline *Cis-/Trans*-Isomerisation at the β3αC Loop

The preferred configuration of a peptide bond with its partial double bond character is *trans*, but *cis-/trans*-isomerisation of peptide bonds is a well-known phenomenon, in particular if proline provides the amide nitrogen atom [36]. Since the process requires breaking of a (partial) double bond, it is by definition a change of the configuration rather than the conformation as it is incorrectly designated in many publications. *Cis-/trans*-isomerisation in polypeptide chains often occurs as part of the protein folding process; CK2α, e.g., always contains a stable *cis*-peptide, namely, in a loop region of its C-terminal helical domain (Pro231 of human CK2α). With one *cis*-peptide among 17 proline residues, the *cis*-proline frequency in human CK2α is close to the average determined from a data set of 571 nonredundant protein structures [37].

Yet, *cis-/trans*-isomerisation happens in mature proteins as well where it is assumed to be an "intrinsic molecular switch" [38] without the need for covalent modifications. Often special enzymes—the peptidyl prolyl isomerases—are required to enable and control the process which is sometimes dependent on the phosphorylation of a neighbouring side chain and which is regarded as an important "molecular timer" [39]. In addition, enzyme-independent "native state proline isomerisation" [38] was observed and is likely to be functionally relevant.

In summary, there is an amazingly large literature about both enzyme-catalysed and spontaneous *cis-/trans*-plasticity at prolyl peptide bonds. Against this background, it was less surprising than we believed when we recently identified a so far unknown *cis*-proline in the N-terminal domain of CK2α (PDB 4IB5, Fig. 4b) [30]. More precisely, it was found at Pro72 within the β3αC loop (magenta-coloured ellipse of Fig. 1) of human CK2α$^{1\text{-}335}$. In all previous CK2α structures, a *trans*-proline had been observed at this position. Noteworthy, the preparation procedure involving bacterial gene expression and chromatographic purification did not significantly differ.

The functional meaning of a *cis-/trans*-plasticity at Pro72 is unclear so far. Pro72 is an absolutely conserved residue of CK2α, and the β3αC loop of CK2α is unconventional among EPKs because of its shortness missing the helix αB of the canonical protein kinase fold [40]. The aforesaid structure [30] suggested a correlation with the occupation of the N-lobe cap by a cyclic peptide mimicking the binding region of CK2β, since only with the *cis*-configuration at Pro72 the preceeding Lys71 side chain could form a hydrogen bond with a critical tyrosine side chain of the cyclic peptide (Fig. 4b). To probe the hypothesis that Pro72 and its ability to switch to the *cis*-configuration play a role in binding of the cyclic peptide or even CK2β, we created a point mutant Pro72Ala of CK2α$^{1\text{-}335}$. Preliminary and unpublished data of this mutant show no significant changes in the binding profile. Unexpectedly, however, it was thermostabilised and folded much more efficiently in the bacterial expression system than the wild type.

5 The Hinge/Helix αD Region: A Case of "Cracking"?

The interdomain hinge/helix αD region of CK2α (green ellipse in Fig. 1, Fig. 5) has two remarkable features otherwise unknown from EPKs:

– The predominant conformation of this zone is different from other EPKs, leaving more space at the ATP-binding site and contributing thus to the "dual-cosubstrate specificity" of the enzyme [41, 42]. This fact was already discussed in the first

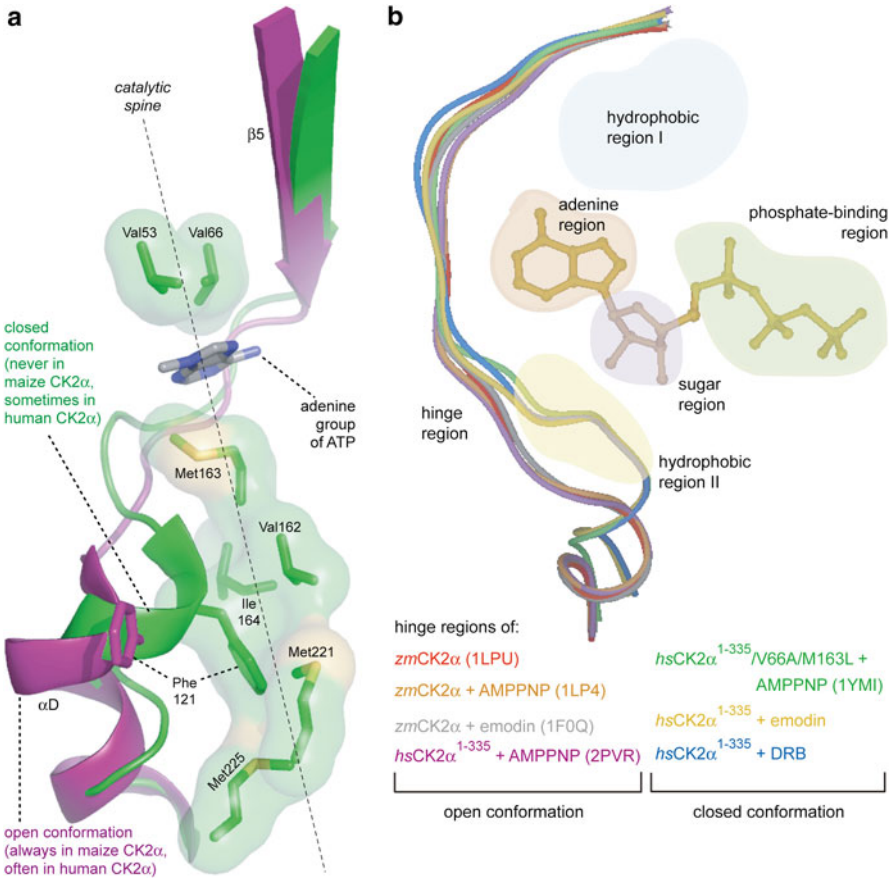

Fig. 5 Conformational ambiguity of the hinge/helix αD region next to the ATP site. (**a**) The hinge/helix αD region of CK2α was found with an open (*purple*) or with a closed conformation (*green/yellow*). The catalytic spine (indicated by a molecular surface) is fully assembled only in the closed conformation which so far occurred exclusively with human CK2α; in this conformation, the Phe121 side chain completes the catalytic spine. (**b**) The closed conformation of the hinge/helix αD region partly occupies the hydrophobic region II, i.e. one of the five sections defined by Traxler and Furet [44] in their protein kinase pharmacophore model. This picture was a part of a talk presented by one of the authors at the 5th International Conference on Protein Kinase CK2 2007 in Padua (Italy)

structure publication of CK2α [7] when only a handful of comparison structures were available, but it received a more profound basis by the spine concept of Taylor and Kornev [4] according to which any fully active protein kinase requires two "spines", i.e. two stacks of hydrophobic side chains crossing the interdomain interface. Consistent to this concept, CK2α has a "regulatory spine" which was never observed in a disassembled state, but Taylor and Kornev [4] left CK2α out when they illustrated the second, the "catalytic spine", which includes a side chain from the hinge/helix αD region. In other words, with respect to the regulatory spine, CK2α is a perfect representative of a typical EPK, but concerning the catalytic spine, it is an outlier [12, 13] (magenta-coloured trace in Fig. 5a) and once again a "challenge to canons" [43].

– The hinge/helix αD region of CK2α is not always stable in its non-canonical conformation; rather, it can switch to the EPK-canonical catalytic spine state (green trace in Fig. 5a). This conformational plasticity was noticed first in 2005 [42] and presented to the community at the 5th International Conference on Protein Kinase CK2 in 2007 in Padua, Italy, where it was discussed in the light of the protein kinase pharmacophore model of Traxler and Furet [44] (Fig. 5b). In 2008, the first report published [9] that CK2α orthologues differ in this respect: while maize CK2α sticks to the non-canonical and space-providing "open" hinge/helix αD conformation (Fig. 5a/b), human CK2α is able to exist with the EPK-typical "closed" conformation as well.

A number of follow-up questions were addressed in the subsequent years: (1) Which internal or external restraints and in particular which ligands favour either the open or the closed hinge/helix αD conformation? (2) Why do some CK2α orthologues exist always and stably with an open hinge/helix αD conformation while others can switch to the closed state? (3) Which of the two conformations is the (more) active one? (4) Can the particular features of the hinge/helix αD region be exploited for the design of CK2α-targeting drug, i.e. for improving the selectivity by addressing the unique open conformation? (5) Is the plasticity of the hinge/helix αD region coupled to the flexibility of other regions like the β4β5 loop or the ATP-binding loop?

One of the problems in this context became recently apparent by an analysis in which we described a strong artificial influence of the crystallisation conditions on the conformation of the hinge/helix CK2α region [15]: CK2α structures going back to crystallisation media dominated by kosmotropic salts like ammonium sulphate or sodium citrate as precipitants always have the "closed" hinge/helix αD conformation; this makes sense insofar as kosmotropic salts typically support hydrophobic interactions which means in this context that they induce the completion of the hydrophobic catalytic spine by burying the otherwise solvent-exposed aromatic side chain of Phe121 in a hydrophobic cavity formed by the other catalytic spine side chains (Fig. 5a). In other words, whenever CK2α was crystallised by a kosmotropic salt as a salting-out agent, the hinge/helix αD region is dominated by this medium and adopts the EPK-canonical conformation irrespective of any ligand. Noteworthy, maize CK2α that seems to have particular sequence adaptations in this region in favour of the open hinge/helix αD conformation [12] was never crystallised by a kosmotropic salt.

Hence, it is not trivial to distinguish between such artificial restraints on the hinge/helix αD conformation and genuine effects of ATP-competitive ligands. Nevertheless, Battistutta and Lolli [12] derived a rule that can be regarded as well established now: ATP-site ligands without polar interactions to the backbone of the hinge region (e.g. the inhibitor emodin [9, 14, 27]) do not prefer either of the two conformations, whereas ligands that exploit such hinge interactions for binding (like ATP as the prototype) have a clear preference for the open hinge/helix αD conformation.

CK2β was a further ligand of CK2α suspected to be capable of stabilising the open hinge/helix αD conformation [11]. This hypothesis was based on the first $CK2\alpha_2\beta_2$ holoenzyme structure [6] in which both CK2α chains have an open hinge/helix αD conformation, but due to the non-proximity of CK2β and the hinge/helix αD region, it lacked a convincing mechanistic rationale. In fact, recent $CK2\alpha_2\beta_2$ tetramer [32] and CK2α/cyclic peptide structures [30] with closed hinge/helix αD conformation ruled out the notion that the occupation of CK2α's N-lobe cap by CK2β or by a CK2β analogue induces the open hinge/helix αD conformation.

Recent molecular dynamic simulations have revealed that the closed hinge/helix αD conformation is energetically even more stable than the open one [16]. The highest activation energy barrier between the two states is about 33.5 kJ/mol and depends largely on the rotation of the aforementioned Phe121 side chain (Fig. 5a) [16]. This is consistent with stable populations in solution coexisting in a dynamic equilibrium and with the possibility to stabilise either of them by specific ligands.

In many human CK2α structures, however, the whole hinge/helix αD region is characterised by a high degree of disorder, i.e. by high crystallographic B-factors and disrupted electron density maps. Noteworthy, this zone is not a typical surface loop for which this kind of disorder is not unusual. Rather, it looks like an example of local protein unfolding or "cracking" [45], a phenomenon that is assumed to play a key role for the change of a protein between two functionally relevant states by lowering the activation barrier via entropy reduction. In the case of epithelial growth factor receptor kinase, molecular dynamic simulations accompanied by hydrogen/deuterium exchange studies suggested that cracking of the hinge/helix αD region is important for the transition to catalytically inactive conformations [46]. How general cracking of the hinge/helix αD region is for EPKs and in particular whether it plays a role in the function of CK2α is an open and a very interesting question.

6 The Glycine-Rich ATP-Binding Loop: The Classical Case of Structural Plasticity

Apart from the hinge/helix αD region, the ATP-binding loop, which connects the β-strands 1 and 2 and stabilises via highly conserved glycine residues the negatively charged triphospho moiety of the cosubstrate, is the second functionally important high-mobility zone (black ellipse in Fig. 1) investigated in the aforementioned molecular dynamics study [16]. The rationale for this selection was a model [11]

according to which CK2β-unbound CK2α might exist in a dynamic equilibrium between three conformationally and functionally different states (Fig. 6a): (i) fully active with open hinge/helix αD region and stretched ATP-binding loop; (ii) fully inactive [10] with a closed hinge/helix αD region, with a collapsed ATP-binding loop blocking the ATP site and with a shortcut of both elements via an ionic interaction between Arg47 and Asp120; and (iii) partially active with a closed hinge/helix αD region but with a stretched ATP-binding loop so that binding of ATP is possible [42] albeit in a not fully productive way.

The computational study [16] confirmed that the two principal states of the ATP-binding loop (stretched and collapsed as illustrated in Fig. 6b) have approximately the same free energy. Although the highest activation barrier along the path between them is only about 13 kJ/mol, a spontaneous collapse of the stretched ATP-binding loop during a 40 ns calculation could not be observed [16]. Nevertheless, among CK2α crystal structures, a collapse of the ATP-binding loop [27] and in particular the bending down of Arg47 into the ATP site (Fig. 6b) were observed more than once. Most recently, it was found (at least in one of three CK2α chains unambiguously) in the complex structure of human CK2α with a cyclic CK2β-mimetic peptide [30].

Insofar the existence of CK2α conformations with a collapsed ATP-binding loop is assured. It is well possible that CK2β by touching the back of the β1/β2 region (Fig. 6c) [6, 32, 33]—noteworthy, Phe54 in strand β2 is one of the "hot spots" of the CK2α/CK2β interaction [47] as visible in Fig. 4a—activates CK2α by stabilising the stretched ATP-binding loop and by preventing Arg47 from blocking the access to the ATP site [10]. In accordance with this notion, a recent study, in which conformational dynamics was derived from a sophisticated redefinition of electron density noise level followed by a systematic analysis of concerted side chain movements in some structures of CK2α and other EPKs, has revealed such an allosteric communication pathway between the ATP site and the CK2β-binding site of CK2α [19] (Fig. 6d).

7 The CMGC-Typical αGH Insert Remains Puzzling in its Function

As a member of the CMGC family of EPKs [48], CK2α contains a CMGC-characteristic helical insertion between the helices αG and αH of the EPK consensus fold [40]. In all CK2α structures, this insert has high B-factors (red ellipse in Fig. 1). Five years ago, we developed some ideas about possible functions of this region in the context of substrate binding and recognition based on homology to other CMGC family EPKs [8]; none of those notions was confirmed since that time, but falsification did not happen either. So the function of this high-mobility region remains enigmatic. For more insight, structures of enzyme/substrate complexes have to be awaited.

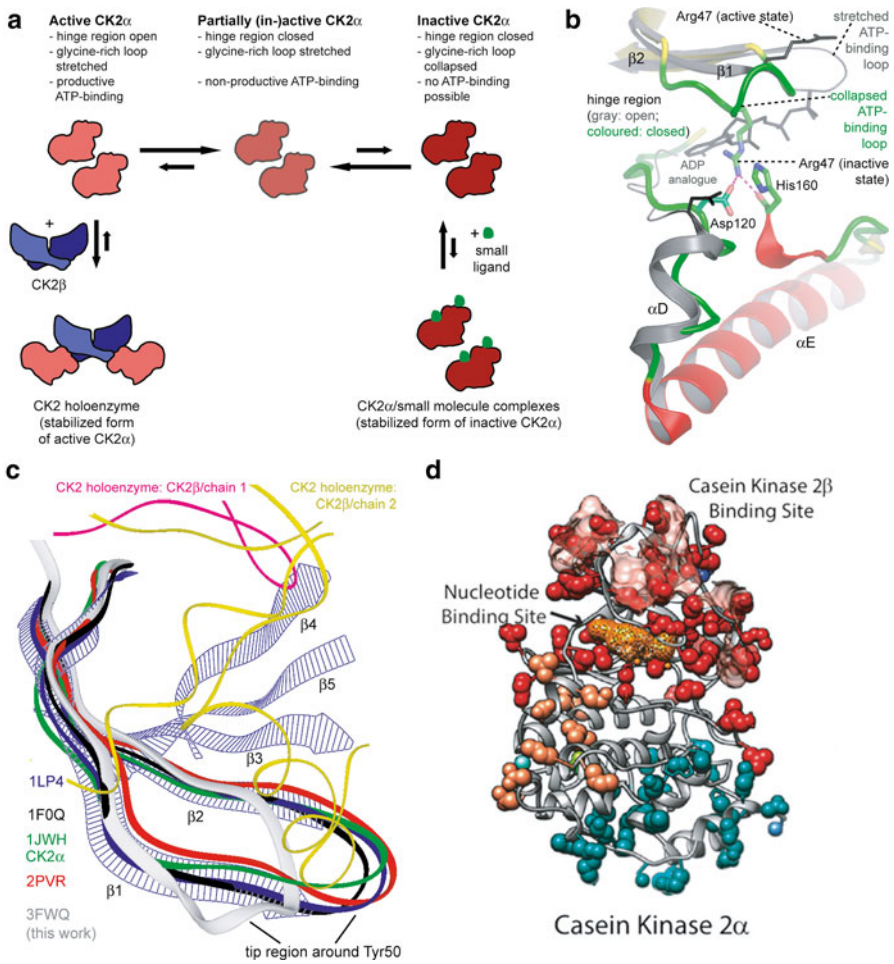

Fig. 6 The plasticity of ATP-binding loop as potential basis of the allosteric activation of CK2α by CK2β. (**a**) Model of CK2α regulation by CK2β and by small metabolites, published in a basic form after a structure of human CK2α with collapsed ATP-binding loop had been discovered [10] and in an extended version (including a partially active state of CK2α) shortly afterwards [11]. Reprinted from Biochimica and Biophysica Acta, Vol. 1804, Niefind, K. and Issinger, O.-G., "Conformational plasticity of the catalytic subunit of protein kinase CK2 and its consequences for regulation and drug design", pp484–492, 2010, with kind permission from Elsevier. (**b**) Shortcut between the ATP-binding loop (after collapse) and the hinge/helix αD region (after switching to the closed conformation) preventing access to the ATP site [10]; the largest conformational change is seen in Arg47 which directly interacts with Asp120 and His160 while it points to the opposite direction in fully active CK2α with extended ATP-binding loop. (**c**) Illustration of the first structure-based hypothesis [10] on how CK2β might activate CK2α by stabilising the ATP-binding loop from behind and thus preventing its collapse into the ATP site. Reprinted from the Journal of Molecular Biology, Vol. 386, Raaf et al., "First inactive conformation of CK2α, the catalytic subunit of protein kinase CK2", pp 1212–1221, 2009, with kind permission from Elsevier. (**d**) Allosteric communication path between the ATP site and the CK2β-binding site suggested by the concerted side chain mobility in that region [19]; the authors of this study describe a sophisticated procedure to detect the side chain polymorphism in protein crystal structures and apply it to CK2α and to some other eukaryotic protein kinases in an apo form and in an ATP-bound form, respectively. Reprinted from the Proceedings of the National Academy of Science USA, Vol. 111, Lang, P. T. et al., "Protein structural ensembles are revealed by redefining X-ray electron density noise", pp 237–242, 2014, with kind permission from the Proceedings of the National Academy of Science USA

8 CK2 Structural Biology: From Basic Insight to Application and Back!

After in an initial phase some fundamental CK2 structures had been published [6, 7, 49], CK2 crystallography entered an "application phase" already several years ago when CK2α/inhibitor structures became more and more important as valuable tools for drug development [50].While efforts in structure-based design of CK2 inhibitors is ongoing, the interest in the structural bases of fundamental properties of CK2 is revitalised. Aggregation and autoregulation phenomena of the CK2 holoenzyme are structurally investigated [31–33]; more species than so far are taken into account in CK2 structural biology [51]. We believe that more information about the conformational and configurational dynamics of the enzyme will enrich these efforts in a most fruitful way.

Acknowledgement The contributions of Anja Asendorf, Anna Köhler and Nicole Splett are gratefully acknowledged. The work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) to KNI (NI 643/4–1).

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