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Isoform Specific Inhibition of Human Protein Kinase CK2α and CK2α' by an Indenoindole Derivative

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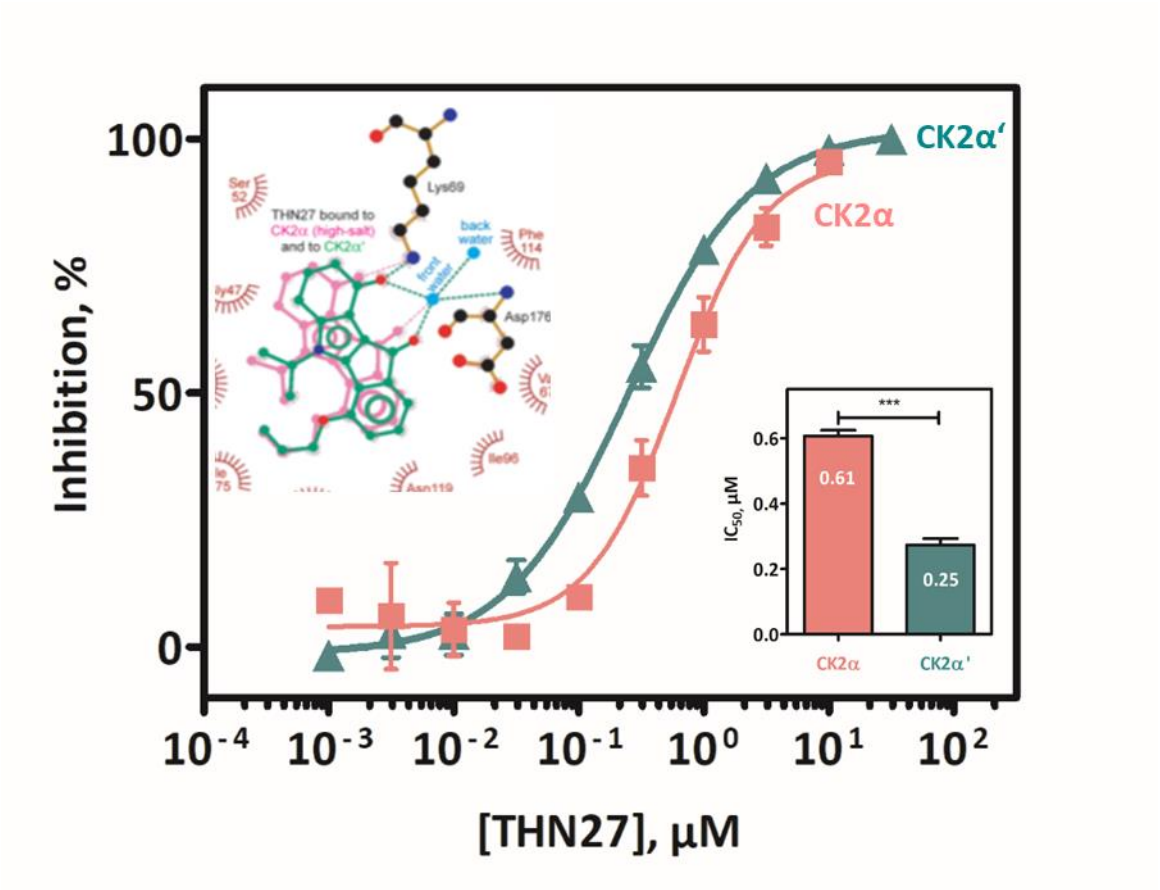

Abstract

The human protein kinase CK2 is an emerging target not only in current cancer research but also in the pathophysiology of viral diseases, such as CoV-2 infection. Two isoforms of the catalytic subunit of human CK2, namely CK2α and CK2α' were identified, exhibiting high similarity but minor functional and structural differences. Further, they differ in their expression profiles, with CK2α being ubiquitously expressed in every tissue while CK2α' being mainly present in brain and testis. In the cell, CK2α and CK2α' exist either as free subunits or in a tetrameric complex bound to a dimer of non-catalytic CK2β subunits. Inhibitors of CK2 which selectively target specific subunits of the kinase are advantageous for the examination of the different functions of the paralogous isoforms.

Here we report on THN27, an indeno[1,2-b]indole derivate that exhibits higher CK2α' inhibitory activity (IC_{50} = 0.25 µM) in comparison to CK2α (IC_{50} = 0.61 µM). Co-crystal structures of CK2α and CK2α' with THN27 reveled a different conformational viability in the interdomain hinge region explaining this behavior. Remarkably, this selective inhibitory behavior was eliminated by the addition of the regulatory subunit ($CK2\alpha_2\beta_2$ IC_{50} = 0.12 µM; $CK2\alpha'_2\beta_2$ IC_{50} = 0.12 µM). These results indicate that the preference of THN27 for CK2α' can be further utilized to study the distinct functions of free catalytic subunit paralogs.

Keywords: Human Protein Kinase CK2; THN27; Isoform specific inhibition

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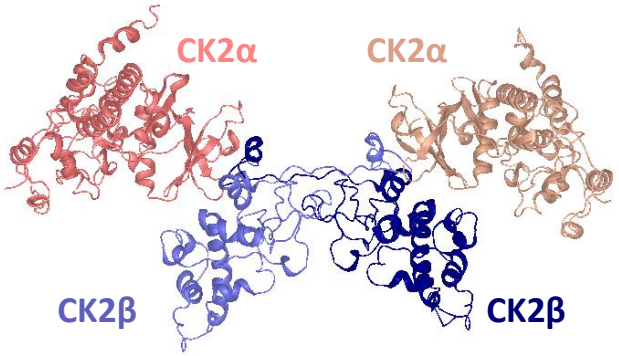
Introduction

Human protein kinase CK2



- Ubiquitous, constitutively active Ser/Thr kinase
- Free catalytic subunits or heterotetrametric complex of two catalytic subunits CK2α and/or CK2α' and two non-catalytic subunits CK2β

Emerging target

- in current cancer research
- in the fight against viral diseases, such as CoV-2

Isoforms CK2α and CK2α'

- Two catalytic subunit isoforms CK2α and CK2α' in humanes, exhibiting high similarity

  but
- Minor structural differences
- Differences in their expression profiles (CK2α - ubiquitously expressed; CK2α' - mainly present in brain and testis)
- Different cell cycle dependent phosphorylation

- **Isoform specific functions**

Isoform specific inhibitors are required to study the distinct functions of free catalytic subunit paralogs and to specifically address isoform associated diseases

Introduction

The two isoforms of human protein kinase CK2

Sequence alignment of human CK2α and CK2α'

```
numb. CK2α'    1        10         20         30         40     45          56  60
                |        |          |          |          |      | Gly-rich loop |   |
CK2α'           mpgpaagSRA RVYaeVNslR sREYWDYEaH VpsWGNQDDY QLVRKLGRGK YSEVFEAINI
CK2α            ~msgpvpSRA RVYtdVNthR pREYWDYEsH VveWGNQDDY QLVRKLGRGK YSEVFEAINI

numb. CK2α'   61          72       80         90         100        110
                |  strand β3 |        |          |          |          | interdom. hinge
CK2α'           TNNErVVVKI LKPVKKKKIK REvKILENLR GGtNIIkLiD tVKDPVSkTP ALVFEyiNNT
CK2α            TNNEkVVVKI LKPVKKKKIK REiKILENLR GGpNIItLaD iVKDPVSrTP ALVFEhvNNT

numb. CK2α'  121       130        140        150        160        170
                |helix αD  |        |          |          | catal. loop | Mg-bind. loop
CK2α'           DFKQLYQiLT DfDIRFYMYE lLKALDYCHS kGIMHRDVKP HNVMIDHqqk KLRLIDWGLA
CK2α            DFKQLYQtLT DyDIRFYMYE iLKALDYCHS mGIMHRDVKP HNVMIDHehr KLRLIDWGLA

numb. CK2α'  181       190        200        210        220        230        240
                |        |          |          |          |          |          |
CK2α'           EFYHPaQEYN VRVASRYFKG PELLVDYQMY DYSLDMWSLG CMlASMIFRr EPFFhGqDNY
CK2α            EFYHPgQEYN VRVASRYFKG PELLVDYQMY DYSLDMWSLG CMlASMIFRk EPFFhGhDNY

numb. CK2α'  241       250        260        270        280        290        300
                |        |          |          |          |          |          |
CK2α'           DQLVRIAKVL GTEeLYgYlk KYhIdLDPhF NDILGqHSRK RWEnFiHSEN rHLVSPEALD
CK2α            DQLVRIAKVL GTEdLYdYid KYnIeLDPrF NDILGrHSRK RWErFvHSEN qHLVSPEALD

numb. CK2α'  301       310        320        330        340        350
                |        |          |          |          |          |
CK2α'           lLDKLLRYDH QqRLTAkEAM EHPYFYpVVK eQsqpcadna vlssgltaar ~~~~~~~~~~
CK2α            fLDKLLRYDH QsRLTArEAM EHPYFYtVVK dQarmgsssm pggstpvrsa nmmsgis...
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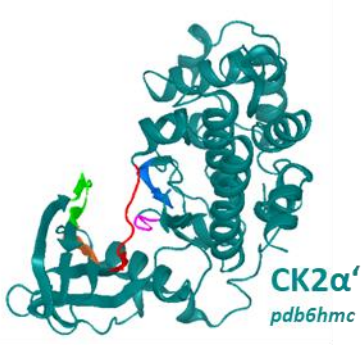
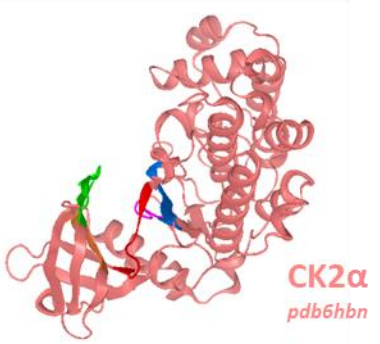


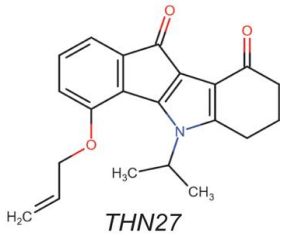


- 86 % identity in the catalytic core
- only variation in ATP-binding site:
  - **His115 and Val116 CK2α**
  - **Tyr116 and Ile117 CK2α'**

ATP-binding site:
glycine-rich loop, β3 strand, interdomain hinge, catalytic loop, magnesium-binding loop

Modified from Lindenblatt *et al*. (2019)

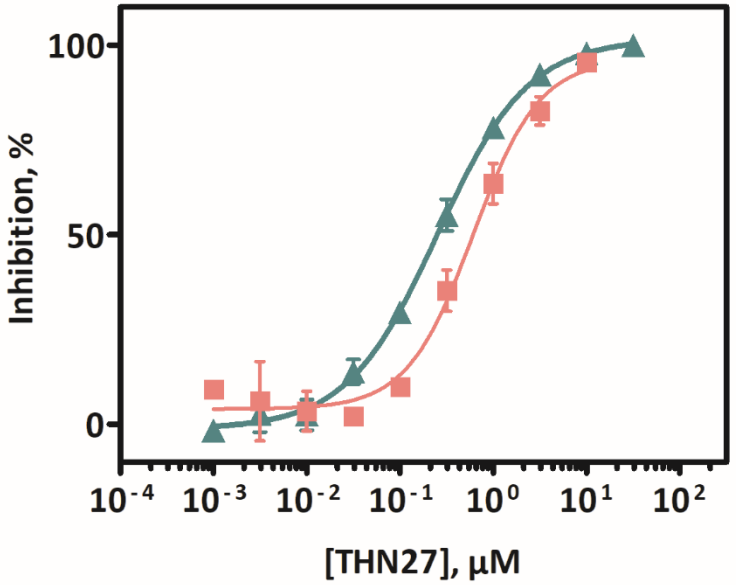
Results and discussion

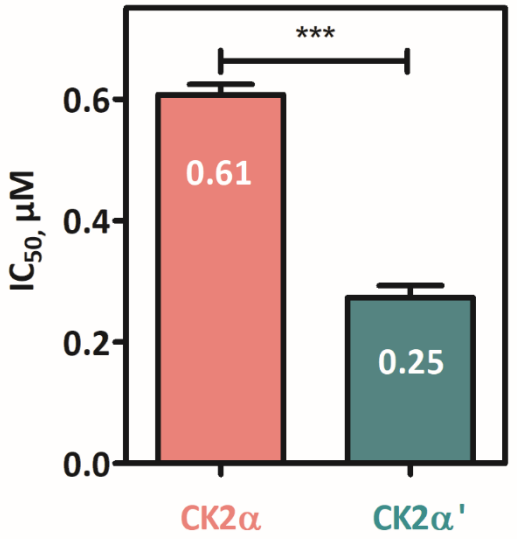


5-isopropyl-4-[(prop-2-en-1-yl)oxy]-5,6,7,8-tetrahydroindeno[1,2-b]indole-9,10-dione

Indenoindole derivate THN27 as inhibitor of free catalytic subunits CK2α an CK2α‘

Capillary electrophoresis analysis of CK2α/α‘ inhibition by THN27. The phosphorylation activities of CK2α and CK2α’ against the substrate peptide (RRRDDDSDDD) were analyzed in presence of different concentrations of THN27 to determine IC_{50} values (CK2α = 0.61 µM; CK2α‘ = 0.25 µM) .





*** p < 0.0001
n = 3

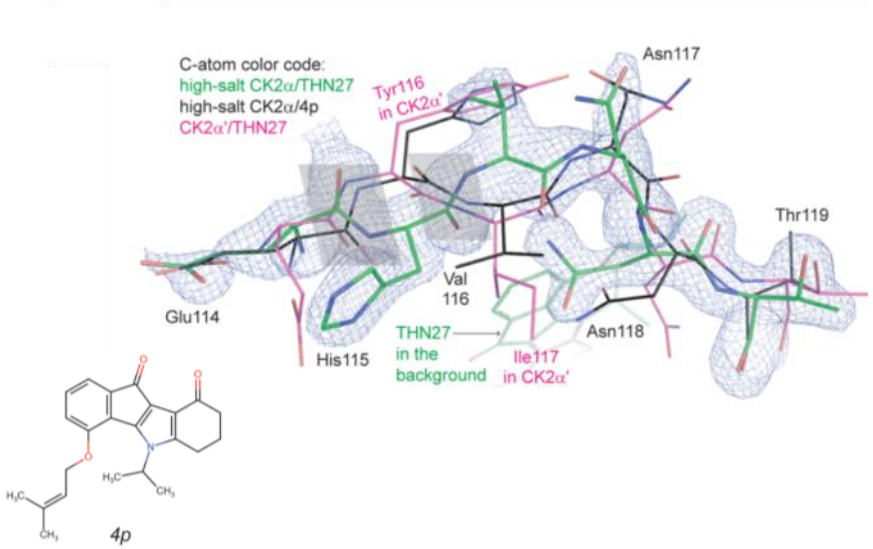
➢ **THN27 inhibits CK2α’ stronger than CK2α**

Modified from Lindenblatt *et al*. (2019)

Results and discussion

Structural explanation for isoform specific inhibition of THN27 – hinge region

High resolution crystal structures of CK2α and CK2α' in complex with THN27 and of CK2α in complex with 4p as control.



Lindenblatt *et al*. (2019)

conformational variability of the hinge region

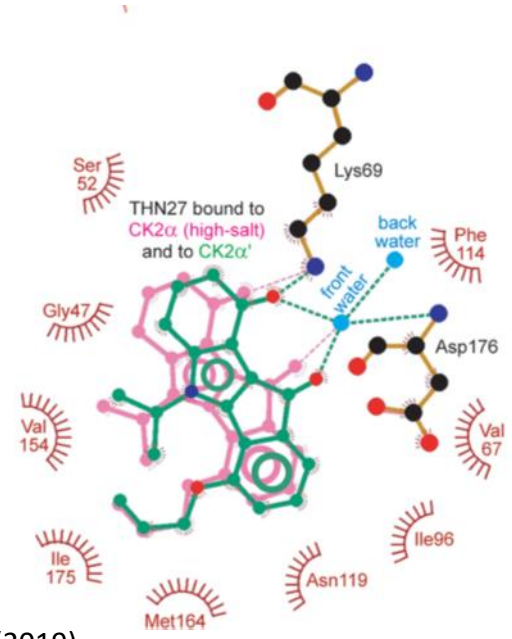
- CK2α **His115** and **Val116** smaller, more flexible → conformationally dynamic hinge region
  (only with THN27 not with 4p)
- CK2α' **Tyr116** and **Ile117** larger, less flexible → stabilized, rigid hinge region

- **Different conformations of the hinge region in the CK2α'/THN27 and CK2α/THN27 complex**
- **Hinge region of CK2α is dynamic and does not provide a preformed conformation for THN27 binding as the hinge region of CK2α' does**

Results and discussion

Structural explanation for isoform specific inhibition of THN27 – ATP binding site

2D-projection of the noncovalent interactions between THN27 and either CK2α or CK2α'.



Lindenblatt *et al*. (2019)

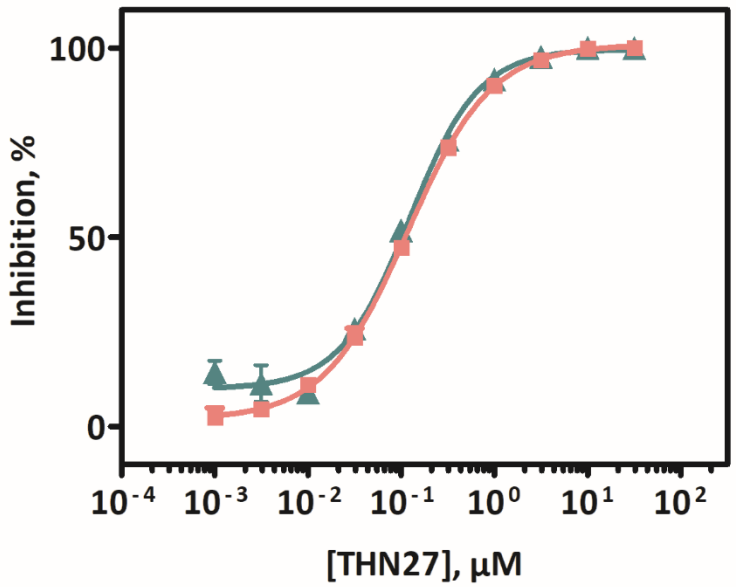
- Two ketonic oxo groups of THN27B in hydrogen bond network with two conserved water molecules, Lys69 and Glu82
- CK2α': THN27B **three** H-bonds
- CK2α: THN27B rotated outward, only **two** H-bonds

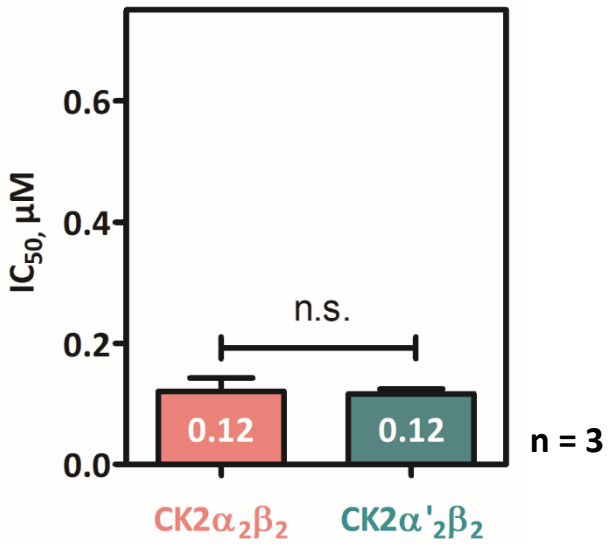
- **Outward rotation of THN27 in the ATP site of CK2α**
- **Loss of one H-bonds between THN27 and CK2α**
- **THN27 binds stronger to CK2α' than to CK2α**

Results and discussion

THN27 inhibition of $CK2\alpha_2\beta_2$/$CK2\alpha'_2\beta_2$ holoenzymes

Capillary electrophoresis analysis of $CK2\alpha_2\beta_2$/$CK2\alpha'_2\beta_2$ inhibition by THN27. The phosphorylation activities of $CK2\alpha_2\beta_2$ and $CK2\alpha'_2\beta_2$ against the substrate peptide (RRRDDDSDDD) were analyzed in presence of different concentrations of THN27 to determine IC_{50} values (CK2α = 0.12 µM; CK2α' = 0.12 µM) .





- **Inhibitory selectivity of THN27 is removed in presence of CK2β**
  - **Most probably, CK2β stabilizes the hinge conformation**

Modified from Lindenblatt *et al*. (2019)

Conclusions

- THN27 inhibits CK2α' stronger than CK2α
  - This effect is explainable by a conformationally stable enzyme environment of CK2α' due to a rigid hinge region and three instead of two (CK2α) H-bonds to THN27
  - In comparison, CK2α offers a dynamic hinge region and does not provide a preformed conformation for THN27 binding
  - THN27 could be utilized to study the distinct functions of free catalytic subunit paralogs
- The formation of the holoenzymes $CK2\alpha_2\beta_2$/$CK2\alpha'_2\beta_2$ removes the difference in THN27 inhibition
  - unlikely that THN27 can be extended toward a CK2 inhibitor with strong isoform selectivity
  - Comparative structural analyses of significant $CK2\alpha_2\beta_2$- and $CK2\alpha'_2\beta_2$-holoenzyme complexes are required to verify a stabilizing effect of CK2β to the hinge conformation

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