

Available online at www.sciencedirect.com





COMMUNICATION

Evolved to Be Active: Sulfate Ions Define Substrate Recognition Sites of CK2α and Emphasise its Exceptional Role within the CMGC Family of Eukaryotic Protein Kinases

Karsten Niefind¹*, Christina W. Yde², Inessa Ermakova¹ and Olaf-Georg Issinger²

¹Universität zu Köln Institut für Biochemie Zülpicher Straße 47 D-50674 Köln Germany

²Syddansk Universitet Institut for Biokemi og Molekylær Biologi Campusvej 55 DK-5230 Odense Denmark $CK2\alpha$ is the catalytic subunit of protein kinase CK2 and a member of the CMGC family of eukaryotic protein kinases like the cyclin-dependent kinases, the MAP kinases and glycogen-synthase kinase 3. We present here a 1.6 Å resolution crystal structure of a fully active C-terminal deletion mutant of human CK 2α liganded by two sulfate ions, and we compare this structure systematically with representative structures of related CMGC kinases. The two sulfate anions occupy binding pockets at the activation segment and provide the structural basis of the acidic consensus sequence S/T-D/E-X-D/E that governs substrate recognition by CK2. The anion binding sites are conserved among those CMGC kinases. In most cases they are neutralized by phosphorylation of a neighbouring threonine or tyrosine side-chain, which triggers conformational changes for regulatory purposes. $CK2\alpha$, however, lacks both phosphorylation sites at the activation segment and structural plasticity. Here the anion binding sites are functionally changed from regulation to substrate recognition. These findings underline the exceptional role of $CK2\alpha$ as a constitutively active enzyme within a family of strictly controlled protein kinases.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: protein kinase CK2; casein kinase 2; CMGC family of eukaryotic protein kinases; constitutive activity; protein crystallography

*Corresponding author

 $CK2\alpha$ is the catalytic subunit of protein kinase CK2 (former name casein kinase 2). Together with $CK2\beta$, a non-catalytic anchor protein (also referred to as regulatory subunit) serving as a docking platform for substrates and other binding partners,¹

E-mail address of the corresponding author: Karsten.Niefind@uni-koeln.de stoichiometry. CK2 is a pleiotropic and acidophilic serine/threonine kinase with more than 300 substrates described in the literature²; its consensus sequence for phosphoacceptor site recognition (S/T-D/E-X-D/E) contains two acidic determinants at the P+1 and the P+3 position, one of which at least must be present in a substrate.² Within the phylogenetic tree of eukaryotic protein kinases (EPK) CK2α is a remote member of the

CK2 α associates to a CK2 holoenzyme with $\alpha_2\beta_2$

kinases (EPK) CK2 α is a remote member of the CMGC family branch^{3,4} (Supplementary Data Figure 1) and contains an extended insert in the C-terminal domain after helix α G (Figure 1(a)), which is a CMGC-typical landmark. The eponymous members of the CMGC family are the cyclin-dependent kinases (CDK), the mitogen-activated kinases (MAPK), glycogen synthase kinase-3 (GSK3) and the cell division control 2 (CDC2)-like kinases

Abbreviations used: AMPPNP, adenylyl

imidodiphosphate; CAPK, cyclo-AMP-dependent protein kinase; CDK2, cyclin-dependent kinase 2; CK2, casein kinase 2; CK2 α , catalytic subunit of protein kinase CK2; CK2 β , non-catalytic subunit of protein kinase CK2; *hs*CK2 α^{1-335} , C-terminal deletion mutant of recombinant human CK2 α ; EPK, eukaryotic protein kinase; GSK3, glycogen-synthase kinase 3; MAPK, mitogen-activated protein kinase; PDB, Protein Data Bank.

(a)		ATP-binding lysine basic stretch
seq. no. CK2a 2 1 hsCK2a ¹⁻³³⁵ SGVPSRA GSK3 (1GNG) CBK2 (12RK) CDK2 (10MZ) CAPK (1ATP) sec. str. CK2a 	20 30 RV YTDVNTHRPR EYWDYESHVV EWGN(40 50 60 70 73 80 85 QDDYQL VRKLGRGKYS EVFEAINITN NEKVVVKILK PVKK KKIRKEIKIL EVSYTD TKVIGNGSFG VVYQAKLCDS GELVAIKKUL PQGK AFKNRELQIM RAVYRD LQPV AVCSAVDGRT GAKVAIKKLY RPF.QSELFA KAYNELQIM GPRYTN LSYIGEGAYG MVCSAYDNLN KVVAIKKIS PFEHQTYC QRTLREIKII MENFQK VEKIGEGTYG VVYKARNKLT GEVVAIKKIR LDT.ETEGVP STAIREISII LDQPDR IKTLGTGSFG RVMLVKHKES GNHYAMKILD KQKVVKLKQI EHTINEKRII Y-LIGEG-YG - V YKAT GVAIKKI
$\begin{array}{llllllllllllllllllllllllllllllllllll$		117 120 124 130 140 150 160 VN NTDFKQL YQTLTDYDIR FYMYEILKAL DYCHSMGIMH RDVKPHNVMI VPE.TV YRVARHYSRA KQTLPVIYVK LYMYQIFRSL AYHSFGICH RDIKRQNLLI MGT.DL GKLMK HEKLGEDRIQ FLVYQMLKGL RYHAAGIIH RDLKPGNLAW MET.DL YKLLK TQHLSNDHIC YFLYQILRGL KYHSANVLH RDLKPSNLLI LHQ.DL KKFMDASA LTGIPLPLIK SYLFQLLQGL AFCHSHRVLH RDLKPENLLI VAGGEM FSHLRR IGRFSEPHAR FYAAQIVLTF EYLHSLDLIY RDLKPENLLI <
phosphory	latable threenines and strain	ned CMGC
$\begin{array}{llllllllllllllllllllllllllllllllllll$	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Coll 210 220 230 240 245 RYPKGP ELLVDYQMYD YSLDMWSLGC MLASMIFRKE PFFHGHDNYD QLVRIAKU RYYRAP ELIFGATDYT SSIDUWSAGC VLAELLL.GQ PIFFGDSGVD QLVEIIKU RWYRAP EVILNWMRYT QTVDIWSVGC IMAEMIT.GK TLFKGSDHLD QLKEIMKU RWYRAP EIMLNSKGYT KSIDIWSVGC ILAEMLS.NR PIFFGKHYLD QLNHILGI LWYRAP EILLGCKYYS TAVDIWSLGC IFAEMVT.RR ALFFGDSEID QLFRIFRI PEYLAP EILLSK.GYN KAVDWWALGV LIYEMAA.GY PFFADQPIQ IYEKIVSGKU RWYRAP E-LLGYVD-WSVGC I-AE> <αF> <αG>
seq. no. CK2α 250 hsCK2α ¹⁻³³⁵ LGTEDLYD GSK3 (IGNG) LGTPTREQ p38γ (ICM8) TGTPPAEF ERK2 (2ERK) LGSPSQED CDK2 (1QM2) LGTPDEVV CAPK (1ATP) R consens. CMGC/RD LGTP sec. str. CK2α <α	260 270 280 XI DKYNIELDPR FNDILGRHSR KRWEI I IR EMNPNTTEFAPP QIKAL VQ RLQSDEAKNYMKGL PELEH LN CIINLKARNYLLSL PHKNI VKPSF PKWAL WP GVTSMPDYKPSF PKWAL	290 300 310 313 320 328 RFVHSE NQHLVSPEAL DFLDKLLRYD HQSRL TAREAMEHPY FYTVV~~~~~ HPWTKV FRPRTPPEAI ALCSRLLEYT PTARL TPLEACAHSF FDELRDPTVK KKDFAS ILTNASPLAV NLLEKMLVLD AEQRV TAGEALAHPY FESLHQVGKY KVPWNR LFPNADSKAL DLLDKMLTFN PHRRI EVEQALAHPY LEQYTDPSDE RQDFSK VVPLDEDGG SLLSQMLHYD PMKRI SAKAALAHPF FQDVTKPVPH FPSHFSSDLK DLLRNLLQVD LTKRFGNLKN GVNDIKNHKW FATTDWIAIY
(b) 1 2 83 85 81 88	N-terminal segment	(c) hsCK2α ¹⁻³³⁵ (this work) hsCK2α ¹⁻³³⁷ in CK2 holoenzyme (1JWH) hsCK2α ¹⁻³³⁵ V66A/M163L (1YMI) hsCK2α ¹⁻³³⁵ (283 K structure; 1PJK) maize CK2α (1LP4) N-terminal segment
αD αE αE αF αG αI CMGC insert	β6 β9 αA1 P+3 sulfate αH P+1 sulfate	e P+1 loop P+1 loop CDK2/cyclin A (1QMZ) partially active CDK2/cyclin A (1QMZ)

Figure 1 (legend on next page)

Download English Version:

https://daneshyari.com/en/article/2188337

Download Persian Version:

https://daneshyari.com/article/2188337

Daneshyari.com