Photo-crosslinking of human protein kinase regulatory subunit CK2β for the identification of CK2 binding partners

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Incorporation of unnatural amino acid p-azidophenylalanine (pAzF) by genetic code expansion during protein biosynthesis

Incubation with cell lysate & Irradiation with UV light

Separation and analysis by mass spectrometry

CK2α CK2β

N3

m/z

N3

N3

N5

CH2

NH

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Abstract:

Human protein kinase CK2 is a heterotetrameric Ser/Thr kinase, consisting of two catalytic (CK2α/α’) and two regulatory (CK2β) subunits. CK2 plays a key role in several physiological and pathological processes. Moreover in cancer cells it was shown that CK2 is upregulated [1]. Although the number of more than 300 substrates is still increasing, the regulation of CK2 remains unclear [2]. It is assumed that several protein-protein interactions are involved in the regulation of CK2. Thereby CK2β modulates the substrate specificity of CK2 and also functions as a docking platform for regulators and substrates. This study aims for the identification of binding partners by photo-crosslinking coupled with mass spectrometry. Therefore the unnatural amino acid p-azidophenylalanine (pAzF) is incorporated into CK2β [3].

Here we report the establishment of the photo-crosslinking procedure with purified CK2β-pAzF with its strongest binding partner CK2α as a proof of principle. The photo-crosslinking product of CK2β-pAzF and CK2α was detected by SDS-PAGE analysis and immunostaining. Furthermore it was shown, that the photo-crosslink reaction is specific for interaction partners and is not affected by other proteins. The site directed photo-crosslinking reaction was compared to the common used homo-bifunctional NHS-ester disuccinimidyl suberate (DSS) that crosslinks primary amino groups.

References:
[1] Tawfic, S. et al.: Histol Histopathol. 2001, 16:573-582.
[2] Meggio, F. and Pinna, L.A.: FASEB J. 2003, 17:349-368.
[3] Chin, J.W. et al.: J. Am. Chem. Soc. 2002, 124, 9026-9027.

Keywords: Protein Kinase CK2; Photo-crosslinking
Introduction

CK2 as a pleiotropic kinase
- key role in several physiological and pathological processes
- regulation of CK2 still unclear
- CK2β as a modulator of substrate specificity of CK2 and as a docking platform for regulators and substrates

Identification of new binding partners of CK2β by photo-crosslinking and mass spectrometry

Proof of principle – photo-crosslinking of CK2α

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Results and Discussion

Influence of pAzF incorporation into CK2β on holoenzyme formation

Capillary electrophoresis analysis of CK2 activity
The phosphorylation of a substrate peptide (SP) by CK2α alone and in addition of CKβ or CK2β_pAzF was analyzed at 37°C.

Incorporation of pAzF into CK2β keeps its function to stabilize CK2α unaffected
Results and Discussion

Photo-crosslinking of CK2β\textsubscript{pAzF} and CK2α

CKβ\textsubscript{pAzF} was incubated with CK2α and irradiated with UV light of 365 nm. The αβ-photo-crosslink (*) was analysed by SDS-PAGE with Coomassie staining and by Western Blot with a primary antibody against CK2α.
Results and Discussion

Specificity of photo-crosslinking reaction in presence of non-interaction partners

Photocrosslinking of CK2β\textsubscript{pAzF} and CK2α in presence of bovine serum albumin (BSA) as a non-binding partner of CK2β

CKβ\textsubscript{pAzF} was incubated with CK2α and a two fold higher concentration of BSA. The proteins were irradiated with UV light of 365 nm and separated by SDS-PAGE. (*) CK2αβ-photo-crosslink; (**) CK2 ββ-photo-crosslink

Photo-crosslinking reaction is not influenced by background proteins like BSA.
Results and Discussion

Non-site-directed crosslinking method in comparison

Crosslinking of CK2β and CK2α with disuccinimidyl suberate (DSS)

CKβ and CK2α were incubated with different concentrations of the homo-bifunctional NHS-ester DSS that crosslinks primary amino groups. Crosslinks were analysed by SDS-PAGE.

One αβ-photo-crosslink with CK2β_pAzF+CK2α

Multiple crosslinks with DSS+CK2β+CK2α
Conclusions

- The unnatural amino acid $p$-azidophenylalanine (pAzF) was incorporated into the regulatory CK2 subunit CK2β.

- This mutant $\text{CK2}_\beta^{p\text{AzF}}$ was still able to increase the activity of CK2α.

- $\text{CK2}_\beta^{p\text{AzF}}$ was successfully photo-crosslinked with CK2α.

- It could be shown, that the photo-crosslinking reaction is not influenced by background proteins like bovine serum albumin.

- Compared to another crosslinking method using DSS, photo-crosslinking with incorporated pAzF offers the advantage of a site directed reaction with only one crosslinking product per $\text{CK2}_\beta^{p\text{AzF}}$ protein.
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