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

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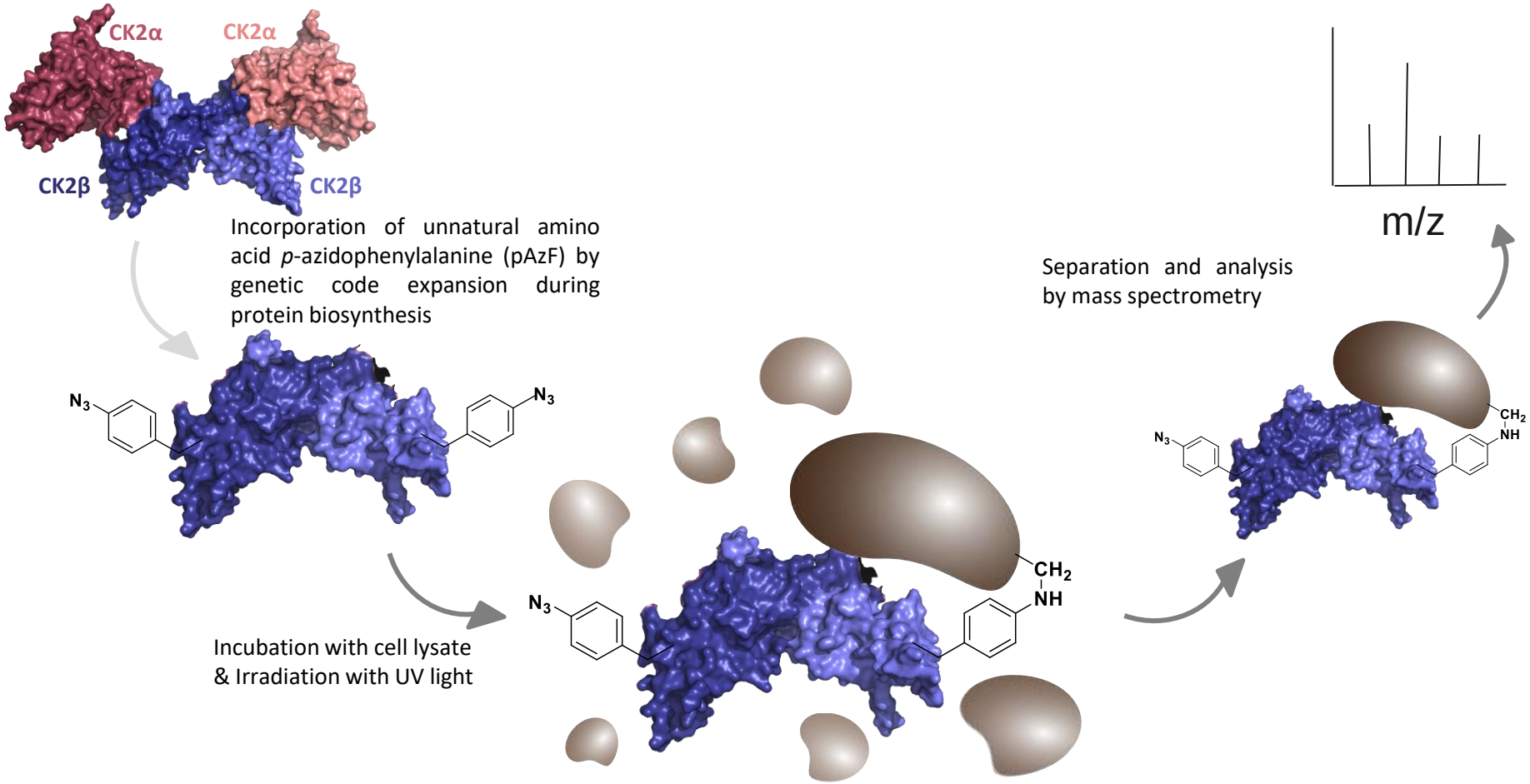


Abstract:

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**.

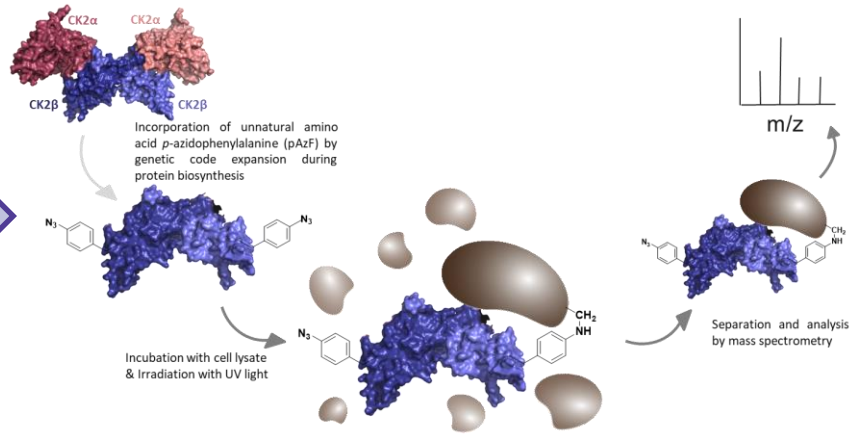
Keywords: Protein Kinase CK2; Photo-crosslinking

Introduction

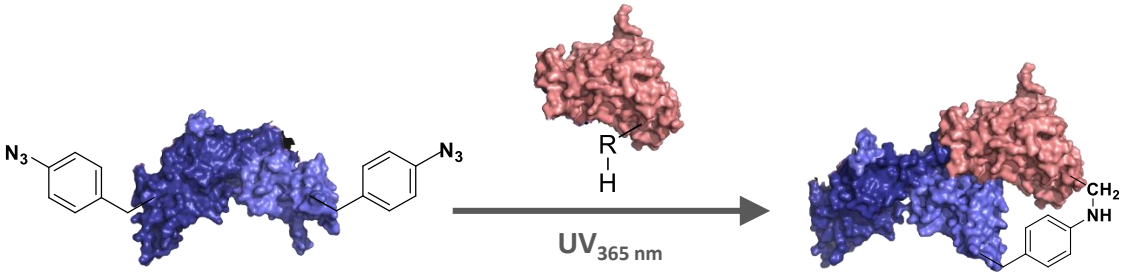
CK2 as a pleiotropic kinase

- 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



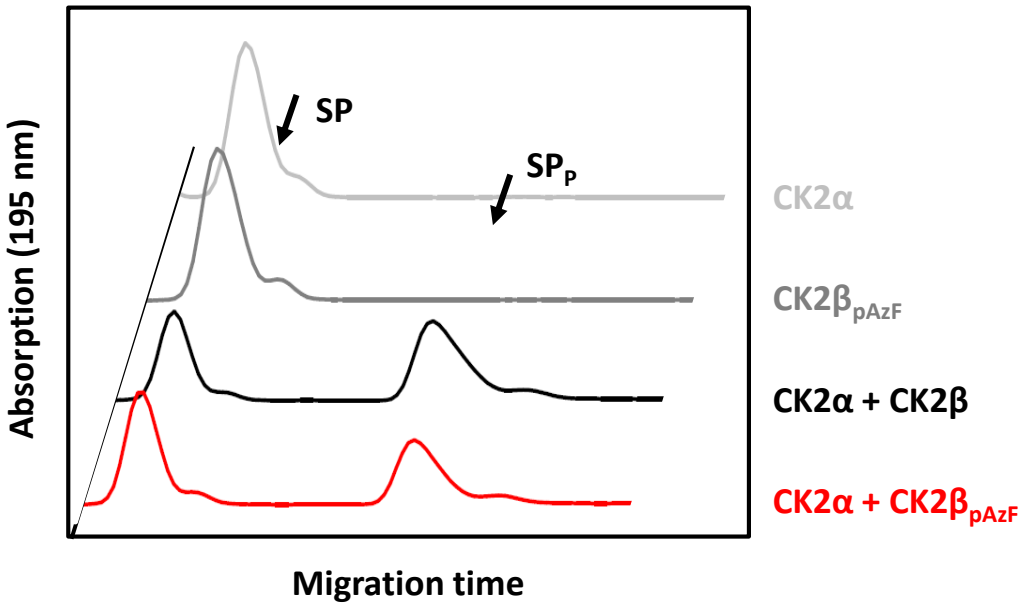
Proof of principle – photo-crosslinking of CK2α



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.

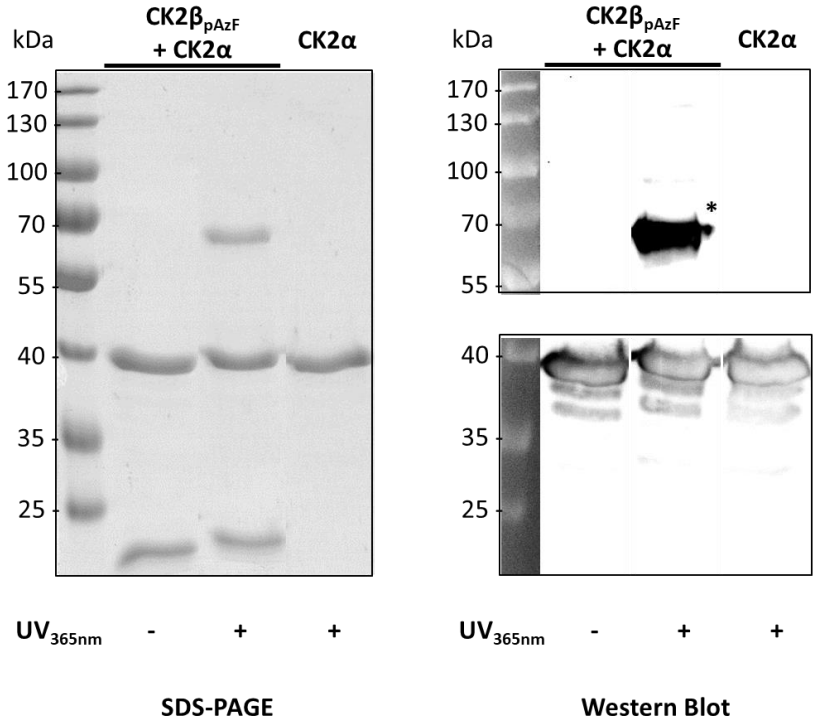


Incorporation of pAzF into CK2β keeps its function to stabilize CK2α unaffected

Results and Discussion

Photo-crosslinking of CK2β_{pAzF} and CK2α

CKβ_{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.



The interaction of CK2α and CK2β_{pAzF} was covalently captured by photo-crosslinking

Results and Discussion

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

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

CKβ$_{pAzF}$ 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

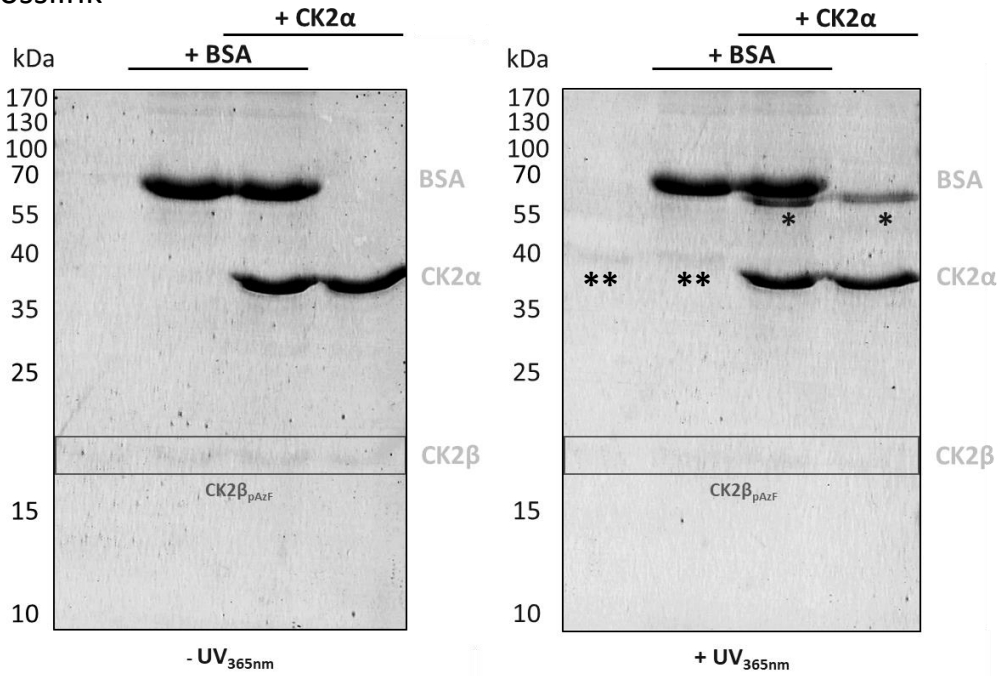
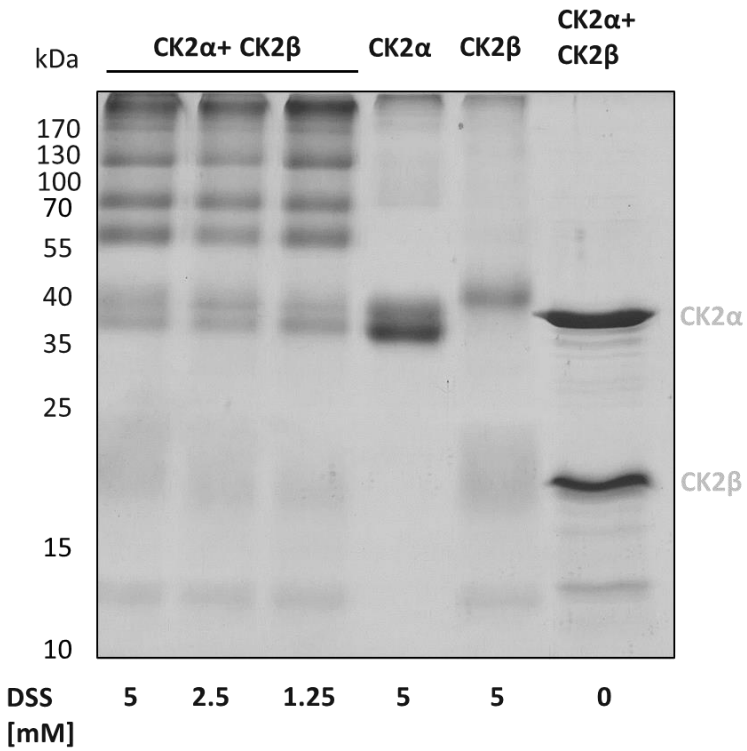


Photo-crosslinking reaction is not influenced by background proteins like BSA

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 $CK2\beta_{pAzF}$+CK2α

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Multiple crosslinks with DSS+CK2β+CK2α

Conclusions

- The unnatural amino acid *p*-azidophenylalanine (pAzF) was incorporated into the regulatory CK2 subunit CK2β
- This mutant $CK2\beta_{pAzF}$ was still able to increase the activity of CK2α
- $CK2\beta_{pAzF}$ 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 $CK2\beta_{pAzF}$ protein

Acknowledgements

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