Experimental evidences of the NO action on a recombinant PrxII F from pea plant and its effect preventing the citrate synthase aggregation

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\textbf{Article Info}

\textbf{Article history:}
Received 5 February 2015
Received in revised form 10 February 2015
Accepted 16 February 2015
Available online 26 February 2015

\textbf{Keywords:}
Citrate synthase
Oligomerization
PrxII F
S-nitrosylation

\textbf{Abstract}

S-nitrosylation is emerging as a key post-translational protein modification for the transduction of NO as a signaling molecule in plants. This data article supports the research article entitled "Functional and structural changes in plant mitochondrial PrxII F caused by NO" [1]. To identify the Cys residues of the recombinant PrxII F modified after the treatment with S-nitrosylating agents we performed the LC ESI-QTOF tandem MS and MALDI peptide mass fingerprinting analysis. Change in $A_{650\text{ nm}}$ was monitored to estimate the thermal aggregation of citrate synthase in the presence S-nitrosylated PrxII F. The effect of the temperature on the oligomerization pattern and aggregation of PrxII F was analysed by SDS-PAGE and changes in absorbance at 650 nm, respectively.

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DOI of original article: http://dx.doi.org/10.1016/j.jprot.2015.01.022

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1. Data, experimental design, materials and methods

PsPrxII F treated with 5 mM GSNO was run in SDS-PAGE and the visualized bands were analysed by TOF QTOF mass spectrometry (Fig. 1). S-nitrosylated PrxII F was subjected to the biotin switch method [2] and immunoprecipitated using anti-biotin antibody and then was subjected to SDS-PAGE. The visualized bands were excised from the gel and digested overnight, in darkness at 37 °C, adding recombinant trypsin (Sigma-Aldrich, St-Louis, MO) at a 1:20 ratio as described in [3]. The digestion was stopped by adding 0.5% trifluoroacetic acid (TFA, Pierce Rockford, IL) and the peptides were extracted immediately, dried by speed-vacuum centrifugation and resuspended in 5 μL of initial ESI solvent solution (100% water + 0.5% formic acid). The visualized bands were digested and the peptides were analysed by nano LC ESI–QTOF tandem MS and MALDI peptide mass fingerprinting analysis. The S-nitrosylation was estimated by changes in the mass of the peptide containing the Cys residues, GVDSVI\text{C}_{84}VAINDPYTVNAWAEK in the monomeric (score 98) and dimeric (73) forms of the PrxII F while KVVIFGLPGAYT\text{G}V_{59}\text{S}SK only in the monomeric form (28). Additionally, the nitrosylation of PrxII F was also verified as the incorporation of a HPDP-biotine on the Cys 59 in the dimeric form (Fig. 2).

The thermal aggregation of CS was evaluated by light scattering at 650 nm during 1800 s at 45 °C, in the presence of GSNO (Fig. 3A) and SNP-treated (Fig. 3B) PsPrxII F. This was assayed at molar ratio 0.5:1 and 2:1 (PsPrx IIIF:CS) observing that it was also prevented at molar ratio 2:1 (PsPrx IIIF:CS) while
no inhibition of CS aggregation was observed at molar ratio 0.5:1 (PsPrx II:CS) and DTT treated-PsPrxII F. Additionally, the samples were separated into a soluble and pellet fraction and visualized on SDS-PAGE gel to identify the CS amount precipitated as consequence of its thermal aggregation. Staining of bands corroborated the results observed at 650 nm, a decrease in the intensity of the bands corresponding to pellet fraction indicated that CS thermal aggregation was prevented by GSNO and SNP-treated PsPrxII F at molar ratios 1:1, 1:2 (PsPrxII F:CS) (Fig. 3C and D). It is noted, that the thermal aggregation of CS assayed on SDS-gel was strongly prevented at molar ratio 2:1 (PsPrxII F:CS) in presence of GSNO-treated PsPrxII F, while SNP-treated PsPrxII F prevented the thermal aggregation of CS to a slow molar ratio of 0.5:1 (PsPrxII F:CS). Experimental controls were carried out to demonstrate that PsPrxII F treated with DTT did not prevent the CS thermal aggregation and to verify that high temperature (45 °C) did not provoke the aggregation of the PsPrxII F (Fig. 4A and B).

**Conflicts of interest**

None.
Fig. 3. Chaperone activity estimated from the ability of PsPrxII F to inhibit the thermal aggregation of citrate synthase (CS). PrxII F treated with 5 mM GSNO (A) and 250 μM SNP (B) was incubated with CS at a molar ratio (0.5:1, 1:1, 2:1). Samples were separated into pellet and soluble fractions and equal volumes were analysed by SDS-PAGE and visualized by silver staining. Thermal aggregation is evident from the amount of CS present in the pellet fraction after incubation with GSNO (C) and SNP (D) at the molar ratios (0.5:1, 1:1, 2:1). The numbers represent the intensity of the bands quantified on an image analyser (Gen Tools, Syngene Frederick, MD). The significance of differences between means values was determined by one-way analysis of variance. Duncan’s multiple range test was used to compare the means when necessary. All error bars represent standard error (SE) of the mean. The asterisk above the bars indicates significant difference ($P < 0.01$).
Acknowledgments

D. Camejo and A. Ortiz were supported by JAE postdoc-CSIC Research program and FPI program from MICINN-Spain, respectively. This work was supported by MICINN (BFU2011-28716, BIO2008-04067) and the Séneca Foundation, Murcia, Spain (04553/GERM/06). Authors thank personnel from CBN and IPBLN, CSIC, Spain belonging to ProteoRed, PRB2-ISCIII for their help in MS analysis. Authors also thank Dr. T Philip for correction of the written English in the manuscript.

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Fig. 4. Electrophoretic mobility on SDS-PAGE visualized by Coomassie staining of the recombinant PsPrxII F treated with 50 mM DTT (PrxII F-SH), 5 mM GSNO (PrxII F GSNO) and 250 μM SNP (PrxII F SNP) incubated at 25 °C and 45 °C during 30 min (A). Light scattering at 650 nm of PrxII F treated with DTT, GSNO and SNP incubated at 45 °C during 1800 s (B).