Neospora caninum 由来 Protein Disulfide Isomerase の同定と機能解析

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Cloning and characterization of a gene encoding a protein disulfide isomerase from *Neospora caninum*

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SUMMARY

A gene encoding a protein disulfide isomerase (PDI) was isolated from a *Neospora caninum* cDNA expression library. The nucleotide sequence of the cDNA clone revealed the presence of an ORF of 1,416 bp, which encoded 461 amino acids, showing a high degree of homology to *Toxoplasma gondii* PDI. The gene was cloned into a pGEX vector and expressed in *E. coli* as a GST fusion protein. The *Nc*PDI was detected in *N. caninum* tachyzoite lysate and ES products with a molecular weight of 50 kDa. IgA antibody in 58.0% of individual cattle tear samples recognized both the recombinant and native *Nc*PDI, which suggests that the PDI-specific antibody may be involved in defense against parasites. In addition, PDI-specific inhibitors showed significant inhibitory effect on the growth of *N. caninum* tachyzoites. The purified recombinant *Nc*PDI demonstrated biological activitiy in vitro by catalysis and refolding of reduced RNase, and assisted in the recovery of native from denatured lysozyme. These findings indicate that the *Nc*PDI possesses specific-PDI enzymatic activity and it offers a putative target for prevention and chemotherapy of neosporosis.

[Aim of Research]

Protein disulfide isomerase is a multifunctional protein of endoplasmic reticulum (ER) known to be involved in the formation, breakage, and rearrangements of disulfide bonds during the folding of protein. The PDIs of some protozoa such as *Toxoplasma gondii*, *Leishamania major*, and *Plasmodium falciparum* have been reported. The targeting of PDI might provide better understanding in parasite-host interaction, and offers new opportunities for the prevention and chemotherapeutic strategies in the control of *N. caninum* and *T. gondii* infection. The objective of this study is to express and characterize *N. caninum* PDI for further studies of its function(s) on the control of neosporosis.

[Materials and Methods]

One Monoclonal antibody (mAb) 9E8, which showed cross-reactivity with both *N. caninum* and *T. gondii* was used to immunoscreen a cDNA library of *N. caninum*. The resulted clone was sequence and analysed

by using macVector software program and BLAST program. One resulted sequence was designed as NcPDI gene due to the high homology with *T. gondii* PDI gene. The sequence was then cloned into pGEX-4T-3 vector and expressed as GST fusion protein in *E. coli*. Analysis of expressed protein was performed by SDS-PAGE and Western Blot analysis. Antiserum against recombinant protein rainsed in mice was used to detect native protein in parasite lysate and excretory-secretory (ES) products of *N. caninum*. IgA antibody in bovine tear was detected with parasite lysate and recombinat protein. The specific PDI inhibitors tocinoic acid and zinc bacitracin in various concentration were used in a vitro assay to detect the inhibitory effects on growth of the parasites. Assays of NcPDI activity in RNase refolding and in lysozyme refolding were performed as descried otherwhere (Gilbert, 1998; Puig and Gilbert, 1994).

[Results and Discussion]

One clone containing 1,416bp ORF, encoding 471 amino acids was obtained by immuniscreening of *N. caninum* cDNA expression library with 9E8 mAb. Sequence analysis showed NcPDI present very high homology (94% identity) with *T. gondii*, containing two Trx-like domains with active site sequence motif CXXC characteristic for PDIs, and a C-terminal ER retention signal (GEEL). Like *T. gondii* PDI, NcPDI also different from all other known eukaryotic PDI sequences in an amino acid substitution of the consensus histidine (H) by a tyrosine (Y) in the C-terminal Trx-like domain. *N. caninum* and *T. gondii* are two very close parasites, they share the similar character in the Trx-like domain. As expected, around 76 kDa GST-NcPDI fusion protein has been expressed in *E. coli* as soluble form with very high-level expression. Both GST-NcPDI and NcPDI reacted with mAb 9E8 and mouse antiserum against *N. caninum* tachyzoites in a Western blot analysis. Anti-GST-NcPDI serum clearly recognized a 50 kDa protein in both *N. caninum* lysate and ES products. Mouse antiserum against recombinant GST-NcPDI protein reacted with excretory-secretory protein from *N. caninum* strongly indicated that this protein can secret out of ER. It has been shown PDI play chaperone function in assistant membrane protein folding. So it is possible that PDI exits the ER bound to its targets function as a chaperone for membrane proteins. The evidence that NcPDI present as one of ES proteins might explain that in our previous work the mAb reacted with putative NcPDI showed high inhibition to the growth of *N. caninum*, although its intracellular localization suggests that PDI is unavailable for binding by the antibody. It is known that PDI of *T. gondii* is targeted by mucosal IgA antibodies in human. In order to determine whether NcPDI could be recognized by mucosal IgA antibodies in bovine tears, 52 tear samples of cattle were incubated with both recombinant NcPDI and parasite lysate, and then incubated with goat anti-bovine IgA in western blot analysis. The result showed IgA antibodies of 32 tear samples out of 52 samples were able to recognize the recombinant NcPDI, and 30 tear samples recognized a 50 kDa protein band in *N. caninum* tachyzoite lysate. The results indicate that PDI-specific antibodies constitute part of the mucosal antibody repertoire presumably involved in defense against parasites. The effect of PDI inhibitors on parasite growth was detected. *N. caninum* tachyzoites expressing GFP were grown in Vero cell in the presence of various concentrations of PDI inhibitors, tocinoic acid and zinc bacitracin. A significant inhibition (more than 50%) was observed at 0.25 mM zinc bacitracin and 1.0 mm tocinoic acid, and almost a 100% inhibition was observed at 2 mM of both inhibitors. PDI specific inhibitors also showed high growth inhibition in *L. major* in vitro. Moreover, the PDI of *P. faciparum* was selectively targeted by anti-malaria compounds inhibiting growth of this parasite. Present findings suggest PDI as a potential suitable target for anti-protozoan drugs. The results of enzymatic activity assay showed NcPDI was able to catalyze the refolding of scrambled RNase and assistant in recovery of native lysozyme. In summary, one biologically active protein the PDI present in the ES products has been identified in *N. caninum*. The fact that PDI specific inhibitors inhibited the growth of *N. caninum* in vitro, and that the NcPDI was recognized by IgA antibody in bovine tear suggest the potential role of PDI in the prevention and control of protozoan diseases.

[References]

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