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Besnoitia besnoiti protein disulfide isomerase (BbPDI): molecular characterization, expression and in silico modelling.

Marcelino E, Martins TM, Morais JB, Nolasco S, Cortes H, Hemphill A, Leitão A, Novo C.

Source

Instituto de Investigação Cientifica Tropical, CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Av. da Universidade Técnica, 1300-477 Lisboa, Portugal.

Abstract

Besnoitia besnoiti is an apicomplexan parasite responsible for bovine besnoitiosis, a disease with a high prevalence in tropical and subtropical regions and re-emerging in Europe. Despite the great economical losses associated with besnoitiosis, this disease has been underestimated and poorly studied, and neither an effective therapy nor an efficacious vaccine is available. Protein disulfide isomerase (PDI) is an essential enzyme for the acquisition of the correct three-dimensional structure of proteins. Current evidence suggests that in Neosporacaninum and Toxoplasmagondii, which are closely related to B. besnoiti, PDI play an important role in host cell invasion, is a relevant target for the host immune response, and represents a promising drug target and/or vaccine candidate. In this work, we present the nucleotide sequence of the B. besnoiti PDI gene. BbPDI belongs to the thioredoxin-like superfamily (cluster 00388) and is included in the PDI\_a family (cluster defined cd02961) and the PDI\_a\_PDI\_a'\_c subfamily (cd02995). A 3D theoretical model was built by comparative homology using Swiss-Model server, using as a template the crystallographic deduced model of Tapasin-ERp57 (PDB code 3F8U chain C). Analysis of the phylogenetic tree for PDI within the phylum apicomplexa reinforces the close relationship among B. besnoiti, N. caninum and T. gondii. When subjected to a PDI-assay based on the polymerisation of reduced insulin, recombinant BbPDI expressed in E. coli exhibited enzymatic activity, which was inhibited by bacitracin. Antiserum directed against recombinant BbPDI reacted with PDI in Western blots and by immunofluorescence with B. besnoiti tachyzoites and bradyzoites.