Available online at www.sciencedirect.com
ScienceDirect
Molecular & Biochemical Parasitology 157 (2008) 137–147
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Plasmodium falciparum signal peptidase is regulated by phosphorylation and required for intra-erythrocytic growth

Renu Tuteja*, Arun Pradhan, Sutikshan Sharma

Malaria Group, International Centre for Genetic Engineering and Biotechnology, P.O. Box 10504, Aruna Asaf Ali Marg, New Delhi 110067, India

Received 31 May 2007; received in revised form 16 October 2007; accepted 18 October 2007
Available online 24 October 2007

Abstract

The human malaria parasite *Plasmodium falciparum* exports a variety of its proteins through its endoplasmic reticulum (ER) based secretory pathway in order to survive in the host erythrocyte. Signal peptidases are membrane-bound endopeptidases and have an important role in the transport and maturation of these parasite proteins. Prokaryotic signal peptidases are indispensable enzymes required for the removal of N-terminal signal peptide from the secretory proteins. Eukaryotic signal peptidases exist as multimeric protein complex in the ER and the catalytic subunit of this complex catalyzes removal of the N-terminal signal peptide from preproteins. All the signal peptidases contain five regions of high-sequence similarity referred to as boxes A–E. Here we report characterization of the catalytic subunit of signal peptidase complex (SPC) from *P. falciparum*. This protein designated as PfSP21 shows homology with the similar subunit from other sources and contains all the conserved boxes A–E. PfSP21 is able to cleave the peptide substrate containing the signal peptidase cleavage site. PfSP21 is phosphorylated by protein kinase C and its enzyme activity was upregulated after this phosphorylation. Immunofluorescence assay studies revealed that PfSP21 is localized in the ER of *P. falciparum*. PfSP21 dsRNA specifically inhibits the growth of *P. falciparum* in culture and this inhibition is most likely due to the decrease in the amount of endogenous PfSP21 protein. These studies demonstrate the characterization of a functional subunit of SPC from *P. falciparum* and should make an important contribution in our better understanding of the complex process of protein translocation in the parasite.

Keywords: Endoplasmic reticulum; *Plasmodium falciparum*; Protein phosphorylation; Protein translocation; Signal peptide

1. Introduction

Malaria is a devastating disease caused by the protozoan parasites of the genus *Plasmodium* and *Plasmodium falciparum* causes the most virulent form of malaria [1]. The parasite undergoes a cycle of asexual replication and division in human erythrocytes and in order to survive in the host, the parasite significantly modifies the host cell. The particular parasite virulence and disease pathology are caused mainly by the parasite-encoded virulence proteins, which are synthesized by the parasite and transported to specific locations within the host cell [2]. These virulence proteins pass through the parasite's secretory pathway and are delivered via the endoplasmic reticulum (ER) to their correct destination [2,3]. Protein targeting in *P. falciparum* is a very complex process and its secretory pathway is almost similar to higher eukaryotes but it contains highly reduced Golgi network and rhoptries, micronemes and dense granules, which represent specialized secretory compartments characteristic of the parasite [2,4–7]. Most of the secreted parasite proteins are synthesized with a classical amino-terminal ER-type signal sequence known as signal peptide while other proteins contain a putative internal signal peptide or do not contain recognizable ER targeting signals [4,5,8]. These secreted proteins are then routed through the ER and the signal peptide is removed in the ER lumen [7]. Some of the parasite proteins such as KAHRP (knob-associated histidine-rich protein) and RESA (ring-expressed surface antigen) contain unusual 'non-canonical' recessed hydrophobic signal sequences beginning 20–50 amino acids from the amino-terminus [4,5]. The translocation machinery of higher eukaryotes does not recognize these recessed signal sequences because KAHRP is not processed correctly in cell-free systems using mammalian microsomes [5]. The major virulence factor, PfEMP1 (*P. falciparum* erythrocyte membrane protein1) also lacks an N-terminal hydrophobic signal sequence

* Corresponding author. Tel.: +91 11 26741358; fax: +91 11 26742316.
E-mail addresses: renu@icgeb.res.in, renu_tuteja@hotmail.com (R. Tuteja).

0166-6851/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2007.10.007

and it has been assumed that its transmembrane region acts as a "start transfer" signal for insertion into the ER membrane [3,4].

The family of structurally related enzymes, which catalyzes the removal of the N-terminal signal peptide from a variety of precursor proteins, is commonly known as signal peptidases [9]. The cleavage of signal peptide by these enzymes helps to release the secreted protein from various biological membranes including the membrane of the ER so that it can be transported to its final destination [9–11]. Signal peptidases have been identified in various organisms including bacteria, archaea and eukarya and these enzymes belong to a novel family of serine proteases [9,12–14]. The bacterial signal peptidase (also known as leader peptidase) represents a novel antibiotic target because the inhibition of its activity leads to cell death due to an accumulation of secretory proteins in the cell membrane [10]. The bacterial signal peptidase usually consists of a single polypeptide but the eukaryotic microsomal signal peptidases of the ER are multimeric protein complexes [9]. The mammalian signal peptidase complex (SPC) was initially purified from canine ER microsomes and the subunits of this complex are named as SPC25, SPC22/23, SPC21, SPC18, and SPC12, according to their apparent molecular masses [15]. Due to the high homology to leader peptidase and also to each other, two of the subunits SPC18 and SPC21, which are located within the lumen of the ER, have been shown to function as catalytic subunits [15,16]. Two other subunits, SPC12 and SPC25 were found to be not essential for the enzymatic reaction [17]. In contrast to the SPC in mammals, the SPC in the yeast *Saccharomyces cerevisiae* has only one catalytic subunit, Sec11p that is the homologue of mammalian SPC18 and SPC21 [18–20].

Although the catalytic subunit of signal peptidase enzymes from various sources share little overall similarity, they all contain five regions of highly significant sequence homology and these are termed as boxes A–E [9,11,21]. Box A corresponds to the transmembrane segment and boxes B–E participates in the catalytic activity [10]. In the bacterial signal peptidase, serine present in box B and lysine present in box D form the catalytic dyad but in eukaryal enzyme the lysine is replaced with a histidine and the catalytic dyad is serine-histidine [10,11]. The consensus recognition site for signal peptidase is alanine-X-alanine, which provides a recognizable cleavage site [21]. *Bacillus subtilis* contains as many as five signal peptidase genes and several signal peptidase-encoding genes in a single genome have been reported previously [9–11]. Recently the gene encoding signal peptidase from the parasite *Leishmania major* and molecular and functional characterization of signal peptidase from human pathogen *Legionella pneumophila* has been reported [22,23].

The bioinformatics analysis of the *P. falciparum* genome revealed that it contains the homologues to almost all the components of the SPC and earlier we have reported the characterization of one of the signal peptidase homologue from *P. falciparum* [24,25]. In this study we report the isolation and functional characterization of a homologue of the catalytic subunit of the SPC18 and SPC21 from *P. falciparum*. The study demonstrates that this *P. falciparum* signal peptidase of 21 kDa (PfSP21) is homologous to other signal peptidases from both prokaryotes and eukaryotes and contains the five conserved domains A–E. The purified protein is catalytically active and the activity is upregulated after phosphorylation with protein kinase C (PKC). Immunofluorescence microscopy of the parasite-infected erythrocytes was used to show that PfSP21 is located within the ER of the parasite. We investigated the effect of PfSP21 specific dsRNA on parasite growth and the results show that growth of the parasite in culture is specifically blocked. These studies demonstrate the characterization of the catalytically active subunit of the SPC, which is an important component of the protein translocation pathway, from the human malaria parasite *P. falciparum*.

2. Materials and methods

2.1. Isolation of genomic DNA, RNA, preparation of cDNA and PCR amplification of signal peptidase gene

P. falciparum (strain 3D7) was cultured using human erythrocytes with 4% haematocrit in RPMI media from Gibco (Invitrogen, Carlsbad, CA, USA) supplemented with 10% human serum using a protocol described earlier [26]. Total RNA was isolated from asynchronous *P. falciparum* parasite lysate using RNeasy kit from Qiagen (GmbH, Germany). Total RNA was used for the preparation of cDNA using cDNA synthesis kit (Superscript first-strand synthesis system from Invitrogen, Carlsbad, CA, USA). The *P. falciparum* signal peptidase gene was amplified from this cDNA preparation using the forward primer PfSPF1 (5′-CG*GGATCC*ATGGATTTTATAAAAGAA-3′) and the reverse primer PfSPR1 (5′-C*AAGCTT*TCATATCCCATTAATATCAT-3′). The restriction sites in the primers have been written in italics. The PCR conditions were one cycle of denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min and extension at 72 °C for 1 min and final extension was carried out at 72 °C for 10 min. The PCR product was analyzed by agarose gel electrophoresis, gel purified and cloned into pGEM-T vector from Promega (Madison, WI, USA) and the obtained DNA clones were sequenced by dideoxy sequencing reactions. The DNA band from the recombinant clone of *P. falciparum* was excised using BamHI and HindIII enzymes and gel purified for subcloning into the expression vectors. The genomic DNA from the parasite culture was isolated using standard protocol [27]. The signal peptidase gene was amplified using *P. falciparum* genomic DNA as template and oligonucleotide primers PfSPF1 and the reverse primer PfSPR1 as described above. The PCR product was gel purified and cloned into pGEM-T vector from Promega (Madison, WI, USA) and the obtained DNA clones were sequenced.

For Southern blotting the *P. falciparum* genomic DNA was digested with the restriction enzymes NdeI and TaqI. The digested DNA was electrophoresed on a 0.8% agarose gel and transferred to nylon membrane according to the standard protocol [27]. The PCR fragment of signal peptidase from *P. falciparum* was labeled and used as a probe for hybridization.