Genomics Proteomics Bioinformatics 11 (2013) 378–384

Genomics Proteomics Bioinformatics

www.elsevier.com/locate/gpb
www.sciencedirect.com

LETTER

Identification of the Phosphorylated Residues in TveIF5A by Mass Spectrometry

Laura Itzel Quintas-Granados [1], César López-Camarillo [1], Jesús Fandiño Armas [1], Guillermo Mendoza Hernandez [2,#], María Elizbeth Alvarez-Sánchez [1,*]

[1] *Genomic Sciences Postgraduate, Autonomous University of Mexico City (UACM), CP 03100 Mexico City, Mexico*
[2] *Department of Biochemistry, Faculty of Medicine, National Autonomous University of Mexico, CP 04510 Mexico City, Mexico*

Received 11 March 2013; revised 27 June 2013; accepted 8 July 2013
Available online 5 December 2013

KEYWORDS

Trichomonas vaginalis;
Initiation factor eIF5A;
Phosphorylation;
Casein kinase 2

Abstract The initiation factor eIF5A in *Trichomonas vaginalis* (TveIF5A) is previously shown to undergo hypusination, phosphorylation and glycosylation. Three different pI isoforms of TveIF5A have been reported. The most acidic isoform (pI 5.2) corresponds to the precursor TveIF5A, whereas the mature TveIF5A appears to be the most basic isoform (pI 5.5). In addition, the intermediary isoform (pI 5.3) is found only under polyamine-depleted conditions and restored with exogenous putrescine. We propose that differences in PI are due to phosphorylation of the TveIF5A isoforms. Here, we have identified phosphorylation sites using mass spectrometry. The mature TveIF5A contains four phosphorylated residues (S3, T55, T78 and T82). Phosphorylation at S3 and T82 is also identified in the intermediary TveIF5A, while no phosphorylated residues are found in the precursor TveIF5A. It has been demonstrated that eIF5A proteins from plants and yeast are phosphorylated by a casein kinase 2 (CK2). Interestingly, a gene encoding a protein highly similar to CK2 (TvCK2) is found in *T. vaginalis*, which might be involved in the phosphorylation of TveIF5A in *T. vaginalis*.

Introduction

Eukaryotic translation initiation factor 5A (eIF5A) is a polyamine-dependent protein present in all eukaryotic cells [1]. The precursor eIF5A undergoes a specific spermidine-dependent post-translational modification known as hypusination for eIF5A activation [2]. During hypusination, a unique amino acid, Nε-(4-aminobutyl-2-hydroxyl) lysine, called hypusine, is generated by transferring the aminobutyl moiety of spermidine to a specific lysine residue in precursor eIF5A [2]. Both the lysine residue and the flanking residues are conserved among eukaryotes [3]. Hypusine is exclusively synthesized in precursor eIF-5A [3]. Besides hypusination, eIF5A proteins are phosphorylated in *Saccharomyces cerevisiae* (eIF5Ab) [4,5] and maize (ZmeIF5A). The phosphorylation in ZmeIF5A occurs at serine residue 2 (S2) [6]. Interestingly, eIF5A is represented by more than one isoforms in *Plasmodium falciparum*, presumably due to post-translational modifications [7]. Expression of

* Corresponding author.
E-mail: maria.alvarez@uacm.edu.mx (Alvarez-Sánchez ME).
Deceased.

Peer review under responsibility of Beijing Institute of Genomics, Chinese Academy of Sciences and Genetics Society of China.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.gpb.2013.07.004

two eIF5A isoforms was decreased modestly during schizont development, while expression of another isoform with a considerably more acidic isoelectric point (pI) was increased 15-fold and demonstrated a significant fold change during the proteomic analysis of *P. falciparum* at various developmental stages [7].

eIF5A from *Trichomonas vaginalis* (TveIF5A) is of archaebacterial origin [8]. T. vaginalis is the protozoan parasite responsible for trichomonosis, which is the most common sexually transmitted infection. Trichomonosis is associated with an increased risk of human immunodeficiency virus (HIV) infection [9], pelvic inflammatory disease [10] and adverse pregnancy outcomes [11]. Our previous studies reported that two spots with different isoelectric points (pI) (5.2 and 5.5) were detected in the T. vaginalis protein extracts. These two spots corresponded to the precursor (pI 5.2) and mature TveIF5A (pI 5.5), respectively [8], and the mature isoform contains the hypusine residue [12]. Interestingly, when putrescine biosynthesis was inhibited in *T. vaginalis* using 1,4-diamino-2-butanone (DAB) and the parasites were then transferred to a medium containing exogenous putrescine, a new TveIF5A spot with a pI of 5.3 was observed [12]. According to sequencing analysis, these three isoforms shared identical primary structures; however, the precursor and intermediate isoforms were 19 kDa, while the mature isoform was 20 kDa, which is phosphorylated and glycosylated [12]. Moreover, in silico analyses of the precursor isoform indicated that serine residues S3, S21, S83 and S153, threonine (T) residues T78 and T114, and tyrosine (Y) residues Y37 and Y96 are potential phosphorylation sites [12]. In this study, we went further to identify the phosphorylated residues in TveIF5A using LC–MS/MS. We found that mature TveIF5A contains four phosphorylated residues, S3, T55, T78 and T82; among which S3 and T82 were also phosphorylated in the intermediary isoforms, whereas no phosphorylated residues were identified in the precursor TveIF5A.

Results

T. vaginalis was grown in TYM-serum medium (control) or transferred into a medium containing exogenous putrescine after DAB treatment (DAB-treated). The extracted proteome was subjected to 2D gel separation and an identical gel was immunoblotted for the identification of the specific spots immunorecognized by the anti-TveIF5A antibodies. In this report, we focused on the 25–15 kDa region to identify phosphorylation sites in the three TveIF5A isoforms (**Figure 1**). For the parasites grown in the control medium, spots that were immunorecognized by the anti-peptide TveIF5A antibody corresponded to the precursor and mature TveIF5A isoforms with pIs of 5.2 and 5.5, respectively (Figure 1A). As expected, for the DAB-treated parasites that were then transferred into medium containing exogenous putrescine, the intermediary TveIF5A isoform was observed with a pI of 5.3 (Figure 1B). These three spots were excised from the gels and digested with trypsin to identify the protein and its possible phosphorylated residues by MS/MS analysis. According to the MS/MS identification, all the three isoforms, with pIs of 5.5, 5.3 and 5.2, respectively, corresponded to TveIF5A (Table S1). According to previous reports, the pI 5.5 isoform corresponds to mature TveIF5A, whereas the pI 5.3 and 5.2 isoforms correspond to the intermediary and precursor TveIF5A, respectively.

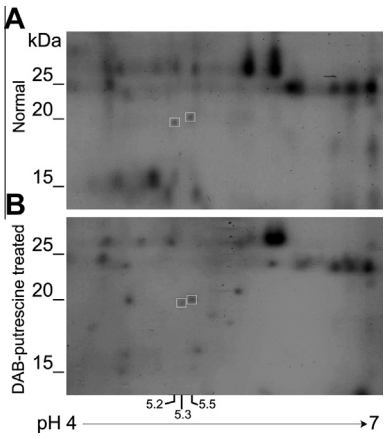

Figure 1 Identification of the three TveIF5A isoforms
Trichomonas vaginalis was grown under normal conditions (**A**) or treated with DAB and then cultured in a medium containing exogenous putrescine for 30 min (**B**). Total proteins were extracted and separated on 15% polyacrylamide 2DE gel. The gel was stained with Sypro-Ruby. Shown here are partial gels containing small proteins (15–25 kDa) including TveIF5A isoforms (boxed). The pI of each TveIF5A spot is indicated at the bottom. Experiments were performed at least in triplicate with identical results.

The MS/MS data were analyzed to identify modifications such as phosphorylated residues. We manually validated the peptides to identify the phosphorylated residues. **Figure 2** shows the phospho-site assignment for one of the phosphorylated peptides as an example. The peptide (SSAEEEVHHDLEIQEVDAGSQEK) found in the mature (pI 5.5) (Figure 2A) and intermediary (pI 5.3) (Figure 2B) TveIF5A had a monoisotopic mass of 3107.3357 and the S2 residue of this peptide represented a phosphorylated residue (**Table 1**). Peptide MSSAEEEVHHDLEIQEVDAGSQEK was identified for the precursor TveIF5A (pI 5.2); however, no phosp horylated residues were revealed (Figure 2C).

The phosphorylation sites were identified by manual validation of the phosphorylated peptides (Tables 1 and S2) using Mascot analysis as described in Material and Methods section. The mature isoform (pI 5.5) contains four phosphorylated residues, S3, T55, T78 and T82, whereas the intermediary isoform (pI 5.3) contains two phosphorylated residues at S3 and T82 (Table 1). No phosphorylated residues were observed in the precursor TveIF5A (pI 5.2).

The position of the phosphorylated residues in the primary sequence of TveIF5A is shown in **Figure 3A**. Multiple sequence alignment analysis indicated that T55 is highly conserved in all the species detected, while S3 and T78 are replaced with acidic residues D or E in most of the remaining species and T82 often is replaced with S, a residue potentially undergoing phosphorylation. K57, the residue for hypusination in TveIF5A [12], and the flanking sequences are highly conserved across all species examined (Figure S1). TveIF5A share 37% identity with eIF5A from *Leishmania mexicana*. We thus generated a 3D model of TveIF5A using eIF5A from *L. mexicana* (PDB: 1XTD) [13] as the template. According to the 3D model, the phosphorylated residues T55 and T82 are near K57 (**Figure 3B**).