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Review

Malaria proteomics: Insights into the parasite–host interactions in the pathogenic space☆,☆☆

José M. Bautista[a,b,*], Patricia Marín-García[c], Amalia Diez[a,b], Isabel G. Azcárate[a,b], Antonio Puyet[a,b]

[a]Department of Biochemistry and Molecular Biology IV, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain
[b]Research Institute Hospital 12 de Octubre, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain
[c]Department of Preventive Medicine, Public Health and Medical Immunology and Microbiology, Faculty of Health Sciences, Rey Juan Carlos University, Alcorcón, Madrid, Spain

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ABSTRACT

Proteomics is improving malaria research by providing global information on relevant protein sets from the parasite and the host in connection with its cellular structures and specific functions. In the last decade, reports have described biologically significant elements in the proteome of Plasmodium, which are selectively targeted and quantified, allowing for sensitive and high-throughput comparisons. The identification of molecules by which the parasite and the host react during the malaria infection is crucial to the understanding of the underlying pathogenic mechanisms. Hence, proteomics is playing a major role by defining the elements within the pathogenic space between both organisms that change across the parasite life cycle in association with the host transformation and response. Proteomics has identified post-translational modifications in the parasite and the host that are discussed in terms of functional interactions in malaria parasitism. Furthermore, the contribution of proteomics to the investigation of immunogens for potential vaccine candidates is summarized. The malaria-specific technological advances in proteomics are particularly suited now for identifying host–parasite interactions that could lead to promising targets for therapy, diagnosis or prevention. In this review, we examine the knowledge gained on the biology, pathogenesis, immunity and diagnosis of Plasmodium infection from recent proteomic studies. This article is part of a Special Issue entitled: Trends in Microbial Proteomics.

Abbreviations: 2D-DIGE, 2-D Fluorescence Difference Gel Electrophoresis; 2D-nanoLC, 2D-nanoflow high-performance liquid chromatography; 2D-PAGE, Two-dimensional gel electrophoresis; 4-HNE, 4-hydroxy-2-nonenal; ASP, Apical sushi protein; G6PD, Glucose-6-phosphate dehydrogenase; GBA, Guilt by association; GPF, Green-fluorescent protein; GPI, Glycosylphosphatidylinositol; HSP, Heat shock protein; HSP70, Heat shock protein-70; iRBCs, Infected red blood cells; MSP, Merozoite surface protein; MudPIT, Multidimensional protein identification; PKA, Protein kinase A; PM, Plasmepsin; PM-II, Plasmepsin II; PTMs, Post-translational modifications; RAMA, Rhoptry-associated membrane antigen; RBC, Red blood cell; ROS, Reactive oxygen species; SERA, Serine repeat antigen.

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☆☆ *Pathogenic space* is here defined as the three-dimensional extent in which parasite and host interact to produce disease, which is developed during their struggle for existence.

* *Corresponding author at:* Department of Biochemistry and Molecular Biology IV, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain. Tel.: +34 91 394 3823; fax: +34 91 394 3824.
E-mail address: jmbau@ucm.es (J.M. Bautista).

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Contents

1. Proteomics strategies in malaria

The parasitic Plasmodium species, causing malaria in vertebrates, are eukaryotic organisms that undergo drastic morphological changes during their complex biological life cycle in two different hosts across sexual and asexual stages (Fig. 1). Early proteomic analyses [1,2] on the life cycle of Plasmodium has demonstrated the existence of a tight-control on protein expression for surviving in both invertebrate and vertebrate environments, for the intracellular and extracellular survival, for invasion of multiple host cell membranes, and for evasion of host immune systems. Hence, numerous and innovative approaches have been required for the detailed proteomic studies on the parasite and to distinguish proteome changes in the host cell upon infection. Below, we focus on major advances generated in the last decade in specific strategies developed for the proteomic analysis of the intra-erythrocytic cycle of Plasmodium, the main carrier of the malaria disease in human and other vertebrates.

1.1. *In vitro approaches*

Several important global questions, which are relevant for the understanding of malaria and its eradication, may be addressed by proteomic approaches, including the ascertaining of the protein populations differentially expressed at each cycle stage, the identification of post-translational protein modifications and the identification of potential vaccine candidates or targets for antimalarial drugs. Proteomics of the malaria parasite began nearly three decades ago with pioneering work though two-dimensional gel electrophoresis (2D-PAGE) of the differential patterns of parasite proteins obtained before and after treatment of the antimalarial drug mefloquine [3]. Proteomics was also used in those years as a mean of identifying natural genetic variation in parasite populations [4]. Proteomic studies were limited at that time by the inability to easily identify proteins of interest on 2D gels, a situation that has dramatically changed in recent years with the completion of the genome project of *Plasmodium falciparum* [5–7] a decade ago and the innovations in mass spectrometry (MS) technology specifically dedicated to proteins [8].

Hence, the completion of the genome sequence of *P. falciparum* [5–7] opened the possibility of performing global transcriptomic and proteomic studies on the parasite life cycle. The first reports on the proteomes at different plasmodial cycles and stages were performed by means of non-gel techniques, such as multidimensional protein identification (MudPIT) [1] or 2D-nanoflow high-performance liquid chromatography (2D-nanoLC) [2] technologies. These studies were later complemented by other approaches allowing in-depth proteomic analysis such as the combination of 2D-PAGE with MALDI-TOF MS. Founding proteome studies in *P. falciparum* have been devoted to the characterization of the set of proteins expressed across developmental parasite stages, such as merozoites or schizonts, requiring the use of synchronic cultures and methods for the enrichment of specific plasmodial forms. Consequently, the improvement of classic protocols of *in vitro* culture of *P. falciparum* to obtain high densities of parasites at short synchronized windows [9,10] enable the isolation of large amounts of parasites and infected erythrocytes for proteomic studies. The presence of several different membrane structures during Plasmodium parasitism complicates isolation and attribution of proteins to specific cellular compartments or organelles during proteomic studies. Thus, the combined use of proteomics with in-depth bio-informatics analysis is facilitating adscription and annotation of subproteomes from membrane compartments [11,12]. In one of the first 2D-PAGE MALDI-TOF MS proteome-wide analysis of *P. falciparum* at intraerythrocytic stages [13], the combination of culture synchronization, schizont enrichment followed by isolation of subcellular vesicles, and the specific processing-protease inhibition to obtain parasitophorous vacuolar membrane-enclosed merozoite structures, allowed the isolation of proteins from late-stage schizonts (total and vesicle fraction), and merozoites suitable for 2D electrophoretic separation and MALDI-TOF MS identification of selected spots. Overall, from 255 protein spots analyzed, only 18% were of non-plasmodial origin, while 20% were not identified. The vesicle and membrane-bound