Data Article

Preferential binding of 4-hydroxynonenal to lysine residues in specific parasite proteins in plakortin-treated *Plasmodium falciparum*-parasitized red blood cells

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A R T I C L E  I N F O

Article history:
Received 13 October 2015
Received in revised form 2 November 2015
Accepted 2 November 2015
Available online 11 November 2015

Keywords:
plakortin endoperoxide antimalarial drug 4-hydroxynonenal post-translational modifications Plasmodium falciparum red blood cell

A B S T R A C T

The data show the frequencies by which the amino acid residues lysine, histidine and cysteine of six proteins of the malaria parasite *Plasmodium falciparum* are post-translationally modified by the lipoperoxidation endproduct 4-hydroxynonenal after challenging the parasitized red blood cell with plakortin. Plakortin is an antimalarial endoperoxide whose molecular anti-parasitic effect is described in Skorokhod et al. (2015) [1]. Plakortin did not elicit hemoglobin leakage from host red blood cells and did not oxidize reduced glutathione.

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S p e c i f i c a t i o n s  T a b l e

| Subject area          | Biology          |
|-----------------------|------------------|
| More specific subject area | Molecular pharmacology |
| Type of data           | Tables, graphs   |

DOI of original article: http://dx.doi.org/10.1016/j.freeradbiomed.2015.10.399

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How data was acquired
MALDI-TOF spectrometer (MALDI micro MX, Waters, Milford, MA, USA);
Luminometer Sirius (Berthold, Pforzheim, Germany)

Data format
Processed data

Experimental factors
Parasitized and non-parasitized red blood cells were kept in in vitro cell culture up to 24 h. Chemical extraction for parameter assessment.

Experimental features
Heme-dependent luminol-enhanced luminescence assay for hemoglobin.
Thiol-reaction with DTNB and OD measurement at 412 nm. Mass spectrometric analysis of peptides after trypsin-digestion of proteins.

Data source location
University of Torino, Torino, Italy

Data accessibility
Data are provided with this article

Value of the data

- The data show how endoperoxide-elicited oxidative stress might specifically target parasite proteins of functional importance by binding the lipoperoxidation endproduct 4-HNE.
- Data on endoperoxide-elicited modifications of proteins of a unicellular parasite as molecular cause for cell death may be of interest for similar studies with other microorganisms.
- Data for lethal modifications of specific microbial proteins with lipoperoxidation endproducts such as 4-HNE may suggest a novel strategy for high throughput drug research, e.g. antimalarials.
- The data on host red blood cells (RBC) intactness may be useful to monitor host cell damage by redox active substances.

1. Data

1.1. Frequencies of lysine residues modified by 4-HNE

In Table 1 we list the number of lysine, histidine and cysteine residues (K, H and C, respectively) that were found conjugated with 4-HNE and compare them with the total number of each of these amino acids in the respective protein. The complete list of 4-HNE modified Plasmodium falciparum proteins detected after plakortin treatment and specific sites of modification are reported in [1].

There is no particular imbalance between the portion of 4-HNE-conjugated amino acids in the proteins extracted from plakortin-treated parasites. We note that Plasmodium proteins contain elevated % of asparagine and lysine residues [2], even more then in vertebrates, where K frequency is 2–3 times higher than that of H and C [3].

Evidently, availability of the amino acid residue for 4-HNE is a crucial factor for binding. C residues engaged in disulfide bridges are no ligands for 4-HNE, while the basic amino acid K might be more frequently exposed to the protein–solvent interface compared to H, and hence be more accessible to 4-HNE binding [4]. The data about unbalanced ratios were published for other proteins such as liver fatty acid-binding protein [5], cytochrome c [6] and alpha-synuclein [7], where 4-HNE binding site distribution between K, H, C was 4:1:1 [5]; 10:1:0 [6] and 2:1:0 [7] respectively.

1.2. Data for hemoglobin release from RBC under plakortin treatment

Culture of RBC infected (parasitized) with trophozoite-stage P. falciparum were treated with 0–10 μM of plakortin and hemoglobin release was measured in culture supernatant (Fig. 1). The concentration is indicated in nmol/l. The concentration in the whole RBC suspension was 1 mM and assessed after complete lysis of RBC in NaOH/Triton: this is the highest maximal achievable concentration of hemoglobin in the supernatant corresponding to 100% RBC lysis.

As shown in Fig. 1 the hemoglobin was not released from RBC even at 10 μM plakortin, the highest applied concentration. Very low lysis was detectable in non-parasitized RBC (npRBC) and a still very modest, although double as high value in trophozoite-parasitized mature forms of P. falciparum. This
| 4-HNE-MODIFIED PROTEIN (NAME, ALIAS; IDs) | 4-HNE BINDING SITES (distribution between K, H, C) | K, H, C count in the protein sequence and the total number of amino acids |
|------------------------------------------|-----------------------------------------------|------------------------------------------------------------------|
| 1 *HSP70-1, HSP70_PLAF7                  | K:17, H:2, C:2                                | K:54, H:7, C:9                                                  |
| Uniprot: Q8IB24_PLAF7                   |                                               | Tot 680 AA                                                     |
| NCBI GI: 124512406                      |                                               |                                                                 |
| PlasmoDB: PF3D7_0818900                 |                                               |                                                                 |
| 2 *V-type proton ATPase catalytic subunit A (EC 3.6.3.14), vacuolar ATP synthase subunit a (vapA) | K:2                                            | K:42                                                          |
| Uniprot: Q76NM6, VATA_PLAF7             |                                               | Tot 611 AA                                                     |
| NCBI GI: 124512982                      |                                               |                                                                 |
| PlasmoDB: PF3D7_1311900                 |                                               |                                                                 |
| 3 *Enolase (EC 4.2.1.11) phosphopyruvate hydratase | K:10                                          | K:38                                                          |
| Uniprot: Q8IJN7, ENO_PLAF7              |                                               | Tot 446 AA                                                     |
| NCBI GI: 124802328                      |                                               |                                                                 |
| PlasmoDB: PF3D7_1015900                 |                                               |                                                                 |
| 4 *Vacuolar protein sorting-associated protein 11, putative, former RING finger protein PFE0100w | K:13                                          | K:165                                                         |
| Uniprot: Q8I480-ZNRF2_PLAF7             |                                               |                                                                 |
| NCBI GI: 124505963                      |                                               |                                                                 |
| PlasmoDB: PF3D7_0502000                 |                                               |                                                                 |
| 5 *DYHC1_PLAF7; Dynein heavy chain-like protein | K:0                                           | K:477                                                         |
| Uniprot: Q 8IBG1                        |                                               | Tot 1272 AA                                                    |
| NCBI GI: 296004907                      |                                               |                                                                 |
| PlasmoDB: PF3D7_0729900                 |                                               |                                                                 |
| 6 *Heat shock protein 70 (HSP70-2); BiP; GRP78 | K:4                                           | K:63                                                          |
| Uniprot: Q8I2 × 4_ PLAF7                |                                               | Tot 4985 AA                                                    |
| NCBI GI: 124506906                      |                                               |                                                                 |
| PlasmoDB: PF3D7_0917900                 |                                               |                                                                 |
elevated value in trophozoites depends on the parasite biology and is plakortin independent. Plakortin has no effect on the barrier function of the host RBC membrane, irrespective whether parasitized or not.

1.3. Levels of reduced glutathione in plakortin-treated RBCs

Reduced glutathione (GSH) was quantified in RBC kept at 5% hematocrit in PBS supplemented with 20 mM glucose and plakortin for 24 h at 37 °C. As shown in Fig. 2 the GSH level in RBC remained unchanged after plakortin supplementation in a wide concentration range from 1 to 100 μM.

**Fig. 1.** Hemoglobin release from incubated trophozoite-stage cultures and npRBC measured as heme concentration in culture supernatant. Hemoglobin release was assessed 12 h after plakortin supplementation by heme-dependent luminol-enhanced luminescence. A single point represents the mean of 3 replicates of one experiment. N=3 independent experiments with 3 different blood donors.

**Fig. 2.** Quantification of GSH in RBC treated or not with plakortin (1–100 μM, final concentration). Columns present mean GSH ± SD values from 2 replicates performed with RBC from two donors.
2. Experimental design, materials and methods

Unless otherwise stated all materials were obtained from Sigma-Aldrich (St. Louis, MO, USA). Plakortin, methyl 4,8-diethyl-6-methyl-3,6-peroxy-9-dodecenoate, was obtained from the organic extract of the Caribbean sponge Plakortis simplex and purified by combination of column chromatography and HPLC as described [8]. The final purity of the compound was > 99%.

2.1. in vitro cultivation of Plasmodium falciparum and stage-specific enrichment of parasitized RBCs

*P. falciparum* (Palo Alto strain, Mycoplasma free) parasites were kept in permanent culture as described [9–11]. RBC that were used for plakortin incubation experiments were from blood samples taken on the same day of the experiment. Cultures were incubated in a humidified CO2 cell incubator under a N2/CO2/O2 atmosphere of 90 vol%:5 vol%:5 vol%.

2.2. Plakortin treatment of parasitized RBC (pRBC) and npRBC

Pure plakortin was dissolved at 50 mM in ethanol (stock solution) and diluted to 10 mM (final concentration) with DMSO prior to use. The stock solution was kept at −20 °C until use. Synchronous cultures were supplemented with a single dose of 10 μM plakortin at ring-stage (12–16 h post-invasion) or at trophozoite-stage (26–30 h post-invasion) and kept under standard culture conditions for 3 h until mass spectrometry analysis. Enriched parasite fractions and npRBCs were treated similarly and kept under the same standard culture conditions.

2.3. Hemoglobin quantification

The hemoglobin concentration in the culture supernatant was assayed by heme-dependent luminol-enhanced luminescence. Luminescence was measured with a double-injector luminometer (Sirius; Berthold, Pforzheim, Germany) as described [12–14]. All assays were performed in triplicate.

2.4. Preparation of protein extracts from pRBC

Washed and 1700 g sedimented npRBC or pRBC were hypo-osmotically lysed in a 10-fold excess (v/v) of ice-cold lysis buffer (10 mM K2HPO4/KH2PO4 (pH 8.0)), supplemented with a protease inhibitor cocktail containing: 1 mM EDTA, 250 μM phenyl-methyl-sulfonyl fluoride (PMSF), 1 mM leupeptin, 3 μM pepstatine, the phosphatase inhibitors sodium orthovanadate and sodium fluoride each at 1 mM and 100 μM Trolox, during 5 min on ice. The ruptured membranes and organelles of parasite origin were sedimented at 16,200 g for 3 min at 4 °C and the supernatant discarded. The pellet was washed 10 times by re-suspension in fresh lysis buffer and subsequent sedimentation at 16,200 g for 1 min. pRBC preparations contained the host cell membrane as well as membranes and organelle debris from the parasite. The proteins were extracted with SDS-containing Laemmli buffer at 95 °C for 5 min. Solubilized proteins were kept at −20 °C prior to use and β-mercaptoethanol (5% v/v) was added to protein samples before loading to the SDS-PAGE. The proteins were quantified using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA, USA).

2.5. Identification of 4-HNE modified proteins by mass spectrometric analysis and peptide mass fingerprinting

For the identification of 4-HNE-conjugated proteins in plakortin-treated parasites, matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometric analysis was performed. Proteins extracted from enriched ring and trophozoite stage pRBCs and npRBCs cultured with or without 10 μM plakortin for 3 h were separated by SDS-PAGE. Gels were stained with colloidal Coomassie, protein bands of interest excised from the gel and in-gel trypsin digested. MS analysis was performed on a MALDI-TOF spectrometer MX; Waters, Milford, MA equipped with a delayed extraction unit, according to the tuning procedures suggested by the manufacturer, operating in
reflectron mode [15,16]. Peak lists were generated by Protein Lynx Global Server (Waters, Milford, MA). The 25 most intense masses were used for database searches against the SwissProt (Uniprot) and NCBI databases using the free search program Mascot (http://www.matrixscience.com). The parameters used for the searches were: taxa P. falciparum, trypsin digestion, 1 missed cleavage, methionine oxidation and 4-HNE as variable modifications with a maximum error allowed of 100 ppm. For P. falciparum, proteins taken on to consideration had a Mascot score higher than 37 for SwissProt searches and higher than 67 for NCBI searches, as suggested by Mascot to be “significant”.

2.6. GSH assay in RBC

Assay of GSH in RBC was performed according to [17]. GSH values were given as mM (µmol/ml packed RBCs).

2.7. Statistical analysis

Non-parametric Mann–Whitney U Test was used to determine the significance of differences between the groups’ means (PASW Statistics 18, SPSS IBM, Chicago, IL).

Acknowledgments

The study was supported by the Italian Ministry for Research (MIUR PRIN2008, Leads ad attività antimalarica di origine naturale. Isolamento, ottimizzazione e valutazione biologica), MIUR Grant 2010C2LKKJ-007 (E.S.), the University of Torino Intramural Funds (E.S.; O.A.S. PROG 101289 2012 and 2013, PROG 102326 2014) and EviMalaR (European Virtual Institute dedicated to Malaria Research) Project no. 242095 (P.A.).

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