Potential Hemoglobin A/F role in clinical Malaria

Vikky Awasthi¹, Debprasad Chattopadhyay² & Jyoti Das*¹

¹Immunology Division, ICMR-National Institute of Malaria Research, Dwarka, New Delhi; Present Address: Regional Medical Research Centre, Belagavi, Nehru Nagar, National Highway No.4, Belagavi-590010, Karnataka, India. ²ICMR Virus Unit, Calcutta, ID & BG Hospital, GB 4, Belaghata, Kolkata, India; Jyoti Das: E-mail - jyoti@mrcindia.org; Telephone: +91-25307203, Fax: +91-25307177; Corresponding author

Received August 5, 2017; Revised August 18, 2017; Accepted August 19, 2017; Published August 31, 2017

Abstract

The Malarial parasite resides in the host RBC during its erythrocytic cycle. Plasmodium meets its entire nutritional requirement from RBC. It scavenges the hemoglobin of RBCs to meet its amino acid requirement. The host hemoglobin is made of different chains and it is dependent on age. Hemoglobin F (HbF), which has two-alpha and two gamma chain persists in children upto six years, and hemoglobin A (HbA) made of two alpha and two beta chains dominates. Therefore, it is of interest to compare the compositional features of HbA with HbF. Isoleucine is present in hemoglobin A (HbA) made of two alpha and two gamma chains dominates. A significant amount of fetal hemoglobin persists upto eight months after birth. Most people have only trace amounts, if any, of fetal haemoglobin after infancy. The combination of two alpha genes and two beta genes forms the normal adult haemoglobin, haemoglobin A whereas 5 Hemoglobin A2, is composed of two alpha chains and two delta chains and constitutes up to 3% of total hemoglobin in adults. The hemoglobin with different chains varies in amino acids composition, their oxygen carrying capacity as well as in their stability. Embryonic hemoglobin (HbE) and HbF has better oxygen carrying capacity as compared to other hemoglobin types however the stability of HbE is lower as compared to adults Hbs. Parasites are totally dependent upon hemoglobin to replicate in the host. Therefore, it is of interest to study importance of Hb in RBC.

Malaria affects all age groups. However, the children are affected the most. According to WHO report there were an estimated 438 000 malaria deaths around the world in 2015, 69% of total deaths due to malaria are known to occur in children aged from 6 months to 5 years [1]. Children are considered to be most vulnerable groups in human population, Severe anaemia,
hypoglycaemia and cerebral malaria are the features of severe malaria more commonly seen in children than in adults [13].

Though the parasite culture in RBC containing adult Hb is routinely performed [14] the studies on parasite culture in RBCs containing HbF by different groups show contradictory results. Some research have suggested that growth of Plasmodium is retarded in RBC containing HbF [15] while some suggests that under conditions of high oxygen the Plasmodium shows preference to RBC containing HbF with vigorous growth [16]. The inhibition of parasite growth in HbF has been due to factors present in mother’s plasma [16].

In this study, we show the abundance of different amino acids in Plasmodium falciparum 3D7 and distribution of different amino acids according to its essential nature in host. We have also compared the amino acid composition of different chains of haemoglobin to determine the difference that leads to preference of RBC containing HbF, which might explain the disease severity in children.

**Methodology:**
The FASTA format of all protein sequence, 5,369 proteins, of Plasmodium was downloaded from PlasmoDB. Composition of protein sequences was completed using ProtParam tool. ProtParam tool is an online tool, which is freely available and determines the sequence composition and predicts other physical parameters [17]. Total amino acid composition was compiled using Microsoft Excel. The Charts and graphs were made using Microsoft excel. MSA of haemoglobin chains was performed using Clustal Omega tool from the EMBL-EBI Web Services [18].

**Table 1:** Dataset. The table lists the amino acid residues present in total protein of Plasmodium falciparum (A), essential amino acids (B), conditionally essential amino acid (C) and non-essential amino acid (D) residues present in Plasmodium falciparum.

| (A) Amino Acid Residue | Number of Residue |
|------------------------|------------------|
| ALA (A)                | 82537            |
| CYS (C)                | 73987            |
| ASP (D)                | 270290           |
| GLU (E)                | 183037           |
| PHE (F)                | 298756           |
| GLY (G)                | 118471           |
| HIS (H)                | 100949           |
| ILE (I)                | 387902           |
| LYS (K)                | 491170           |
| LEU (L)                | 318735           |
| MET (M)                | 91797            |
| ASN (N)                | 600456           |
| PRO (P)                | 83223            |
| GLN (Q)                | 115618           |
| ARG (R)                | 110769           |
| SER (S)                | 267090           |
| THR (T)                | 171384           |
| VAL (V)                | 159380           |
| TRP (W)                | 20830            |
| TYR (Y)                | 238546           |

| (B) Essential amino acid | Number of residues |
|--------------------------|-------------------|
| Histidine (H)            | 100949            |
| Isoleucine (I)           | 387902            |
| Leucine (L)              | 318735            |
| Methionine (M)           | 91797             |
| Phenylalanine (F)        | 298756            |
| Threonine (T)            | 171384            |
| Tryptophan (W)           | 20830             |
| Valine (V)               | 159380            |
| Lysine (K)               | 491170            |

| (C) Conditionally essential amino acid | Number of residues |
|---------------------------------------|-------------------|
| Arginine (R)                          | 110769            |
| Asparagine (N)                        | 600456            |
| Glutamine (Q)                         | 183037            |
| Glycine (G)                           | 118471            |
| Proline (P)                           | 83223             |
| Serine (S)                            | 267090            |
| Tyrosine (Y)                          | 238546            |

| (D) Non-essential amino acid | Number of residues |
|-----------------------------|-------------------|
| Alanine (A)                 | 82537             |
| Aspartate (D)               | 270290            |
| Cysteine (C)                | 73987             |
| Glutamate (E)               | 183037            |
Figure 1: Abundance of amino-acid in total protein of Plasmodium. Sequences of all the 5639 proteins of Plasmodium were downloaded and abundance of amino acids were determined for individual protein by ProtParaman tool. The pie chart represents the abundance of the amino acids (A). The distribution of amino acids in Plasmodium proteins on the basis of essential (B), conditionally essential (C) and non-essential amino acids (D).

Figure 2: Multiple sequence alignment of amino acid sequence of different chains of hemoglobin gamma (NP_000550.2), Epsilon (NP_005321.1), Delta (NP_000510.1), Beta (NP_000509.1), and Alpha (P69905.2), and Zeta (3W4U:E). Identical and similar amino acids are highlighted with similar font colors.

Results:
Abundance of Amino acids in parasite:

Complete protein sequences of 5369 were downloaded from PlasmoDB for Plasmodium falciparum 3D7. The amino acid
compositions of total 5369 proteins are represented by pie chart (Figure 1A). As The amino-acid composition of Plasmodium falciparum proteins would suggest the co-relation between the amino-acids composition of haemoglobin and parasite proteins. The most incorporated amino acid in Plasmodium protein is N (13%), K (12%) and I (9%). We further analysed amino acids composition of Plasmodium protein according to the amino acid requirement by the host i.e. essential amino acids (Figure 1B), conditionally essential amino acids (Figure 1C) and non-essential amino acid (Figure 1D). It was observed that the essential and conditionally essential amino acids are widely incorporated in the Plasmodium proteins as compared to non-essential amino acids.

Distribution of amino acids in Haemoglobin chains:
The protein sequence of the haemoglobin chain was downloaded from NCBI and the composition of amino acid was determined using ProtParaman tool [17] as described above.

The fasta formats for different chain of the haemoglobins were obtained from NCBI. The assertion number of different chains of haemoglobin were as follows:

NP_000500.2 hemoglobin subunit gamma: MGHFTFEDKATITSSLWKGVNVEDAGGETLGRLLVYPWTQR FFDSFGNLSASASSAIMGPKVAKHHKVLTSGLDATKHLDDLK GTFAQLSELHCDKLHVDPENFKALLGNVLTVLAIHFGKEFTEP VQASWQKMTVATAVASLSRYH

NP_005321.1 hemoglobin subunit epsilon: MVHFTAEKAAVTSLSWMKNVEAAGGEALGRLLVYPWTQ RRDFSGNLLSSPSAILGNPKVKAKHGKVLTSFSGDAIKNMDNLK PAFAKLSELHCDKLHVDPENFKALLGNVMVILALHFGKEFTEP VQAAWQKLSVAVAIHALAHYH

NP_000510.1 hemoglobin subunit delta [Homo sapiens]: MVHLTPEEKAVTALWKGVNVEDAGGEALGRLLVYPWTQ RRDFSFGDSTPDAVMGNPKVAKHHKVLGAFSDGHLHLDN LKGTFSQSLHCDDKLVHDPENVFRLLGNVLVCALRNFGEFT TQPQMAAYQKVAGVANALAHKYH

Table 2: Table showing the distribution of different amino acids in different chains of haemoglobin.

| HB SUBUNIT | A | C | D | E | F | G | H | I | K | L | M | N | P | Q | R | S | T | V | W | Y |
|------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GAMMA      | 12| 1 | 8 | 8 | 12| 7 | 3 | 12| 17| 3 | 5 | 4 | 4 | 3 | 11| 11| 13| 3 | 2 | 147|
| EPSILON    | 17| 1 | 5 | 9 | 9 | 7 | 5 | 14| 6 | 7 | 6 | 6 | 2 | 2 | 9 | 6 | 13| 3 | 2 | 147|
| DELTA      | 15| 2 | 7 | 7 | 8 | 13| 7 | 0 | 11| 18| 3 | 8 | 6 | 5 | 4 | 6 | 5 | 17| 2 | 3 | 147|
| Beta       | 15| 2 | 7 | 8 | 8 | 13| 9 | 0 | 11| 18| 2 | 6 | 7 | 3 | 5 | 7 | 18| 2 | 3 | 147|
| Alpha      | 21| 1 | 8 | 4 | 7 | 7 | 10| 0 | 11| 18| 3 | 4 | 7 | 1 | 2 | 11| 9 | 13| 1 | 3 | 141|
| Zeta       | 16| 1 | 8 | 6 | 7 | 6 | 7 | 9 | 17| 2 | 1 | 5 | 3 | 6 | 13| 12| 11| 2 | 3 | 142|

Discussion:
Plasmodium parasite, scavenges the haemoglobin to meet its amino acid requirements while lipids are obtained from RBCs membrane. The distribution of amino acids in the proteins of Plasmodium shows incorporation of all the 20 amino acids. However, the amino acids, which are essential and conditionally essential to the host, are present in higher percentage than non-essential amino acids.

Among the essential amino acids leucine is required most followed by isoleucine. Among the conditionally essential amino acids N is required the most. N is present in parasites as repeat which might have role in immune evasion by antigenic variation. Other amino acids like arginine, which is essential for polyamine synthesis is important for robust growth of the parasite.

I make up to 9% of total amino acids in Plasmodium falciparum has to be obtained exogenously from blood, as it is absent in adult haemoglobin. Istvan et al. has shown that the absence of I affects parasite growth and I analogue inhibits the parasite growth [19]. The gamma chain of foetal haemoglobin (HbF) contains all the amino acids including I. Hence, the parasite residing in the foetal...
RBC does not require exogenous supply of isoleucine, which in turn might aid parasite to multiply exponentially. Study by Sauerzopf et al., has shown that *P. falciparum* are equally permissive to growth in HbF and HbA in *vitro* further confirms that the growth of parasite will be aided by the presence of isoleucine. The percentage of HbF has been shown to be highest in foetus and 18% up to the age of 9 while in the age group of 10-20 years its percentage falls to 8% [20, 21]. The presence of significant percentage of HbF up to the age of 9 might explain the vulnerability to malaria and death due to malaria.

**Conclusion:**
As Plasmodium solely depends upon the degradation of haemoglobin for its amino acid requirements it meets most of the nutritional requirements from haemoglobin. As HbF contains all the amino acids including isoleucine (absent in HbA) required by the parasite hence, this might be aid growth of parasite in HbF containing RBC. Besides at the age of 9 up to, 18% of RBC contains HbF. Hence, we hypothesise that presence of HbF would aid parasite growth leading to disease severity in children.

**Acknowledgement:**
We would like to thank, NIMR (ICMR) for funding the study.

**Conflict of Interest:**
Authors declare no conflict of Interest

**References:**
[1] http://www.who.int/malaria/publications/world_malaria_report/en/
[2] Daneshvar C et al. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 2009 49: 852 [PMID 19635025]
[3] Prudêncio M et al. Nat. Rev. Microbiol. 2006 4: 849 [PMID 17041632]
[4] Cowman AF & Crabb BS. Cell 2006 124: 755 [PMID: 16497586]
[5] Liu J et al. Proc. Natl. Acad. Sci. 2006 103: 8840 [PMID: 16731623]
[6] Martin RE & Kirk K. Blood 2007 109: 2217 [PMID: 17047158]
[7] Nagaraj VA et al. Nat. Commun. 2015 6: 8775 [PMID: 26531182]
[8] Cobbold SA et al. Int. J. Parasitol. 2011 41: 125 [PMID: 20851123]
[9] Awasthi V et al. J Vector Borne Dis. 2017 54: 139 [PMID: 28748834]
[10] Babbitt SE et al. Proc. Natl. Acad. Sci. 2012 109: E3278 [PMID: 23112171]
[11] Bunn HF & Forget BG. Hemoglobin: Molecular, Genetic and Clinical Aspects. 1986.
[12] Stamatoyannopoulos JA & Nienhuis AW. Annu. Rev. Med. 1992 43: 497 [PMID: 1374600]
[13] Schumacher RF & Spinelli E. Mediterr. J. Hematol. Infect. Dis. 2012 4(1): e2012073 [PMID: 23205261]
[14] Frederick L. Schuster Clin Microbiol Rev. 2002 15(3): 355. [PMID: 12097244]
[15] Amaratunga C et al. Malaria. PLOS ONE 2011 6: e14798 [PMCID: PMC3075246]
[16] Sauerzopf U et al. Malar. J. 2014 13: 436 [PMID: 25406504]
[17] http://web.expasy.org/protparam/
[18] http://www.ebi.ac.uk/Tools/msa/clustalo/
[19] Istvan ES et al. Proc. Natl. Acad. Sci. 2011 108: 1627 [PMID: 21205898]
[20] Ender KL et al. J. Pediatr. Hematol. Oncol. 2011 33: 496 [PMID: 21941141]
[21] Meier ER et al. PLOS ONE 2015 10: e0136672 [PMID: 26366562]

**License statement:** This is an Open Access article which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. This is distributed under the terms of the Creative Commons Attribution License

Edited by P Kangueane

Citation: Awasthi et al. Bioinformation 13(8): 269-273 (2017)