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Invited Review

Structural architecture and interplay of the nucleotide- and erythrocyte binding domain of the reticulocyte binding protein *Py*235 from *Plasmodium yoelii*

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ABSTRACT

Human malaria is caused by the cyclical invasion of the host's red blood cells (RBCs) by the invasive form of the parasite, the merozoite. The invasion of the RBC involves a range of parasite ligand receptor interactions, a process which is under intensive investigation. Two protein families are known to be important in the recognition and invasion of the human erythrocyte, the erythrocyte-binding like (EBL) proteins and the reticulocyte binding like proteins, of which the *Py*235 family in *Plasmodium yoelii* is a member. Recently the nucleotide binding domain (NBD94), that plays a role in ATP sensing, and the erythrocyte binding domain (EBD) of *Py*235, called EBD_{1-194}, have been identified. Binding of ATP leads to conformational changes within *Py*235 from *P. yoelli* and results in enhanced binding of the protein to the RBC. Structural features of these domains have been obtained, providing the platform to discuss how the structural architecture creates the basis for an interplay of the sensing NBD and the EBD domain in *Py*235. In analogy to the receptor-mediated ligand-dimerization model of the EBL proteins *Pv*DBP and *Pf*EBA-175 from *Plasmodium vivax* and *Plasmodium falciparum*, respectively, we hypothesise that *Py*235 of *P. yoelii* binds via its EBD_{1-194} domain to the RBC receptor, thereby inducing dimerization of the *Py*235-receptor complex.

1. Introduction

The complex life cycle of the malaria parasite is characterised by distinct invasive forms of the sporozoite and merozoite that invade hepatocytes and erythrocytes in the vertebrate host, respectively, and the ookinetes inside the insect vector that penetrate the mosquito midgut epithelium (Vanderberg, 1974; Huber et al., 1991; Meis et al., 1992; Gaur et al., 2007). Invasion of red blood cells (RBCs) by the merozoite and the subsequent cyclical replication of the parasite is the cause of malaria-associated pathology. The merozite has specialized organelles (rhoptries and micronemes) at its apical end, which are responsible for the storage and release of the ligands required for host cell invasion. The different steps in merozoite invasion appear to involve different ligand-receptor interactions (Cowman and Crabb, 2006; Iyer et al., 2007). Initial binding of the merozoite is via low affinity interactions, proposed to be initiated by the parasite proteins, MSP1 and AMA1 (Peterson et al., 1989; Chitnis and Blackman, 2000; Holder, 2009; Kadekoppala and Holder, 2010; Peng et al., 2010). The subsequent stronger interaction between the merozoite and the RBC involves at least two protein families, known to be important in recognition and invasion of the human erythrocyte. The first family includes erythrocyte-binding like (EBL) proteins, which are thought to play a crucial role in erythrocyte recognition, junction formation and invasion (Adams et al., 1992, 2001; Cowman and Crabb, 2006). Members of this protein family contain (i) one or two extracellular cysteine-rich Duffy Binding Like (DBL) domains (called region II or RII), with each DBL domain mediating binding to a single receptor on the RBC, (ii) a second extracellular cysteine-rich domain (region VI), (iii) a type I transmembrane domain and a short cytoplasmic domain (Tolia et al., 2005). *Plasmodium falciparum* has four functional EBL proteins, called *Pf*EBA-175, *Pf*EBA-140, *Pf*EBA-181 and *Pf*EBL-1 (Adams et al., 1992, 2001). In comparison, *Plasmodium knowlesi* and *Plasmodium vivax* genomes encode three *Pk*DBP and a single *Pv*DBP protein, respectively (Aikawa et al., 1978; Carlton et al., 2008; Pain et al., 2008).

The second family important in merozoite invasion is the reticulocyte binding like (RBL) proteins that includes *P. vivax* reticulocyte binding proteins 1 and -2 (*Pv*RBP-1 and -2) (Galinski et al., 1992) and the *Py*235 family in *Plasmodium yoelii* (Keen et al., 1990, 1994; Borre et al., 1995). Unlike the EBLs, there is no direct identifiable domain structure in RBLs, making it difficult to identify functional domains within these large proteins. In *P. falciparum* the RBL family includes *Pf*Rh1, *Pf*Rh2a, *Pf*Rh2b, *Pf*Rh4 and *Pf*Rh5 (Rayner et al., 2000; Kaneko et al., 2002; Duraisingh et al., 2003; Triglia et al., 2005). The erythrocyte binding domains (EBDs) of *Pf*Rh1, *Pf*Rh4 and *Pf*Rh5 have been mapped, which show limited overall

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sequence conservation between them (Gaur et al., 2007; Gao et al., 2008; Hayton et al., 2008; Rodriguez et al., 2008; Baum et al., 2009; Tham et al., 2009). In *P. falciparum* different members of the RBLs are able to recognise different receptors on the RBC and the combination of different RBLs and EBLs expressed in the merozoite define unique invasion pathways (Tham et al., 2012).

In the case of *P. yoellii*, the RBLs are coded by the 235 kDa rhopty protein (*Py*235) multigene family and have been shown to play an important role in parasite virulence, host cell adaptation and immune evasion (Freeman et al., 1980; Holder and Freeman, 1981; Snounou et al., 2000; Khan et al., 2001; Grüner et al., 2004). The single member, *Py*01365, of the *Py*235 family has been shown to directly bind to RBCs (Ogun and Holder, 1996) and is dominantly expressed in both virulent and avirulent parasite strains (Iyer et al., 2007). Genetic disruption of *Py*01365 reduces the overall virulence of the *P. yoelii* YM line by reducing the total repertoire of RBCs that the parasite is able to invade (Bapat et al., 2011; Ogun et al., 2011). The EBD of *Py*01365 has recently been identified and its low resolution solution structure has been determined (Grüber et al., 2011). In addition, *Py*235 has been shown to contain an ATP/ADP nucleotide binding domain (NBD), proposed to be involved in the sensing of extracellular ATP levels in the process of invasion (Ramalingam et al., 2008; Basak et al., 2011).

Here we review the recent structural features of the NBD and EBD of *Py*235 from *P. yoelii*. We will further discuss the possible role of ATP/ADP binding in initial sensing and the interplay of the EBD and the NBD in the subsequent interaction of the RBL member with its corresponding receptor.

2. Structural features of the NBD of *Py*235

In addition to the availability of the appropriate receptors on the surface of the RBC, intracellular ATP is a requirement for merozoite invasion (Olson and Kilejian, 1982; Dluzewski et al., 1983a, b) with erythrocytes that have been depleted of ATP being refractory to invasion. ATP has recently been shown to play an important role as a signalling molecule (Sprague et al., 1996; Ellsworth et al., 1995; Ellsworth, 2004) and mechanical deformation of erythrocytes, encountered in capillaries forming the microvasculature, leads to increased ATP release (Sprague et al., 1996). It is therefore a reasonable assumption that merozoites are able to sense ATP levels in RBCs to identify suitable cells for invasion. *Py*235 of *P. yoelii* binds strongly to erythrocytes in the presence of ATP, while weaker binding occurs in the presence of ADP or the absence of nucleotides, indicating a nucleotide-dependent rearrangement that modulates the binding domain of *Py*235 for *Py*235-receptor interaction (Ramalingam et al., 2008). Such a nucleotide induced change has been observed in the 94 kDa NBD, (NBD94), of *Py*235, in which ATP binding causes alterations in the C-terminal hinge region (Ramalingam et al., 2008). The recombinant subdomain, $NBD94_{444–547}$, of NBD94 has been identified as the smallest segment of NBD94 still able to bind nucleotides with a preference of ATP- over the ADP analogue, important for sensing the signal for receptor binding of *Py*235 (Grüber et al., 2010). The low resolution solution structures show $NBD94_{444–547}$ as a 134 Å long molecule (Fig. 1), comprised of two globular segments, connected by a spiral region of approximately 73.1 Å in length with an high α-helical content (83%). $NBD94_{444–547}$ includes the $_{483}$FNEIKEKLKHYNFDDFVKEE$_{502}$ peptide ($NBD_{483–502}$), observed to bind the nucleotide-analogue 8-N_3-3′-biotinyl-ATP (Basak et al., 2011) and located in the spiral region of $NBD94_{444–547}$. The solution structure of $NBD_{483–502}$ reveals an α-helix between amino acid residues 485 and 491 (Fig. 1; Basak et al., 2011). The N- and C-terminal segments of the $NBD_{483–502}$ structure bend at tyrosine 493, a residue important for ATP/ADP binding (Fig. 1). $NBD_{483–502}$ has been shown to decrease *Py*235 binding to the erythrocytes (Basak et al., 2011), indicating a competitive event of the peptide and the NBD of *Py*235 in ATP-binding and/or an ATP-dependent *Py*235 binding to erythrocytes, making this peptide or modified forms a potential inhibitor of *Py*235-erythrocyte receptor complex formation.

It appears that nucleotide–binding to the $NBD94_{444–547}$ region is able to transfer a signal that alters the conformation and/or the accessibility of the binding region to the receptor. To achieve this, a structural element similar to a hinge that transfers the signal should be present in *Py*235. The crystallographic structure of the domain $NBD94_{566–663}$ of *Py*235, which is downstream of the $NBD94_{444–547}$ region, represents two helices with lengths of 97.8 and 48.6 Å, respectively, that are linked by a loop (Fig. 1; Grüber et al., 2010). Two kinks, formed by the residues Y60-K62 and S85-E87, spread both helices apart by an angle of 40.2° and cause a hinge-like element. It has been proposed that the residues G61 and S85 are in very close proximity of about 9.8 Å (Grüber et al., 2010). These structural features would allow $NBD94_{566–663}$ to move up and down like a hinge, thereby transmitting ATP-ADP binding in $NBD94_{444–547}$ with up and down movements in $NBD94_{566–663}$, which is coupled to the very C-terminal region of NBD94 (Fig. 1). This C-terminal domain, made up of residues 674 to 793 of NBD94, called $NBD94_{674–793}$, contains α-helices and its low resolution structure shows a chair–like shape with three domains of 96, 35 and 20 Å in length (Fig. 1; Grüber et al., 2010). The longer domain has a spiral feature and the very C-terminal 20 Å long segment turns away from the middle part by 104.5°. We propose that this turn of the C-terminal 20 Å segment of NDB94 enables the proper structural arrangement with its neighbouring C-terminus of *Py*235 and its membrane-embedded part (Fig. 3A). Importantly, independent to the different orientation(s) of $NBD94_{444–547}$, $NBD94_{566–663}$ and the C-terminal segment $NBD94_{674–793}$ relative to each other, the hinge element $NBD94_{566–663}$ forms the central coupling element, able to mediate the sensing of ATP-ADP binding in $NBD94_{444–547}$ with concerted conformational changes in $NBD94_{566–663}$ and $NBD94_{674–793}$, whose subdomains might undergo structural rearrangements, transferred to downstream events in the binding region of *Py*235, thereby facilitating the linkage of nucleotide signaling and *Py*235-erythrocyte binding (Fig. 3B).

3. Conservation of structural features of the *Py*235 EBD

Although none of the receptors that are bound by members of *Py*235 have been identified, at least one member of *Py*235 binds specifically to a neuraminidase-resistant, chymotrypsin- and trypsin-sensitive erythrocyte receptor (Ogun and Holder, 1996; Ogun et al., 2000). Antibodies targeting *Py*235 of *P. yoelii* have been shown to successfully protect the host against the virulent strain of this parasite (Freeman et al., 1980; Holder and Freeman, 1981). In contrast to the DBL domains of the erythrocyte-binding proteins, the large size and limited sequence homology has made it difficult to identify functional domains in *Py*235. However, recently the EBD of the single member of *Py*235, *Py*01365, has been described (Grüber et al., 2011). The domain responsible for erythrocyte binding has been identified as a 194 amino acid region ($EBD_{1–194}$) at the very N-terminus of NBD94, which is similar in size to the 170 residue minimal binding domain of region II of the *Pv*DBP protein (Batchelor et al., 2011). The recombinant form of the $EBD_{1–194}$ domain of *Py*235 binds mouse RBCs with the same specificity as the full-length *Py*235 (Grüber et al., 2011). The low resolution structure of $EBD_{1–194}$ reveals this protein as an elongated homodimer in solution with a larger domain of