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HLA-DR allele reading register shifting is associated with immunity induced by SERA peptide analogues

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ABSTRACT

SERA protein is a leading candidate molecule to be included in an antimalarial vaccine. Conserved high activity binding peptides (HABP) binding to red blood cells (RBC) have been identified in this protein. One of them (6762) localising in the 18-kDa C-terminal fragment was used to induce protective immunity with negative result. Critical RBC binding residues (assessed by glycine-analogue scanning) were replaced by others having the same mass, volume and surface but different polarity, rendering some of them immunogenic as assessed by antibody production against the parasite or its proteins and protection-inducing against challenge with a highly infectious *Aotus* monkey-adapted *Plasmodium falciparum* strain.

A shift in binding to purified HLA-DR allelic molecules from the same haplotype and in their reading register was found, suggesting that modified molecules had adopted a different ^{1}H NMR 3D structure allowing a better fit into the MHCII–pept-TCR complex, thereby representing a new mechanism for inducing immune protection.

Several proteins from *Plasmodium falciparum* malaria parasite (the most lethal form of this disease), implicated in merozoite maturation, processing of other molecules and invasion of RBCs, have been identified as leading candidates for an antimalarial vaccine.

One of them is the serine repeat antigen (SERA-5) encoded by one of the members of the SERA gene family which is an antigenic and structurally very conserved 989-amino acid-long protein containing 35 repeated serines [1,2].

SERA is expressed during late trophozoite and schizont maturation, were it undergoes proteolytic processing during merozoite release and invasion, giving rise to a 47-kDa N-terminal, a 50-kDa inner domain with significant serine-protease active homologous regions and a 18-kDa C-terminal. The 47- and 18-kDa fragments contain cysteine-rich domains associated via a disulphide bridge, forming a soluble 73-kDa hybrid protein fragment in non-reducing conditions [3]. The 47-kDa fragment is processed in turn into two 25-kDa fragments, one of which remains bound to the 18-kDa by a disulphide bridge and associated to the merozoite membrane (Fig. 1A).

Although its exact function is still not very clear, SERA is the target of *in vivo* parasite's antibodies before and after merozoite release; and its recognition results in merozoite's agglutination and subsequent obstruction of their dispersion. Such evidence strongly supports considering SERA as a good candidate for being included in a multi-antigenic, minimal subunit-based chemically synthesised antimalarial vaccine.

Bearing in mind such objective, 20-amino-acid-long sequential peptides covering the complete SERA sequence (native peptides) were synthesised and studied in highly robust, specific and sensitive RBC binding assays, for determining those protein regions involved in red blood cell (RBC) invasion. Such methodology allowed identifying those peptides having high binding activity, namely high activity binding peptides (HABPs) [4]; amongst which was the native HABP 6762 corresponding to residues 901–920 in the SERA protein and whose critical RBC binding residues were determined via glycine-analogue scanning.

Immunogenicity studies revealed that conserved SERA HABPs were, not immunogenic nor protection-inducing in *Aotus* monkeys (a primate species highly susceptible to human malaria) since they did not induce antibody production nor protection against experimental challenge with an adapted *P. falciparum* strain (FVO) highly virulent for *Aotus* monkeys. To solve this problem, analogues having their critical RBC binding residues changed for amino acids with similar mass but opposite polarity were thus synthesised and assayed in monkeys to test their antibody inducing ability and protective effect against experimental challenge [5,6].

However, it is known that the immune response against malaria is under the genetic control of the major histocompatibility com-

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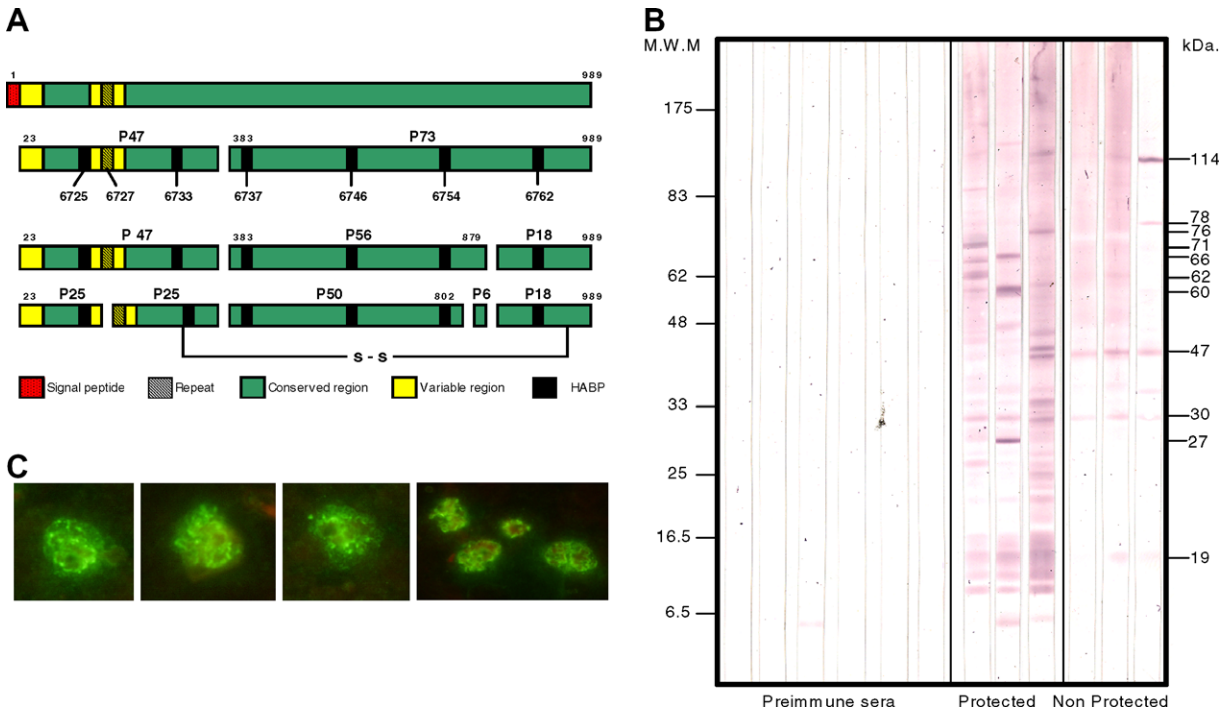

Fig. 1. (A) Schematic diagram of the SERA protein showing its processing as well as the localisation of the different conserved HABPs (in black bars). Green bars: conserved regions, yellow bars: variable regions and dashed bars: repeated region. (B) Immunofluorescence recognition pattern of antibodies induced by modified HABPs reacting with structures present on the parasitophorous vacuole as well as small dots inside infected RBCs. (C) Immunoreactivity of sera from *Aotus* monkeys immunised with modified HABPs with *P. falciparum* FCB-2 lysate proteins by Western blot analysis.

plex (MHC), specifically of the region encoding Class II molecules and particularly the HLA-DR region. It is known that the HLA-DRβ1* region encodes 16 different alleles named HLA-DRβ1*0101 to HLA-DRβ1*16, with more than 300 known variants. These alleles have been serologically, molecularly and phylogenetically grouped into five large groups or haplotypes each of which include several alleles: HLA-DR1 (including HLA-DRβ1*01, 10, 103, 104 alleles), HLA-DR51 (HLA-DRβ1*15 and 16), HLA-DR52 (HLA-DRβ1*03, 11, 12, 13, 14), HLA-DR8 (exclusively including HLA-DRβ1*08) and HLA-DR53 (HLA-DRβ1*04, 07 and 09) [7].

Therefore the ability of 6762 conserved HABP and its modified analogues for binding to purified HLA-DRβ1* molecules was assayed seeking for an association between their binding ability to these molecules and type of immune response induced by them.

Three-dimensional structure molecular models were thus obtained based on the ^{1}H NMR spectral parameters of native HABP 6762 and its analogue peptides in the search for correlating their three-dimensional structure with their immunological function, trying to design a rational approach towards synthetic, multi-component subunit-based, antimalarial vaccine development.

Materials and methods

Peptide chemical synthesis. Native peptide 6762 and its **13502**, **13780**, **14104**, **12756**, **13782**, **24210** and **24310** modified analogues (shown in bold type throughout the paper) were synthesised by the standard solid-phase peptide synthesis method [8], purified by reverse-phase HPLC and their molecular mass were determined by MALDI-TOF mass spectrometry (Autoflex Bruker Daltonics). Glycine–cysteine (GC) was added to each peptide's C- and N-terminal during synthesis to allow polymerisation following oxidation.

Animals. Spleen-intact *Aotus* monkeys from the Colombian Amazon basin, in our monkey colony in the Amazon jungle (Leticia), were used for this trial. This species has proved to be very susceptible to experimental infection with the *Aotus* adapted *P. falciparum* FVO strain [9] and tested by immunofluorescence for presence of anti-*P. falciparum* parasite antibodies at 1:20 dilution. Animals were handled according to National Institute of Health (NIH) animal guidelines.

Immunisation. Groups of 5–9 *Aotus* monkeys were immunised with 125 μg peptide as described in previous works [5]. Blood samples were obtained before each immunisation (days 0, 20, and 40) and 20 days following the third immunisation for immunology assays.

Challenge. Immunised and control *Aotus* monkeys were infected intravenously 20 days after the last immunisation with 100,000 *P. falciparum* FVO-strain infected RBCs, a dose known to be 100% infective for these monkeys [9].

Protection was defined as the total absence of parasites in blood during the 15 days of the experiment. Non-protected monkeys developed patent parasitaemia on day 5 or 6, reaching $\geqslant$5% levels between days 8 and 10. They then received treatment with antimalarial drugs and were kept in quarantine until ensuring complete cure, to be later returned into the jungle.

Parasitaemia assessment. Parasitaemia was measured daily for each monkey, starting on day 5 after challenge using immunofluorescence for reading percentage of parasitised RBCs on Acridine Orange-stained slides [9].

Immunofluorescence antibody (IFA) testing and Western blotting. Immunofluorescence antibody (IFA) testing and Western blotting were assessed as thoroughly described [10].

HLA-DR molecule affinity purification. Purified human molecules were obtained from DR1, WT100BIS (DRβ1*0101), DR3, COX (DRβ1*0301), DR4, BSM (DRβ1*0401), DR7 EKR (DRβ1*0701) and DR11 BM21 (DRβ1*1101) homozygous EBV-B cell lysates by affinity chromatography using anti-HLA-DR mAb L-243 cross-linked to protein A Sepharose CL-4B (Amersham Pharmacia Biotech AB) as affinity support.

Peptide-binding competition assays. Peptide-binding competition assays were conducted for measuring unlabelled peptides' ability to compete with biotinylated indicator peptides for binding to purified HLA-DR molecules, as previously described [11]. Biotinylated-labelled hemagglutinin HA 306–318 (PKYVKQNTLKLAT) peptide was used as control peptide for DRβ1*0101, DRβ1*0301, DRβ1*0401 and Gly-Phe-Lys-$(Ala)_7$ ($GFKA_7$) for DRβ1*1101 and DRβ1*0701. Relative binding affinities were determined by competition assays, where a good competitor was a peptide inhibiting indicator peptide's binding to the HLA molecule being tested by more than 50%.

Circular dichroism (CD). Measurements were taken at room temperature on nitrogen-flushed cells using a Jasco J-810 spectropolarimeter (Jasco, Japan). Spectra were performed and registered at 190–250 nm wavelength intervals using a 1-mm path-length rectangular cell.

NMR analysis and structure calculations. Ten milligrams of pure peptide were dissolved in 600 μl TFE–water (30/70 v/v) for NMR experiments. NMR spectra were recorded on a Bruker DRX-600 spectrometer (Bruker, Billerica, USA) at 295 K. Double-quantum filter correlation spectroscopy (DQF-COSY) [12], total correlation spectroscopy (TOCSY) [13] and nuclear overhauser enhancement spectroscopy (NOESY) experiments were used for assigning spectrum [14] and data was processed on an Indy computer (Silicon Graphics) equipped with XWINNMR-updated software (Bruker). Distance Geometry (DGII) software was used for providing a family of 50 structures. These structures were refined by using simulated annealing protocol (DISCOVER software). Structures having reasonable geometry and few violations were then selected.

Results

Peptide characterisation

HPLC results showed that peptide purity was sufficiently high to be analysed by ^1H NMR. Mass spectroscopy experimentally determined masses were equal to theoretical masses (data not shown). The polymers used for immunisation had molecular weights in the 8–24 kDa range, as assessed by size exclusion chromatography (SEC).

Immunogenicity studies

Conserved HABPs have been broadly proven to be not immunogenic, nor protection-inducing [5,6], and conserved 6762 HABP was not an exception since immunisation of *Aotus* monkeys with this peptide did not induce antibody production against this protein as assessed by IFA or Western blot (respectively) nor was induced protection against experimental challenge with the parasite [9].

When peptides **13780** and **13782** were modified (by changing K_{17} 6762 for M and E, respectively), an induced immunogenicity was observed as assessed by the presence of high levels of IFA antibodies and Western blot reactivity but no protection was induced (Table 1, group B). On the contrary, immunogenicity (Fig. 1B) and protection were observed when K_{14} was replaced by M (modified HABP **24210**), and such effect was even more noticeable when an extra modification was performed by changing I_{12} for N in **24310** analogue peptide. This experiment was repeated once more with this modified HABP, leading to identical results (Table 1, group A).

Western blot assays

Whilst pre-immune and negative sera did not show any reactivity with schizont lysate, sera from **24310** immunised and protected monkeys (obtained on the day previous to challenge) displayed reactivity with 73-, 72-, 62-, 60-, 27- and 19-kDa molecules, whereas the only monkey immunised with **24210** (which was protected, developing low antibody titres by the 3rd immunisation (1:160)) produced antibodies against 114-, 76-, 48-, 47-, 30- and 19-kDa molecules. Similarly, sera from non-protected monkeys against experimental challenge were reactive against 114-, 78-, 47-, 30- and 19-kDa molecules (Fig. 1C).

Table 1
Humoral immune response and protective efficacy induced by native 6762 and its modified analogues

Polimerised Peptide No.	Sequence (P1 P4 P6 P9)	PI	IFA ≥1:160 post2nd	IFA ≥1:160 post3rd	Prot.	Group
6762	**N E V** S E R V H V **Y** H I **L** **K** **H** I K **D** G K	0	0/5	0/5	0/5	
24310a	**N M V** S E R V H V **Y** H **N** L **M** H I K D G K	0	1(320)	ND	1/6	A
24310b	**N M V** S E R V H V **Y** H **N** L **M** H I K D G K	0	1(640)	ND	1/6	A
24210	**N M V** S E R V H V **Y** H I **L** **M** H I K D G K	0	0/8	1(160)	2/8	A
13782	**V M N** S E R V H V **Y** H I **L** **K** H I **E** D G K	0	2(320)	2(640)	0/5	B
13780	**V M N** S E R V H V **Y** H I **L** **K** H I **M** D G K	0	3(160)	2(320)	0/4	B
12756	**V I N** S E R V H V **Y** H I **L** **K** H I K D G K	0	0/4	0/4	0/4	C
13502	**V E N** S E R V H V **Y** H I **L** **K** H I K D G K	0	0/6	0/6	0/6	C
14104	**I M N** S E R V H V **Y** H I **L** **K** H I **M** D G K	0	0/6	0/6	0/6	C
24932	**N E V** S E R V H V **Y** H **H** **L** **M** H I K D G K	0	0/9	0/9	0/9	C
27670	**N E V** S E R V H V **Y** H I **L** **M** **L** I K D G K	0	0/8	0/8	0/8	C
29630	**N E V** S E R V H V **Y** H **H** **L** **M** H I K D G K	0	0/8	0/8	0/8	C
29632	**N E V** S E R V H V **Y** H **H** **L** **M** H **H**		0/8	0/8	0/8	C
CONTROLS		0	0/50	0/50	0/50	0/30

The amino acid sequence of peptides used for immunising *Aotus* monkeys is shown in one letter code (numbered according to our Institute's serial system). IFA reciprocal antibody titres (shown in brackets) determined from serum samples taken before the 1st immunisation (PI = Pre-immune) and 20 days following post 2nd and 3rd immunisations. The prefix corresponds to the total number of *Aotus* presenting these antibody titres. Prot. = total number of *Aotus* protected against experimental challenge from those presenting the antibody titres. Groups A: immunogenic and protection-inducing modified HABPs, B: only immunogenic and C: not immunogenic nor protection-inducing.

Binding to HLA-DRβ1 purified molecules*

Native HABP 6762 did not bind to any of the studied molecules (which were representative alleles of the HLA-DR1, DR52 and DR53 haplotype); however, it was found that **24310** bound with high affinity to HLA-DRβ1*0301 and that **24210** bound with similar affinity (although not as high) to HLA-DRβ1*0301 and DRβ1*1101, both belonging to the HLA-DR52 haplotype. Immunogenic but non-protection-inducing HABP **13782** bound to HLA-DRβ1*1101 as well as non-immunogenic, non-protection-inducing **12756**, although the latter bound also with some affinity to HLA-DRβ1*0301 (Table 2).

Binding motifs and reading registers

HABP **24310** displayed characteristic HLA-DRβ1*0301 binding motifs and reading registers in its sequence: V_9 in Pocket 1, N_{12} in Pocket 4, M_{14} in Pocket 6 and K_{17} in Pocket 9 [7,15]. By the same token, **24210** presented characteristic HLA-DRβ1*1101 binding motifs, such as Y_{10} in Pocket 1, L_{13} in Pocket 4, H_{15} in Pocket 6 and D_{18} in Pocket 9; and although induced antibodies were lower, 2/8 monkeys were protected against experimental challenge.

Modified HABPs **13780** and **13782** displayed similar binding activity as **24210**; the same HLA-DRβ1*1101 binding motifs showed an extra modification in K_{17} M/E, suggesting that this residue is probably making contact with the TCR and could be playing a critical role in inducing protective antibodies.

Peptide 6762 and its analogues' secondary structure conformation as determined by (CD)

Both monomeric and polymeric 6762 and its analogues' solution structures determined by circular dichroism (CD) in 30% TFE presented one spectra maximum at 193 nm wavelength and two minimum spectra curve points at 208 and 222 nm wavelength, indicating their predominantly stable α-helix conformation (Fig. 2I).

NMR analysis

^{1}H-signals were assigned according to Wüthrich's standard procedure [14]; TOCSY and DQF-COSY spectra were used to correlate side chains spin systems with HN-CHα cross-peaks. The peptides' NOESY spectra showed stronger αN $(i,i+1)$ sequence signals than intra-residue cross-peaks. The presence of d_{NN} cross-peaks indicated a significant conformation population in the α region of the φψ space. Some medium-range d_{NN} $(i,i+1)$, $d_{\alpha\beta}$ $(i,i+3)$, $d_{\alpha N}$ $(i,i+3)$, $d_{\alpha N}$ $(i,i+4)$ cross-peaks indicated the presence of a typical helical fragment in all peptides between residues 2–17.

Structure calculations and 3D structural analysis

Set of 50 independently produced structures were obtained from peptide 6762, satisfying experimental constraints when using 323 NOEs-derived distance restraints previously classified according to signal-strength (including 77 inter-residue constraints). Nineteen ω dihedral angle restraints were used, six hydrogen bond constraints were introduced for residues having them. A family of 22 low-energy conformers was accepted which did not have a distance violation larger than 0.23 Å. Peptide structures of 6762 were helical between residues 2–17. Structure calculations for peptides **24310**, **24210**, **13782**, **13780**, **12756** and **13502** showed that all of them displayed also an α-helical structure between residues 2 and 17 (Fig. 2II), thus confirming CD structural analysis (Fig. 2I).

Native, modified non-immunogenic, simply immunogenic and immunogenic protection-inducing HABPs displayed similar secondary structure but different HLA-DR binding characteristics, therefore suggesting important but very subtle structural differences.

The best immunogenic and protection-inducing modified HABP (**24310**) binding with high capacity to the HLA-DRβ1*0301 displayed a 19.8 Å distance between the furthest atoms fitting into Pockets 1–9 of this molecule. Immunogenic protection-inducing modified HABP **24210** (HLA-DRβ1*1101), displayed different reading register and binding motifs. It would have been very informative to determine the role of polarity shift of residue 17 (M and E) in antibody induction and protection when comparing **13780** (insoluble at ^{1}H NMR concentration conditions) and **13782** with the other modified HABPs that elicited immunogenity and protective activity.

Looking at the 3D structures of **12756** and **13502**, it is not surprising that the former displayed promiscuous binding activity and the latter no binding activity at all.

Table 2
Immunological properties of SERA native HABPs and their corresponding modified analogues, such as antibody (Ab) production and protection-induction

Peptide	Ab titres and protected				% Binding HLA-DRb1* alleles					Ref for 3D struct.
					DR1	DR52		DR53		
	PI	II20	III20	Protection	0101	0301	1101	0401	0701	
6725	0	0	0	0/5	2	**62**	36	37	ND	[10]
23422	0	2(640)	ND	2/17	3	37	**83**	41	ND	[10]
6737	0	0	0	0/5	2	**78**	2	38	ND	[20]
12748	0	1(250)	11(2560)	1/4	10	8	**52**	20	ND	[20]
22834	0	1(2560)	ND	2/9	7	0	**54**	37	15	[20]
22796	1	5120	ND	2/9	12	0	**52**	30	18	[20]
6746	ND	ND	ND	ND	1	**71**	**55**	5	ND	[21]
24216	0	5(350)	ND	2/9	−27	37	**44**	5	10	[21]
23230	0	1(320)	ND	1/9	23	**84**	37	13	25	[21]
6762	0	0	0	0	2	−5	3	3	12	
24310	0	2(320)	ND	2/12	−1	**60**	35	17	12	
24210	0	0	1(160)	2/8	1	**51**	**50**	11	8	
13782	0	2(320)	2(640)	0/5	10	21	**47**	10	ND	
12756	0	0/4	0/4	0/4	18	**54**	**51**	17	15	
13502	0	0/6	0/6	0/6	2	39	**47**	8	21	

Peptides are grouped according to their corresponding binding activity to different purified HLA-DRβ1* haplotype molecules (values are shown in bold type whenever affinity was ⩾50%). ND, not determined. Ref, reference for 3D structure.

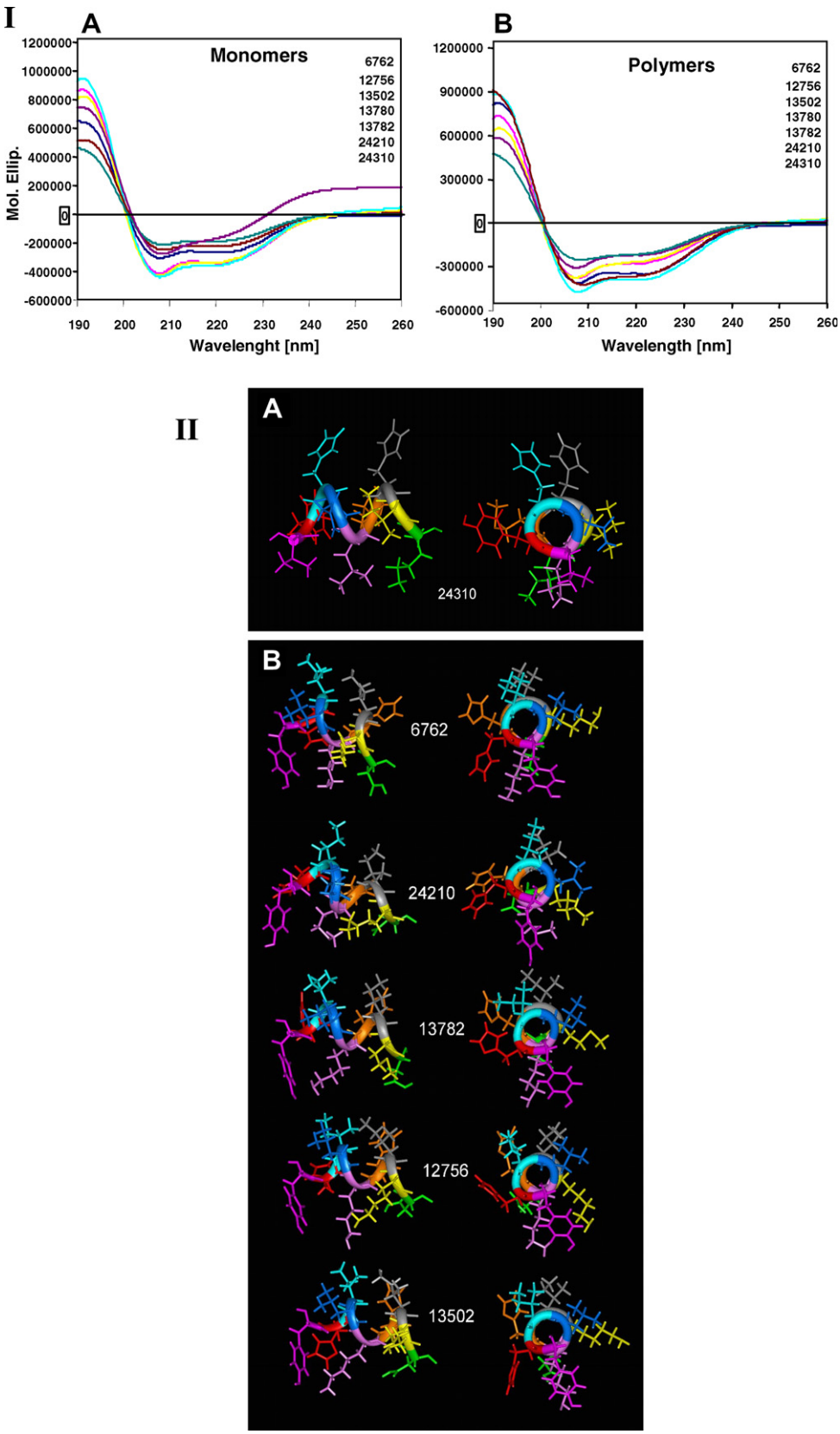

Fig. 2. (I) CD spectra of peptide 6762 and its analogues. The shape of the curve in the spectra shows a typical α-helix behaviour in (A) peptides monomers and (B) peptides polymers. (II) Ribbon 3D structure representation of peptide 6762 and its analogues. The consensus 3D structure of each family of calculated structures is shown with the colour code for their corresponding nine amino acid-reading register established according to HLA-DRβ1* binding activities of modified HABPs: fuchsia (Pocket 1), red (P2), turquoise (P3), dark blue (Pocket 4), rose (P5), brown (Pocket 6), grey (P7), yellow (P8), green (Pocket 9). The frontal 3D view is shown on the right-hand panel whilst the lateral view is shown in the left-side panel. (A) Modified HABP **24310** showing high binding activity to HLA-DRβ1*0301 and a reading register beginning in V_9. (B) Theoretical structure of peptide 6762 which did not bind to any of the purified HLA-DRβ1* molecules; and **24210** and **13782** which bound to HLA-DRβ1*1101 with a reading register beginning in Y_{10}. **12756** and **13502** display very bizarre structures.

Discussion

Antibodies directed against N- and C-terminal regions and against the complete SERA protein have been shown to inhibit merozoite invasion of erythrocytes *in vitro* [16,17], being some of the recognised epitopes located within residues 17–165 [18]. Several SERA fragments have been proved being immunologically important. For instance, murine inhibitory monoclonal antibody (mAb 43E5) recognising the P47 domain as well as epitopes contained within P47 have conferred significant protection against experimental challenge with *P. falciparum* in *Aotus* monkeys and squirrel monkeys [16]. Seemingly, monkeys immunised with the SERA-1 fragment (encompassing residues 24–285 which include our HABPs 6725 and 6727), or with the 18-kDa fragment including our HABP 6762, have induced protection against experimental infection with *P. falciparum* in *Aotus* monkeys [16,17] (Fig. 1A). Additionally, recent studies with detergent-resistant membranes (DRM) using Triton X-100, have shown that SERA proteins are expressed on merozoite membranes in association with membrane-anchored lipid-rafts proteins by a still unestablished mechanism [19], thus renewing the interest for SERA as a critical protein for RBC invasion.

Our studies with synthetic peptides have led to recognised 7 peptides within the SERA peptidic sequence binding specifically and with high affinity to erythrocytes (namely HABPs) (Fig. 1A). Peptides 6725, 6727 and 6733 are localised within the 47-kDa fragment. The segment corresponding to the 50-kDa fragment contains peptides 6737, 6746 and 6754 whilst peptide 6762 is located in the 18-kDa protective-inducing fragment (Fig. 1A). Peptides 6725 and 6737 interact with 17 and 35-kDa erythrocyte surface proteins [4]. Based on previously established principles where critical binding residues were replaced by others having same mass and volume but opposite polarity, modifications were made to 6762 HABPs as well as to other SERA HABPs (6725, 6737, 6746) for rendering them immunogenic and protection-inducing peptides (Table 2). Although the 3D structure of peptides 6725, 6737 and 6746 had been reported previously [10,20,21], their binding ability to HLA-DR purified molecules had not been determined by then and is now being shown in Table 2.

Several characteristics are very prominent in these SERA HABPs. All native HABPS bind with high affinity to RBCs, regardless of their molecular localisation in any of the cleavage fragments and they all display an α-helical structure when assessed by CD (Fig. 2I) and ^{1}H NMR [10,20,21]. Similarly, all native SERA native HABPs belonging to this protein are non-immunogenic and non-protection-inducing, and all bind to HLA-DR52 haplotype associated allelic molecules, except for 6762, which could be binding to other allelic molecules from this haplotype such as HLA-DRβ1*12, 13 or 14 or molecules not available when performing this study.

However, the purpose of this manuscript is the shiftment in HLA preferential binding ability from one HLA-DRβ1* haplotype allele molecule to another allele of the same haplotype, when conserved HABPs are modified to render them immunogenic and protection-inducing. This was clearly observed in 6762 which did not bind to any of the allele molecules studied here when modified into immunogenic, protection-inducing modified HABPs **24310** and **24210** they acquired the ability to bind to HLA-DRβ1*0301 and HLA-DRβ1*1101 purified molecules, respectively (Table 2).

This ability to shift their binding preference when modified and render immunogenic, protection-inducers was also observed in SERA conserved HABPs 6725, 6737 and 6746 (Table 2), the first of which shifted from binding to HLA-DRβ1*0301 (6725) to HLA-DRβ1*1101 (**23422**). It is worth stressing that whilst native HABP 6737 bound with high affinity to HLA-DRβ1*0301, all immunogenic protection-inducing modified HABPs (such as **12748**, **22834** and **22796**) bound to HLA-DRβ1*1101 with high affinity. By the same token, promiscuous 6746 binding simultaneously to HLA-DRβ1*0301 and HLA-DRβ1*1101, strongly bound to HLA-DRβ1*0301 (i.e. **23230**) or HLA-DRβ1*1101 (i.e. **24216**) when modified to become immunogenic and protection-inducing, although the latter's immunogenic and protection-inducing ability was higher [21]. This shift occurs within alleles of the same HLA-DR haplotype since no binding to HLA-DR1 or HLA-DR53 haplotype molecules was observed.

Some peptides can be read within the context of different Class II molecule isotypes in the same individual. For example, in the murine system, the 64-76 Hb (Haemoglobin b) peptide can be read by mouse I-E^k molecules (similar to human HLA-DR) from I_{68} to K_{76} but can also by I-A^K molecules (more similar to HLA-DQ) be read from V_{67} to I_{75}. The same Class II molecule can also read the same peptide in two different registers, as occurs with OVA 323–339 peptide where the I-A^d molecule can be read either from 323–335 or from 325–336, being the former incapable of activating T-cell hybridome cells [22]. Similarly, the Myelin Basic Protein (MBP 84–102) peptide can also be read in two different registers by HLA-DRβ1*1501 and HLA-DRβ5*0101, both resulting in totally different immunological responses with tremendous impact in autoimmune responses [23,24].

However, the phenomenon described here is different, since a non-immunogenic, non-protection-inducing native HABP shifts its binding ability and thus its reading register for one allele to another allele from the same haplotype when appropriately modified to render it immunogenic and protection-inducing; this change is biologically and structurally documented in this manuscript.

Although all peptides displayed an α-helical structure by CD and ^{1}H NMR, their lateral chain orientation was differ depending on the HLA-DRβ1* molecule they bind to and their corresponding reading register. Such small variations could thus be allowing for a better fit into the Pockets of other allele molecules and therefore a preferential TCR usage capable of inducing an appropriate protective immune response, clearly documented here for these SERA derived HABPs.

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