J. Biosci., Vol. 17, Number 1, March 1992, pp 45–53. © Printed in India.

Expression of selected domains of the circumsporozoite antigen of *Plasmodium knowlesi*

SAUDAMINI S DEODHAR, SOWMINI SAMPATH and SHOBHONA SHARMA*

Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha Road, Bombay 400 005, India

MS received 1 October 1991; revised 13 January 1992

Abstract. The circumsporozoite antigen of the simian malarial parasite, *Plqsmodium knowlesi,* consists of tandemly repeated immunodominant peptide units which may play a role in evading the immune system. To study the immunogenicity of this antigen in the absence of the immunodominant repeats, the whole of the non-repetitive region of this antigen has been expressed in *Escherichia coli.* The entire amino-terminal region up to the start of the repeats, and the full non-repetitive carboxyl region starting from the end of the repeats up to the termination codon, have been expressed separately, as fusion proteins with a 26 kD glutathione-S-transferase protein of *Schistosomq japonicum.* A repeat-less truncated antigen has also been expressed as the same fusion protein. The amino-terminal fusion protein (GST-CSN), is a soluble protein of a molecular weight of 38 kD, which could be purified by affinity chromatography on immobilized glutathione. The carboxyl-terminal fusion protein (GST-CSC), is insoluble, migrates with an anomalous molecular weight of 32 kD, and binds to the affinity matrix weakly. The truncated repeat-less fusion protein (GST-CSNC) is also an insoluble protein of molecular weight of 48 kD. Unlike the two separate domains, GST-CSNC is an extremely unstable protein in *Escherichia coli.*

Keywords. Circumsporozoite antigen; *Plasmodium knowlesi*; non-repetitive domains.

1. Introduction

The major sporozoite surface antigen, the CS-protein, has been implicated as a protective antigen in conferring immunity against malaria in a number of animals (Nussenzweig and Nussenzweig 1989). The complete nucleotide sequence and the deduced protein structure of the CS-gene for a number of species of malarial parasites have been documented so far (Kemp *et al* 1987). In the simian malarial parasites, a remarkable diversity in the repetitive region of the CS-genes, is observed amongst different strains of the parasite (Sharma *et al* 1985; Galinski *et al* 1987). The diverse structure of the CS-proteins from two different strains of *Plasmodium knowlesi,* led us to examine the immunogenic cross-reactivity of these antigens (Sharma *et al* 1986). By using various synthetic peptides it was found that multiple antigenic sites are present on the CS-antigen (Vergara *et al* 1985; Sharma *et al* 1986).

The diversity of the repetitive region indicates that it may serve the purpose of an immune evasion epitope. Recent reports (Link *et al* 1990; Schofield and Uadia 1990) have shown that the sporozoites elicit a T-cell independent antibody response towards the repetitive regions, and it is postulated that this may be to mask the adjacent non-repetitive regions of the protein from eliciting an immune response. So far the immunogenicity and protectivity of the CS-antigen in the absence of the repeat region has not been studied. The immunogenicity of two small stretches of amino acids (region I and II), which were found to be conserved in the CS-genes of

*Corresponding author.

a number of species, have been assessed using synthetic peptides (Ballou *et al* 1985, Vergara *et al* 1985). The repeat region of CS-antigens, along with short stretches of the non-repetitive parts have been expressed in *Escherichia coli* before (Young *et al* 1985; Barr 1987). The entire CS-gene of *P. knowlesi,* has been expressed earlier in yeast (Sharma and Godson 1985). Recently there has been a report on the expression of different regions of the CS-gene of *P. falciparum* in E. *coli* as a fusion protein of mouse dihydrofolate reductase (Stuber *et al* 1990). Here we report the expression of the entire non-repetitive domains of the CS-antigen of *P. knowlesi,* as fusion proteins in *E. coli.*

2. Materials and methods

2.1 *Construction of the recombinant plasmids*

The construction of the recombinant plasmids for the expression of the two domains is shown in figure 1. A 1·4 Kb XmnI fragment of the CS-gene, comprising of nearly all of the coding region of the CS-protein, was subcloned from the λKN7 clone of the *Nuri* strain (Sharma *et al* 1985), into the SmaI site of plasmid pUC9. In all the cloning steps, wherever a blunt-end ligation was performed, the orientation of the insert in the resultant plasmid was determined with suitable restriction analysis. From this recombinant pUC9 plasmid, a 300 bp BamHI-HpaII fragment of DNA coding for the entire amino-terminal region of the protein up to the repeats, was filled in and subcloned in the SmaI site of the expression system pGEX-3X (Smith and Johnson 1988). For the carboxy-terminal region, an RsaI-EcoRV fragment from the pKMz plasmid, containing the CS-gene of the H strain (Ruiz *et al* 1987), was introduced at the SmaI site of pUC9 vector. From the recombinant pUC9, the

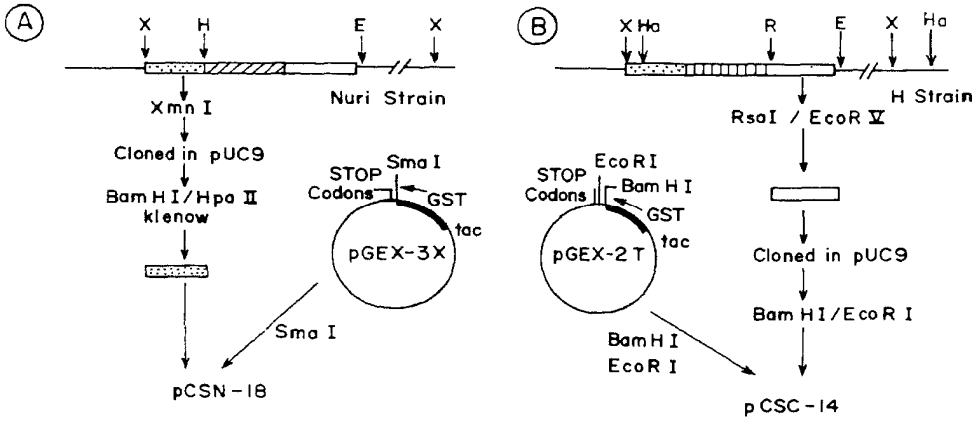

Figure 1. Construction of the recombinant plasmids for the expression of the (**A**) amino-terminal domain, and (**B**) carboxyterminal domain of the CS-gene. Restriction enzymes: E, EcoRV; H, HpaII; Ha, HaeIII; R, RsaI; X, XmnI. The vertical and the oblique bars represent the central repeat regions; while the stippled and the unshaded regions depict the amino-terminal and the carboxy-terminal non-repetitive regions of the CS-gene, respectively.

BamHI-EcoRI fragment was subcloned in the expression vector pGEX-2T. The difference between the pGEX-3X and pGEX-2T is mainly that in the reading frame at the cloning sites.

The construction of the plasmid for the expression of the repeat-less CS-fusion protein is shown in figure 2. The amino-terminal domain was obtained from the *Nuri* strain using XmnI-HpaII restriction, and subcloned in to the SmaI site of pUC8. A positive clone was selected such that the orientation of the CS-domain was the same as the ß-galactosidase gene of pUC8, with the 5'-end towards the EcoRI side. The RsaI-EcoRV fragment of the H strain gene, defining the carboxy terminal domain was also subcloned in to pUC8, but at the HincII site, which is situated 3' to the SmaI site of pUC8. From the sequences of the two domains, it was assessed that the linker between the SmaI and HincII sites would join the two fragments in the proper reading frame, provided the orientation of the fragments are in the same direction (as the ß-galactosidase gene of pUC8 in this case). The orientations of the two fragments were determined in the respective pUC8 recombinant clones, pHX5 and pRRV2 (figure 2) with the help of internal restriction site HindiIII. Subsequently, the EcoRI-BamHI fragment of the pHX5 plasmid containing the amino-terminal domain, was subcloned in to the recipient pRRV2 plasmid giving rise to the recombinant plasmid pERP112, containing the

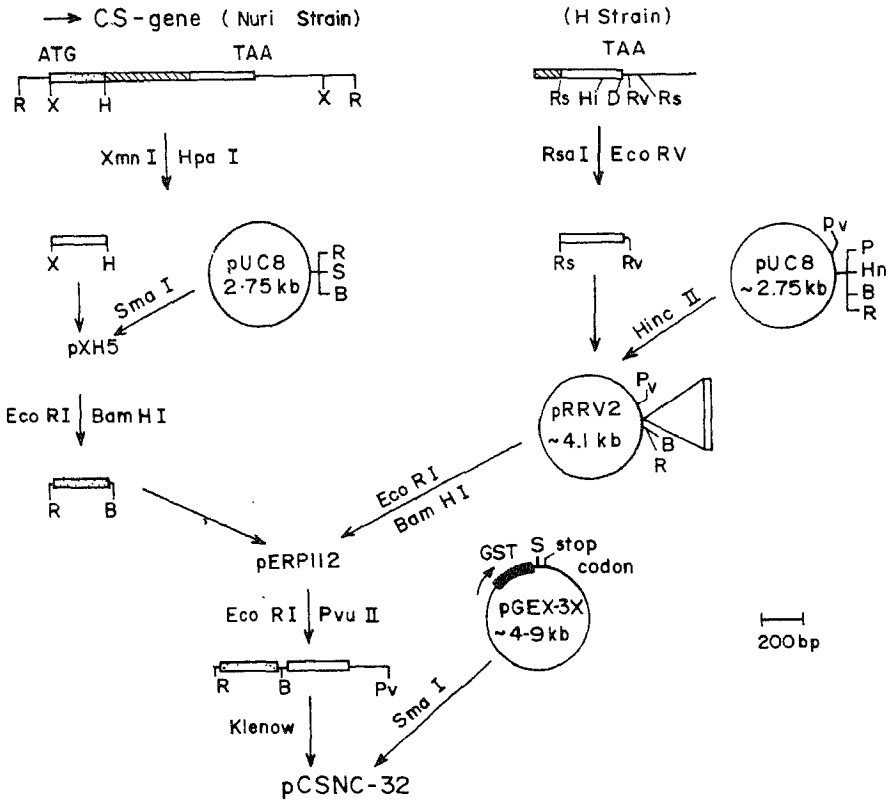

Figure 2. Construction of the recombinant plasmid for the truncated repeat-less CS-gene. Restriction sites used: B, BamHI; D, DdeI; H, HpaII; Hi, HindIII; Hn, HincII; P, PstI; Pv, PvuII; R, EcoRI; Rs, RsaI; RV, EcoRV; S, SmaI; X, XmnI.

truncated CS-gene. The EcoRI-PvuII fragment was then subcloned in to pGEX-3X for expression. The PvuII site is from the pUC8 plasmid, situated about 200 bp 3'-to the termination codon of the CS-gene.

E. *coli* cells were transformed with the recombinant pGEX plasmids, tested for the fusion protein formation, and the positive clones pCSN-18, pCSC-14 and pCSNC-32 were identified

2.2 *Expression and purification of fusion proteins*

E. coli cells transformed with the recombinant plasmids were diluted 1 in 10 from overnight cultures into 10 ml of fresh medium and grown for 1 h at 37°C. After inducing with isopropyl-D-thiogalactopyranoside (1 mM for pCSN and 5 mM for pCSC and pCSNC), they were grown for another 2 h. The cells were pelleted and lysed in 1 ml of MTPBS (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4, pH 7·3, 1% Triton-X 100). The crude extract was mixed with 100 µl of 50% glutathione agarose beads (Sigma) and placed on a rotating platform. After absorption for 20 min, the beads were pelleted and washed repeatedly in MTPBS. The proteins were eluted either by boiling in equal volumes of gel loading buffer or by elution with 5 mM glutathione, and analyzed by SDS-PAGE.

3. Results and discussion

The two domains of the CS-antigen that were expressed are shown in figure 1. The amino terminal domain was taken from the *Nuri* strain, as it contained a convenient HpaII site at the beginning of the repeat region. This site cuts at the last proline residue of the five contiguous conserved amino acids of the region I (Sharma *et al* 1985). The XmnI enzyme restricts at a site 7 bp 3'-to the ATG codon. Thus the XmnI-HpaII fragment contained 95 amino acids comprising all of the amino-terminal non-repetitive domain of the CS-protein excepting the first three amino acid residues. The carboxy-terminal domain was taken from the H strain because of the existence of an RsaI site that cuts into the last amino acid of the last repeat unit. For expression, a 372 base pair fragment, extending 10 base pair beyond the termination codon, containing all of the 121 amino acids of the non-repetitive carboxy-terminal region, was used. The same two domains were used in the truncated protein construct (figure 2) with a linker region of pUC8, 11 bp from Smal site to HincII site, connecting the two. This resulted in the addition of aspargine, serine and histidine at the junction of the two domains. The EcoRI-PvuII fragment, used for cloning in to the pGEX expression vector (Smith and Johnson 1988), contained about 200 bp extra from the pUC8 plasmid. However, this extra DNA was situated 3'-to the termination codon of the CS-gene. The plasmid pGEX has a glutathione-S-transferase (GST) gene into which the gene under study can be cloned, resulting in a fusion protein. The fusion proteins of both the carboxy-terminal domain and the truncated protein were terminated at the CS-gene termination, while the fusion protein for the amino-terminal domain was terminated by the stop codon of the GST protein, provided in all reading frames in the pGEX vectors (Smith and Johnson 1988). The plasmids constructed should have proper reading frames as assessed from the respective DNA sequences. An

incorrect reading frame would result in the termination of the fusion protein after 20–25 amino acids, as predicted by the sequence (Sharma *et al* 1985). This would result in a fusion protein of about 28 to 29 kD. Since the fusion proteins are of much higher molecular weight (see below), it is likely that the reading frames are proper.

There is a sequence and hence protein divergence between the *Nuri* and the H strain CS-proteins over 60 base pairs at the end of the repeat region (Sharma *et al* 1985). The amino terminal domain on the other hand is 98% conserved between the two strains. Thus the two expressed domains, as well as the truncated CS-protein virtually represent a subset of the *H* strain protein.

The GST fusion protein can be induced by IPTG and can be affinity purified to > 80% purity by using immobilized glutathione (Smith and Johnson 1988). Figure 3 shows the production of the amino-terminal fusion protein (GST-CSN) in the crude extracts, and after affinity purification. Maximum amounts of protein are obtained with 3 h of induction, after which it is saturated. The yield of the fusion protein is around 25-30 mg/litre of culture. This protein is soluble, has a molecular weight of 38 kD, which is expected from the addition of the 98 amino acids to the 26 kD GST protein.

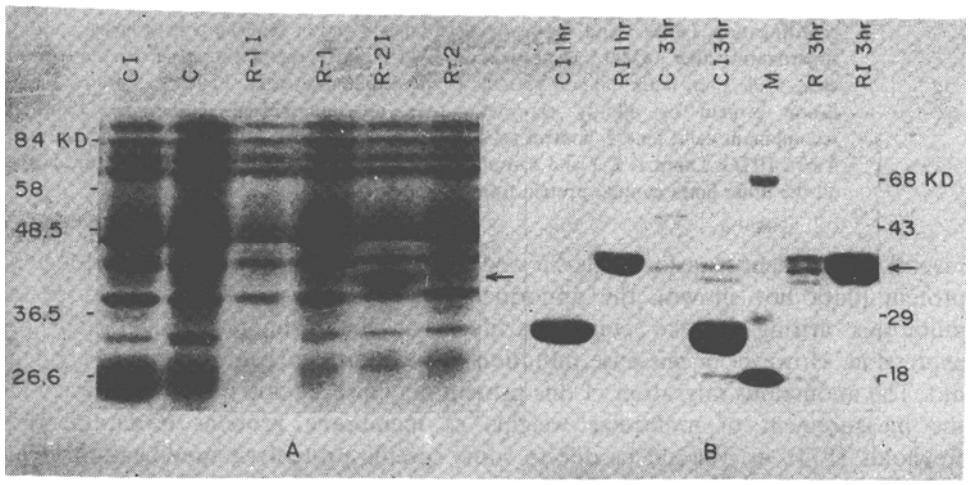

Figure 3. Expression of the amino-terminal region of CS-antigen as a fusion protein. Both the panels A and B show a Coomassie stained profile of proteins separated on a 10% SDS-PAGE. Panel A: total proteins. Lanes C, R-1 and R-2 are *E. coli* transformants with the pGEX-3X plasmid and two clones for the amino-terminal fusion protein construct, containing the insert in the wrong orientation and the correct orientation (pCSN-18), respectively. Each lane has protein corresponding to about 0·2 ml of the original culture. Panel B: proteins purified on glutathione-agarose beads. Lanes C: Cells transformed with vector pGEX-3X; R, recombinant-2 (pCSN-18) of panel A; M, molecular weight markers. Cultures were harvested 1 and 3 h after induction. Each lane represents protein from about 1·5 ml of the original culture. Arrow indicates the fusion protein. I indicates cultures that were induced with **1** mM IPTG.

Figure 4 shows the production of the carboxy-terminal fusion protein (GST-CSC). The expected molecular weight of this protein is 40 kD, but that observed is 32 kD. To eliminate the possibility of DNA deletion, the size of the insert in pCSC clone was examined, and was found to be of the appropriate size of 370 bp. Since

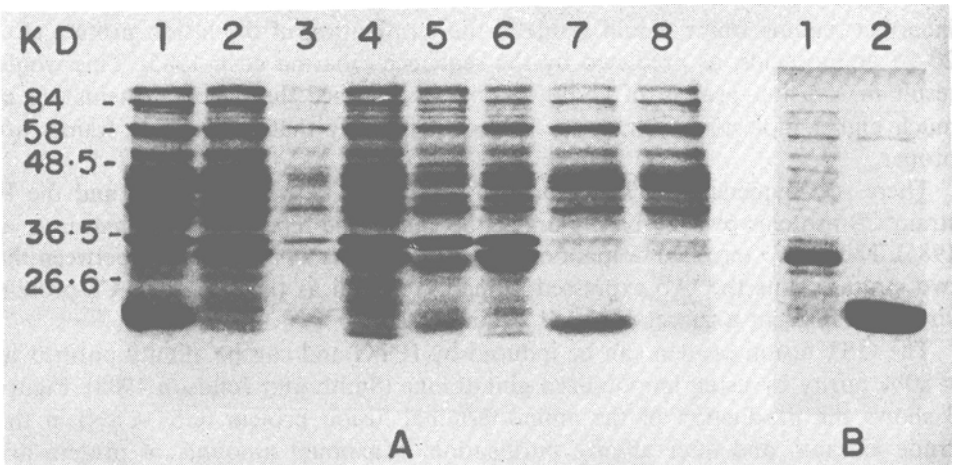

Figure 4. Expression of the carboxy-terminal region of the CS-antigen as a fusion protein (GST-CSC). Both the panels A and B show Coomassie stained profiles of proteins separated on a 12·5% SDS-PAGE. Panel A: Lanes 1 and 2 are crude extracts of *E. coli* transformed with the control vector pGEX-2T plasmid and the recombinant plasmid pCSC-14, respectively. Lanes 3–8 show the distribution of the fusion protein on differential centrifugation. Lanes 3 and 4, 600 *g* pellets; lanes 5 and 6, 16,000 *g* pellets; lanes 7 and 8, supernatants after 16,000 *g* centrifugation. Odd lanes represent extracts from the control cells, while even lanes contain extracts from recombinant cells. Panel B: Purification of fusion protein by affinity chromatography on immobilized glutathione. Lane 1, recombinant cells; lane 2, control cells. Cultures were grown for 2 h after induction with 5 mM IPTG. Lanes 1, 2, 7 and 8 contain protein equivalent from 0·2 ml of culture, while all the other lanes contain protein from 1·0 ml of culture.

several independent transformants showed the same molecular weight for the fusion protein (data not shown), the abnormality is unlikely to be due to missense mutations arising due to cloning artifacts at the ultimate cloning step for expression. However, a missense mutation in the source plasmid cannot be ruled out. The anomalous migration of this protein may also be due to the inaccuracy in the measurement of molecular weights of membrane proteins (Grefrath and Reynolds 1974), or it could be due to some specific proteolytic modification in *E. coli.* Protein degradation during the extract preparation is unlikely to occur *in vitro,* because all preparations have always shown an identical protein band, with no evidence of any other lower bands. All CS-proteins have been found to migrate with anomalous molecular weights, and it is attributed to its unusual structure (Nussenzweig and Nussenzweig 1989).

The GST-CSC protein was found to be largely particulate as shown in figure 4. Most of the protein came down with the 650 *g* and 16,000 *g* pellets. This protein bound to the affinity matrix rather weakly, and perhaps because of its particulate nature, pulled down a number of non-specific proteins with it (figure 4B). Maximum amount of GST-CSC protein was obtained at 2 h after induction, with lower yield of the fusion protein beyond this period of induction. The yield of the GST-CSC protein is around 10–15 mg/litre of culture.

Figure 5 shows the expression of the truncated repeat-less CS-protein fused to the GST-protein. This protein (GST-CSNC) was found to be extremely labile and the induction of the protein was variable. Most of the time the fusion protein could not

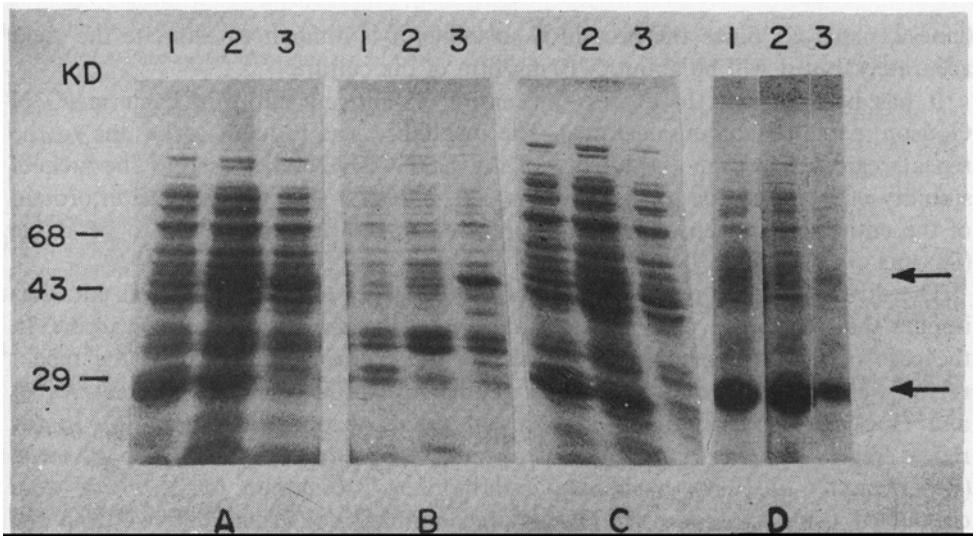

Figure 5. Expression of the repeat-less truncated CS-gene as a fusion protein. Figure shows Coomassie stained profiles of proteins separated on a 10% SDS-PAGE. Panel A: Crude extracts; B: 16,000 *g* pellet; C: 16,000 *g* supernatant; D: Fusion protein affinity purified on glutathione-agarose beads. In each of the panels, lane 1 represents the cells transformed with vector plasmid pGEX-3X, while lanes 2 and 3 represent two independent culture tubes of the recombinant cells. The arrows indicate the recombinant protein at 48 kD and the GST-protein at 26 kD. Protein samples are equivalent to: Panels A and C: 0.15 ml culture; Panel B: 0·3 ml culture and Panel D: 1·5 ml culture.

be detected properly in crude extracts. Figure 5 illustrates the behaviour of the fusion protein in two independent culture tubes (lanes 2 and 3). In one tube containing the recombinant cells (lane 2), the growth was much more than the other tube (lane 3), as well as the control cells producing only GST (lane 1); even though identical conditions for growth and induction were used. This is an indication of non-induction of the fusion protein in this tube (lane 2). Since protein samples corresponding to equivalent culture volumes were loaded on the gel, the protein content per lane reflects this difference in growth. Not only did the cells in lane 2 not induce the fusion protein properly, the protein was probably degraded *in vivo,* as most of the fusion protein was found to be degraded to a protein of 26 kD molecular weight even in the crude extract, whereas the production of the fusion protein in the other tube (lane 3) can be seen distinctly in the crude extract, as well as in the 16,000 *g* pellet, with no evidence of degradation (panels A and B). However, after the extra steps for the affinity purification, which takes about an hour to perform, even the other recombinant sample shows a considerable amount of degradation to a 26 kD protein (lane 3, panel D). The use of certain protease inhibitors, to stabilize the fusion protein, did not help significantly. Thus the fusion protein appeared to be very unstable both *in vivo.* and *in vitro.*

GST-CSNC is also a particulate protein, as most of the protein was found to be associated with the 16,000 *g* pellet, rather than the supernatant (figure 5, panels B and C). The molecular weight of the protein is 48 kD, 4 kD smaller than the expected molecular weight. Much of the degraded protein is presumably the GST protein as a lot of it was recovered in the 16,000 *g* supernatant as a 26 kD product

(lane 2, panel C). Since the protein is so labile, it is difficult to estimate the yield accurately, but it will be about 5–10 mg/litre of the culture.

It has been difficult to express the entire CS-antigen stably in bacteria (G N Godson, personal communications). The instability was thought to be due to the repeat region. However, the lability of the GST-CSNC indicates that the lack of stability may not be due to the repeat region. Indeed a galactosidase fusion protein of the entire CS-antigen of the *Nuri* strain of *P. knowlesi* was found to be stable (Sharma *et al* 1986).

The diversity of the repeat regions of the CS-genes of simian malarial parasites implies that the repeat units are not suitable for the use of an effective vaccine. In the case of the human malarial parasites, *P. falciparum* and *P. vivax,* the repeat region was found to be invariant in a number of strains (Arnot *et al* 1985; Weber and Hockmeyer 1985). However, a recent report demonstrates variation in the repeat region of over 14% of clinical cases in western Thailand for *P. vivax* (Rosenberg *et al* 1989). In human and monkey vaccination trials, which were conducted using synthetic peptide and recombinant constructs derived from the repeat region of the *P. falciparum* and *P. vivax* CS-antigens, the success has been limited so far (Ballou *et al* 1987; Herrington *et al* 1987; Collins *et al* 1989). Furthermore field studies have shown that naturally acquired antibodies against the repeat regions, do not provide total protection from infections in persons living in malaria endemic areas (Hoffman *et al* 1987).

Antibodies to the non-repetitive regions of the CS-gene of *P. knowlesi* have been detected earlier in sera of protected monkeys and rabbits immunized with sporozoites (Vergara *et al* 1985; Sharma *et al* 1986). Some of these epitopes have been detected on the surface of the sporozoites and hence are accessible to the immune system (Vergara *et al* 1985). In case of *P. falciparum* and *P. vivax,* other B-cell epitopes have been detected in the non-repetitive regions (Romero *et al* 1987; Stuber *et al* 1990). The high expression of the entire non-repetitive domains of the CS-protein in *E. coil* will help in the assessment of the immunogenicity and protectivity of these regions, in the absence of the immunodominant immune evasive repeat region. Since *P. knowlesi* and the human malarial parasite *P. vivax* are extremely homologous, it would be of use to assess the protectivity of the *P. knowlesi* non-repetitive regions.

Acknowledgement

We wish to thank Dr Robert White for the gift of the pGEX plasmids during his visit to our Institute on a British Council Exchange Programme.

References

Arnot D E, Barnwell J W, Tam J P, Nussenzweig R S and Enea V 1985 Circumsporozoite protein in *Plasmodium vivax*: gene cloning and characterization of the immunodominant epitope; *Science* **230** 815-818

Ballou W R, Rothbard J, Wirtz R A, Gordon D M, Williams J S, Gore R W, Schneider I, Hollingdale M R, Beaudoin R L, Maloy W L, Miller L H and Hockmeyer W T 1985 Immunogenicity of synthetic peptides from circumsporozoite protein of *Plasmodium falciparum*; *Science* **228** 996-999

Ballou W R, Sherwood J A, Neva F A, Gordon D M, Wirtz R A, Wasserman G F, Diggs C L, Hoffman S L, Hollingdale M R, Hockmeyer W T, Schneider I, Young J F, Reeve P and Chulay J D 1987 Safety and efficacy of a recombinant DNA *Plasmodium falciparum* sporozoite vaccine; *Lancet* 1277-1281

Barr P J, Gibson H L, Enea V, Arnot D E, Hollingdale M R and Nussenzweig V 1987 Expression in yeast of a *Plasmodium vivax* antigen of potential use in a human malaria vaccine; *J. Exp. Med.* **165** 1160-1171

Collins W E, Nussenzweig R S, Ballou W R, Ruebush T K, Nardin E H, Chulay J D, Marjarian W R, Young J F, Wasserman G F, Bathurst I, Gibson H L, Barr P J, Hoffman S L, Wasserman S S, Broderson J R, Skinner J C, Procell P M, Filipski V K and Wilson C L 1989 Immunization of *Saimiri sciureus boliviensis* with recombinant vaccines based on the circumsporozoite protein of *Plasmodium vivax*; *Am. J. Trop. Med. Hyg.* **40** 455-464

Galinski M R, Arnot D E, Cochrane A H, Barnwell J W, Nussenzweig R S and Enea V 1987 The circumsporozoite gene of the *Plasmodium cynomolgi* complex; *Cell* **48** 311-319

Grefrath S P and Reynolds J A 1974 The molecular weight of the major glycoprotein from the human erythrocyte membrane; *Proc. Natl. Acad. Sci. USA* **71** 3913-3916

Herrington D A, Clyde D F, Losonsky G, Cortesia M, Murphy J R, Davis J, Baqar S, Felix A M, Heimer E P, Guillessen D, Nardin E, Nussenzweig R S, Nussenzweig V, Hollingdale M R and Levine M M 1987 Safety and immunogenicity in man of a synthetic peptide and malaria vaccine against *Plasmodium falciparum* sporozoites; *Nature* (*London*) **328** 257-259

Hoffman S L, Oster C N, Plowe C V, Woollett G R, Beier J C, Chulay J D, Wirtz R A, Hollingdale M R and Mugambi M 1987 Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications; *Science* 237 639-641

Kemp D J, Coppel R L and Anders R F 1987 Repetitive proteins and genes of malaria; *Annu. Rev. Microbiol.* **41** 181-208

Link H T, Ballou R and Krzych U 1990 Immune response to *Plasmodium berghei* sporozoite antigens; *Am. J. Trop. Med. Hyg.* **43** 452-463

Nussenzweig V and Nussenzweig R S 1989 Rationale for the development of an engineered sporozoite malaria vaccine; *Adv. Immunol.* **45** 283-334

Romero P, Heimer E P, Herrera S, Felix A M, Nussenzweig R S and Zavala F 1987 Antigenic analysis of the repeat domain of the circumsporozoite protein of *Plasmodium vivax*; *J. Immunol.* **139** 1679-1682

Rosenberg R, Wirtz R A, Lanar D E, Sattabongkot J, Hall T, Waters A P and Prasittisuk C 1989 Circumsporozoite protein heterogeneity in the human malaria parasite *Plqsmodium vivax*, *Science* **245** 973-976

Ruiz A R, Ozaki L S, Gwadz R W and Godson G N 1987 Organization and expression of the *Plasmodium knowlesi* circumsporozoite antigen gene; *Mol. Biochem. Parasitol.* **23** 233-245

Schofield L and Uadia P 1990 Lack of *Ir* gene control in immune response to malaria; *J. Immunol.* 144 2781-2788

Sharma S and Godson G N 1985 Expression of the major surface antigen of *Plasmodium knowlesi* sporozoites in yeast; *Science* **228** 879-882

Sharma S, Gwadz R W, Schlesinger D H and Godson G N 1986 Immunogenicity of the repetitive and non-repetitive peptide regions of the divergent CS-protein of *Plasmodium knowlesi*; *J. Immunol.* 137 357-361

Sharma S, Svec P, Mitchell G H and Godson G N 1985 Diversity of circumsporozoite antigen genes from two strains of the malarial parasite *Plasmodium knowlesi*; *Science* **229** 779-782

Smith D B and Johnson K S 1988 Single-step purification of polypeptides expressed in *E. coli* as fusions with glutathione S-transferase; *Gene* **67** 31-40

Stuber D, Bannwarth W, Pink J R L, Meloen R H and Matile H 1990 New B cell epitopes in the *Plasmodium falciparum* malaria circumsporozoite protein; *Eur. J. Immunol.* **20** 819-824

Vergara U, Gwadz R, Schlesinger D, Nussenzweig V and Ferreira A 1985 Multiple non-repeated epitopes on the circumsporozoite protein of *Plasmodium knowlesi*; *Mol. Biochem. Parasitol.* **14** 283–292

Vergara U, Ruiz A, Ferreira A, Nussenzweig R S and Nussenzweig V 1985 Conserved group-specific epitopes of the circumsporozoite proteins revealed by antibodies to synthetic peptides; *J. Immunol.* **134** 3445-3448

Weber J L, Hockmeyer W T 1985 Structure of the circumsporozoite protein gene in 18 strains of *Plasmodium falciparum*; *Mol. Biochem. Parasitol.* **15** 305-316

Young J F, Hockmeyer W T, Gross M, Ballou W R, Wirtz R A, Trosper J H, Beaudoin R L, Hollingdale M R, Miller L H, Diggs C L and Rosenberg M 1985 Expression of *Plasmodium falciparum* circumsporozoite proteins in *Escherichia coli* for potential use in a human malaria vaccine; *Science* **228** 958-962