

photopolymerized, catalyzed by 0.01% w/v ammonium persulfate and 0.0008% w/v riboflavin, under fluorescent lamps. Cationic terminating buffer was 202 mM glycine, 190 mM acetic acid pH 4.5. For both systems, samples were loaded in each leading buffer. Samples consisted of 300 µl of 1 × leading buffer, 3% Sucrose, 1.32 mg IEF markers (BioRad, USA) and either 1.3 or 2.0 mg of dialyzed human serum (anionic and cationic respectively). Electrophoresis was performed at 0.25 mA constant current for 12 or 30 hours (anionic or cationic), prior to the elution of 80×(1 min) 64 µl fractions.

Fraction analysis. Fractions were analyzed by SDS-PAGE⁹, and purity and yields quantitated by densitometry (Molecular Dynamics Computing Densitometer and ImageQuant software, USA). Fractions of highest purity were pooled, dialyzed 1 in 500,000 against 150 mM Tris-HCl pH 7.5, 0.05% w/v SDS at 4°C. Spectral and amino acid analysis was performed to determine concentrations and product modifications.

Monomer acrylamide assays and free SDS determination. To determine the levels of monomer acrylamide, 25 µl samples were loaded onto a Whatman Partisil 10 ODS-2 HPLC analytical column with a C4 precolumn and eluted with water at a flow rate of 2 ml/min with a detector sensitivity of 0.002 absorbance units at 208 nm. Concentration of acrylamide present in the sample was determined by comparison with acrylamide standards. Between runs, the precolumn was regenerated with a 0–100% acetonitrile gradient. This permitted the assay of acrylamide in protein solutions by ensuring proteins were removed before the ODS-2 column. Rates of acrylamide scavenging by thioglycolic acid were determined by incubating 100 µg/ml acrylamide in thioglycolic buffer at 50, 40 and 30°C and assaying aliquots for free acrylamide at various time points. Determination of the SDS concentration associated with the protein in the dialyzed pools was done according to the procedure of Waite and Wang¹⁰.

Protein sequencing. Protein samples were sequenced using a model 473A Applied Biosystems protein sequencer, either directly by loading a sample aliquot into a Prospin cartridge (Applied Biosystems, USA), or sequenced after SDS-PAGE and electro-blotting to polyvinylidene difluoride membranes (Immobilon-psq, Millipore, USA) and excising band of interest⁸.

Amino acid analysis. Proteins were hydrolyzed in vapor phase 6M HCl, 0.1% phenol at 110°C for 24 hours then analyzed by pre-column derivatization with phenylisothiocyanate. Proteins purified on acrylamide, which had not been pre-electrophoresed, contained a novel peak in the amino acid analysis which eluted slightly behind carboxymethyl cysteine. A standard for this peak was prepared by reacting glutathione with acrylamide, purifying the adduct by reverse phase HPLC, followed by acid hydrolysis. A new peak, assumed to be carboxyethyl cysteine, was present in the analysis of the hydrolysate. This co-eluted with the novel peak in protein hydrolysates. A relative absorbance for this carboxyethyl cysteine was determined by comparison with the peak heights of the glycine and glutamate present in the modified glutathione hydrolysates.

Acknowledgments

We would like to gratefully acknowledge Drs. Dieter Gillessen and Bela Takacs of F. Hoffmann La-Roche, Basel for supplying the recombinant RAP-1; Dr. Peter Kennedy of Biotech Australia, Sydney and Peter Schoofs of CSL Ltd, Melbourne for their supplies of recombinant RAP-1 and RAP-2. This work was financially supported by grants provided by the Australian malarial vaccine development joint venture SARAMANE, AMRAD Ltd, and the Australian National Health and Medical Research Council.

References

- Ridley, R. G., Takacs, B., Lahm, H., Delves, C. J., Goman, M., Certa, U., Matile, H., Woollett, G. R. and Scaife, J. G. 1990. Characterisation and sequence of a protective rhopry antigen from *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* **41**:125–134.
- Saul, A., Cooper, J., Hauquitz, D., Irving, D., Cheng, Q., Stowers, A. and Limpitboon, T. 1992. The 42-kilodalton rhopry-associated protein of *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* **50**:139–150.
- Ridley, R. G., Takacs, B., Etlinger, H. and Scaife, J. G. 1990. A rhopry antigen of *Plasmodium falciparum* is protective in *Saimiri* monkeys. *Parasitology* **101**:187–192.
- Baumann, G. and Chrambach, A. 1976. Gram-preparative protein fractionation by isotachopheresis: Isolation of human growth hormone isohormones. *Proc. Nat. Acad. Sci. USA* **73**:732–736.
- Holloway, C. J. and Battersby, R. V. 1984. Preparative Isotachopheresis. *Methods Enzymol.* **104**:281–301.
- Stowers, A., Prescott, N., Cooper, J., Takacs, B., Stueber, D., Kennedy, P. and Saul, A. 1995. Immunogenicity of recombinant *Plasmodium falciparum* rhopry associated proteins 1 and 2. *Parasit. Immunol.* *In press.*
- Righetti, P. G., Faupel, M. and Wenisch, E. 1992. Preparative electrophoresis with and without immobilized pH gradients, p. 159–200. *In: Advances in Electrophoresis.* Chrambach, A., Dunn, M. J. and Radola, B. J. (Eds.). VCH Publishers, New York, NY.
- Moos, M., Nguyen, N. Y. and Liu, T.-Y. 1988. Reproducible high yield sequencing of proteins electrophoretically separated and transferred to an inert support. *J. Biol. Chem.* **263**:6005–6008.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**:680–682.
- Waite, J. H. and Wang, C.-Y. 1975. Spectrophotometric measurement of dodecyl sulfate with basic fuchsin. *Anal. Biochem.* **70**:279–280.

Erratum

Due to a printing error, Figure 5 in Boublik, Y., Di Bonito, P. and Jones, I.M., 1995, "Eukaryotic virus display: Engineering the major surface glycoprotein of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) for the presentation of foreign proteins on the virus surface." *Bio/Technology* **13**:1079–1084, was incorrect. The correct figure and legend are reproduced below.

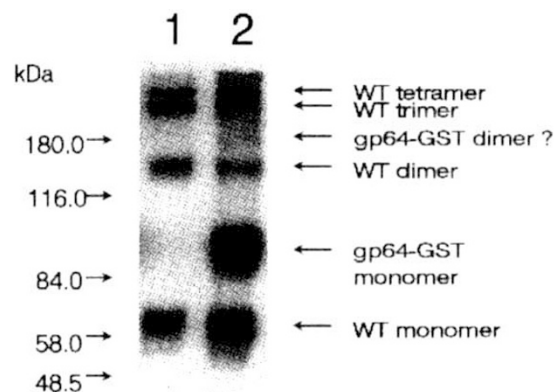


FIGURE 5. Oligomers of gp64. Samples were processed for Western blot as described. Lane 1: AcNPV wild type. Lane 2: AcGSGP-1. The positions of the wild type oligomer forms are indicated, as are the molecular weight markers (kDa). The Western blot was developed with the AcV5 anti-gp64 MAb.

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