Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 2259–2262, 1994.
Printed in Great Britain.

Role of cell surface carbohydrates in malaria

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Abstract. Metabolic labelling of Plasmodium falciparum malarial parasites with [^{3}H]-glucosamine, [^{3}H]-galactose, [^{3}H]-mannose, [^{3}H]-ethanolamine and [^{3}H]-myristic acid and subsequent purification in SDS-PAGE yielded, amongst other proteins, the 195 KDa glycoprotein, which is the major merozoite surface antigen. Reductive ß-elimination of the glycoprotein in the gel released labelled sugars. Processing of the reaction products and acid hydrolysis of the derived sugars suggested the presence of N-acetylglucosaminitol, N-acetylglucosamine and other components. Acid hydrolysis of the labelled glycoprotein and examination of the products by chromatography indicated the presence of glucosamine, galactose, mannose, ethanolamine and myristic acid. The 195 KDa glycoprotein was adsorbed by wheat germ agglutinin and desorbed with N-acetylglucosamine. Labelled galactose was incorporated in the glycoprotein by treatment with [^{3}H]-UDP-galactose and bovine milk galactosyl tranferase. The externally glycosylated glycoprotein released labelled galactose on treatment with ß-galactosidase. The carbohydrate chains in the 195 KDa glycoprotein are linked to the protein core through O-glycosyl linkage and N-acetylglucosamine and serine residues are involved in the linkage region.

Malaria is an important parasitic disease of tropical and subtropical countries with an increasing toll of morbidity and mortality each year. Plasmodium falciparum is the protozoan parasite which causes malignant tertian malaria of major medical concern. Malarial infection progresses through various stages of plasmodial development along a complex life cycle with the expression of distinct antigenic proteins and glycoproteins. The immune responses elicited against these antigens are stage-specific and involve both cell and antibody mediated mechanisms (1). High molecular weight antigens have been recognised on the surface of the merozoite stage of development (2-4). These macromolecules mediate merozoite invasion of erythrocytes (5,6). It has been observed that antibodies against some of the high molecular weight

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glycoproteins inhibit cell growth in culture. There is considerable interest in developing a vaccine against malaria (7). However, the efforts have been hampered because of diversity of the parasite antigens.

Recently, extensive interest has been developed to investigate the role of sugar residues present in the glycoprotein antigens that are synthesized by P.falciparum and exposed on merozoite surface (8,9). The presence of carbohydrates on these surface antigens has been demonstrated by in vitro labelling and enzymic release of sugar residues from the parasite cells (10,11), lectin binding (12) and inhibition studies (13). It has also been shown that intact carbohydrate chains help maintain the antigenicity of the glycoprotein (10). Because of the strong immunological behaviour of the sugar incorporated molecules and lack of information as to the nature of the incorporated carbohydrates, investigations to explore the role of carbohydrates in malaria were initiated.

The parasite cells of strain M25/ZAIRE were cultured in asynchronous mode in RPMI medium containing 10% normal human serum, 0.1% glucose and 50 mCi/ml of the radiolabelled sugars, amino alcohol and myristic acid, separately or in admixture. The metabolically labelled parasites were in good morphological condition and the surface coat was preserved. The labelled proteins and glycoproteins were extracted from the parasite cells in the non-ionic NP-40 detergent. From the NP-40 extract the labelled 195 KDa glycoprotein was immunoprecipitated using the monoclonal antibody 310 (14). The NP-40 extracted and/or immunoprecipitated glycoprotein was analysed by SDS-PAGE (15) and by autoradiography. The gel pattern in autoradiography suggested predominant incorporation of glucosamine in comparison with mannose and galactose in the 195 KDa glycoprotein.

Depolymerization of the 195 KDa glycoprotein with acid and subsequent chromatography of the products of acid hydrolysis showed the presence of labelled molecules comigrating with glucosamine, mannose, galactose, ethanolamine and myristic acid. The presence of glucosamine in the glycoprotein was further confirmed by conversion of glucosamine with ninhydrin to arabinose (16). Reductive alkali treatment of the material in the gel strip corresponding to the 195 KDa glycoprotein solubilized 80% of the radioactivity suggesting that the oligosaccharides which contain labelled sugars are O-glycosidically linked to the peptide. Acid hydrolysis of the ß-eliminated labelled material yielded compounds comigrating with N-acetylglucosaminitol, N-acetylglucosamine, mannose, galactose and trace amounts of N-acetylgalactosaminitol. In an accompanying experiment the ß-eliminated products were reduced in situ with sodium borotritide, and subsequent acid treatment showed therein the presence of labelled alanine arising from serine, suggesting O-glycosidic linkage between glucosamine and serine. Hexosaminidase treatment of 195 KDa glycoprotein liberated a sugar that migrated as N-acetylglucosamine.

The 195 KDa glycoprotein, isolated from the gel in phosphate buffer, was

treated with [^{3}H]-UDP-galactose and bovine milk galactosyl transferase to obtain externally galactosylated glycoprotein. The galactosylated glycoprotein released labelled galactose residues on sequential treatment with alpha- and ß-galactosidases suggesting alpha- and ß-anomery of the galactose residues transferred to the glycoprotein. ß-linked galactose residues were in abundance. The glycoprotein appears to possess two sites to accept galactose residues, in particular terminal N-acetylglucosamine residues. This observation was further supported when this glycoprotein was adsorbed by wheat germ agglutinin and desorbed by N-acetylglucosamine. The enzymic studies on the labelled glycoprotein suggested the presence of terminal galactose residues in addition to N-acetylglucosamine. The sequential degradation with enzymes indicated the presence of oligosaccharide chains. However, it is not possible to assess at this stage whether the enzymes are eliminating radiolabelled sugars from the surface of the protein or from the anchor region glycan.

The O-linked terminal N-acetylglucosamine is now clearly shown to be present in the 195 KDa glycoprotein. The glycoprotein also possesses galactose and mannose residues in addition to N-acetylglucosamine. The evidence available so far suggests only the presence of O-glycosyl linkage in the glycoprotein. This finding is consistent with our earlier observations (17,18). Evidence for the absence of N-glycosyl linkage between the sugar moiety and the protein core has been proposed. The coexistence of N-linked oligosaccharides or N-linked N-acetylglucosamine in a glycoprotein bearing O-linked chains or O-linked N-acetylglucosamine needs to be clarified. Mannose residues were incorporated in the glycoprotein. To establish whether these residues are present in the glycoprotein in N-linked chains or in the anchor glycan (19,20) will require further studies. However, it has been observed that the incorporation of glucosamine in the 195 KDa glycoprotein is much more intense compared to that of mannose.

The 195 KDa glycoprotein and its processed products, the 14 and 16 KDa glycoproteins, under conditions of metabolic labelling similar to those used for glucosamine, mannose and galactose, incorporate glucosamine with much more specific activity as compared to mannose and galactose. Galactose was incorporated in the glycoprotein with least abundance under the metabolic condition used (17). From the results of metabolic labelling experiments, wheat germ agglutination, reductive ß-elimination and galactose incorporation, it is clear that in the glycoprotein the carbohydrate chains mainly contain N-acetylglucosamine residues linked to serine and, as described for other glycoproteins (20), glucosamine, mannose and galactose, in addition to ethanolamine and myristic acid in the anchor region. The presence of O-linked N-acetylglucosamine in cell surface glycoproteins (21), subcellular organelles (21) and in Schistosoma mansoni (23) is known. The biological and immunological role of O-linked glycoproteins in P.falciparum is not yet known. However, this study

demonstrates that the carbohydrate chains of the immunodominant 195 KDa glycoprotein are O-linked, that N-acetylglucosamine is present in it as terminal sugar and that ethanolamine and myristic acid are among its components.

Acknowledgment. This investigation received, in part, financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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