The Korean Journal of Parasitology
Vol. 34, No. 3, 211-213, September 1996

☐ **Brief Communication** ☐

Electrophoretic variations of enzyme, GDH (NADP-dependent glutamate dehydrogenase)(EC. 1.4.1.4) in characterizing clones and isolates of Malaysian *Plasmodium falciparum*

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Abstract: Malaysian, African and Thai *Plasmodium falciparum* isolates were cultured *in vitro* by the Trager and Jensen method (1976; 1977) and were later cloned by the limiting dilution method (Rosario, 1981). Forty-eight clones were obtained and were characterized by electrophoretic variations of GDH (NADP-dependent glutamate dehydrogenase)(EC. 1.4.1.4). It was found that they were pure clones because they possessed either GDH-1 or GDH-2 unlike their parent isolates which exhibited both GDH-1 and GDH-2.

Key words: *Plasmodium falciparum*, isolates, clones, NADP-dependent glutamate dehydrogenase, electrophoretic variations

Electrophoresis of isoenzymes is a valuable technique in characterizing parasites because enzymes are products of genes and most of them are under the control of nuclear genes. Hence, enzymes can provide basic information on the organization of parasite genome. Enzyme forms are also remarkable for their stability over many generations. In malaria parasites, this technique is extremely valuable in providing markers by individual clones and mixture of diverse clones can be recognized in materials produced by cloning experiments, where the starting material is heterogenous with regards to enzyme types. In the present study, we examined the electrophoretic variations of GDH (NADP-dependent glutamate dehydrogenase)(EC. 1.4.1.4) on the Malaysian isolates and clones.

Blood samples infected only with *P. falciparum* were used in this study. Gombak A, Gombak C, ST 9, ST 12, ST 85 and ST 148 isolates were obtained from nomadic malaria patients residing in central and northeast of Peninsular Malaysia whilst Gambian and TGR isolates were obtained from patients in West Africa and Thailand respectively (Ang *et al.*, 1995a). These isolates were then cultured *in vitro* following the method of Trager and Jensen (1976; 1977) at the School of Pharmaceutical Sciences, University of Science Malaysia, Minden, Penang and Filaria and Malaria Division, Institute for Medical Research, Kuala Lumpur, Malaysia. Clones were obtained from these isolates using the limiting dilution method (Rosario, 1981) and both the isolates and their clones were prepared for electrophoresis using the method as previously described (Sanderson *et al.*, 1981; Thaithong *et al.*, 1981).

Variant forms of GDH in each isolate and clone were identified by starch gel electrophoresis at 3-5°C at 240 V for 4 hours.

• Received Dec. 9 1995, accepted after revision Aug. 26 1996.

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Table 1. GDH isoenzyme variants for *Plasmodium falciparum* isolates and clones

Isolates	GDH[a)]	Clones	Frequency
Gombak A	GDH-1	A/C1, A/D3	2/6
	GDH-2	A/D5, A/G4, A/H2, A/H7	4/6
Gombak C	GDH-1	C/C4, C/C8, C/C10	3/6
	GDH-2	C/A2, C/B4, C/B7	3/6
ST 9	GDH-1	ST 9/D9, ST 9/D10	2/6
	GDH-2	ST 9/A4, ST 9/A7, ST 9/B5, ST/D8	4/6
ST 12	GDH-1	ST 12/A3, ST 12/E8, ST 12/F8	3/6
	GDH-2	ST 12/A4, ST 12/D5, ST 12/D7	3/6
ST 85	GDH-1	ST 85/A2, ST 85/B4, ST 85/D3	3/6
	GDH-2	ST 85/A5, ST 85/B3, ST 85/D7	3/6
ST 148	GDH-1	ST 148/A5, ST 148/A6, ST 148/A7, ST 148/F7, ST 148/F8	5/6
	GDH-2	ST 148/A4	1/6
Gambian	GDH-1	Gm/Al, Gm/H5, Gm/H7	3/6
	GDH-2	Gm/B2, Gm/C5, Gm/C6	3/6
TGR	GDH-1	TGR/C4	1/6
	GDH-2	TGR/A2, TGR/B4, TGR/B7, TGR/C7, TGR/H2	5/6

[a)]Relative mobilities of GDH-1 and GDH-2 are 0.7 and 1.0 respectively.

The electrode and gel buffers used were 0.013 M citrate-0.155 M Na_2HPO_4 at pH 7.0 and 0.0054 M citrate-0.032 M Na_2HPO_4 at pH 7.0 respectively. The substrate used was 10 mg/ml lithium lactate (Sigma Chemical, USA) and the cofactor was 5 mg/ml NAD (Sigma Chemical, USA). The band positions of the isoenzymes were visualized after electrophoresis using the method as previously described (Ang *et al.*, 1995b) and the isoenzyme forms of GDH were classified following Sanderson *et al.* (1981).

Table 1 shows GDH electrophoretic variants of *P. falciparum* isolates and their respective clones. Both variants which were identified as GDH-1 and GDH-2 migrated to the anode. However, none of them possessed the third variant, GDH-3, despite that this was found in the *P. falciparum* isolates obtained from patients in Africa (Sanderson *et al.*, 1981).

GDH-1 and GDH-2 of Gombak A, Gombak C, ST 9, ST 12, ST 85, ST 148, Gambian and TGR isolates were characterized as having both GDH-1 and GDH-2 with relative mobilities of 0.7 and 1.0 respectively. Gambian and TGR isolates were incorporated into the present study mainly to serve as references of isoenzyme analysis of GDH compared to those of the local isolates.

However, the clones prepared from these eight isolates exhibited monovariant GDH. From the 6 local isolates, 18 clones out of 36 clones, A/C1, A/D3, C/C4, C/C8, C/C10, ST 9/D9, ST 9/D10, ST 12/A3, ST 12/E8, ST 12/F8, ST 85/A2, ST 85/B4, ST 85/D3, ST 148/A5, ST 148/A6, ST 148/A7, ST 148/F7 and ST 148/F8 clones possessed GDH-1 with relative mobility of 0.7 whilst the rest, A/D5, A/G4, A/H2, A/H7, C/A2, C/B4, C/B7, ST 9/A4, ST 9/A7, ST 9/B5, ST 9/D8, ST 12/A4, ST 12/D5, ST 12/D7, ST 85/A5, ST 85/B3, ST 85/D7 and ST 148/A4 clones possessed GDH-2 with relative mobility of 1.0.

For Gambian isolates, only three out of six clones, Gm/A1, Gm/H5 and Gm/H7 possessed GDH-1 with relative mobility of 0.7 whilst the rest, Gm/B2, Gm/C5 and Gm/C6 possessed GDH-2 with relative mobility of 1.0. In contrast, for TGR isolate, only one clone, TGR/C4 possessed GDH-1 with relative mobility of 0.7 whilst the rest, TGR/A2, TGR/B4, TGR/B7, TGR/C7 and TGR/H2 clones possessed GDH-2 with relative mobility of 1.0.

Isoenzyme typing which was based strictly on the genotype of the organisms and recorded from the position of each distinguishablly stained bands of enzyme activity on gels has been widely used in studying the variety of enzyme forms in trypanosoma, leishmania,

babesia, rodent species of plasmodia as well as species infecting primates and human.

The aim of the present paper was to summarize an electrophoretic variation of GDH on a total of 48 clones of which 36 clones were obtained from Malaysian *P. falciparum* isolates, whilst the rest, were from Africa and Thailand, using the limiting dilution method (Rosario, 1981). The *P. falciparum* GDH is responsible in the production of alpha ketoglutarate and NADPH in amino acid metabolism (Sherman, 1977a; 1977b; 1979; Roth *et al.*, 1982).

The present results confirmed that these isolates are genetically heterogeneous with respect to isoenzyme pattern of GDH, GDH-1 and GDH-2, with relative mobilities of 0.7 and 1.0 respectively. However, the clones were monovariant with respect to this enzyme. This study showed that isoenzyme can be used as a genetic marker to identify clones from a heterogenous population of isolates because samples of *P. falciparum* taken on one occasion from a single patient frequently contains mixtures of genetically diverse clones, due either to heterogeneity of the parasite in a single mosquito, or to infection by parasites from several mosquitoes.

Further results showed that these isolates and their respective clones did not possess GDH-3, which was found in the *P. falciparum* parasites isolated from patients in Africa (Sanderson *et al.*, 1981).

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