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Research Note

Mitochondrial Cytochrome *c* Oxidase III (COIII) Gene of *Plasmodium vivax*

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Over the past decade, more evidence has accumulated to suggest an active role of the intraerythrocytic *Plasmodium* mitochondrion in the energy metabolism and pyrimidine biosynthesis in the parasite (Fry, 1991). In addition, recent efforts to define the mitochondrial DNA of malaria parasites have revealed unique features of the genome. It is a circularly permuted tandem repeat of 6 kilobase pairs (kb) that encodes fragmented large and small subunit ribosomal RNAs (rRNAs) (Feagin *et al.*, 1992). Mitochondrial genomes of *Plasmodium* have received much interest as they encode some components of the respiratory chain, including cytochrome *c* oxidase subunit I and III (COI and III) and cytochrome *b*, as well as rRNAs, and the respiratory chain has been suggested to be a target for antimalarial compounds (Vaidya *et al.*, 1993). However, sequence data for the 6 kb element of human malaria is available only from *Plasmodium falciparum* (Feagin *et al.*, 1992), although sequence data from murine (Vaidya *et al.*, 1989) and avian (Aldritt *et al.*, 1989) parasites have been reported. Due to the limited amount of starting material, isolation of pure mitochondrial DNA and sequence analysis are difficult in the case of other human malaria, *P. vivax*, *P. ovale*, and *P. malariae*. In this study, primers specific for *P. falciparum* COIII gene were used in polymerase chain reaction (PCR) to amplify a part of *P. vivax* COIII gene which was then compared with that of *P. falciparum* and other organisms.

P. falciparum specific primers (primer A: 5' CTAGAGATTTCAAAACTCATTCC 3', primer B: 5' GTTTCATATCCTGCAATTAACATC 3'), which amplify 374 nucleotides coding the partial peptide of COIII (from Val 15 to Ser 137) (Feagin *et al.*, 1992), were synthesized. No PCR products was observed by this primer set when human total DNA was used as template. Total DNA of *P. vivax* was isolated from two patients, one from an Orang Asli (aboriginal) from Gombak Hospital, Selangor Darul Ehsan in Malaysia and the other from a Malaysian student with the infection acquired in India. Single infection of these patients with *P. vivax* were confirmed by the microscopic analysis and PCR using two different primer sets specific for *P. vivax* and *P. falciparum* rRNA genes, respectively. Infected erythrocytes were treated with 0.15% (w/v) saponin at 37°C for 20 min and then washed four times with phosphate-buffered saline, pH 7.2. DNA was extracted by phenol-chloroform. PCR reactions were run with 0.01–0.1 μg of template DNA, 1 μM of each primer, at 94°C for 15 sec, 50°C for 30 sec and 72°C for 30 sec for 30 cycles in a GeneAmp PCR system 9600 (Perkin-Elmer Ceutus). PCR products

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with expected size (~370 bp) were obtained from both *P. vivax* DNAs, and were then cloned into the PCRII vector (TA cloning kit, Invitrogen). The sequence analysis were done with automated DNA sequencers, Applied Biosystems model 373A and Shimadzu DSQ-1. Nucleotide sequence data reported in this paper are available in the GSDB, DDBJ, EMBL and NCBI data bases under the accession number D45369. Sequence similarities were maximized by the program GENETYX (SDC).

Figure 1 shows the partially determined nucleotide and deduced amino acids sequences of *P. vivax* COIII gene. Alignment of the nucleotides of *P. vivax* COIII with that of *P. falciparum* COIII (Feagin *et al.*, 1992) showed nucleotide homology of 88% while amino acid sequence comparison gave a homology of 91%. The partial sequence of *P. vivax* COIII gene appeared to be AT rich (77%). It is interesting to note that sequence data of COIII for both Malaysian and Indian isolates of *P. vivax* are identical, although relatively higher frequency of mutation in the mitochondrial DNA from various species has been suggested even in the coding regions (Brown *et al.*, 1979).

COIII is the second-largest subunit of cytochrome *c* oxidase, and has a physiologically important function in the correct assembly of the enzyme complex (Wikström *et al.*, 1985). This subunit is very hydrophobic and is predicted to contain several transmembrane helices. Figure 2 shows a comparison of

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     primer B
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         10         20         30         40         50         60
GTTTCATATCCTGCATTAACATCATTATATGGAACATCTTTAAAATATTTTTCTGTGGGT
V  S  Y  P  A  L  T  S  L  Y  G  T  S  L  K  Y  F  S  V  G

         70         80         90        100        110        120
ATCTTATTTACATCTAATCCTATTATTTTTATAATATTTGTATATTCTATTAGAGAAAGT
I  L  F  T  S  N  P  I  I  F  I  I  F  V  Y  S  I  R  E  S

        130        140        150        160        170        180
TTTTATTCTATATTTTCATCATTAGTATCAGGAATGTTATCTATAATTATATCTGAAGCA
F  Y  S  I  F  S  S  L  V  S  G  M  L  S  I  I  I  S  E  A

        190        200        210        220        230        240
ATATTATTTATTACATATTTTTGGGGTATTTTACATTTTAGTTTATCTCCATATCCATTA
I  L  F  I  T  Y  F  W  G  I  L  H  F  S  L  S  P  Y  P  L

        250        260        270        280        290        300
TATAATGAAGGTATTATTTTAACATCATCAAGAATGTTAATTCTAACTATTACATTTATT
Y  N  E  G  I  I  L  T  S  S  R  M  L  I  L  T  I  T  F  I

        310        320        330        340        350        360
TTAGCAAGTGCATCATGTATGACAGCATGTTTACAATTTCTAATAGAAAAAGGAATGAGT
L  A  S  A  S  C  M  T  A  C  L  Q  F  L  I  E  K  G  M  S
                                                    <--------

        370
TTTGAAATCTCTAG
F  E  I  S
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 primer A
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Fig. 1 Partial nucleotide and deduced amino acid sequences of *P. vivax* COIII gene. Numbers indicate the first nucleotide residue of the cloned PCR product.

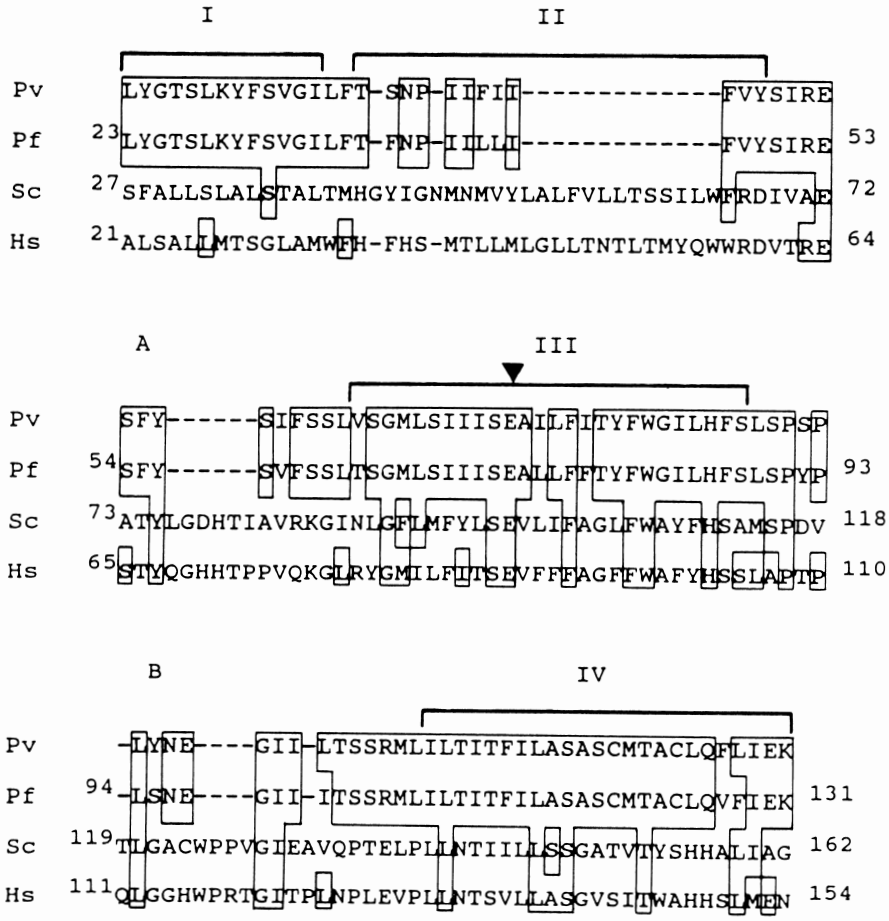

Fig. 2 Comparison of the partial amino acid sequence of COIII from various species. Pv, *P. vivax* (this study. The regions deduced from the PCR primers are not shown); Pf, *P. falciparum* (Feagin *et al.*, 1992); Sc, *Saccharomyces cerevisiae* (Thalenfeld and Tzagoloff, 1980); Hs, *Homo sapience* (Anderson *et al.*, 1981) are presented. I to IV indicate transmembrane helices, A and B indicate hydrophilic domains, and an arrow head indicates the invariant glutamate residue as described in the text.

the partial amino acid sequence of the *P. vivax* COIII with those from other species. In contrast to the great similarity between two *Plasmodium* species, similarity between *P. vivax* COIII and human COIII (28%) was much lower than that between yeast COIII and human COIII (40%). The partial amino acid sequence of *P. vivax* COIII determined in the present study includes transmembrane helices I–IV and two hydrophobic regions A and B in the folding model. Deletions were observed in the transmembrane helix II and two hydrophilic regions for both *P. vivax* and *P. falciparum* as shown in Figure 2. These deletions are found only in the *Plasmodium* peptides, suggesting that tertiary structure of COIII and interaction between the subunits in *Plasmodium* cytochrome *c* oxidase may be different from those of other organisms. In general, most of the conserved residues in COIII are found in helix III and in the two C-terminal helices, helix VI and helix VII (Wikström *et al.*, 1985). Helix III contains the invariant glutamate residue that reacts with dicyclohexyl carbodiimide (Wikström *et al.*, 1985) and has been

in the focus of interest because proton translocation in this coupling site is inhibited by chemical modification. Comparing to the other helices, conservation of amino acid sequence in the helix III as well as the glutamate residue was found in both *P. vivax* and *P. falciparum* indicating a functional importance of this segment.

In conclusion, the partial nucleotide sequence of COIII gene encoded on the mitochondrial DNA of the human malaria parasite, *P. vivax*, was determined after the successful amplification of DNA using primers designed from the sequence of *P. falciparum*. This is the first report on the sequence of *P. vivax* mitochondrial DNA, although the sequence determined is partial. The unique feature of the respiratory component found in the present study is common in both *Plasmodium spp.*, making this enzyme a target for specific diagnosis and antimalarial compounds. Further analysis including full length sequence of mitochondrial DNA from *P. vivax* and other pathogens of humans are now in progress.

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