Tokai J Exp Clin Med., Vol. 23, No. 2, pp.89-90, 1998

Mitochondrial Respiratory Chain of Malaria Parasite

Kiyoshi KITA, Yoh-ichi WATANABE, Satoru TAKEO, Shigeru MINEKI, Hiroko HIRAWAKE, Hisako AMINO, Emi ASO, Junichi WATANABE and Somei KOJIMA

Department of Parasitology, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Parasites exhibit greater diversity in energy metabolism than do the host animals, and many have exploited unique respiratory chains as adaptation to their natural habitat. During the past decade, biochemical and molecular biological studies on unique features of helminth mitochondria have progressed using *Ascaris suum* [1]. These include the elucidation of the molecular structures of the components involved and an understanding of the evolution of the energy transducing system and of the developmental changes that occur during the life cycle. In contrast to the helminth mitochondria, only little information has been available about *Plasmodium* mitochondria because of the difficulty in preparing intact mitochondria from the parasite. Therefore, we have started molecular biological approach to *Plasmodium* mitochondria for the first step of the study.

I) Cytochrome c oxidase III (COIII) gene of Plasmodium vivax

In malaria parasites, there is the 6 kb DNA element which encodes cytochrome c oxidase subunit I, subunit III (COIII), apocytochrome *b* and fragmented ribosomal RNAs genes. Previously, we have amplified a partial DNA fragment of the COIII gene of *Plasmodium vivax*, the human malaria parasite, using PCR primers derived from *P. falciparum* sequence [2]. In this study, we amplified two other DNA fragments of *P. vivax* using PCR primers derived from either *P. falciparum* or *P. vivax* sequences to cover the whole COIII gene region. The possible open reading frame in the determined sequence is 792-nucleotides long. The complete COIII sequences of *P. vivax* and *P. falciparum* are 71% nucleotide and 73% amino acid identical, while the *P. vivax* and *P. yoelii* sequences are 81% nucleotide and 82% amino acid identical. We have detected RT-PCR products using COIII gene specific primers and oligo (dT)- or random-primed cDNA from poly (A)+RNA from the erythrocytic stage of *P. vivax*. The results suggest that the COIII gene of *P. vivax* is functional in its erythrocytic stages and that the COIII gene transcript has the 3'-poly (A) sequence.

The recent results of crystallographic studies on bacterial and bovine cytochrome *c* oxidase revealed the 3 dimensional structure of this subunit. The sequence homology of COIII of *Plasmodium* of those of other organisms suggested the presence of similar, but not identical structure. The deletion specifics for *Plasmodium* COIII were found in the N-terminal region, helix I and hydrophilic region between helices III and IV. Such difference especially from the counterpart of human, the host organism, may relate to the different interactions with other components involved in electron transport [3].

II) Complex II (succinate-ubiquinone oxidoreductase/ fumarate reductase)

Complex II is well known marker enzyme for mitochondria and functions as succinate dehydrogenase (SDH) in the respiratory chain of aerobic energy metabolism. In addition, complex II of many parasites exhibit high fumarate reductase activity (FRD), reverse reaction of SDH, and play a key role as a terminal enzyme in the anaerobic electron-transport chain, the NADH-fumarate reductase pathway. Complex II is generally composed of four polypeptides and appears to be a highly conserved enzyme complex. The largest flavoprotein subunit (Fp) with a molecular weight of about 70 kDa contains covalently bound flavin (FAD; flavin adenine dinucleotide) as cofactor, and the second largest subunit with a molecular weight of about 30 kDa contains three distinctive iron-sulfur clusters, S-1 [2Fe-2S], S-2 [4Fe-4S], S-3 [3Fe-4S], and is referred to as the

Kiyoshi KITA, Department of Parasitology, Institute of Medical Science, University of Tokyo, Shiroganedai 4-6-1, Minato-ku, Tokyo 108-0071, Japan

iron-sulfur subunit (Ip). These subunits forms rather hydrophilic catalytic portion of the enzyme complex, transferring reducing equivalents from succinate to water-soluble dyes such as DCIP (SDH) or reduced methyl viologen to fumarate (FRD).

In the present study, the amino acid sequences of Fp and Ip subunit in *P. falciparum* mitochondria were deduced from genomic clones, and compared with those of other parasites such as *A. suum* as well as that of human host. The deduced amino acid sequences show that N-terminal domains of both Fp and Ip are rich in lysine, threonine and serine residues, which are typical characteristics of mitochondrial targeting sequence. Comparison to other species shows the basic structure including domains essential for enzyme-activity is well conserved. However, detailed sequence comparison also reveals unique sequence in *P. falciparum* Fp; one is deletion near C-terminus and the other is insertion near the domain interacting with AMP portion in the FAD. Sequences analyzed so far show that the deletion near C-terminus is common only amongst complex II of unicellular organisms; bacterial-, yeast mitochondrial- and that of *P. falciparum*. Insertion near the AMP-interacting domain is found in genome-DNA of four *P. falciparum*-isolates (K1, FCR3, THAI-K and SOLOMON-A). RT-PCR confirms the presence of this insertion sequence in mRNA, indicating the expression of this inserted region as peptide in the final product.

REFERENCES

1) Kita, K., Hirawake, H., and Takamiya, S. (1997) Cytochromes in the respiratory chain of helminth mitochondria. Int. J. Parasitol. in press.
2) Lim, P. K. C., Kita, K., Tan, S. K., Furuta, T., Aoki, T., Watanabe, Y., and Mak, J. W. (1995) Mitochondrial cytochrome *c* oxidase III (COIII) gene of *Plasmodium vivax*. Jpn. J. Parasitol. 44, 40-43.
3) Lim, P. K. C., Watanabe, Y., Tan, S. K., Furuta, T., Aoki, T., Kojima, S., Kita, K., Sharma, Y. D., and Mak, J. W. (1996) Cytochrome *c* oxidase III (COIII) gene of *Plasmodium vivax*: Complete coding sequence and its expression in the erythrocytic stage of the parasite. Jpn. J. Parasitol. 45, 373-383.