โครงสร้างจีโนมไมโทคอนเดรียของปลาการ์ตูนอานม้า (*Amphiprion polymnus*)

Mitochondrial Genome Structure of Saddleback Anemonefish (*Amphiprion Polymnus*)

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บทคัดย่อ

คณะผู้วิจัยได้ทำการหาลำดับนิวคลีโอไทด์ของจีโนมไมโทคอนเดรียในปลาการ์ตูนอานม้า (*Amphiprion polymnus*) ซึ่งมีความยาว 16,903 คู่เบส ประกอบด้วย ยีนที่แปลรหัสเป็นโปรตีน 13 ยีน ไรโบโซมอลอาร์เอ็นเอ 2 ยีน และทรานสเฟอร์อาร์เอ็นเอ 22 ยีน โดยมีลำดับของยีนบนจีโนมไมโทคอนเดรียเหมือนกับปลาในวงศ์ Pomacentridae เมื่อเปรียบเทียบความเหมือนของลำดับนิวคลีโอไทด์กับปลาการ์ตูนส้มขาว (*A. ocellaris*) และปลาการ์ตูนสองแถบ (*A. bicinctus*) พบว่ายีน *ND6* มีลำดับนิวคลีโอไทด์แตกต่างมากที่สุด ส่วนยีน *COIII* มีลำดับนิวคลีโอไทด์เหมือนกันมากที่สุด จึงแสดงว่ายีน *ND6* อาจใช้ในการศึกษาความสัมพันธ์ของปลาในวงศ์ Pomacentridae ได้เหมาะสมที่สุด

ABSTRACT

The complete sequence of the saddleback anemonefish (*Amphiprion polymnus*) mitochondrial genome has been determined. The entire sequence is 16,903 base pairs (bp) in length, with 13 protein-coding, two ribosomal RNA and 22 transfer RNA genes, and the gene order is highly similar to that of Pomacentridae species. Comparison of sequence similarity of each protein-coding sequence among *A. polymnus*, *A. bicinctus*, and *A. ocellaris* revealed that the sequences of *ND6* gene had the lowest similarity, whereas those of *COIII* gene shared the highest sequence similarity. This suggests that the *ND6* gene might be suitable for molecular phylogenetic study in the Pomacentridae fishes.

คำสำคัญ: ปลาการ์ตูน, นิวคลีโอไทด์, จีโนมไมโทคอนเดรีย, ยีน *ND6*

Keywords: anemonefishes, nucleotide, mt genome, *ND6* gene

INTRODUCTION

Vertebrate mitochondrial genome (mt genome) is a circular molecule with size varying among 16–19 kb. It contains 37 genes encoding 13 protein-coding genes, 2 ribosomal RNAs, 22 transfer RNAs, and a variable control region (CR) or D-loop (Avise, 1994). The mtDNA has been widely used in molecular evolution and phylogeny studies since its mutation rate is higher than that of nuclear DNA, and the ordering of mitochondrial genes often remains unchanged. However, the gene coding for the subunits of cytochrome oxidase and cytochrome b are conserved gene, whereas the most variable ones are the NADH dehydrogenase and ATPase genes. These findings suggest that the rate of mutation in each mitochondrial gene is different based on activities and functions.

Anemonefishes or clownfishes (*Amphiprion* and *Premnas*, Pomacentridae) are found along the coral reef with 28 species (27 species of *Amphiprion* and 1 species of *Premnas*). Seven species inhabit Thailand (Allen, 1997). Anemonefishes are identified commonly by their color patterns, even though morphological characters are highly variable. The sibling species could exhibit extensively overlapped features. Recently, molecular phylogenetic studies of *Cytb*, 16S rRNA, and control region revealed that the origin of anemonefishes is monophyletic (Elliott *et al*., 1999; Santini and Polacco, 2006). However, the phylogeny revealed two subclades: first clade comprises *A. ocellaris* and *P. biaculeatus*; and another clade comprises *A. polymnus*, *A. frenatus*, *A. sandaracinos*, and *A. clarkii* (Elliott *et al*., 1999). *A. polymnus* was positioned at the terminus of the tree, and *A. ocellaris* was located at the basal position of the relationship. However, these data were derived from a few markers, with different mutation rate. Analyses of such sequences in additional mitochondrial genes should provide more conclusive evidence. Nevertheless, only complete mt genome of *A. ocellaris* and *A. bicinctus* has been reported, but those of terminal clade have not yet been performed. In this study, the complete sequence of mt genome of saddleback anemonefish (*A. polymnus*) and its structure were determined. The mt genome data were also compared with those of other anemonefishes as the preliminary data.

MATERIALS AND METHODS

One sample of *A. polymnus* was collected from Rayong Coastal Fisheries Research and Development Center (Thailand). Total genomic DNA was extracted from muscle tissues and fin clip of *A. polymnus* following a standard phenol-chloroform protocol. A primer set which covered all regions of the mt genome from caecilian amphibians was used to perform PCR based method (San Mauro *et al*., 2004). Standard PCR reaction was performed using 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM specific primers, 0.50 U of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), and 50 ng genomic DNA in a final reaction volume of 20 μl. PCR cycling conditions contained the initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30s, primer annealing at 50–60°C for 35s, primer extension at 72°C for 1 min, then postcycling extension at 72°C for 10 min. Amplified products were examined by electrophoresis on 1% agarose gel, and the nucleotide sequences of the DNA fragments determined using First Base service (Seri Kembangan, Malaysia). Genome assembly was conducted using CAP3 (Huang and Madan, 1999) with manual annotation, and the sequences were searched with the National Center for Biotechnology Information (NCBI) database using the blastx and blastn programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All tRNAs were recognized using the tRNAscan-SE 1.21 (Lowe and Eddy, 1997), and all mitogenome sequences of *A. polymnus* were compared with those of *A. ocellaris* (AP006017) and *A. bicinctus* (JQ030887) to characterize their mt genome structure.

RESULTS AND DISCUSSION

The complete mitochondrial sequence of *A. polymnus* was 16,903 bp in length, and the gene order was similar to that of *A. ocellaris*, and *A. bicinctus*. The overall nucleotide base composition was A, 29.6%; C, 29.0%; G, 15.5%; and T, 25.9% for *A. polymnus*. The AT content (55.5%) was slightly higher than the GC content (44.5%), as observed in other anemonefishes (*A. ocellaris*, AT 54.6%; GC 45.4% and *A. bicinctus*, AT 55.2%; GC 44.8%). Twenty-two tRNA genes were interspersed along the genome with the two copies of $tRNA^{ser}$ and $tRNA^{Leu}$. All tRNA genes lengths ranging from 67 to 77 bp were predicted to the typical cloverleaf structures (Lowe and Eddy, 1997) except for the $tRNA^{Ser}$ showing the deviated secondary structure, as also found in *A. bicinctus*. Comparison of protein-coding gene among *A. polymnus*, *A. ocellaris*, and *A. bicinctus* revealed that *COIII* had the highest similarity between *A. polymnus* and *A. ocellaris*for 91.97%, whereas *ATPase8* had the highest value for *A. polymnus* and *A. bicinctus* at 97.62%. However, the comparison of *ND6* sequence showed the lowest similarity between *A. polymnus* and *A. ocellaris*: 83.91%, *A. polymnus* and *A. bicinctus*: 92.34%, and *A. ocellaris* and *A. bicinctus*: 85.06%. The lowest average similarity was found in *ND6* gene with the value of 87.1%. These results collectively suggest that *ND6* could be used to identify anemonefish species.

The common start codon of all protein coding genes was ATG except for *COI* and *ATPase6* genes, having GTG as the initiation codon. These results were consistent with those of *A. ocellaris* and *A. bicinctus*. Seven of thirteen protein coding genes showed a terminally incomplete stop codon with T or TA, which appeared to be commonly created by posttranscriptional polyadenylation in vertebrate mtDNA genome (Table 2) (Ojala *et al*., 1980). The remaining protein-coding genes end with complete stop codon (TAA and AGA). Codon usage of all protein coding genes of *A. polymnus* was analyzed. At the third position of amino acids with fourfold degenerate sites, codons ending in A (38.54%) were often found, followed by C (34.33%), T (21.37%), and G (5.76%), whereas those of twofold degenerate codons, C (35.71%) appears to be used more than T (24.24%) in pyrimidine codon family. Purine codon family ends mostly with A (31.31%) and G (6.73%) in the third position nucleotide of all codon families. All these features are very similar to those observed in *A. ocellaris* and *A. bicinctus*. A control region (CR) size of *A. polymnus* was 1,061 bp, which was larger than those of *A. ocellaris* (917 bp) and *A. bicinctus*with (898 bp). It was located in the region between $tRNA^{Pro}$ and $tRNA^{Phe}$. Three domains (TAS, central conserved region, and CSB1–CSB3) were found in CR of *A. polymnus*.

CONCLUSION

The organization and gene content of *A. polymnus* mt genome was similar to those of *A. ocellaris* and *A. bicinctus*. The *ND6* gene of *A. polymnus* had the lowest sequence similarity, whereas *COIII* gene showed the highest similarity among the protein-coding sequences. These results suggest that the *ND6* gene might be suitable for molecular phylogenetic study of Pomacentridae fishes.

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