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Short communication

IgM, IgD and IgY and their expression pattern in the Chinese soft-shelled turtle *Pelodiscus sinensis*

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ABSTRACT

Three Ig isotypes, IgM, IgD, and IgA, were previously known in reptiles. Here, in this report we describe IgM, IgD and a novel immunoglobulin heavy-chain isotype upsilon (IgY) in Chinese soft-shelled turtle (*Pelodiscus sinensis*). The IgM and IgY constant domains are characteristically similar to their counterparts described in other vertebrates. The expression of IgM and IgD were detected at mRNA level early during embryonic development, and their expression increased during further development. However, the IgY expression was not detected in larval turtles until 90 days after hatching-out. The increase in the transcription of these three Ig molecules was analyzed by using real-time PCR in spleen, kidney and blood following the injection of inactivated *Aeromonas hydrophila*. The primary increase in the expression of these three Igs was observed 1 week after the first injection, although not statistically significant, and the second injection 2 weeks after the first injection provoked a significant increase in the expression of these Igs, revealing a pattern of primary and secondary antibody response in the turtle. The present study represents the first report on reptile IgY and the pattern of IgM, IgD and IgY transcription in reptiles.

1. Introduction

Immunoglobulins (Igs) are important and essential components in humoral immune system of vertebrates. The basic structure of the Igs is composed of a four-chain unit in which two heavy chains are associated with two light chains, and the constant domains define the isotype of Ig heavy chains (Flajnik, 2002). In mammals, five Ig isotypes, IgM, IgD, IgG, IgA and IgE have been reported, and three isotypes, IgM, IgY and IgA in avians (Lundgvist et al., 2006; Schaerlinger et al., 2008). However in anuran amphibians, five isotypes have so far been reported, namely, IgM, IgY, IgX, IgD and IgF, and studies on amphibian Igs have mostly resulted from the model animal *Xenopus*. *Xenopus* IgY, which is consisted of four constant region domains, is regarded as a functional homologue of mammalian IgG (Warr et al., 1995). IgX has been considered to be an analogue of mammalian IgA because a large number of IgX-positive B cells were preferentially expressed in the gut epithelium (Mussmann et al., 1996). The *Xenopous* δ gene (IgD, possess eight C-domains) locates in the same gene cluster, following immediately 3′ end of the IgM gene, as in mammals (Ohta and Flajnik, 2006). IgF heavy chain with only two constant domains, is similar to amphibian IgY in sequence, but its gene contains a hinge exon making it the earliest example in evolution of an Ig isotype with a separately encoded genetic hinge (Zhao et al., 2006).

Reptiles are of great interest in evolution since they are the link between the amphibians, and birds, and mammals (Deza et al., 2007). However, the knowledge of immunoglobulins in reptiles is very limited when compared with other vertebrates. At present, only three immunoglobulin heavy chain classes, IgM, IgA and IgD, have been described in reptiles. The immunoglobulin M heavy chain gene with similar characteristics to those described in other species, was initially reported in turtle (*Pseudemys scripta*), and also has been found in gecko (*Eublepharis macularius*) (Turchin and Hsu, 1996; Deza and Espinel, 2008). Recent studies have revealed two other kinds of Ig isotypes, IgA and IgD in gecko. IgA is similar to *Xenopus* IgX and bird IgA, suggesting that IgA has an evolutionary relationship with avian and mammalian IgA (Deza et al., 2007). IgD is made up of 11 domains without any evidence of intragenic duplications of exons as described in IgD genes of *Xenopus* (Deza and Espinel, 2008). Any other Ig isotypes are not known to reptiles.

In mainland China, Taiwan and Japan, the Chinese soft-shelled turtle (*Pelodiscus sinensis*) is commercially cultured due to its nutritional and medical values. To date, little research has been carried out on the immune system of the Chinese soft-shelled turtle. In this

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article, we demonstrated three Ig isotypes, IgM, IgD and one unique Ig heavy chain gene designated as IgY in the Chinese soft-shelled turtle. The transcription of these three Ig was examined following bacterial stimulation.

2. Materials and methods

2.1. *Chinese soft-shelled turtle and* Aeromonas hydrophila

Chinese soft-shelled turtles *P. sinensis* weighing about 400–500 g each, were purchased from a research base of the Institute of Hydrobiology, Chinese Academy of Sciences. The animals were acclimatized in plastic aquaria in freshwater for 10 days before experiments.

Aeromonas hydrophila strain T4, originally isolated from clinically diseased Chinese soft-shelled turtle, was kindly provided by Prof. Chengping Lu from the College of Veterinary Medicine, Nanjing Agriculture University, China. The bacterium was cultured in 100 ml Luria Bertani (LB) liquid medium with gentle agitation at 25 °C for 24 h. The bacteria were inactivated in 0.5% (v/v) formalin, before being centrifuged at 10,000 × *g* for 10 min. The sediment was then washed three times with sterile phosphate buffered saline

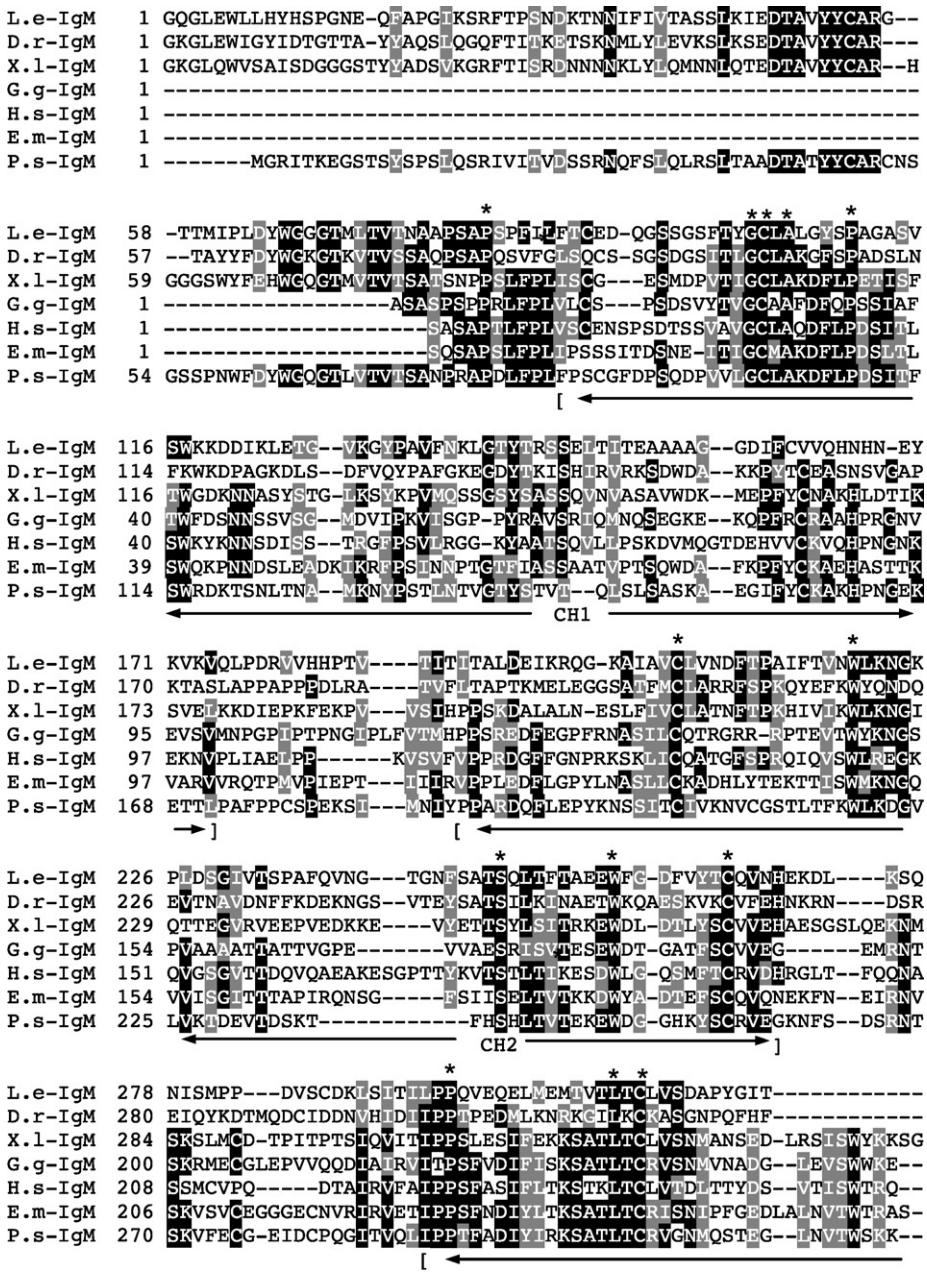

Fig. 1. Alignment of the amino acid sequences of IgM (a), IgD (b) and IgY (c) heavy chain. *L.e*: *Leucoraja erinacea*, *D.r*: *Danio rerio*, *X.l*: *Xenopus laevis*, *G.g*: *Gallus gallus*, *E.m*: *Eublepharis macularius*, *H.s*: *Homo sapiens*, *P.s*: *Pelodiscus sinensis*, *A.c*: *Anolis carolinensis*, *P.w*: *Pleurodeles waltl*. The identical residues are indicated with asterisks (*) and shaded in black, and the missing amino acids are marked by dashes. The similar amino acids are shaded in gray. The arrow indicates the Ig domain. GenBank accession numbers are as follows: skate *Leucoraja erinacea* IgM (AAA49547); zebrafish *Danio rerio* IgM (AAK69167); frog *Xenopus laevis* IgM (AAH84123); chicken *Gallus gallus* IgM (CAA25762); human *Homo sapiens* IgM (X14940); gecko *E. macularius* IgM (ABY74510); Chinese soft-shelled turtle *P. sinensis* IgM (secreted form, FJ605150); anole *Anolis carolinensis* IgD (ABV66130); IgD2 (ABY89145) and IgD (ABY55154) in *E. macularius*; Chinese soft-shelled turtle *P. sinensis* IgD (secreted form, FJ605149); anole *Anolis carolinensis* IgY (secreted form, ABV66132); chicken *Gallus gallus* IgY (secreted form, NP989594); *Pleurodeles waltl* IgY (secreted form, CAE02686) and Chinese soft-shelled turtle *P. sinensis* IgY (secreted form, FJ605148).