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Toxoplasma gondii: Identification and characterization of bradyzoite-specific deoxyribose phosphate aldolase-like gene (*Tg*DPA)

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*Tg*DPA, *Toxoplasma gondii* deoxyribose phosphate aldolase
RT-PCR, reverse transcription-polymerase chain reaction
IFAT, indirect immuno fluorescent antibody test

ABSTRACT

Toxoplasma gondii undergoes stage conversion from tachyzoites to bradyzoites in intermediate hosts. There have been many reports on bradyzoite-specific genes which are thought to be involved in stage conversion. Here, we described a novel *T. gondii* deoxyribose phosphate aldolase-like gene (*Tg*DPA) expressing predominantly in bradyzoites. The *Tg*DPA gene encodes 286 amino acids having a predicted molecular weight of 31 kDa. Sequence analysis revealed that *Tg*DPA had a deoxyribose phosphate aldolase (DeoC) domain with about 30% homology with its *Escherichia coli* counterpart. RT- and quantitative PCR analyses showed that the *Tg*DPA gene was more expressed in bradyzoites and that its expression gradually increased during *in vitro* tachyzoite-to-bradyzoite stage conversion. A polyclonal antibody against recombinant *Tg*DPA protein was raised in rabbits, and immunofluorescent analysis demonstrated that *Tg*DPA was expressed in bradyzoites *in vivo* and *in vitro*. These findings indicate that the *Tg*DPA gene is a new bradyzoite-specific marker and might play a role in bradyzoites.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite affecting most warm-blooded animals and one of the most common human and veterinary pathogen world-wide (McLeod and Remington, 1987; Tenter et al., 2000). It is an important opportunistic pathogen of immunocompromised hosts such as HIV-AIDS and transplant patients (Luft and Remington, 1992; Wong and Remington, 1993).

Toxoplasmosis is usually transmitted through oocyst-contaminated food or water and tissue cysts in raw or undercooked meat. It is present in about 13% of the human population, with a prevalence ranging from 10% to 90% (Dubey, 1986). The parasite has two forms of replication, namely sexual and asexual. The sexual stage occurs exclusively in feline animals in which oocysts are produced. The asexual stage occurs in a wide variety of intermediate hosts such as livestock and humans, which is characterized by two forms (i.e tachyzoite and bradyzoite forms). Tachyzoites are responsible for acute toxoplasmosis and congenital neurological birth defects. As a response to the host immune system attack during the progression of acute illness, the tachyzoites differentiate into encysted bradyzoites, which grow slowly and remain latent within the tissues for many years, representing a threat to immunocompromised patients such as AIDS and transplant patients (Navia et al., 1986; Dubey et al., 1998).

During the course of infection in intermediate hosts, a small fraction of the tachyzoites differentiate to form bradyzoites. Replication of these parasites is greatly slowed and they begin to express differentiation-specific markers (Yahiaoui et al., 1999; Ferguson 2004) and establish a cyst wall (Dubey et al., 1998). Therefore, an understanding of the mechanisms governing the interconversion between tachyzoites and bradyzoites might lead to new strategies for preventing tissue cyst formation and/or parasite re-emergence in immunocompromised patients. We have

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therefore taken advantage of the sequence information from the *T. gondii* expressed sequence tag (EST) database and focused on one bradyzoite-specific gene herein referred to as the *T. gondii* deoxyribose phosphate aldolase-like gene (*Tg*DPA; EST ID, TgESTzz32g04 and gene ID, 641.m01505). This gene has been previously reported as a DRPA-like gene (Ctoxoqual Contig No. 4436), which is developmentally regulated in tachyzoite-to-bradyzoite differentiation (Manger et al., 1998; Cleary et al., 2002). Since those studies, further investigations have not yet been conducted to elucidate the function of this gene. 2-Deoxyribose 5-phosphate aldolase (DERA) catalyzes the reversible aldol reaction of acetaldehyde and glyceraldehyde 3-phosphate from the sugar phosphate, deoxyribose 5-phosphate, which is the main sugar produced during deoxynucleoside catabolism (Valentin-Hansen et al., 1982; Sgarrella et al., 1997; Heine et al., 2001). It was expected that *Tg*DPA has enzymatic activity and plays an important role in utilization of deoxyribose as a carbon and energy source in the bradyzoite stage. We thought that the elucidation of this bradyzoite-specific gene would allow us to understand the molecular mechanisms of differentiation in *T. gondii*.

In the present study, expression of *Tg*DPA in tachyzoites and encysted bradyzoites was determined by RT- and quantitative PCR analyses. Anti-*Tg*DPA polyclonal antibody was produced in a rabbit by full length GST-fused *Tg*DPA protein, and the polyclonal antibody was used in IFAT to examine the localization of *Tg*DPA in bradyzoites *in vivo* and *in vitro*. Expression kinetics of the *Tg*DPA gene in *in vitro*-induced bradyzoites was also examined.

2. Materials and methods

2.1. Enzymes and chemicals

Restriction endonucleases were purchased from Toyobo Co., Ltd. (Osaka, Japan) and Promega (USA). Other DNA-modifying enzymes and RNase-free DNase I were purchased from TaKaRa Shuzo Co., Ltd. (Kyoto, Japan). All reagents used were commercially available and of analytical grade. Collagenase was purchased from Wako Pure Chemical Industries, Ltd., Japan.

2.2. Animals

Six to eight-week-old female ICR mice and a 3-month-old male white Japanese rabbit used in our experiments were purchased from Clea, Japan. The mice were used for maintenance of the *T. gondii* cysts through monthly passage, whereas the rabbit was used for polyclonal anti-*Tg*DPA antibody production. All experiments were conducted according to the guidelines issued by Obihiro University of Agriculture and Veterinary Medicine.

2.3. Parasites

The high virulent Type I strain of RH and the low virulent and cyst-forming Type II strains of Beverley, PLK and ME49 of *T. gondii* were used. RH strain was used for Western blotting described below as tachyzoite lysate samples. Beverley strain was used for chronic infection in mice and *in vivo* bradyzoite samples. PLK and ME49 strains were used for *in vitro* differentiation as described below. Tachyzoites of Beverley, PLK and ME49 strains were maintained in our laboratory through serial passage in Vero or human foreskin fibroblast (HFF) cells grown in modified Eagle's medium (Sigma–Aldrich, UK) supplemented with 5% foetal calf serum (FCS). Beverley strain cysts were obtained from the brains of orally infected ICR mice. The cysts from brain tissues were extracted as previously described with minor modifications (Makala et al., 2003). In brief, brains were removed from *T. gondii*-infected mice 1 month after infection and were homogenized in RPMI-1640 medium (Sigma–Aldrich, UK). The brain homogenates were resuspended in 50 ml of RPMI-1640 medium and finally the cysts were purified from the brain homogenate using 25% (w/v) Gum Arabic (Sigma–Aldrich, UK). Briefly, 2 ml of the Gum Arabic of 1.07 sg (specific gravity) were added to each glass tube and 2 ml of the Gum Arabic of 1.05 sg were dispensed slowly to each tube, followed by the addition of 5 ml of the above brain homogenates. After 10 min of centrifugation at 2100*g* at 15 °C, the pellets from all the tubes were added together and washed 3 times with 1× phosphate buffered saline (PBS). The purified cysts were then used for total RNA extraction.

2.4. In vitro differentiation of Toxoplasma gondii

In vitro differentiation was conducted by alkaline treatment of confluent Vero or HFF cells grown in 12-well plates. Confluent Vero or HFF cells were infected with tachyzoites of PLK or ME49 strains and differentiation was induced by culture in sodium bicarbonate-free RPMI 1640 containing 1% FCS, 50 mM Hepes (pH 8.1) at 37 °C without CO_2 (Fux et al. 2007). Cyst wall was stained with FITC-conjugated *Dolichos biflorus* lectin (DBL) as described previously (Fux et al., 2007). For the time course study, the start day of *in vitro* differentiation was day 0. Parasite cells were collected every day and used for total RNA extraction after treating with 15 U of collagenase at 37 °C for 30 min to rupture the cyst wall (Omata et al., 1995).

2.5. TgDPA cloning and sequencing analysis

*Tg*DPA cDNA containing an entire coding region was RT-PCR amplified from Beverley strain total RNA as a template using prim-

Table 1
Oligonucleotide sequences of primers and probes used in this study.

Name	Oligonucleotide sequence (5′–3′)[a]
RT-PCR analysis	
DPA forward	CGTATTCGGCTCCTCGTTAG
DPA reverse	CTTTCGTTTTCACGGACCAT
GRA1 forward	ACAGGGCAGGGATTAGGAAT
GRA1 reverse	AACGCACGAAGGAAAATGTC
GRA2 forward	AGAGGCAACAAGAGCCAGAA
GRA2 reverse	TTCTTTGGCCACCTTGAAAC
SAG1 forward	ACGGGGGATTCTGCTAGTCT
SAG1 reverse	CTTCCGCAGACAACTTGACA
BAG1 forward	ACAACGGAGCCATCGTTATC
BAG1 reverse	GTAGAACGCCGTTGTCCATT
Real-time PCR analysis	
GRA1 forward	TCACTGCATCTTCCAGTTGCA
GRA1 reverse	GAAGACGTGGCTCAAGCAGAA
GRA1 probe	(6-FAM)-TTGCTCCGAATTAAG-(NFQ-MGB)
SAG1 forward	AACGATCAACAAGGAAGCATTTC
SAG1 reverse	TCCCCCTGTGCATCCAATA
SAG1 probe	(6-FAM)-CCGAGTCAAAAAGCGTCA-(NFQ-MGB)
BAG1 forward	CTGAATCCTCGACCTTGATCGT
BAG1 reverse	GAATCAGTGCGGCAATGGA
BAG1 probe	(6-FAM)-ACACGTAGAACGCCG-(NFQ-MGB)
DPA forward	CCCACGCGACTATGTTTACAGTT
DPA reverse	CGTTTTCACGGACCATGTACTC
DPA probe	(6-FAM)-ACTTGATTTCCATAGCGCTT-(NFQ-MGB)
Cloning of the TgDPA gene	
DPA forward	TTGAATTCATGGCGACAGAGCAATTACT
DPA reverse	TTCTCGAGTCACGGGTTCGTGTCTGGAGA
Sequencing analysis	
pGEX 5′	GGGCTGGCAAGCCACGTTTGGTG
pGEX 3′	CCGGGAGCTGCATGTGTCAGAGG

[a] FAM, reporter dye 6-carboxifluorescein; NFQ, non-fluorescent quencher; MGB, minor groove binder.