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学　位　論　文　要　旨

博士後期　課程　畜産衛生学　専攻
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論文題目：Functional characterization of aspartate aminotransferase from *Toxoplasma gondii*（トキソプラズマのアスパラギン酸アミノ基転移酵素の機能解析）

要旨

Toxoplasma gondii is a protozoan parasite of which cats are the final host and humans or other animals are the intermediate hosts. Toxoplasmosis, caused by *T. gondii*, is an important zoonotic disease spreading worldwide, that threatens human health and causes economic losses in livestock industry. However, there is no effective vaccine, and a combination of compounds, pyrimethamine and sulfadiazine, is commonly used for treatment, but there are concerns about side effects and drug resistance problems. Hence, there is a strong demand for the development of safer and more effective therapeutic drugs. In this study, the function of aspartate aminotransferase (AAT) of *T. gondii* was analyzed, and the potentials of reported inhibitors of AATs for treating toxoplasmosis were assessed.

In chapter 1, *AAT* gene was characterized in *T. gondii* type I RH and type II PLK strains. The results showed that *TgAAT*s were 1,794 bp nucleotide sequences coding 597 amino acid sequences. The sequencing analysis indicated that *Tg*AAT has distant identities of 46% with that in *Homo sapiens* and *Mus musculus*. Furthermore, the ability of r*Tg*AAT for using the L-aspartate and α-ketoglutarate as the substrates in the transamination reaction was confirmed and this is consistent with the predictions and with the results in *Plasmodium*. Importantly, two compounds, hydroxylamine (HYD) and carboxymethoxylamine (CAR), known as AATs inhibitors were found that they could

indeed inhibit the activity of this enzyme. These data suggest that these two compounds could have potential to inhibit the growth of *T. gondii* through the pathway of AAT, but it remains to be confirmed by further experiments.

In chapter 2, the deficient lines of the *AAT* gene in *T. gondii* type I RH and type II PLK strains were generated and characterized. The *AAT* genes were identified from the *T. gondii* genome database, and the deficient strains (*Δaat*s) targeted were developed using the CRISPR/Cas9 genome-editing system. In addition, the functionally complemented strains (ComAATs) in which *AAT* was restored were also prepared. Through a series of experiments, data demonstrated that the *AAT* gene was deleted as expected in the *TgΔaat* strains, and that the *AAT* gene was restored as expected in the ComAAT strains. Furthermore, the functions of the *AAT* gene were identified by comparing with wild-type strains, *Δaat* strains, and ComAAT strains. Results showed that *AAT* gene-deficient parasites had a markedly reduced growth rate *in vitro* compared with the wild-types, and this growth damage in *Δaat* lines was restored by reversion to *AAT*. Importantly, this study found that the defect of growth caused by loss of *AAT* could be rescued by supplementary α-ketoglutarate, suggesting that AAT plays a role in α-ketoglutarate metabolism in *T. gondii*. On the other hand, no significant reduction was observed in the pathogenicity of *AAT* gene-deficient strains *in vivo*. Overall, these results suggest that the metabolic system controlled by AAT plays an important role in the *in vitro* growth of parasites.

In chapter 3, the inhibitory effects of HYD and CAR, were investigated on the growth of wild-type strains. This study found that HYD and CAR impaired the lytic cycle of *T. gondii in vitro*, and had the ability to control acute toxoplasmosis *in vivo*. However, even in *AAT* gene-deficient parasites, these growth-inhibitory effects were not significantly attenuated, suggesting that the inhibitory actions of HYD and CAR are AAT-independent.

In the future, it will be required to search for other inhibitors of AAT and to elucidate the anti-*T. gondii* mechanisms of HYD and CAR.

In summary, the *AAT* gene was identified for the first time in *T. gondii* and its function was analyzed using gene-editing system, and that *AAT* plays an important role in the growth of parasites. On the other hand, the current data suggest that HYD and CAR can be safe and effective drug candidates for inhibiting the growth of *T. gondii*. Further studies targeting the mechanisms of action of HYD or CAR are warranted, which is expected to contribute to the development of new therapeutic methods for toxoplasmosis.

備考　1　論文題目が英語の場合には，（　）書きで和訳を付す。

2　博士論文については，日本語の場合１８００～２２００字，英語の場合１０００～１４００語とする。修士論文については，それ以下でもかまわない。

3　図表は，要旨には記載しないこととする。

4　枚数は１枚を超えても差し支えない。