Dissertation Evaluation Report

Report No.	Diploma Number: D-BIO 1397	Applicant's Name	Rajib Acharjee

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Evaluation Report of Dissertation

1. Evaluation of the research purpose.

Development of new drugs against *Toxoplasma gondii* is imperative, and electron transport chain (ETC) of mitochondria in this parasite can be a promising drug target. Among ETC enzymes, malate:quinone oxidoreducatase (MQO) is an essential enzyme, since it plays important roles in three metabolic pathways – ETC, tricarboxylic acid cycle, and fumarate cycle. For evaluation of drug candidates and determination of cross-sensitivity among different pathogens, it is important to expand the spectrum of MQO inhibitors. Prior to the screening of drug candidates against *T. gondii* MQO (TgMQO), the applicant sought to examine whether or not TgMQO could serve as a novel drug target, in which TgMQO was purified and characterized biochemically. Therefore, the research purpose is appropriate.

2. Evaluation of the research methods.

The TgMQO gene, lacking the mitochondrial targeting signals was expressed using two different bacterial expression systems. FN102(DE3)TAO is the new bacterial expression system developed for the present study. Solubilization of membrane-bound TgMQO from *E. coli* was done with *n*-octyle-β-D-glycopyranoside and the enzyme was purified using affinity column chromatography. The optimization of the assay condition was carried out spectrophotometrically using colorimetric assay, while steady-state kinetics measured at direct consumption of ubiquinones. Inhibition of the purified TgMQO was done with ferulenol, a sesquiterpenoid coumarin compound. Therefore, the research methods are valid.

3. Evaluation of the analysis, interpretation and discussion.

The new expression system, FN102(DE3)TAO, developed in the present study, consolidated the hypothesis of underestimation of enzyme activity. The quality of the purified TgMQO was enough to be used for biochemical studies and screening of inhibitors. The oligomeric state, assay conditions, and steady-state kinetics of the purified TgMQO may provide insights into the mechanism of the mitochondrial-type MQO. The inhibition kinetics of the purified TgMQO with ferulenol showed a mixed-type inhibition which is a desirable feature for drug designing.

As stated above, the dissertation will greatly contribute to development of new drugs against *Toxoplasma gondii*, and the evaluators uniformly agree that the dissertation is worthy of being approved for a Doctor of Philosophy in Medical Science.