Inhibitory Effects of Tetragalloylglucose on the Complex II of Mitochondrial Respiratory Chain of *Ascaris* Muscle

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The effects of tetragalloylglucose (1,2,3,6-tetra-*O*-galloyl-β-D-glucose) on purified complex II (succinate–ubiquinone oxidoreductase) of the mitochondrial electron transport system of *Ascaris* muscle were studied. Both succinate–ubiquinone-1 (Q_1) oxidoreductase, and succinate dehydrogenase measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in the presence of phenazine methosulfate (PMS) were inhibited by tetragalloylglucose. The inhibitions of both reductase activities of complex II were of competitive type, and the inhibitor constant (K_i) for *Ascaris* complex II (148 nM) was lower than that for rat liver complex II (1.5 μM). Thus, *Ascaris* complex II is much more sensitive to this inhibitor than the mammalian counterpart.

Keywords tetragalloylglucose; *Ascaris*; complex II; succinate dehydrogenase

Tannic acids contained in Chinese gall and in Turkish gall are polyphenols, being composed of glucose and gallic acid.[1] Commercial tannic acid is a very complex and non-uniform mixture,[2-3] and has a wide variety of biological activities, including antibacterial effect.[1,4] Recently we have shown that galloylglucose purified from tannic acid inhibited the growth of *Photobacterium phosphoreum*, and that the target sites of galloylglucose were reduced nicotineamide adenine dinucleotide (NADH) dehydrogenase and terminal oxidase complex (cytochrome bd) of the respiratory chain.[5]

We have also shown that galloylglucose inhibits NADH dehydrogenase and succinate dehydrogenase of the mitochondrial electron transfer system of rat liver (H. Adachi, K. Konishi, and I. Horikoshi, *Chem. Pharm. Bull.*, **37**, 1341 (1989)). In this work, we show that galloylglucose is a potent inhibitor of complex II (succinate–ubiquinone oxidoreductase) of the mitochondrial respiratory chain of *Ascaris* adult muscle.

Materials and Methods

Preparation of Complex II of *Ascaris* The procedure for purification of complex II of *Ascaris* was as described previously.[6]

Assay of Succinate Dehydrogenase Activity Succinate–ubiquinone-1 (Q_1) reductase was assayed by monitoring the reduction of Q_1 according to the method of Takamiya *et al.*[6] The succinate–phenazine methosulfate (PMS)/3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reductase activity was determined spectrophotometrically by measuring the absorbance change of MTT at 570 nm ($\varepsilon = 17\ mM^{-1}\ cm^{-1}$). The concentrations of MTT and PMS were 60 μg/ml.

Assay of Fumarate Reductase Activity The assay of fumarate reductase activity was carried out under anaerobic conditions by the method of Kita *et al.*,[7] by monitoring of the oxidation of reduced methylviologen (550 nm).

Other Method Protein was determined by the method of Lowry *et al.*[8] with bovine serum albumin as a standard.

Chemicals Pure tetragalloylglucose (1,2,3,6-tetra-*O*-galloyl-β-D-glucose) isolated from Turkish gallotannin was a generous gift from Drs. I. Nishioka and G. Nonaka. Ubiquinol-1 was kindly provided by Eisai Co., Ltd. Other chemical reagents used were of the highest purity commercially available.

Results and Discussion

We have shown previously that tannic acid inhibits succinate dehydrogenase activity in rat liver mitochondria.[9] First, we examined the effect of tetragalloylglucose, which is one of the major components of tannic acid,[3] on the succinate–ubiquinone reductase activity of mitochondria of *Ascaris*. The inhibition of the activity was of competitive type (the inhibitor constant (K_i) was below 1 μM). The same activity of rat liver mitochondria was also inhibited by tetragalloylglucose, and the K_i value was about 5 μM. These data indicate that the succinate–ubiquinone reductase activity of mitochondria of *Ascaris* is more sensitive than that of rat liver. To study these phenomena in more detail, we used the purified complex II of *Ascaris*.

Dose-dependent inhibitory effects of galloylglucose were observed (50% inhibitory concentration (IC_{50}): succinate–ubiquinone, 150 nM; succinate–PMS/MTT reductase, 170 nM). Dialysis experiments showed the inhibition of succinate dehydrogenase to be reversible.

We measured the inhibitory effect of tetragalloylglucose on succinate–Q_1 reductase activity of complex II of *Ascaris* at various concentrations of the substrate. As is clear from

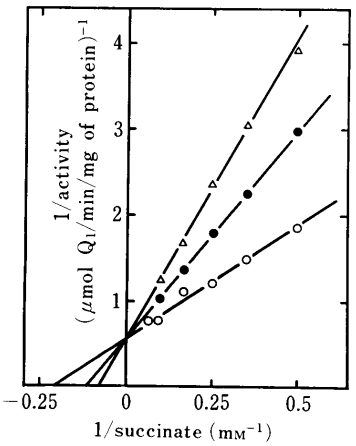

Fig. 1. Double-Reciprocal Plots of the Effects of Tetragalloylglucose on Succinate Dehydrogenase Activity in Complex II of *Ascaris*

The assay mixture, in a total volume of 2 ml, contained complex II of *Ascaris*, 30 mM phosphate buffer (pH 7.5), various concentrations of succinate, 30 μM Q_1, and/or tetragalloylglucose. (○), no addition; (●), in the presence of 100 nM inhibitor; (△), 200 nM inhibitor. The plots are typical of competitive inhibition. The K_i values were calculated to be 121 nM. From the data in the absence of galloylglucose, the K_m for succinate and V_{max} values were estimated to be 4.35 μM and 1.79 μmol Q_1/min/mg of protein, respectively.

the double-reciprocal plot, tetragalloylglucose is an inhibitor of competitive type for succinate–Q_1 (Fig. 1) and succinate–PMS/MTT reductase activities (not shown). This inhibitory pattern was the same as in the case of rat liver. From this experiment, the K_i values on succinate–Q_1 and succinate–PMS/MTT reductase were estimated as 121 nM and 148 nM, respectively. These values are much lower than those for rat liver complex II (1.5 μM) (data not shown).

These results indicated that the *Ascaris* enzyme is sensitive to galloylglucose. In the case of thenoyltrifluoroacetone (TTFA), which is a potent inhibitor of complex II in mammalian mitochondria, a difference in sensitivity among the preparations of complex II of different organisms was reported.[10] The succinate–ubiquinone reductase activity of *Ascaris* complex II was not inhibited by TTFA, even at concentrations as high as 100 μM.[10]

The complex II also acts as a fumarate reductase (the reverse reaction of succinate dehydrogenase) in the anaerobic respiration of *Ascaris* adult muscle, and plays a very important role in anaerobic energy metabolism.[6,7] The IC_{50} of tetragalloylglucose for fumarate–methylviologen oxidoreductase was estimated to be 29 μM (data not shown), which is lower than that of succinate dehydrogenase.

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