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Mendeleev Commun., 2024, **34**, 523–524

Mendeleev Communications

Quercetin and dihydroquercetin are the activators of galactonolactone oxidase from *Trypanosoma cruzi*

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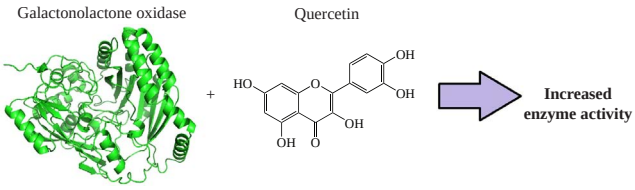
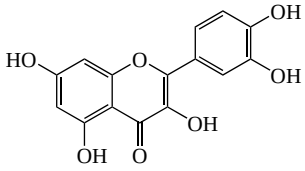
DOI: 10.1016/j.mencom.2024.06.017

The enhancing effect of natural flavonoids, quercetin and dihydroquercetin, on the catalytic activity of galactonolactone oxidase from *Trypanosoma cruzi* has been established.

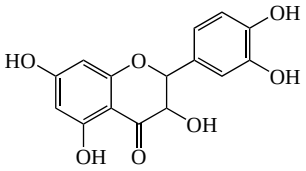


Keywords: galactonolactone oxidase, *Trypanosoma cruzi*, flavonoids, quercetin, dihydroquercetin.

Quercetin (QC) and dihydroquercetin (DQC) are natural compounds belonging to flavonoid group that poses a wide range of pharmacological properties, ranging from anti-inflammatory and antioxidant activities to antiviral effects (including the treatment of Middle East respiratory syndrome coronavirus MERS-CoV infection).[1–3] Being biologically active compounds, QC and DQC influence enzymes playing an important role in living organisms. Thus, QC suppresses the activity of xanthine oxidase, a drug target in the treatment of hyperuricemia, and is a promising basis for drug development.[4] Flavonoids can not only suppress, but enhance the catalytic activity of enzymes, for example, DQC has been shown to increase the activity of ATP phosphohydrolase which determines anti-inflammatory activity of DQC.[5]



Quercetin



Dihydroquercetin

Galactonolactone oxidase from microorganism *Trypanosoma cruzi* (TcGAL), which causes Chagas disease, is a membrane enzyme that catalyzes *in vivo* the final stage of the synthesis of vitamin C (Scheme 1). Vitamin C is an antioxidant that *T. cruzi* cannot consume from the outside[6] and which is necessary for the microorganism to survive when it penetrates host macrophages.[7] Therefore, TcGAL is considered as a possible drug target for the treatment of Chagas disease,[8] but the effect of natural compounds (including activators and electron acceptors) on TcGAL is not fully understood. Quercetin and its analogues inhibit the growth of *T. cruzi*,[9] and, at the same time, they suppress the activity of enzymes vital for the microorganism, for example E-NTPDase.[10]

In this work, the effect of QC and DQC on the TcGAL activity was studied using the reverse micelles system based on bis(2-ethylhexyl) sulfosuccinate (AOT),[8] which provides refolding of TcGAL from inclusion bodies and allows one to measure the enzyme activity (TcGAL is a membranotropic enzyme and functions at the interface).† It was found that the addition of QC or DQC to the reaction system enhances the TcGAL activity (Figure 1), which may be a consequence of the action of these compounds as enzyme activators or as electron acceptors (acting as a second substrate).

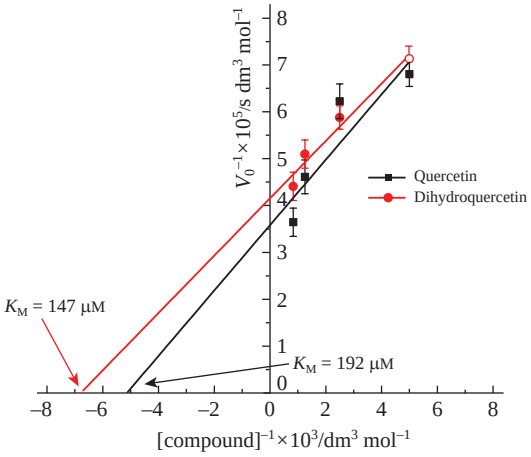
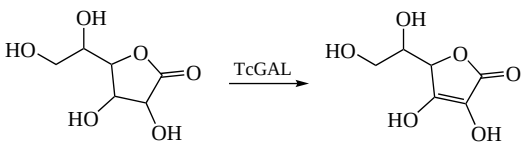


Figure 1 Double reciprocal plots for TcGAL in case of QC or DQC. Conditions are the same as in Scheme 1. K_M is the Michaelis constant.



Scheme 1 *Reagents and conditions*: 1 mM D-arabinono-1,4-lactone, 120 μM 2,6-dichlorophenolindophenol, 35 nM TcGAL, concentrations of QC and DQC are 200–1200 μM, 0.1 M AOT in *n*-octane, W_0 = 22, pH 8.8, 25 °C.

† The control of the quercetin loading in the micelles and quality of micelles has been performed using FTIR and AFM microscopy (FTIR microscope MICRAN-3 and AFM microscope NTEGRA II).

Table 1 Catalytic parameters of TcGAL and AtGALDH for different effectors.[a]

Enzyme	Effector	$V_{max} \times 10^{-7}$/M s^{-1}	K_M/μM
TcGAL	QC	28 ± 2	192 ± 4
	DQC	24 ± 1	147 ± 6
AtGALDH[b]	PMS	12 ± 1	28 ± 1
	BQ	5 ± 1	400 ± 30
	Cytochrome *c*[c]	13 ± 6	71 ± 2

[a] Conditions as in Figure 2. [b] According to published data.[11] [c] For aqueous medium.

Compared with other compounds enhancing the activity of TcGAL, QC and DQC have advantages: the possibility of their use in micelles (natural EA, cytochrome *c*, denatures in micelles[12]), absence of background reaction at concentrations up to 400 μM, functioning at the enzyme pH optimum 8.8 (in contrast to 1,4-benzoquinone (BQ)[12] functioning at pH 7.2). In addition, QC and DQC impact the activity of the model plant enzyme, namely, L-galactono-1,4-lactone dehydrogenase from *Arabidopsis thaliana* (AtGALDH) (a homologue of TcGAL). Thus, QC and DQC provide two time higher maximum catalytic activity compared to phenazine methosulfate (PMS) and BQ and five times higher compared to cytochrome *c* (Table 1). At the same time, the K_M values for QC and DQC are higher than those for PMS and cytochrome *c*, but lower than for BQ by 2.5 times.

To clarify the role of QC and DQC (activators or EA), their UV spectra before and after full reduction with $NaBH_4$ and UV spectra of the reaction mixture containing QC or DQC (before and after the reaction) were recorded [Figure 2(*a*),(*b*), respectively; data for DQC are not shown]. Initial spectrum (black line) corresponds to the reduced form of QC.[13,14] Spectrum depicted in red line (part *a*) according to literature data corresponds to the radical anion in position 3′–7[13] (calculated spectrum). Therefore, it can be concluded that the $NaBH_4$ reduction of QC (or DQC) leads to formation of this radical anion. After the enzymatic reaction in micellar system, only slight changes in the UV spectra were observed [see Figure 2(*b*)], which manifested as small peak at 425 nm. This peak is also observed in the spectrum of $NaBH_4$-reduced QC [see Figure 2(*a*)]. Such slight difference means that just a marginal part of QC (or DQC) was reduced in the reaction system. In balance, it may be concluded that QC and DQC act rather as activators of TcGAL.

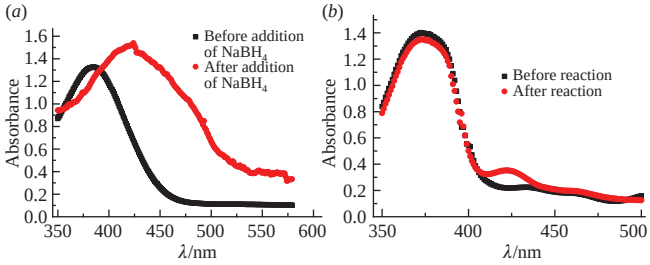


Figure 2 (*a*) Spectra of QC in water before and after addition of excess of $NaBH_4$, T = 25 °C. (*b*) Spectra of reaction mixture, containing 200 μM QC (before and after reaction, 5 min), 1 mM D-arabinono-1,4-lactone, 120 μM 2,6-dichlorophenolindophenol, 35 nM TcGAL, 200 μM QC, medium: 0.1 M AOT in *n*-octane, W_0 = 22, pH 8.8, T = 25 °C.

In summary, quercetin and dihydroquercetin were found to enhance TcGAL activity, so they may be proposed as new activators for TcGAL and AtGALDH. In general, flavonoid antioxidants, used pharmacologically for their antioxidant activity to support cardiac muscle function, have proven to be suitable activators for both plant (AtGALDH) and protozoan (TcGAL) mitochondrial galactonolactone dehydrogenases.

The work was performed using FTIR microscope MICRAN-3, Jasco J-815 CD spectrometer, AFM microscope NTEGRA II of the program development of Moscow State University.

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Received: 19th February 2024; Com. 24/7400