QUERCETIN, AN ENERGY TRANSFER INHIBITOR IN PHOTOPHOSPHORYLATION

Yasuo MUKOHATA, Shoji NAKABAYASHI and Mitsuhiro HIGASHIDA
Department of Biology, Faculty of Science, Osaka University, Toyonaka, 560 Japan

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1. Introduction

Quercetin, 3,3′,4′,5,7-pentahydroxyflavone, was found to bind to either the α- or the β-subunits [1] of isolated chloroplast coupling factor 1 (CF_1).

It was also shown to inhibit the ATPase of mitochondria [2] or chloroplasts [3], but not oxidative phosphorylation [2].

In this report, we show that quercetin inhibits photophosphorylation as an energy transfer inhibitor in competition with the substrate nucleotide. Two quercetin molecules cooperatively bind to sites on the β-subunits of CF_1 in situ and thus inhibit phosphorylation.

2. Materials and methods

Chloroplasts were isolated from market spinach leaves in a choline medium by the procedure described [4]. Chlorophyll was determined by the method [5]. Quercetin (Wako Pure Chemicals, Osaka) was purified by repeated precipitation from ethanol–water.

The electron transport activity from H_2O to methylviologen (50 μM) was measured as oxygen uptake with an oxygen electrode (Beckmann; Field Lab). Phosphorylation was assayed by the method in [6] using 20 μM PMS. A typical reaction mixture contained: 0.1 M sucrose, 5 mM $MgCl_2$, 10 mM Tricine–NaOH at pH 8.3 and chloroplasts containing 20 μg chlorophyll/ml. ADP, ATP, GDP, IDP, 1,N^6-ethenoAMP and P_i ($[^{32}P]P_i$ for phosphorylation assay) were added as required, together with methylviologen or PMS. The ethanol solutions of quercetin were added to the reaction mixture maintaining the ethanol concentration in the mixture constant and less than 5%. The reaction mixture was illuminated by white light (5×10^4 lux) for 12–60 s at 15°C.

Abbreviations: CF_1, chloroplast coupling factor 1; PMS, phenazine methosulfate

3. Results and discussion

Figure 1 shows that quercetin inhibits electron transport under phosphorylation conditions (ADP + P_i), but not in the presence of ATP and P_i (i.e. 'basal electron transport' [7]). The electron transport activity in the absence of any adenylate is little affected by quercetin. Thus, quercetin can not take the place of nucleoside di- or tri-phosphate [7–11] as

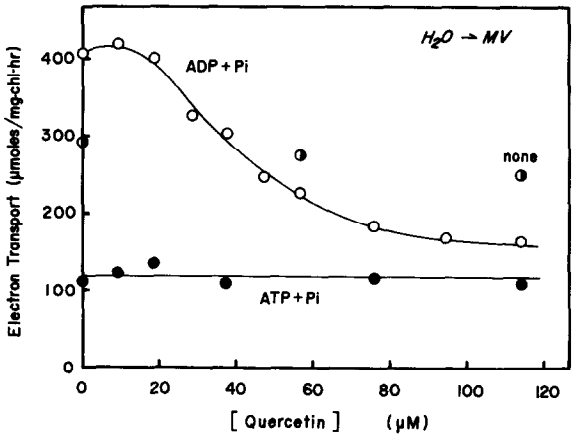

Fig.1. The effect of quercetin on the rate of electron transport from H_2O to methylviologen. The activity was measured under phosphorylation conditions (200 μM ADP + 1 mM P_i) or basal electron transport conditions (200 μM ATP + 1 mM P_i) or without addition (none).

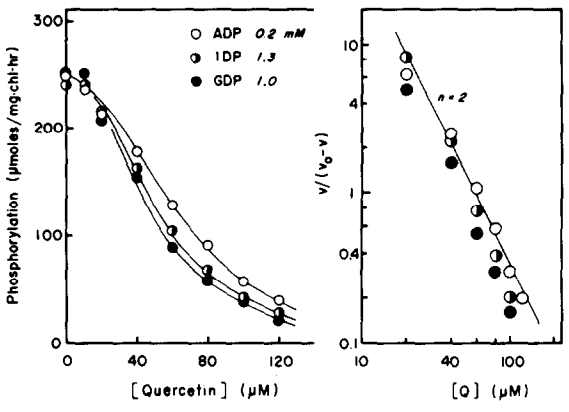

Fig.2. Inhibition of photophosphorylation by quercetin. The concentration of nucleotides was as indicated, with P_i and PMS at 2.5 mM and 20 μM, respectively. (A) inhibition profiles. (B) Hill plots, where v_0 is the rate of phosphorylation in the absence of quercetin, and the line is calculated for a slope of 2.

the effector which binds to the α-subunits [12] of CF_1 altering its conformation [13,14]. Electron transport in the presence of quercetin can be assayed from H_2O to methylviologen or from reduced dichlorophenolindophenol to methylviologen, but not with other electron acceptors such as dimethylquinone or ferricyanide because of the latter's reaction (oxidation) with quercetin.

Figure 2 shows that quercetin inhibits photophosphorylation of nucleoside diphosphate in a sigmoidal fashion with respect to inhibitor concentration, as expected from fig.1. This inhibition profile resembles that of (modified CF_1) ATPase [3]. The inhibition was greater for GDP and IDP than ADP when these nucleotides were examined at the same concentration. However, when these nucleotides were used at a concentration of 10 × K_m (fig.2), the inhibition profiles became almost coincident with each other. The small difference in profiles may be due to inaccuracies in the K_m values used [11]. Figure 2B shows that the apparent Hill constant for the inhibition by quercetin is two for ADP and somewhat larger for GDP and IDP.

A slight enhancement of phosphorylation (and also coupled electron transport (fig.1)) was sometimes observed at low quercetin concentrations around 10 μM. A small enhancement was also found when dimethylsulfoxide [3] was used as the solvent of quercetin. At very low concentrations, the inhibitor may activate the reaction [15] by replacing the nucleotide. Also, the solvents themselves were found to give a small enhancement of phosphorylation.

Figure 3A shows that quercetin inhibition of ADP phosphorylation is competitive with ADP. Figure 3B shows that [ADP]/rate of phosphorylation (the slope of the lines in fig.3A) is linearly related to the square of quercetin concentration. This relationship obeys the equation [15]:

$$v/V_{max} = [ADP]/(K_m + [ADP] + K_m[Q]^2/K_{i_1}\cdot K_{i_2})$$

or after rearrangement,

$$[ADP]\,(1/v - 1/V_{max}) = K_m(1 + [Q]^2/K_{i_1}\cdot K_{i_2})/V_{max}$$

where [ADP] and [Q] represent concentrations of ADP and quercetin, respectively, and K_{i_1} and K_{i_2} the (intrinsic) inhibition constants of the first and the second quercetin, respectively. Although $K_{i_1} \gg K_{i_2}$ (fig.2 and 3), if we assume $K_{i_1} = K_{i_2}$, we can obtain the apparent inhibition constant, K_i = 33 μM, using K_m = 35 μM and V_{max} = 333 μmol/mg·chl-h (fig.3A). This K_i value agrees with the dissociation constant of 34 μM [1] which was determined physico-chemically

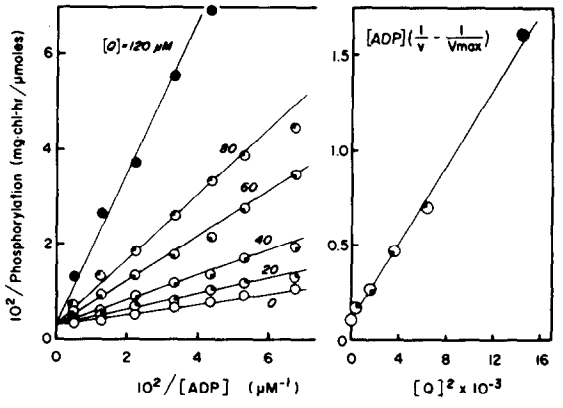

Fig.3. Competitive inhibition of phosphorylation by quercetin with respect to ADP. The concentration of quercetin was as indicated, those of P_i and PMS being 1 mM and 20 μM, respectively. The apparent values of K_m for ADP and V_{max} are 35 μM and 333 μmol/mg·chl-h, respectively. (A) Lineweaver-Burk plots. (B) Plots of the slopes in fig.3A versus the square of quercetin concentration.

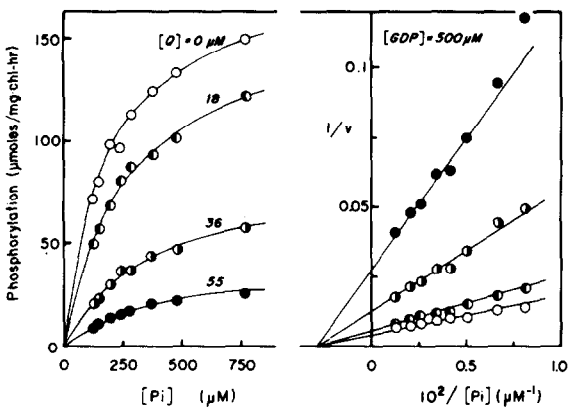

Fig.4. Non-competitive inhibition of phosphorylation by quercetin with respect to phosphate. The concentrations of GDP and quercetin were as indicated. PMS was used at 20 μM. The apparent value of K_m for P_i is 330 μM. (A) Inhibition profiles. (B) Lineweaver-Burk plots.

for quercetin binding to isolated CF_1 in competition with 8-anilino-1-napthalenesulfonate.

Figure 4 shows that quercetin is a non-competitive inhibitor of phosphorylation with respect to P_i. Therefore, contrary to phlorizin which was shown to compete with P_i [16], quercetin is an energy transfer inhibitor which competes with the substrate nucleoside diphosphate.

The binding sites of quercetin were assumed to be located on either the α- or the β-subunits of CF_1 [1]. $1,N^6$-EthenoAMP is a competitive [10] of nucleotide(s) which bind to the so-called tight binding site(s) [8–11] on the α-subunit(s) [12]. When $1,N^6$-ethenoAMP was also present with quercetin, the sigmoidity of the curves of fig.2A was slightly decreased but the $[Q]^2$ relationship still held. Furthermore, the negligible effect of quercetin on the electron transport activities either in the presence of ATP (effector for the α-subunit(s)) or in the absence of nucleotide (fig.1) implies that quercetin does not interact, at least functionally, with the effector binding site(s) on the α-subunit(s). Therefore, the binding sites for quercetin are most likely located on the β-subunits. Two quercetin molecules may be considered to bind cooperatively to the sites on the β-subunits, block the binding of nucleoside diphosphate and so inhibit phosphorylation.

In the absence of quercetin, two nucleoside diphosphate molecules bind to these two sites [17] on the β-subunits, and the initial binding of these two nucleotides to vacant sites on the β-subunits would also be expected to be cooperative. Such cooperative binding of two nucleotides has indeed been shown with isolated CF_1 [18]. A pair of nucleotide-binding sites also fits the mechanism [19] proposed for quasi-arsenylation [20] in which an ATP–AMP pair is considered to be functionally equivalent to an ADP–ADP pair. Preliminary experiments indicate that quercetin inhibits quasi-arsenylation in competition with AMP.

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