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INHIBITION OF PHOSPHORYLASE KINASE, AND TYROSINE PROTEIN KINASE ACTIVITIES BY QUERCETIN

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SUMMARY: Quercetin, a naturally occurring bioflavonoid inhibited the activities of phosphorylase kinasa and a partially purified tyrosine protein kinase from rat lung. The inhibition was rapid and concentration dependent. Quercetin at 100 μM inhibited the activities of phosphorylase kinase and tyrosine protein kinase by about 95 and 80-90 percent respectively. ATP reversed the quercetin mediated inhibition of tyrosine protein kinase but not of phosphorylase kinase. These data suggest that quercetin has differential effect on different protein kinase activities and it may be used as a tool to probe the role of various protein kinases in cell function.

Quercetin, a bioflavonoid, is widely distributed in plant kingdom and occurs in free or conjugated form in many fruits and vegetables (1). Recent studies have indicated that quercetin inhibits the activities of several enzymes including cAMP-independent-protein kinases (2-4), calcium-phospholipid-dependent protein kinase (C-kinase) (5) and tyrosine protein kinases associated with mammary tumors (6) and $pp60^{Src}$ (7, 8). Quercetin, however, has no effect on the activity of cAMP-dependent protein kinase (8). It was, therefore, tempting to determine (a) whether the effect of quercetin was restricted to tyrosine protein kinases associated with viruses and tumors or all the tyrosine protein kinases were affected by this bioflavonoid and (b) whether quercetin exerts its inhibitory effect on another calcium-dependent serine/threonine protein kinase: phosphorylase kinase. The data presented here indicate that quercetin has differential effects on the two protein kinases.

MATERIALS AND METHODS

Materials Poly (Glu: Tyr, 80:20), Dimethyl Sulfoxide, (Me_2SO), phosphatidyl serine, diolein, histone IIA, histone III S, phosphorylase b, and phosphorylase kinase and quercetin were obtained from Sigma Chemical Co., St-Louis, MO, USA.

0006-291X/85 $1.50

Rat lung tyrosine protein kinase was partially purified as described previously (11). All other chemicals were purchased from commercial sources as described previously (12). Quercetin was dissolved in Me_2SO, the final concentration of Me_2SO in the reaction mixture was 3% (V/V). This concentration of Me_2SO had no effect on phosphorylase kinase activity but slightly stimulated the tyrosine protein kinase activity (11). The control reaction mixture contained 3% (V/V) Me_2SO.

Assay of phosphorylase kinase activity Phosphorylase kinase activity was assayed at pH 6.8. The assay mixture contained 50 mM Tris/50 mM beta-glycerophosphate pH 6.8, 5 mg/ml phosphorylase b, 10 mM Mg acetate, 0.2 mM [γ-^{32}P] ATP (specific activity, 300-1000 cpm/pmole) and 1 µg/ml phosphorylase kinase. The reaction was started by the addition of [γ-^{32}P] ATP and incubation was done at 30°C. The kinase activity was determined by following the incorporation of ^{32}P into phosphorylase by using filter paper assay of Reimann et al (13).

Assay of tyrosine protein kinase activity Tyrosine protein kinase activity was assayed in a final volume of 50 µl containing 50 mM Tris-HCl pH 7.5, 30 mM $MgCl_2$, 10 µM sodium orthovanadate, 0.2 mM [γ-^{32}P] ATP (1000-3000 cpm/pmole), 2 mg/ml of exogenous substrate poly (Glu: Tyr, 80:20) and suitably diluted enzyme (5-10 µg protein). The reaction was initiated by the addition of [γ-^{32}P] ATP at 30°C. The kinase activity was determined by following the incorporation of ^{32}P into the exogenous substrate by using filter paper assay as described previously (11).

RESULTS

Effect of quercetin on protein kinase activities The data presented in Figure 1 shows the effect of various concentrations of quercetin on the activities of phosphorylase kinase (Figure 1A) and tyrosine protein kinase (Figure 1B). Quercetin inhibited the activities of both kinases in a concentration dependent manner, however the degree of inhibition was different for the two kinases. Quercetin at 100 µM inhibited phosphorylase kinase and tyrosine protein kinase activities by about 95 and 80-90 percent with apparent Ki values of 3 and 10 µM respectively.

Time course of quercetin mediated inhibition of protein kinase activities Figure 2 shows the effect of quercetin on phosphorylase kinase and tyrosine protein kinase activities as a function of time of incubation. Both phosphorylase kinase and tyrosine protein kinase activities were inhibited by about 95 and 80 percent as early as 5 minutes after the addition of 100 µM quercetin and this inhibition remained unaltered during the entire incubation period tested. These data indicated the rapidity of the inhibitory effect of quercetin.

Effect of ATP on quercetin mediated inhibition of phosphorylase kinase and tyrosine protein kinase Since quercetin has been shown to inhibit the tyrosine

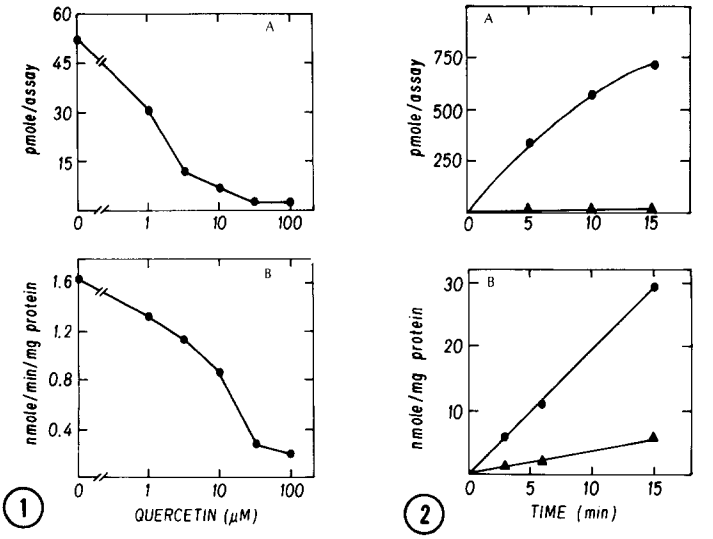

Figure 1: Effect of various concentrations of quercetin on phosphorylase kinase (A) and tyrosine protein kinase (B) activities. The protein kinase activities were determined as described in "Materials and Methods".

Figure 2: Effect of time of incubation on inhibition of phosphorylase kinase (A) and tyrosine protein kinase activities by quercetin. The protein kinase activities were determined in absence (●) or presence (▲) of 100 μM quercetin as described in "Materials and Methods".

protein kinase activity of $pp60^{Src}$ by competing for the nucleotide binding site of the kinase (8), it was interesting to know if a similar effect is observed in the case of phosphorylase kinase and tyrosine protein kinase. Figure 3 shows

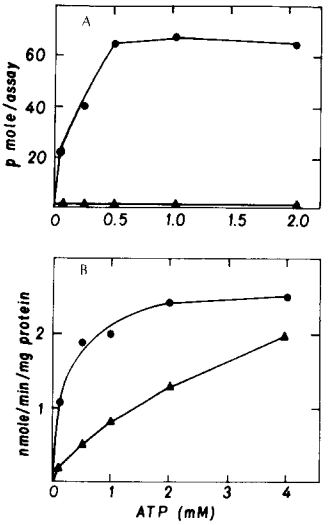

Figure 3: Effect of various concentrations of ATP on quercetin mediated inhibition of phosphorylase kinase (A) and tyrosine protein kinase (B) activities. Phosphorylase kinase and tyrosine protein kinase activities were determined in absence (●) or presence of (▲) of 100 μM quercetin at indicated concentrations of ATP as described in "Materials and Methods".

the effect of various concentrations of ATP on quercetin mediated inhibition of phosphorylase kinase and tyrosine protein kinase activities. ATP did not alter the inhibitory effect of quercetin on phosphorylase kinase activity (Figure 3A), however, higher concentration of ATP reversed quercetin mediated inhibition of tyrosine protein kinase (Figure 3B). For example, 85 percent inhibition observed at 0.1 mM ATP was decreased to 20 percent by 4 mM ATP. These data suggest that quercetin may be competing for the ATP binding site of tyrosine protein kinase from rat lung as has also been demonstrated for $pp60^{Src}$ (8). Futhermore, quercetin has no significant effect on the Km of the substrate for both phosphorylase kinase or tyrosine protein kinase activities (data not shown).

DISCUSSION

This is the first study demonstrating an inhibitory effect of quercetin on phosphorylase kinase, and normal rat lung tyrosine protein kinase activities. Quercetin inhibited phosphorylase kinase to a greater extent as compared to tyrosine protein kinase indicating that different protein kinases have different affinities for quercetin. The inhibitory effect of quercetin on rat lung tyrosine protein kinase is similar to its effect on the $pp60^{Src}$ and rat mammary tumor associated tyrosine protein kinases suggesting that quercetin may be a general inhibitor of all the tyrosine protein kinases. Of special interest was the fact that higher concentration of ATP caused reversal of inhibitory effect of quercetin on the lung tyrosine kinase but not on the phosphorylase kinase. This suggested that in case of rat lung tyrosine protein kinase, quercetin was competing for the ATP binding sites, whereas in case of phosphorylase kinase it was modifying some component(s) other than the ATP binding site. This notion is further supported by the observation that cAMP-dependent protein kinase, despite having an ATP binding site homologous to that of $pp60^{Src}$ (14) is insensitive to inhibition by quercetin (8). Also, quercetin did not appear to change the affinity for the substrate of the kinases (data not shown).

The data presented here do not attempt to elucidate the mechanism by which quercetin exerts its inhibitory effect. However, these data demonstrate that quercetin has differential effects on different protein kinase and thus it may

be used as a tool to probe the role of various protein kinases in the control of cellular homeostasis.

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