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Synergetic inhibition of genistein and D-glucose on α-glucosidase

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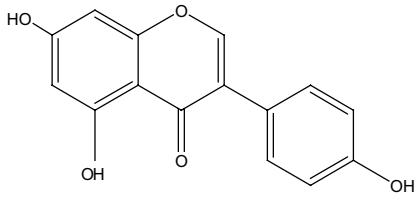
Abstract—Synergetic inhibitory effect of genistein (I_2) and D-glucose (I_1) on α-glucosidase has been studied with kinetics method. It was concluded that the inhibitory effect was much greater when I_2 and I_1 were both added to the reactant solution simultaneously than that they were added, respectively, which suggesting the inhibitors bind to the different sites of α-glucosidase at the same time, and demonstrating synergetic inhibition.

There has been widespread interest in glycosidases in recent years, largely due to their role not only in a multitude of biological systems[1] such as carbohydrate digestion, the processing of glycoproteins and glycolipids, but also in a variety of metabolic disorders and other disease, for example, diabetes, viral attachment, as well as cancer formation.[2] Because of their importance, inhibitors of glycosidase have been considered as compounds of interest for a long time, since they can be vital tools for studying glycosidase mechanisms of action and act as therapeutic agents for some degenerative diseases.[3] α-Glucosidase (EC. 3. 2. 1. 20) are located in the brush-border surface membrane of intestinal cells,[4] and are the key enzymes of carbohydrate digestion.[5] α-Glucosidase inhibitors can delay the digestion of oligosacchride and disaccharide to monosaccharide by inhibiting α-glucosidase, thus reduce the rate of glucose absorption. Some researchers have reported that oral administration of specific α-glucosidase inhibitor could effectively improve hyperglycemia as well as diabetic complications.[6a–d]

Genistein (5,7,4′-trihydroxy-isoflavone, shown in Scheme 1) belongs to the isoflavonoid family[7a] and is the isoflavone of greatest interest in soy protein.[7b] Most of the studies have focused on the pharmacological activities of genistein as a tyrosine kinase inhibitor, and its chemoprotectant activities against cancers and cardiovascular disease, as well as its phytoestrogen activity.[7c] Recently, Dong-Sun et al. has reported that genistein could be a potent α-glucosidase inhibitor.[2]



Scheme 1. The structure of genistein (5,7,4′-trihydroxyisoflavone).

In this paper, we reported, for the first time, the synergetic inhibitory mode of noncompetitive α-glucosidase inhibitors, when reacted against α-glucosidase in the presence of competitive inhibitors such as D-glucose. Since genistein are associated with a broad variety of beneficial properties on human health,[7d,e] it was examined as the counterpart of D-glucose while the synergetic inhibitory reaction was taking place. In order to elucidate the phenomenon and collect some information on where these inhibitors bind to the enzymes or whether these inhibitors bound to the same site simultaneously, thus inhibited the enzyme, we performed the following experiment, which could glean useful information on how these inhibitors influence the activity of the enzyme as to prevent the substrate binding to the enzyme.

The noncompetitive inhibitory effect was determined from the double-reciprocal plots of the inhibition

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kinetics of yeast α-glucosidase by genistein (shown in Fig. 1), showing a K_i value of genistein was 0.0104 mM (as shown in Fig. 1) obtained at 10.2 μM of genistein. The inhibitory effects of daidzein (7,4′-dihydroxy-isoflavone, shown in Scheme 2), another major isoflavone in soybeans, were also examined. (The result not shown.) Both genistein and daidzein were proved to be excellent noncompetitive α-glucosidase inhibitors.

Synergetic inhibitory phenomenon was observed when D-glucose and isoflavones were added to the reactant solution containing substrate and enzyme. As shown in Figure 2, α-glucosidase activity was significantly inhibited by genistein and the IC_{50} (the concentration required for 50% inhibition) was 10.4 μM. When we added 0.78 mM (<IC_{50}, D-glucose) D-glucose to the reaction mixture, the IC_{50} value was dropped to one-fourth (about 2.61 μM). (Shown in Fig. 3.) Stronger inhibition was observed when genistein and D-glucose were added to the reaction solution, and the value of K_i decreased from 0.0113 to 0.00521 mM (K_i'). (Shown in Fig. 3.) Meanwhile, the IC_{50} value 4.17 mM of glucose, decreased to 0.36 mM when added 3.5 μM genistein to the solution (Fig. 3), and the value of K_i decreased, from 12.17 to 1.90 mM (K_i').

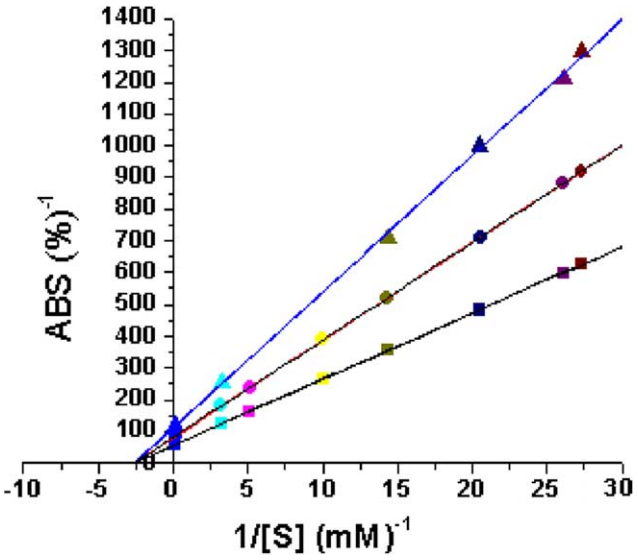
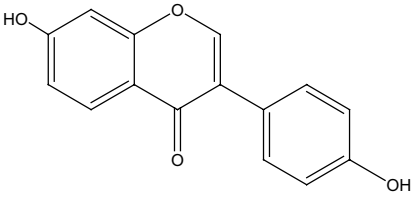


Figure 1. Double-reciprocal plots of the inhibition kinetics of yeast α-glucosidase by genistein (that was treated first with genistein for 1 h at 37 °C of each designed concentration to initiate the enzyme reaction). Without inhibitor (■), with inhibitor, (●, ▲).



Scheme 2. The structure of daidzein (7,4′-dihydroxyisoflavone).

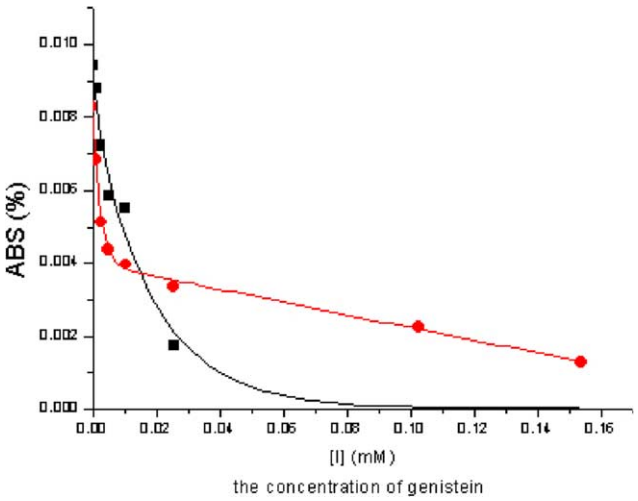


Figure 2. The IC_{50} value for the inhibition of yeast α-glucosidase by genistein and D-glucose (pH = 7.0 and enzyme: 26 μg/mL, [pNPG] = 0.073 mM); genistein (■), genistein and D-glucose (0.78 mM) (●).

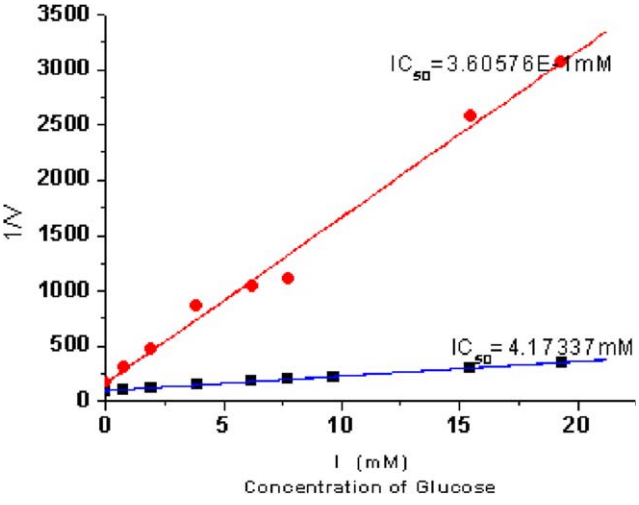


Figure 3. The IC_{50} value for the inhibition of yeast α-glucosidase by genistein and D-glucose (pH = 7.0 and enzyme: 26 μg/mL, [pNPG] = 0.073 mM); D-glucose (■), D-glucose and genistein (3.52 μM) (●).

Based on the data mentioned above, we suggest that there are some kind of cooperation between genistein and D-glucose and genistein binds to a different site of α-glucosidase from glucose.

The synergetic inhibition phenomenon could be conformed to the plots in Figure 4, which may led to the equation $1/V = K_m''(1/[S]) + 1/([I_1]V_m')$. Since the value of K_m'' (with genistein and D-glucose) was much more than that of either K_m or K_m' (only with genistein), we can deduce that the correlation between genistein or glucose and enzyme is much more stronger, whereas the

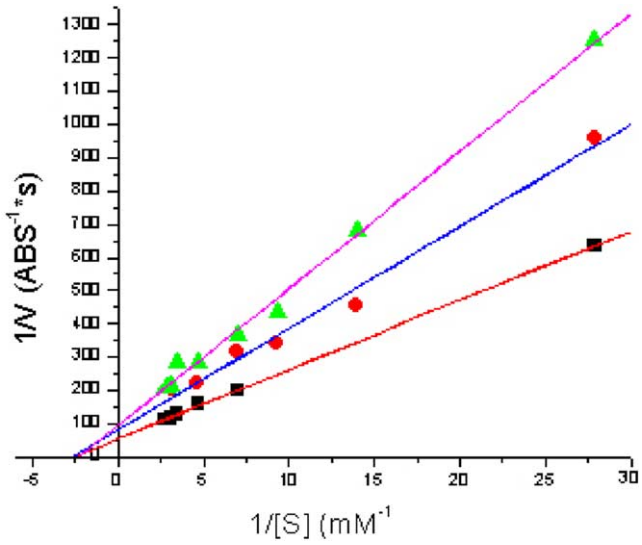


Figure 4. Double-reciprocal plots of the inhibition kinetics of yeast α-glucosidase by genistein and D-glucose (that was treated first with genistein for 1 h at 37 °C of each designed concentration of PNP-α-glucopyranoside to initiate the enzyme reaction). Without inhibitor (■), with genistein as inhibitor (●), with genistein and D-glucose as concerted inhibitors (▲).

substrate bind to the enzyme less tightly when both D-glucose and genistein were added to the solution.

Different IC_{50} values of genistein could be obtained when α-glucosidase was inhibited with or without D-glucose and daidzein were showed in Figure 5, which indicated that the least value was obtained when reversible noncompetitive inhibitor, genistein and competitive reversible inhibitor, D-glucose, were added to the reactant mixtures. However, as genistein and daidzein were both added to the mixture with D-glucose, the IC_{50} value increased a little. Thus we can assume

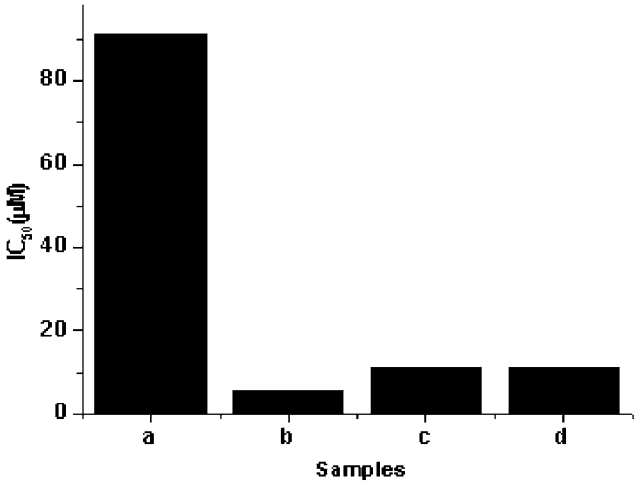


Figure 5. IC_{50} values of genistein with or without other inhibitors, (a) with genistein as inhibitor, (b) with genistein and D-glucose as synergetic inhibitors, (c) with daidzein and D-glucose as synergetic inhibitors, (d) with genistein, daidzein, and D-glucose as synergetic inhibitors.

that genistein and daidzein may bind to the same site of α-glucosidase, but a different one from D-glucose. Additionally, the number of the hydroxyl groups in their molecular structures seems to take an important role in the process of inhibition.

It was showed in our project that both of the inhibitors, competitive α-glucosidase inhibitor I_1 and noncompetitive α-glucosidase inhibitor I_2 could bind to the enzyme. I_1 may bind to the substrate binding site on the enzyme. Since they competed against each other, they could not bind to α-glucosidase simultaneously and led to the fact that the complex EI_1S could not be produced. Whereas inhibitor I_2 could bind to the different binding site from that of the substrate, then the complex EI_2S could be formed. Moreover, the enzyme could bind with inhibitor I_1 and I_2 at the same moment and the complex EI_1I_2 was formed, thus the substrate could not bind to the active site of α-glucosidase.

In this study we found the phenomenon of synergetic inhibition of genistein and daidzein (I_2) and D-glucose (I_1) of α-glucosidase for the first time. It was found that both genistein and daidzein inhibited α-glucosidase with D-glucose greater than that they behaved in the same manner without D-glucose, and inhibitor I_2 can bind to the binding site of α-glucosidase other than that of substrate and I_1, when they reacted against α-glucosidase in the presence of inhibitors I_1, and two kinds of inhibitors could bind to the binding site of the enzyme at the same time. The findings mentioned above provides some useful information about the active site topography and helps to design new potent inhibitors for α-glucosidase, and drugs for diabetes mellitus.

Acknowledgements

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