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Genistein inhibits activities of methylenetetrahydrofolate reductase and lactate dehydrogenase, enzymes which use NADH as a substrate

Michał Grabowski [a], Bogdan Banecki [a], Leszek Kadziński [a], Joanna Jakóbkiewicz-Banecka [b], Rajmund Kaźmierkiewicz [a], Magdalena Gabig-Cimińska [c], Grzegorz Węgrzyn [b], Alicja Węgrzyn [c], Zyta Banecka-Majkutewicz [d, *]

[a] Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland
[b] Department of Molecular Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland
[c] Laboratory of Molecular Biology (Affiliated with the University of Gdańsk), Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Wita Stwosza 59, 80-308 Gdańsk, Poland
[d] Department of Neurology, Medical University of Gdańsk, Dębinki 7, 80-211 Gdańsk, Poland

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ABSTRACT

Genistein (5, 7-dihydroxy-3- (4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) is a natural isoflavone revealing many biological activities. Thus, it is considered as a therapeutic compound in as various disorders as cancer, infections and genetic diseases. Here, we demonstrate for the first time that genistein inhibits activities of bacterial methylenetetrahydrofolate reductase (MetF) and lactate dehydrogenase (LDH). Both enzymes use NADH as a substrate, and results of biochemical as well as molecular modeling studies with MetF suggest that genistein may interfere with binding of this dinucleotide to the enzyme. These results have implications for our understanding of biological functions of genistein and its effects on cellular metabolism.

1. Introduction

5, 7-Dihydroxy-3- (4-hydroxyphenyl)-4*H*-1-benzopyran-4-one, commonly known as genistein, is a natural isoflavone, occurring mostly in leguminous plants [1]. This compound, preliminarily identified as a phytoestrogen, has been subsequently demonstrated to possess surprisingly high spectrum of biological activities. Among them, genistein was found to restore the metabolic balance of bone formation and resorption [2], to alleviate metabolic problems in obesity and type 2 diabetes, mostly due to its anti-oxidant and anti-inflammatory features [3], to protect central nervous system against oxidative stress and neuroinflammation [4], to cause cancer cell growth arrest and apoptosis and to inhibit angiogenesis and metastasis [5], to halt the growth of some bacteria, including human pathogens [6], and to inhibit viral infection [7]. Therefore, phytoestrogenic, anti-inflammatory, antiangiogenesis, antiproliferative, antioxidant, immunomodulatory, pain relief, antibacterial, antiviral and joint protection properties of genistein led to many proposals of the use this isoflavone in treatment of various disorders, including cancer as well as metabolic, inflammatory, infectious, neurological and even genetic diseases [8–12].

The molecular mechanisms of genistein actions are connected mainly to its binding to estrogen receptors [13,14], inhibition of tyrosine kinase activities resulting in either enhancement or impairment of expression of hundreds of genes [15,16], and direct interaction with topoisomerase II causing modulation of its functions [17,18]. On the other hand, some clinical studies indicated that genistein may influence the plasma levels of homocysteine [19–21], an amino acid that is considered to be a risk factor in cardiovascular diseases and stroke [4]. Since such effects of the tested isoflavone on homocysteine levels could be hardly explained by already known mechanisms of its action, we were searching for possible explanation of this phenomenon. Therefore, we aimed to test if genistein can influence activities of enzymes involved in the homocysteine metabolism. One of main enzymes of homocysteine metabolism is methylenetetrahydrofolate reductase (MTHFR), (EC 1.5.1.20), catalyzing conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor in the remethylation of homocysteine to methionine [22].

* Corresponding author.
E-mail address: zytabm@gumed.edu.pl (Z. Banecka-Majkutewicz).

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This enzyme has been conserved during evolution to such extent that specific mutations in the gene coding for a bacterial homolog (MetF) of the human methylenetetrahydrofolate reductase (MTHFR) correspond to common polymorphisms in the human gene [23]. Moreover, products of the wild-type and mutated *Escherichia coli* (*metF*) and human (*MTHFR*) genes are very similar both structurally [23] and functionally [24]. Therefore, in our studies, we have employed the *E. coli* MetF protein as a model.

2. Materials and methods

2.1. Proteins and small molecules

The MetF protein was purified as described previously [25]. Lactate dehydrogenase (LDH) was purchased from Sigma–Aldrich. Genistein, NADH, menadione and buffer ingredients were obtained from Sigma–Aldrich.

2.2. MetF activity test

MetF activity assay was performed as described previously [25], by measuring a decrease in absorbance of NADH, consumed during the reaction. The reaction mixture consisted of 50 mM phosphate buffer containing 10% glycerol and 0.3 mM EDTA, 400 μM NADH, and 1.4 mM menadione (vitamin K3 is used as an artificial substrate for MetF). The activity of MetF was determined by measurement of the kinetics of the reaction at 37 °C. The reaction mixture was prepared without the enzyme, and incubated for 5 min. Following reaction initiation by the addition of the 0.3 μM enzyme, the measurement was carried out for 30 min, by monitoring the absorbance at a wavelength of 340 nm.

2.3. Effect of genistein on the MetF activity

MetF activity assay in the presence of genistein was performed according to the standard assay (described above), but genistein was added to the reaction mixture to final concentrations from 0 to 500 μM. In control experiments, DMSO (a solvent used for preparation of genistein stock solution) was added to the reaction mixture in the amount equivalent to that used in the assay with 500 μM genistein. In order to investigate the mechanism of the enzyme activity inhibition by genistein, the reaction mixture was titrated with increasing concentrations of this isoflavone, from 0 to 400 μM. The concentrations of NADH were between 0 and 600 μM. Enzyme reaction kinetics was determined according to Michaelis–Menten and Lineweaver–Burk equations and plots.

2.4. Influence of reaction initiation factor on genistein-mediated inhibition of MetF activity

Three variants of the test have been developed. First variant assumed preincubation of genistein, MetF and menadione for 5 min, then NADH was added. Second variant assumed preincubation of genistein, NADH and menadione for 5 min, then enzyme was added. Third variant assumed preincubation of genistein, MetF, and NADH for 5 min. Reaction was started by the addition of menadione. All tests were performed in reaction buffer (50 mM phosphate buffer pH 7.2 with 0.3 mM EDTA).

2.5. Effects of genistein on the activity of lactate dehydrogenase

The commercially available enzyme (lactate dehydrogenase, LDH) was used. LDH activity was measured by estimation of the decrease in absorbance of NADH consumed during the reaction. The reaction mixture consisted of the 0.1 μM enzyme, genstein, NADH, 1 mM pyruvate acid and 50 mM phosphate buffer containing 10% glycerol and 0.3 mM EDTA and genistein in concentration 0–500 μM. The reaction was conducted at 37 °C. Absorbance was monitored at 340 nm. In order to investigate the mechanism of the enzyme activity inhibition by genistein, reaction mixture was titrated with increasing concentrations of genistein. The experiment was performed with concentrations of genistein from 0 to 400 μM, while the concentrations of NADH were between 0 and 1200 μM. Enzyme reaction kinetics was determined according to Michaelis–Menten and Lineweaver–Burk equations and plots.

2.6. Molecular modeling

The crystal structure of 1ZP3 of *E. coli* methylenetetrahydrofolate reductase/FAD complex, which was previously deposited [26] in the Protein Data Bank (PDB) [27] was used in our docking experiments. The model of genistein molecule for the docking procedure was constructed using the Avogadro [http://avogadro.openmolecules.net/] molecular editing software. We used also the FAD molecule model, which was already present in the crystal structure of 1ZPT3. The starting geometries of the ligand molecule models were optimized using the built-in Avogadro minimization algorithm based on the MMFF94 force field employing the Steepest Descent Algorithm with 500 steps of minimization. AutoDock Vina [28] was used to perform the molecular docking experiments with the default optimization parameters offered by the program. The ADT [29,30] program from the MGLTools removed non polar

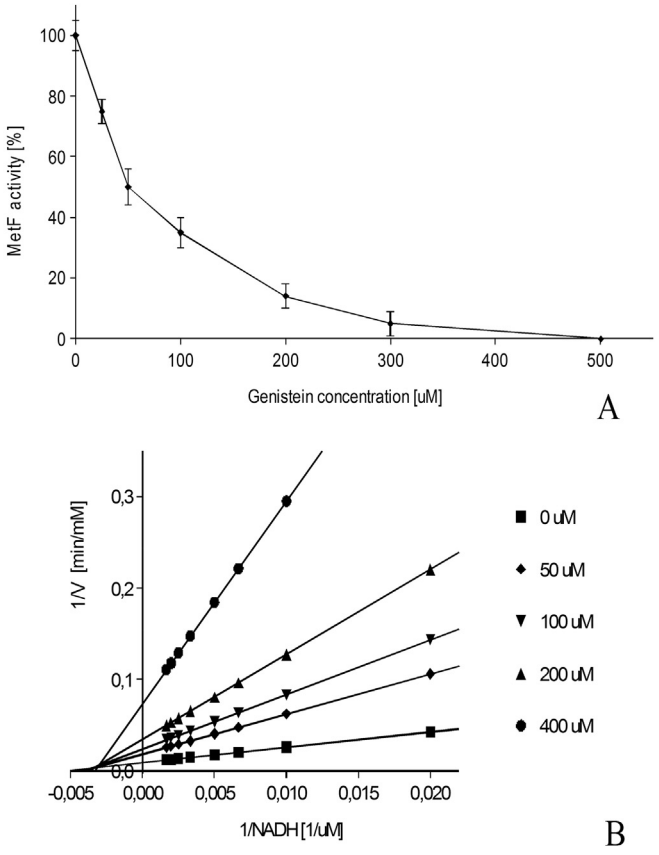

Fig. 1. Panel A MetF activity in the presence of increasing concentrations of genistein. In the experiment shown on panel A, 0.3 μM enzyme was used for each reaction. The activity measured in the control experiment (without genistein) was assumed to be 100% and other values reflect this value. The presented results are mean values from three independent experiments. Error bars represent standard deviation (SD). Panel B represents the kinetics of MetF-catalyzed reaction as a Lineweaver–Burk plot.