International Journal of Biological Macromolecules 46 (2010) 478–486

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Dietary bioflavonoids inhibit *Escherichia coli* ATP synthase in a differential manner

Nagababu Chinnam, Prasanna K. Dadi, Shahbaaz A. Sabri, Mubeen Ahmad, M. Anaul Kabir [1], Zulfiqar Ahmad *

Department of Biological Sciences, Box 70703, East Tennessee State University, Johnson City, TN 37614, United States

ARTICLE INFO

Article history:
Received 24 January 2010
Received in revised form 16 March 2010
Accepted 17 March 2010
Available online 25 March 2010

Keywords:
E. coli ATP synthase
F_1F_o ATP synthase
F_1-ATPase
ATP synthesis
Bioflavonoids
Biological nanomotor

ABSTRACT

The aim of this study was to determine if the dietary benefits of bioflavonoids are linked to the inhibition of ATP synthase. We studied the inhibitory effect of 17 bioflavonoid compounds on purified F_1 or membrane bound F_1F_o *E. coli* ATP synthase. We found that the extent of inhibition by bioflavonoid compounds was variable. Morin, silymarin, baicalein, silibinin, rimantadin, amantidin, or, epicatechin resulted in complete inhibition. The most potent inhibitors on molar scale were morin ($IC_{50} \sim 0.07$ mM) > silymarin ($IC_{50} \sim 0.11$ mM) > baicalein ($IC_{50} \sim 0.29$ mM) > silibinin ($IC_{50} \sim 0.34$ mM) > rimantadin ($IC_{50} \sim 2.0$ mM) > amantidin ($IC_{50} \sim 2.5$ mM) > epicatechin ($IC_{50} \sim 4.0$ mM). Inhibition by hesperidin, chrysin, kaempferol, diosmin, apigenin, genistein, or rutin was partial in the range of 40–60% and inhibition by galangin, daidzein, or luteolin was insignificant. The main skeleton, size, shape, geometry, and position of functional groups on inhibitors played important role in the effective inhibition of ATP synthase. In all cases inhibition was found fully reversible and identical in both F_1F_o membrane preparations and isolated purified F_1. ATPase and growth assays suggested that the bioflavonoid compounds used in this study inhibited F_1-ATPase as well as ATP synthesis nearly equally, which signifies a link between the beneficial effects of dietary bioflavonoids and their inhibitory action on ATP synthase.

1. Introduction

Membrane bound F_1F_o ATP synthase from mitochondria, chloroplasts, and bacteria is responsible for ATP production through oxidative phosphorylation or photophosphorylation. This enzyme is structurally identical and highly conserved in different species. In its simplest form the ~530 kDa *Escherichia coli* F_1F_o ATP synthase contains eight different subunits namely $\alpha_3\beta_3\gamma\delta\varepsilon ab_2c_{10\text{-}15}$. F_1 corresponds to $\alpha_3\beta_3\gamma\delta\varepsilon$ and F_o to ab_2c_{10}. ATP hydrolysis and synthesis occur on three catalytic sites in the F_1 sector, whereas proton transport occurs through the membrane embedded F_o [1,2]. The γ subunit is part of the "rotor" which is composed of γ, ε, and a ring of c subunits. The "stator" is composed of $b_2\delta$. The function of the stator is to prevent co-rotation of catalytic sites as well as the a subunit with the rotor [3,4]. Proton gradient-driven clockwise rotation of γ (as viewed from the membrane) leads to ATP synthesis and anticlockwise rotation of γ results from ATP hydrolysis. The mechanism is essentially a rotary motor and in fact it is the smallest known biological nanomotor. Detailed reviews of ATP synthase structure and function may be found in references [5–11].

ATP synthase is implicated directly or indirectly in several human diseases such as Leigh syndrome, ataxia, Batten's diseases, Alzheimer's, angiogenesis, and increased blood pressure, etc. ([11] and references therein). This enzyme is not only implicated to many disease conditions but is likely to contribute to new therapies for multiple diseases such as, cancer, heart disease, mitochondrial diseases, immune deficiency, cystic fibrosis, diabetes, ulcers, and tuberculosis that affect both people and animals [12,13]. The presence of ATP synthase on the surfaces of multiple cell types, and its involvement in a number of cellular processes, makes this enzyme an attractive molecular target, in the development of treatments for numerous diseases. A wide range of natural and synthetic products are known to bind and inhibit ATP synthase [11,13–15] and biochemical and structural studies of ATP synthase have so far revealed about ten different inhibitor binding sites. A detailed list of known inhibitors and their actions on ATP synthase in relation to human heath and disease is discussed in reference [11].

Bioflavonoids/polyphenols are a class of plant secondary metabolites. The beneficial effects of many fruits, vegetables, and

Abbreviations: NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1, 3-diazole; Mbr, membrane containing ATP synthase; IC_{50}, corresponds to the concentration of inhibitor where 50% of maximal inhibition was observed.

* Corresponding author. Tel.: +1 423 439 6931; fax: +1 423 439 5958.
E-mail address: ahmadz@etsu.edu (Z. Ahmad).
[1] Present address: School of Biotechnology, National Institute of Technology Calicut, Calicut 673601, Kerala, India.

0141-8130/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2010.03.009

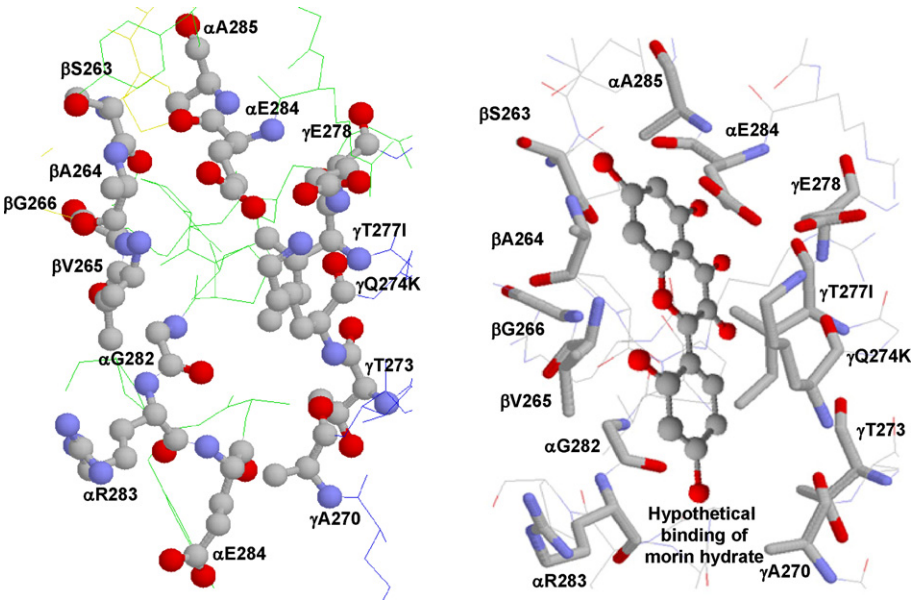

Fig. 1. X-ray crystallographic structure of polyphenol binding site of ATP synthase. (A) Empty and (B) hypothetical binding of morin hydrate at the polyphenol binding pocket. Residues from α, β, and γ subunits involved in interaction with polyphenols are identified. In bovine two variants, Q274K and T277I, occur in the γ subunit and are identified in the figure. PDB file 2jj1 [16] with RasMol [55] was used to generate this figure.

tea have been attributed to the presence of bioflavonoid compounds in them. Bioflavonoids are known to exhibit antioxidants, chemopreventive, and chemotherapeutic properties [16–20]. They have been shown to have anti-allergic, anti-inflammatory [21], and antimicrobial activity [22–24]. Their mode of action is not clear, but some dietary bioflavonoids are known to block the action of enzymes and other substances that promote the growth of cancer cells by binding to the multiple molecular targets in the body including ATP synthase [11,13,16,25,26]. For example one of the most common dietary polyphenol resveratrol has been shown to have multiple uses, with multiple benefits in humans, including but not limited to increased life span, anticancer/antitumor effects, and antimicrobial activities [26]. Resveratrol was also shown to induce apoptosis via mitochondrial pathways [25,27]. Aziz et al. [28] demonstrated the chemopreventive properties of resveratrol against prostate cancer. They found that treatment with resveratrol concentrations of up to 50 μmol/L/day resulted in stimulation of apoptosis in androgen-responsive human prostate carcinoma cells (LNCaP). At similar concentrations resveratrol had no effect on the rate of cell death in normal human prostate cells.

Earlier Zheng and Ramirez [15] studied the inhibitory effects of several naturally occurring polyphenolic phytochemicals on rat brain and liver mitochondrial F_1F_o ATP synthase. They demonstrated that ATP synthase is molecular target for resveratrol and other aglycone isoflavones. Lately, the polyphenols resveratrol, piceatannol, quercetin, quercetrin, or quercetin-3-β-D glucoside, were shown to prevent synthetic or hydrolytic activities of *E. coli* and bovine mitochondrial ATP synthase [13,16]. The proposed mode of action was binding of polyphenols at the polyphenol binding pocket of ATP synthase and blockage of clockwise or anticlockwise rotation of the γ-subunit [16] (see Fig. 1).

The question arises (i) whether the dietary bioflavonoids have differential inhibitory actions on *E. coli* ATP synthase and (ii) what kind of effect dietary bioflavonoids have on the intact *E. coli* cell growth which will indicate their effect on ATP synthesis. Thus we studied the inhibitory effect of seventeen bioflavonoid/polyphenol compounds illustrated in Fig. 2 on *E. coli* ATP synthase using both purified F_1-ATPase and membrane bound F_1F_o ATP synthase preparations. This study shows that dietary bioflavonoids bind and inhibit *E. coli* ATP synthase in differential manner. Our results also reaffirm that the beneficial effect of dietary polyphenols as antitumor or antimicrobial agents may be at least in part are through their inhibitory action on ATP synthase.

2. Materials and methods

2.1. Source of bioflavonoids and other chemicals

Ultra pure bioflavonoid compounds were purchased from Sigma-Aldrich Chemical Company. Catalog numbers for all bioflavonoids used in this study are presented in Table 1. Silymarin used in this study was a mixture of anti-hepatotoxic flavonolignans from the fruit of *Silybum marianum* while silibinin, a pure compound, is the principal component of silymarin. Also, we followed the supplier's directions in the handing of all compounds such as kaempferol was light sensitive so it was protected from light. All the compounds were resuspended in DMSO immediately before use for the desired concentration and were stored in −20 °C. In ATPase assays the final volume of DMSO was not more that 25%. Earlier we noted that up to 40% DMSO has no effect on membrane bound F_1F_o of *E. coli* ATP synthase [13]. All other chemicals used in this study were ultra pure analytical grade, and purchased from either Sigma–Aldrich Chemical Company or Fisher Scientific Company.

2.2. Measurement of growth yield in limiting glucose medium; preparation of E. coli membranes; purification of E. coli F_1; assay of ATPase activity of membrane bound F_1F_o or purified F_1

Both membrane bound F_1F_o and purified F_1 were isolated from the E. *coli* strain pBWU13.4/DK8 [29]. Growth yield in limiting glucose was measured as in Ref. [30]. *E. coli* membrane bound F_1F_o or purified F_1 were prepared as in Ref. [31]. It should be noted that this procedure involves three washes of the initial membrane pellets. The first wash is performed in buffer containing 50 mM TES pH 7.0, 15% glycerol, 40 mM 6-aminohexanoic acid, and 5 mM p-aminobenzamidine. The following two washes are performed in buffer containing 5 mM TES pH 7.0, 15% glycerol, 40 mM 6-aminohexanoic acid, 5 mM p-aminobenzamidine, 0.5 mM DTT, and 0.5 mM EDTA. Prior to experiments, membranes were washed twice more by resuspension and ultracentrifugation in