International Journal of Biological Macromolecules 101 (2017) 153–164

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Understanding the link between antimicrobial properties of dietary olive phenolics and bacterial ATP synthase

Amon Amini, Mason Liu, Zulfiqar Ahmad*

Department of Biochemistry, Kirksville College of Osteopathic Medicine, A.T. Still University, Kirksville, MO 63501, USA

ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form 16 March 2017
Accepted 16 March 2017
Available online 18 March 2017

Keywords:
E. coli F_1F_o ATP synthase
Olive phenolics
Tyrosol
Hydroxytyrosol
Dihydroxyphenylglycol
Oleuropein
Enzyme inhibition

ABSTRACT

The naturally occurring olive phenolics tyrosol, hydroxytyrosol, dihydroxyphenylglycol (DHPG), and oleuropein are known to have antioxidant, antitumor, and antibacterial properties. In the current study, we examined whether the antimicrobial properties of tyrosol, hydroxytyrosol, DHPG, and oleuropein were linked to the inhibition of bacterial ATP synthase. Tyrosol, hydroxytyrosol, DHPG, and oleuropein inhibited *Escherichia coli* wild-type and mutant membrane-bound F_1F_o ATP synthase to variable degrees. The growth properties of wild-type, null, and mutant strains in presence of above olive phenolics were also abrogated to variable degrees on limiting glucose and succinate. Tyrosol and oleuropein synergistically inhibited the wild-type enzyme. Comparative wild-type and mutant F_1F_o ATP synthase inhibitory profiles suggested that αArg-283 is an important residue and olive phenolics bind at the polyphenol binding pocket of ATP synthase. Growth patterns of wild-type, null, and mutant strains in the presence of tyrosol, hydroxytyrosol, DHPG, and oleuropein also hint at the possibility of additional molecular targets. Our results demonstrated that ATP synthase can be used as a molecular target and the antimicrobial properties of olive phenolics in general and tyrosol in particular can be linked to the binding and inhibition of bacterial ATP synthase.

1. Introduction

ATP synthase is the major source of ATP, the energy required to perform biological functions in almost all organisms from bacteria to man [1,2]. The location of ATP synthase in bacteria is the plasma membrane and in humans is the inner membrane of the mitochondria. ATP is generated through the transmembrane electrochemical gradient by coupling ADP and Pi [3–5]. The lesser-known ectopic ATP synthase is found on the surface of numerous cell types where it serves as a ligand receptor and participates in various cellular processes, including angiogenesis, lipid metabolism, and the cytolytic pathway of tumor cells [6].

Cell survival and growth depends on an unhindered supply of ATP. As such, targeted cell death can be achieved through selective inhibition of ATP synthase. Thus, ATP synthase can be an effective and selective molecular target for treatment of various diseases, including microbial infections [6–8]. More than 300 natural and synthetic compounds are known to bind on the F_1 or F_o sectors of ATP synthase, causing complete or partial inhibition. Along with antioxidants, chemotherapeutic, and antimicrobial properties, one of the major ATP synthase inhibitor categories is that of phenolic phytochemicals [6,9–13].

Worldwide antimicrobial resistance is increasing at an alarming rate. By 2050, antibiotic resistant microbial infections are projected to cause millions of additional deaths and cost taxpayers about $100 trillion [14]. The World Health Organization's global report on surveillance of antimicrobial resistance reports that antibiotic resistance is one of the main reasons for this alarming situation [15]. Finding new ways to kill microbes is an urgent global matter. Therefore, phenolic phytochemicals that can selectively bind and inhibit ATP synthase present an excellent opportunity for preventing and combating antibiotic resistant microbial infections.

The demand for evidence-based phytochemicals in general and plant-based phenolic constituents in particular as alternative remedies has increased [7,13,16–20]. A large number of dietary phenolic compounds from a variety of sources have been shown to have potential antitumor or antimicrobial properties [21–25]. Olives and its constituent phenolic compounds have nutraceutical properties and have been assessed for their strong antimicrobial properties at low concentrations against human intestinal and respiratory tract infections, such as those caused by Gram positive bacteria (*Bacillus cereus*, *B. subtilis*, and *Staphylococcus aureus*), Gram negative bac-

* Corresponding author at: Department of Biochemistry, Kirksville College of Osteopathic Medicine, A.T. Still University, 800 W. Jefferson St Kirksville, MO, 63501 USA.
E-mail address: zahmad@atsu.edu (Z. Ahmad).

http://dx.doi.org/10.1016/j.ijbiomac.2017.03.087
0141-8130/

teria (*Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae*), and fungi (*Candida albicans* and *Cryptococcus neoformans*) [26].

For centuries, olives from the *Olea europaea* (European olive) have been widely enjoyed in foods throughout the world. Only about 10% of harvested olives are used as table olives and the rest is turned into oil [27]. Traditionally *O. europaea* has been used for the treatment of several infectious disorders of bacterial, fungal, and viral origin. As such, the antimicrobial and antiviral potential of *O. europaea* has been confirmed in multiple studies [28]. Because olives are a rich source of phenolic compounds, olives and its products have been used to treat a variety of disease conditions, such as inflammation; diarrhea; hemorrhoids; and respiratory, urinary, and intestinal ailments. Moreover, olive constituents have been suggested to possess antimicrobial, anticancer, antidiabetic, antioxidant, antihypertensive, antiinflammatory, and antinociceptive properties and contribute to cardioprotective, gastroprotective, and neuroprotective activities ([27] and references therein). The structures of phenolic constituents of olive—tyrosol, hydroxytyrosol, dihydroxyphenylglycol (DHPG), and oleuropein—are shown in Fig. 1.

Tyrosol is a naturally occurring polyphenol found in olives and extra virgin olive oil [29]. In in vitro studies, tyrosol was shown to be absorbed in a dose-dependent manner from virgin olive oil and indicated antioxidant activity. The bioavailability of tyrosol from virgin olive oil was enough to bind human low-density lipoprotein, suggesting that the phenolic constituents of olives are effective in preventing lipid peroxidation and atherosclerotic processes [29]. Tyrosol was also shown to have protective effects against ethanol-induced oxidative stress in HepG2 cells and prevent ethanol-induced liver damage [30]. Further, tyrosol has been shown to have comparable antiinflammatory effects in an endotoxin-induced uveitis rat model with prednisolone, a well-known antiinflammatory drug, which supports the potential of tyrosol in the treatment of intraocular inflammatory diseases [31]. Finally, the antimicrobial properties of tyrosol were observed in the inhibition of single- and mixed-species biofilm formation by the oral pathogen *Streptococcus mutans* [32].

Hydroxytyrosol is another naturally occurring phenolic phytochemical found in olives and extra virgin olive oil that has antiinflammatory, antioxidant, anticancer, and antimicrobial properties [33]. It was shown to have antidiabetic and antioxidant properties in alloxan-induced diabetic rats [34]. Administration of 8–16 mg/kg body weight of *O. europaea* leaf extracts containing oleuropein and hydroxytyrosol for four weeks resulted in significant reductions of serum cholesterol and glucose levels of the diabetic rats along with restoration of antioxidant enzymatic activities [34]. Hydroxytyrosol also possesses powerful antioxidant effects [35], inhibiting the H_2O_2 –induced DNA damage while indicating a correlation with antiproliferative activities of hydroxytyrosol on breast (MDA and MCF-7), prostate (LNCap and PC3), and colon (SW480 and HCT116) cancer cell lines from the effect of H_2O_2. Hydroxytyrosol was shown to act as a chemopreventive agent for the initiation and progression phases of carcinogenesis [35]. Furthermore, hydroxytyrosol and other phenolics from olive oil have been shown to eradicate in vitro microbial infections caused by *Helicobacter pylori*, commonly linked to peptic ulcers and gastric cancer [36]. Hydroxytyrosol is considered a unique HIV-1 inhibitor [37] and has been shown to prevent HIV from entering host cells and binding to the catalytic site of HIV-1 integrase [38].

DHPG is a major phenolic compound present in table olives and is a naturally occurring hydroxytyrosol derivative and metabolite of norepinephrine [39,40]. DHPG has important implications as a biomarker and has been shown to fluctuate in concentration in rat testis after methamphetamine intake, which may result in male reproductive dysfunction [41]. DHPG was also shown to have some antioxidant activity [40,42].

Oleuropein is one of the most abundantly found secoiridoid glycosides in olives and olive leaves [27]. Oleuropein is responsible for giving immature and unprocessed olives their bitter taste [43,44]. Hydroxytyrosol is a metabolite of oleuropein and, thus, shares many similar structural and beneficial health properties with oleuropein [27]. Oleuropein was found to act as a potent antioxidant and reduced oxidative stress in alloxan diabetic rabbits. The treatment of diabetic rabbits with oleuropein doses of 20 mg/kg body weight for up to 16 weeks resulted in blood glucose values near the normal values of control rabbits, suggesting that oleuropein was a potent antihyperglycemic and antioxidative agent [45]. Oleuropein has also been shown to have cardioprotective properties through its antiischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits [44,46] and it has been shown to have neuroprotective effects [44]. Investigation of the effect of oleuropein on human colon adenocarcinoma cells (HT-29) suggested that oleuropein restricts cell growth and causes apoptosis of HT-29 cells by downregulation of HIF-1α [47]. Moreover, oleuropein was effec-

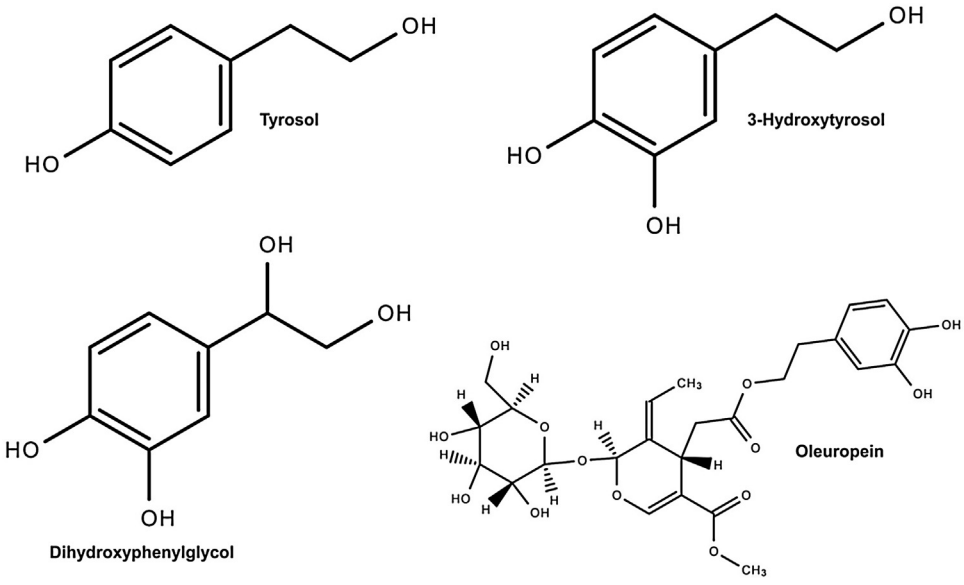

Fig. 1. Structures of olive phenolics tyrosol, hydroxytyrosol, dihydroxyphenylglycol (DHPG), and oleuropein.