ELSEVIER

Process Biochemistry 40 (2005) 2123–2134

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

Antioxidant enzyme response studies in H_2O_2-stressed porcine muscle tissue following treatment with oregano phenolic extracts

Reena Randhir, Dhiraj Vattem, Kalidas Shetty*

Department of Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst, MA 01003, USA

Received 12 July 2004; accepted 10 August 2004

Abstract

The effect of oregano (*Origanum vulgare*) extracts rich in phenolics in ameliorating the adverse effects of oxidative stress caused by H_2O_2 by mediating an antioxidant enzyme response in porcine muscle tissue was investigated. The changes in the total phenolic content, antioxidant activity, proline content, malondialdehyde (MDA) content, activity of glucose-6-phosphate dehydrogenase (G6PDH), which is the first commited step of pentose phosphate pathway, antioxidant enzymes including total peroxidase (TPX), superoxide dismutase (SOD), and catalase (CAT) activity in stressed porcine muscle tissue was determined. The total phenolic content of control and H_2O_2 treated porcine tissue remained constantly low throughout the study. In the oregano treated tissues the phenolic content increased four-fold immediately indicating that the porcine cells had imbibed the oregano phenolics efficiently. In the case of H_2O_2 + oregano treatment the values were higher than control and H_2O_2 alone but less than oregano alone. This indicates that the oregano phenolics inside the porcine cell were potentially reactive and likely being utilized for counteracting the harmful effects of H_2O_2. In the oregano and H_2O_2 + oregano treated tissues the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging antioxidant activity almost doubled from 35% to 75% by 8 h. This also further indicates the high free radical scavenging, antioxidant potential of the oregano phytochemicals in reducing the harmful effects of H_2O_2. In the oregano and H_2O_2 + oregano treatments the G6PDH activity increased seven-fold compared to stressed and control tissue, which coincides with stimulated TPX activity. The H_2O_2 stress alone enhanced G6PDH activity slightly and this response occurred concomitantly with an increase in SOD and CAT activity. The oregano phenolics were able to counter the H_2O_2 effects with reduced need for higher SOD expression. In the present research the highest antioxidant enzyme activity was observed in the stressed H_2O_2 treatment at 8 h incubation indicating the active role of CAT in modulating the harmful effects of H_2O_2, which directly correlated to higher SOD activity. In the case of oregano and oregano + H_2O_2, proline levels were higher compared to H_2O_2 alone, again suggesting the ameliorating effect of oregano on oxidative stress and this may involve proline-linked stimulation of pentose phosphate pathway. The MDA content in the case of oregano + H_2O_2 treatment was comparatively lower than H_2O_2 alone showing the protective effects of oregano against reactive oxygen species and eventually from membrane damage. The major implication from this study is evidence that oregano being rich in phenolics is an effective direct quencher of free radicals. Further, the same phenolics play a beneficial role in protecting porcine muscle tissue from the harmful effects of reactive oxygen species through regulation of antioxidant enzyme response through the phenolic-dependent peroxidases with dependency on pentose phosphate pathway but with reduced dependency on SOD and CAT.

Keywords: Antioxidant enzymes; Oregano; Pentose phosphate pathway (PPP); Reactive oxygen species (ROS); Glucose-6-phosphate dehydrogenase (G6PDH); Total peroxidase (TPX); Superoxide dismutase (SOD); Catalase (CAT); Malondialdehyde (MDA); Proline; Phytochemicals; Phenolics; Porcine; Antioxidant; H_2O_2

1. Introduction

Epidemiological studies consistently indicate that the consumption of phytochemical-enriched fruits, vegetables and herbs lower the incidence of disease in humans. This beneficial effect is attributed to the presence of phenolic phytochemicals [1]. Plants synthesize and accumulate a spectrum of secondary metabolites such as phenolics in response to physiological stimuli and stress [2]. They are synthesized from phenylalanine, via the shikimate pathway, phenylpropanoid pathway and flavonoid pathway (Fig. 1).

* Corresponding author. Tel.: +1 413 545 1022; fax: +1 413 545 1262.
E-mail address: kalidas@foodsci.umass.edu (K. Shetty).

0032-9592/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2004.08.009

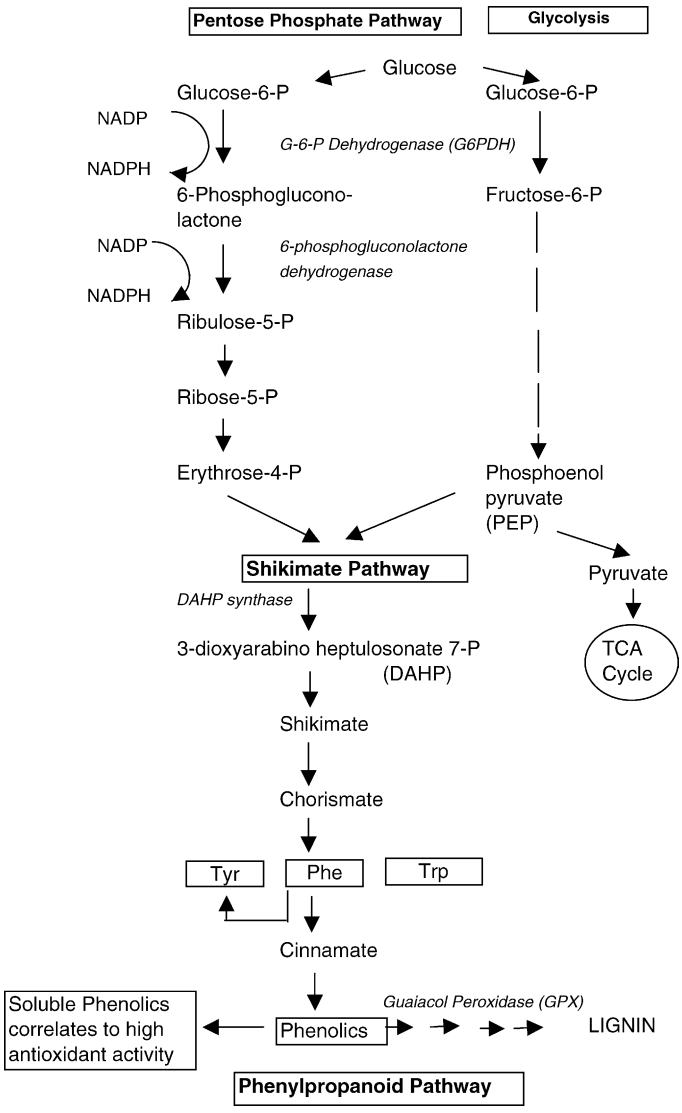

Fig. 1. Pentose phosphate pathway for synthesis of phenolic compounds in plants.

They play a vital role in plant growth, regulation of plant metabolism and lignin synthesis [2,3]. Research has indicated the therapeutic role of phenolics in the prevention of cancer [4], stroke [5], coronary heart disease [6], breast cancer and osteoporosis [7]. Phenolics have also been reported to exhibit pharmacological properties such as antitumour, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity [8–10].

Reactive oxygen species (ROS) are byproducts of regular cell metabolism ($^1O_2$: singlet oxygen, H_2O_2: hydrogen peroxide, $OH^{\bullet}$: hydroxyl radical, $O_2^{\bullet -}$: superoxide radical) and are capable of oxidizing cellular proteins, nucleic acids and lipids [11] (Fig. 2). They contribute to cellular aging [12], mutagenesis [13], carcinogenesis [14], and coronary heart disease [15] possibly through destabilization of membranes [16], DNA damage [13], and oxidation of low-density lipoprotein [17]. They also participate in many cellular events including signal transduction and antibacterial defence [11]. Hence, the maintenance of a balance between oxidants and antioxidants is of significance for cellular homeostasis. The protective effects of phenolics in biological systems are ascribed to their capacity to transfer hydrogen to free radicals, chelate metal catalysts [18], activate antioxidant enzymes [19], reduce alpha-tocopherol radicals [20], and inhibit oxidases [21]. Oxidative stress is defined as oxygen radical-mediated damage to biological macromolecules (proteins, lipids, carbohydrates, and DNA) caused by either increased generation and build up of the oxygen radicals (superoxide radical, hydrogen peroxide, and the hydroxyl radical), and/or the diminished removal or inadequate protection against these ubiquitously present oxygen radicals. In aerobic animals, powerful antioxidant enzymes (catalase, peroxidase, superoxide dismutase) and radical scavengers (glutathione, Vitamin E) are present to remove or scavenge these oxygen radicals in cell organelles and membranes [22–26].