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RESEARCH ARTICLE

Effect of Nitric Oxide on the Interaction Between Mitochondrial Malate Dehydrogenase and Citrate Synthase

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Abstract

Mitochondrial malate dehydrogenase (mMDH) and citrate synthase (CS) are sequential enzymes in Krebs cycle. mMDH, CS and the complex between mMDH and CS (mMDH+CS) were treated with nitric oxide solution. The roles of notric oxide (NO) on the secondary structures and the interactions between mMDH and CS were studied using circular diehroism (CD) and Fourier transform surface plasmon resonance (FT-SPR), respectivley. The effects of NO on the activities of mMDH, CS and mMDH+CS were also studied. And the regulations by NO on mMDH and CS were simulated by PyMOL software. The results of SPR confirmed that strong interaction between mMDH and CS existed and NO could significantly regulate the interaction between the two enzymes. NO reduced the mass percents of α-helix and increased that of random in mMDH, CS and mMDH+CS. NO increased the activities of CS and mMDH+CS, and inhibited the activity of mMDH. Graphic simulation indicated that covalent bond was formed between NO and Asn242 in active site of CS. However, there was no direct bond between NO and mMDH. The increase in activity of mMDH+CS complex depended mostly on the interaction between NO and CS. All the results suggested that the regulations by NO on the activity and interaction between mMDH and CS were accord with the changes in mMDH, CS and mMDH+CS caused by NO.

Key words: Krebs cycle, nitric oxide, surface plasmon resonance, protein-protein interaction, citrate synthase, malate dehydrogenase

INTRODUCTION

The tricarboxylic acid (TCA) cycle, also known as the Krebs cycle or the citric acid cycle, is at the center of cellular metabolism, playing a starring role in both the process of energy production and biosynthesis. At present, the TCA cycle is considered as not only a circle but also flux modes playing important roles in physiological processes in animals, plants and bacteria (Sweetlove *et al*. 2010; Meeks 2011; Zhang and Bryant 2011; McCarthy 2013; Nunes-Nesi *et al*. 2013). The intermediates of the TCA cycle also involve in regulating physiological and biochemical processes, and supplement of the TCA cycle intermediates protects against cell death induced by high glucose/palmitate (Choi *et al*. 2011; Peti-Peterdi 2013). Those results suggest that the protection depends on the flux of the intermediates of the TCA cycle. Activities of the enzymes in the TCA cycle contribute to the flux of the intermediates. Malate dehydrogenase (MDH) and citrate synthase (CS) are two sequential enzymes in the TCA cycle. MDH catalyzes malic acid to oxaloacetic acid which is converted to citric acid by CS. In most ripe fruits, malic and citric acids are the

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main organic acids contributed to fleshy fruit acidity which is an important component of fruit organoleptic quality, and affects postharvest softening (Centeno *et al*. 2011; Etienne *et al*. 2013).

In recent years, mitochondrial malate dehydrogenase (mMDH) and CS are increasingly valued for their important roles in the plant TCA cycle (Sweetlove *et al*. 2010; Nunes-Nesi *et al*. 2013). Recently, complexes between several TCA cycle enzymes were identified *in vivo* interaction analyses in *Bacillus subtilis* (Meyer *et al*. 2011). New evidences confirm that mMDH lowers leaf respiration and alters photorespiration in *Arabidopsis* (Tomaz *et al*. 2010). The overexpression of mMDH gene can improve phosphorus acquisition by tobacco (Lü *et al*. 2012). Antisense inhibition of mMDH can not only enhance photosynthetic activity and the rate of carbon dioxide assimilation (Nunes-Nesi *et al*. 2005), but also alter root growth and architecture in tomato plant (van der Merwe *et al*. 2009). The enhanced photosynthetic performance and growth in transgenic tomato plants are also considered to be a consequence of decreasing mMDH activity (Nunes-Nesi *et al*. 2005). Overexpression of CS can improve plant growth under nutritional stress, such as aluminum and phosphorus tolerance (Koyama *et al*. 1999, 2000; Deng *et al*. 2009), and antisense repression of CS can inhibit the flower formation in transgenic potato plants (Landschutze *et al*. 1995). Structure and expression of CS from higher plants have been studied widely (La Cognata *et al*. 1996). Recently, the function of a citrate synthase gene (*MaGCS*), which is constitutively expressed in all organs with high levels in the fruit, during postharvest banana fruit ripening, is reported (Liu *et al*. 2013). The expression of *MaGCS* can be induced by ethylene and inhibited by the ethylene receptor inhibitor, improved by oxaloacetic acid and suppressed by citric acid, suggesting that *MaGCS* is associated with ethylene biosynthesis and plays an important role in postharvest banana fruit ripening (Liu *et al*. 2013). mMDH and CS of strawberry fruit are purified and the genes are cloned and identified (Iannetta *et al*. 2004). mMDH cDNA clones are also isolated from grape berries and the expression pattern are analysed throughout berry development (Or *et al*. 2000). Isolation and functional characterization of genes encoding citrate synthase are also studied in *Malus* (Han *et al*. 2012), pear fruit (Lu *et al*. 2011). As two sequential enzymes in the TCA cycle, the interaction between mMDH and CS has aroused the interests of the researchers. The interactions between mMDH and CS have been studied widely in animals, plants and microbes (Tompa *et al*. 1987; Morgunov and Srere 1998; Pettersson *et al*. 2000; Iannetta *et al*. 2004; Chow *et al*. 2005). Recently, it is found that bioconjugates formed by adding CS to the Au nanoparticles before MDH addition exhibits higher specific activities for both enzymes than those formed by adding the enzymes in the reverse order. These bioconjugates also have 3-fold higher per-particle sequential activity for conversion of malate to citrate (Keighron and Keating 2010). Those results suggest that exogenous treatments can affect the activities and interaction between mMDH and CS.

As a small biomolecular, nitric oxide (NO) is considered to be a potent inhibitor of the mitochondrial electron transport chain (Brown and Borutaite 2002; Wang *et al*. 2010; Sarti *et al*. 2012), regulates the ripening processes of fruits (Manjunatha *et al*. 2010; Zhu *et al*. 2010), and affects fruit quality during storage (Duan *et al*. 2007; Sun *et al*. 2011). NO can regulate energy metabolism *via* the TCA cycle (Dai *et al*. 2013). Exogenous NO can decrease leaf citrate content and increase root citrate content of citrus, and inhibit CS activity in citrus leaves (Yang *et al*. 2012). Our previous research also found that NO makes a dramatic promotion of mMDH activity and slight increase in CS activity in peach fruit during storage (Ma *et al*. 2011). However, little works have been done to study the roles of NO in the interaction between mMDH and CS. In this paper, the effects of NO on the secondary structures and interaction between mMDH and CS were studied to explore the possible mechanism by which NO regulates the activities of mMDH and CS.

RESULTS

Effects of NO on interactions between mMDH and CS

Fig. 1 depicted the SPR response of the interactions between mMDH and CS in 10% PEG. The SPR wavenumber shift after step V means the change of the film's thickness, which can reflect the protein-protein interactions. It could be found that there was a shift in the SPR resonance after NO treatment. The shift in