Gene 568 (2015) 1–7

Contents lists available at ScienceDirect

Gene

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

Research paper

Characterization of the interaction between superoxide dismutase and 2-oxoisovalerate dehydrogenase

Chongfu Song [a,b], Hebin Li [c], Liangquan Sheng [b], Xiaobo Zhang [a,*]

[a] Key Laboratory of Conservation Biology for Endangered Wildlife of Ministry of Education and College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China
[b] School of Chemistry and Material Engineering, Fuyang Teachers College, Fuyang 236037, People's Republic of China
[c] Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, People's Republic of China

ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form 25 March 2015
Accepted 4 May 2015
Available online 7 May 2015

Keywords:
Thermoadaptation
Protein interaction
Thermus thermophilus
Superoxide dismutase
2-Oxoisovalerate dehydrogenase α subunit

ABSTRACT

Thermophiles are attractive microorganisms to study the adaptation of life in high temperature environment. It is revealed that superoxide dismutase (SOD) is essential for thermoadaptation of thermophiles. However, the SOD-mediated pathway of thermoadaptation remains unclear. To address this issue, the proteins interacted with SOD were characterized in *Thermus thermophilus* in this study. Based on co-immunoprecipitation and Western blot analyses, the results showed that 2-oxoisovalerate dehydrogenase α subunit was bound to SOD. The isothermal titration calorimetry analysis showed the existence of the interaction between SOD and 2-oxoisovalerate dehydrogenase α subunit. The bacterial two-hybrid data indicated that SOD was directly interacted with 2-oxoisovalerate dehydrogenase α subunit. Gene site-directed mutagenesis analysis revealed that the intracellular interaction between SOD and 2-oxoisovalerate dehydrogenase α subunit was dependent on their whole molecules. Therefore our study presented a novel aspect of SOD in the thermoadaptation of thermophiles by interaction with dehydrogenase, a key enzyme of tricarboxylic acid cycle.

1. Introduction

Thermophiles (optimal growth 70–80 °C) and hyperthermophiles (optimal growth 85–100 °C) are adapted to survive at high temperature (Niehaus et al., 1999; Li et al., 2010; Nath and Bharathi, 2011; Song et al., 2012). Due to their great application values and developmental prospects on protein project, genetic engineering, fermented project, and development and utilization of mineral resources, thermophiles and hyperthermophiles have attracted intensive investigations in recent years (Kashefi and Lovley, 2003; Sazanov and Hinchliffe, 2006; Cava et al., 2009; du Plessis et al., 2010; López-López et al., 2010; Wang and Zhang, 2010; Berdygulova et al., 2011; Rocha et al., 2011; Wu et al., 2012; Rabinovitch-Deere et al., 2013; Thompson et al., 2013). The genus *Thermus*, with a growth temperature ranging from 45 °C to 85 °C, belongs to one of the oldest branches of bacteria and forms a phylum together with the genus *Deinococcus* (Fuciños et al., 2011). To date, hundreds of strains from the genus *Thermus* have been isolated from neutral or slightly basic thermal effluents, self heating piles of organic matter and industrial heating systems (Henne et al., 2004; Cava et al., 2009). All of them are thermophilic Gram-negative bacteria that grow aerobically with high growth rates and good cell yields on complex medium, but do not require specific amino acids or vitamins (Cava et al., 2009). As a matter of fact, the thermophilic bacterium *Thermus thermophilus* HB27 has become a model microorganism for biotechnology and genetic engineering of extremophilic microorganisms (Fuciños et al., 2011). Because of their aerobic characters, rapid growth rates and the biotechnological applications of their thermophilic enzymes, different isolates from *Thermus* genus are currently being studied as putative hosts for the expression of multimeric thermophilic enzyme complexes and genetic studies (Cava et al., 2009; Li et al., 2010).

Based on the sequence analysis of 16S rRNA and other genes, most of thermophiles and hyperthermophiles belong to the deepest branches of their respective bacterial or archaeal phylogenetic trees, which supports that these organisms can be the closest living representatives of the last bacterial or archaeal common ancestors. Thus the studies on thermophiles will reveal biological traits of primitive life on earth

Abbreviations: SOD, superoxide dismutase; ΔSOD, the SOD mutant; DHα, 2-oxoisovalerate dehydrogenase α subunit; GST, S-transferase; IPTG, isopropyl-β-D-thiogalactoside; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; Co-IP, co-immunoprecipitation; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MALDI-TOF, Matrix-assisted Laser Desorption Ionization-time-of-flight; ORF, open reading frame; LB-CTCK, LB agar with carbenicillin (250 mg/L), tetracycline (12.5 mg/L), chloramphenicol (34 mg/L) and kanamycin (50 mg/L); LB-TCK, LB medium containing tetracycline, chloramphenicol, and kanamycin; ITC, isothermal titration calorimetry; pBT-SOD, the SOD gene was cloned into plasmid pBT; pTRG-DHα, the DHα gene was cloned into pTRG plasmid; BSA, bovine serum albumin; TCA, tricarboxylic-acid-cycle; SOD-dehydrogenase-TCA, SOD-mediated tricarboxylic-acid-cycle.

* Corresponding author.
E-mail address: zxb0812@zju.edu.cn (X. Zhang).

http://dx.doi.org/10.1016/j.gene.2015.05.008
0378-1119/

(Cava et al., 2009). Thermophiles are attractive microorganisms to study the adaptation of life in high temperature environments. To adapt to hot environments, thermophiles have developed specific strategies for thermoadaptation by their physiological, nutritional and metabolic requirements including the cell components such as lipids, nucleic acids and proteins, thermal resistance of the DNA helixes and the structure of ribonucleic acids (Li et al., 2010). In *T. thermophilus*, the thermophile proteins take great effects on thermoadaptation by their involvements in metabolic pathways (Li et al., 2010). As reported, the sequences of 115 proteins from extremely thermophilic archaeon *Methanococcus jannaschii* are compared with their homologues from mesophilic *Methanococcus* species (Haney et al., 1999). Although the temperatures for the growth of mesophiles are less than that of *M. jannaschii*, their genomic G + C contents are nearly identical. The properties correlated with the proteins of the thermophile include higher residue hydrophobicity, more charged amino acids (especially Glu, Arg and Lys), and fewer uncharged polar residues (Ser, Thr, Asn and Gln) (Haney et al., 1999). The documented data indicate that there is a significant relationship between amino acid composition and protein thermostability at the level of whole prokaryote protein sequences (Gao and Wang, 2012). In addition, the proteins from thermophiles tend to use more charged amino acids to adapt to high temperature environments. The differences of amino acid composition between thermophiles and mesophiles may be beneficial for the maintenance and stability of proteins at high temperatures (Gao and Wang, 2012). Analyses of structures and sequences of several hyperthermostable proteins from various sources reveal two major physical mechanisms of their thermostabilization, namely "structure-based" and "sequence-based" mechanism of their thermal stabilization (Berezovsky and Shakhnovich, 2005). Our previous study revealed that the SOD mutant (ΔSOD) demonstrated a decrease of thermoadaptation, decreasing from 80 °C, the maximal growth temperature of wild-type strain, to 75 °C (Li et al., 2010). At the same time, the optimal growth temperature of ΔSOD had a decrease of 5–10 °C. The extracellular addition of the recombinant SOD in the media of ΔSOD culture significantly increased the growth temperature of ΔSOD. These findings indicated that SOD was essential for thermoadaptation of thermophiles (Li et al., 2010). However, the SOD-mediated pathway of thermoadaptation is not clear.

To address this issue, the proteins interacted with SOD was investigated in *T. thermophilus* in this study. The results showed that the interaction between SOD and dehydrogenase, a key enzyme in tricarboxylic acid cycle, played an important role in thermoadaptation of thermophiles.

2. Materials and methods

2.1. Culture of T. thermophilus

As described previously (Li et al., 2010), *T. thermophilus* was cultured at 70 °C with shaking in TM (Thayer Martin) broth medium (0.1% NaCl, 0.2% yeast extract, 0.4% tryptone and basal salt, pH 7.5). The overnight culture of *T. thermophilus* was diluted at 1:100 with TM medium and inoculated to fresh TM medium for continuous growth to late log phase.

2.2. Recombinant protein expression in Escherichia coli and antibody preparation

The SOD- and DHα (2-oxoisovalerate dehydrogenase α subunit)-encoding genes of *T. thermophilus* were respectively cloned into pGEX-4T-2 vector (Novagen, Germany) and expressed in *E. coli* BL21 (DE3) as glutathione S-transferase (GST)-tagged fusion proteins. The SOD and DHα genes were amplified with sequence-specific primers (SOD, 5′-TGTGGATCCATGCCGTACCCGTTCAA-3′ and 5′-CTGGAATTCTCAGGCC TTCTTGAAGAAC-3′; DHα, 5′-GATGGATCCAT GGTCAAGGAGACCCATCG GT-3′ and 5′-CGAGAATTCCTAGAGCTCCTCCTT GAGGA-3′). The recombinant plasmid containing the SOD or DHα gene was confirmed by sequencing. After incubation at 37 °C overnight, the recombinant bacteria were inoculated into new media at a ratio of 1:100. When the OD_{600} was 0.6, the bacteria were induced with isopropyl-β-D-thiogalactoside (IPTG). After further incubation for 4 h at 37 °C, the induced cells were harvested by centrifugation at 6000 ×*g* for 10 min. The recombinant protein was purified by affinity chromatography using Glutathione Sepharose resins under native conditions according to the recommended protocol (Qiagen, Germany).

The purified recombinant fusion protein was used as antigen to immunize mice. The immunoglobulin G (IgG) fractions of the antiserum were purified with protein A-Sepharose (Bio-Rad, USA) and stored at −80 °C until use. As determined by enzyme-linked immunosorbent assay (ELISA), the titers of antisera were 1:10,000 (Li et al., 2010). Antigen was replaced by PBS in negative control assays. The specificity of antibody was confirmed using Western blot with the recombinant protein.

2.3. Co-immunoprecipitation (Co-IP)

The *T. thermophilus* was collected by centrifugation at 7000 ×*g* for 10 min. The precipitate was resuspended in 0.1 M phosphate buffered saline (PBS, pH 7.5). After sonication for 5 min, the suspension was centrifuged at 13,000 ×*g* for 15 min at 4 °C. The appropriate antibody was added to the supernatant and incubated for 1 h at 4 °C. Protein A Sepharose slurry (Bio-Rad) was subsequently added, followed by incubation for 2 h at 4 °C. Nonspecific binding proteins were removed by five successive rinses with PBS. The Protein A Sepharose was finally eluted with 0.1 M glycine solution (pH 1.8). The eluate was collected and analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or Western blot.

2.4. SDS-PAGE

Samples were heated for 10 min in boiling water, followed by protein separation with 12% SDS-PAGE. The protein bands were visualized by staining with Coomassie Blue R-250.

2.5. Mass spectrometry analysis

The protein was identified using Matrix-assisted Laser Desorption Ionization-time-of-flight (MALDI-TOF) Mass Spectrometry (Bruker Autoflex, Germany) as described before (Li et al., 2010). Data mining was performed using Mascot search engine (version 2.1; Matrix Science, London, UK) against the open reading frame (ORF) database of *T. thermophilus* HB27. A mass deviation of 100 ppm and modifications such as carbamidomethyl, oxidation and Pyro-glu were usually allowed in the database searches.

2.6. Western blot

Proteins separated by SDS-PAGE were transferred to nitrocellulose membrane (Bio-Rad) in electroblotting buffer (25 mM Tris, 190 mM glycine, 20% methanol; pH 8.5) for 70 min. The membrane was immersed in blocking buffer (0.1% skimmed milk, 20 mM Tris, 0.9% NaCl, 0.1% Tween 20, pH 7.2) at 4 °C overnight, followed by incubation with a polyclonal mouse anti-GST-DHα IgG for 3 h. Subsequently, the membrane was incubated in alkaline phosphate-conjugated goat anti-mouse IgG (Pierce Biotechnology, USA) for 1 h and detected with the substrate solution (0.48 mM 5-bromo-4-chloro-3-indolyl phosphate/0.51 mM nitroblue tetrazolium chloride) (Amresco, USA).