Food Chemistry 130 (2012) 254–260

Contents lists available at ScienceDirect

Food Chemistry

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

Significant longevity-extending effects of a tetrapeptide from maize on *Caenorhabditis elegans* under stress

Hang Wu [a], Yan Zhao [b], Yi Guo [a], Li Xu [a,*], Baolu Zhao [c,*]

[a] Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, College of Life Science, Jilin University, Changchun 130012, China
[b] Department of Food Sciences and Technology, Harbin Institute of Technology, Weihai 264209, China
[c] State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 29 June 2011
Accepted 11 July 2011
Available online 21 July 2011

Keywords:
Bioactive peptide
ROS
C. elegans
Oxidative stress
Heat stress
Ageing

ABSTRACT

A tetrapeptide (Leu-Asp-Tyr-Glu) from maize (TPM) is a bioactive peptide. Here we reported that TPM extended the lifespan of *Caenorhabditis elegans* under heat and oxidative stress. Specifically, TPM (10 mM) increased the average longevity of *C. elegans* by 36.9% and 27.6% under heat stress (35 °C) and oxidative stress, respectively. Further studies demonstrated that the significant longevity-extending effects of TPM on *C. elegans* could be attributed to its *in vitro* and *in vivo* free radical-scavenging effects and its up-regulation of stress-resistance-related proteins, including superoxide dismutase-3 (SOD-3) and heat shock protein-16.2 (HSP-16.2). Real-time PCR results showed that the up-regulation of ageing-associated genes such as daf-16, sod-3 and hsp-16.2, in addition to skn-1, ctl-1 and ctl-2, could also contribute to the stress-resistance effect of TPM. These results indicate that TPM can (or has the potential to) protect against external stress and extend lifespan under stress.

1. Introduction

Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage the components of the cell, including proteins, lipids, and DNA.

Extensive studies suggest that ROS, by-products of cellular respiration, play a role in normal ageing by causing random deleterious oxidative damage to a variety of tissue (Harman, 1956; Muller, Lustgarten, Jang, Richardson, & Van Remmen, 2007). Resistance to ROS, due to high activities of ROS-detoxifying enzymes and/or low intrinsic ROS-production levels, characterise commonly long-lived *Caenorhabditis elegans* mutants and these findings are taken to substantiate the free radical theory of ageing (Tanja, Kai, Hannelore, & Uwe, 2010). Longevity genes also mediate increased resistance to oxidative stress; long-lived dauer mutants show increased resistance to oxidative stress and up-regulation of antioxidant genes (Sang-Kyu, Patricia, & Thomas, 2009).

Maize is a plant whose food value and wide variety of uses make it one of the most important crops in the world. With the development of science and technology, as well as the need of using food for health promotion, the understanding of the significance of corn nutrition has constantly advanced for the past century.

A tetrapeptide from maize (TPM), characterised as Leu-Asp-Tyr-Glu, is a bioactive peptide (Xu et al., 2004). In this study, we investigated the anti-ageing effects of TPM in *C. elegans* and found that TPM could significantly improve the longevity of *C. elegans* under stress conditions. Our results suggest that TPM may provide strong protection against stress and extend the longevity of *C. elegans* by scavenging reactive oxygen species and up-regulating the expression of stress resistance-associated genes, such as daf-16, sod-3, skn-1, ctl-1, ctl-2 and hsp-16.2. We believe these findings will provide insight regarding anti-ageing research into bioactive peptides and corn.

2. Materials and methods

2.1. Reagents

FUDR (5-fluoro-2′-deoxyuridine), 98%, was bought from Sigma (St. Louis, MO). ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonate)] (Sigma) and pyrogallol (Sigma) were used as free radical providers. H_2DCF-DA (2′,7′-dichlorodihydro fluorescein

* Corresponding authors. Address: Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, College of Life Science, Jilin University, No. 2699 Qianjin Street, Changchun 130012, China (L. Xu), Institute of Biophysics, Academia Sinica, 15 Datun Road, Chaoyang District, Beijing 100101, China (B. Zhao).
E-mail addresses: xuli@jlu.edu.cn (L. Xu), zhaobl@sun5.ibp.ac.cn (B. Zhao).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.07.027

diacetate) (Sigma) was used as a fluorescent probe. Juglone (5-hydroxy-1,4-naphthoquinone), a reactive oxygen species-generating compound, was used to induce oxidative stress in worms.

TPM was prepared *via* protease hydrolysis of zein catalysed by Alcalase®, an alkaline protease, followed by isolation and purification (Xu et al., 2004).

2.2. Worm strains and maintenance

Standard nematode growth medium (NGM) was used for *C. elegans* growth and maintenance at 20 °C. Unless stated otherwise, plates were seeded with live *Escherichia coli* OP50 bacteria. Bristol N2 (Caenorhabditis Genetics Center; CGC) was used as the wild-type strain. The transgenic strain CF1553 (muIs84, CGC) containing the SOD-3::GFP-linked reporter was used to visualise SOD-3 expression. The CL2070 (dvIs70) strain, containing the HSP-16.2::GFP-linked reporter used to visualise HSP-16.2 expression, was a generous gift from Y. Luo of the University of Maryland (College Park, MD).

2.3. Stress resistance assay

Heat-shock assays were performed at 35 °C using 2-day-old adult worms. The worms were treated with TPM (10 mM) for 2 days and then transferred to an incubator at 35 °C. The number of dead worms was recorded every hour (Hansen, Hsu, Dillin, & Kenyon, 2005; Lithgow, White, Melov, & Johnson, 1995).

The expression of HSP-16.2::GFP in CL2070 worms was investigated by fluorescence microscopy. The worms were treated for 2 days with or without 10 mM TPM, followed by heat shock (treatment at 25 or 30 °C for half an hour and then 35 °C for an hour) and recovery for 24 h (Rea, Wu, Cypser, Vaupel, & Johnson, 2005).

Juglone sensitivity was assessed at 20 °C using 2-day-old adult worms. The worms were incubated with TPM (10 mM) for 2 days and then transferred to plates with 500 μM juglone. The number of dead worms was counted and recorded every hour.

For all life span assays, every experiment was repeated three times and conducted in a double-blind manner.

2.4. ABTS assay

ABTS (38.4 mg) and $K_2S_2O_8$ (6.6 mg) were dissolved in 5 mL water. The solution was kept at room temperature for 12–16 h to form $ABTS^+$ solution by oxidising ABTS with potassium persulfate. The $ABTS^+$ solution was diluted 1:100 in absolute ethanol to prepare the working solution. Subsequently, 1.8 mL of $ABTS^+$ working solution was mixed with 0.2 mL of TPM solution, and the absorbance at 734 nm was measured after a 20-min delay (Beckman UV–Vis spectrophotometer, Model DU640B; Beckman Coulter Inc., Fullerton, CA). The final concentrations of TPM used were 0.3125, 0.625, 1.25, 2.5, and 5 mM.

2.5. Pyrogallol self-oxidation assay

The *in vitro* superoxide anion-scavenging effects of TPM were measured by monitoring the chemiluminescence in the pyrogallol luminol system. All reagents were equilibrated in a water bath at a constant temperature (25 °C) and then added to a glass luminescence tube (1 × 5 cm) in a water bath in the following order: 10 μL 3 mM pyrogallol, 80 μL 4 mM NaOH, 10 μL TPM, and 900 μL 0.1 mM luminol (in sodium carbonate buffer, pH 10.2). The final concentrations of TPM were 0.001, 0.003125, 0.00625, 0.0125, 0.025, 0.05, 0.1 mM. Light emission was observed after a 15-s delay at 25 °C.

2.6. Fenton's reaction

The *in vitro* hydroxyl free radical-scavenging effects of TPM were measured by monitoring the chemiluminescence in the Fenton's reaction system. All reagents were equilibrated in a water bath at a constant temperature (25 °C) and then added to a glass luminescence tube (1 × 5 cm) in a water bath in the following order: 10 μL 3% H_2O_2, 10 μL 0.1 mM Fe^{2+}, 10 μL TPM, and 970 μL 0.1 mM luminol (in sodium carbonate buffer, pH 10.2). The final concentrations of TPM were 0.0925, 0.185, 0.925, 1.85, 3.7 mM. Light emission was observed after a 15-s delay at 25 °C.

2.7. Measurement of intracellular ROS in C. elegans

Intracellular ROS in *C. elegans* were measured using molecular probe H_2DCF-DA. For ROS detection under normal culture conditions, worms that had just reached adulthood were treated with or without TPM (10 mM) for 2 days. For the ROS test under oxidative stress, worms that had just reached adulthood were treated with 300 μM juglone for 1 h and then treated with or without TPM (10 mM) for 2 days. After 48 h of exposure to the respective compounds, animals were washed off the plates with cold M9 buffer. Bacteria were removed by three repeated washes and subsequent centrifugation at low speed. Animals were resuspended in M9 buffer, and a 50-μL volume of the suspension was pipetted in four replicates into the wells of a 96-well plate with opaque walls and transparent bottom and allowed to equilibrate to room temperature. In the meantime, a fresh 100 μM H_2DCF-DA solution from a 100 mM stock solution in DMSO was prepared in M9 buffer, and a volume of 50 μL was pipetted to the suspensions, resulting in a final concentration of 50 μM. On each plate, control wells containing nematodes from each treatment without H_2-DCF-DA and wells containing H_2-DCF-DA without animals were prepared in parallel (Schulz et al., 2007). Immediately after addition of H_2-DCF-DA, basal fluorescence was measured in a microplate reader at excitation/emission wavelengths of 485 and 520 nm. Plates were measured at 20 °C every 30 min for 2 h.

2.8. Fluorescence quantification and visualisation

Overall GFP fluorescence of GFP-expressing populations was assayed using a Thermo Labsystems Fluoroskan Ascent microplate reader (Thermo Fisher, Waltham, MA). Adult worms were treated with or without 10 mM TPM for 2 days. Twenty control or treated adult animals of the indicated age were transferred into 100 μL of M9 buffer to a well of a Costar 96-well microtitre plate (black, clear, flat-bottom wells), and total GFP fluorescence was measured using 485 nm excitation and 530 nm emission filters. Quadruple populations were used for each determination. For fluorescence microscopy, the worms were mounted with a drop of levamisole (10 mM) and placed on a cover slip covered with 3% agarose. The GFP pictures of transgenic worms were taken using an AXIO Imager M2 microscope system (Zeiss).

2.9. Quantitative real-time PCR

Adult worms were treated with or without 10 mM TPM for 2 days. Total RNA was extracted from adult worms with TRIzol reagent (Invitrogen, Carlsbad, CA), and cDNA was produced by oligo(dT) priming. The RT-PCR primers were as follows: daf-2 (NM_065249), 5′-GGCCGATGGACGTTATTTTG-3′ and 5′-TTCCACAGTGAAGAAGCCTGG-3′; daf-16 (NM_001026247), 5′-TTTCCG TCCCCGAACTCAA-3′ and 5′-ATTCGCCAACCCATGATGG-3′; sod-3 (NM_078363), 5′-AGCATCATGCCACCTACGTGA-3′ and 5′-CACCAC CATTGAATTTCAGCG-3′; skn-1 (NM_171347), 5′-AGTGTCGG CGTTCCAGATTTC-3′ and 5′-GTCGACGAATCTTGCGAATCA-3′;

hsp-16.2, 5′-CGTCGAAGAGAAATCTGCTGAA-3′ (left) and 5′-TGCAG CGAACAATACTGTAATTTATG-3′ (right) ctl-1, 5′-GCGGATACC GTACTCGTGAT-3′ and 5′-GTGGCTGCTCGTTAGTTGTGA-3′; ctl-2, 5′-TCCGTGACCCTATCCACTTC-3′ and 5′-TGGGATCCGTATCCATT-CAT-3′; ama-1, 5′-CTGACCCAAAGAACACGGTGA-3′ and 5′-TCCAATT CGATCCGAAGAAGC-3′.

mRNA expression was assessed by quantitative real-time PCR on a Rotor-Gene 6000 real-time PCR detection system (Qiagen, Düsseldorf) using SYBR green. The gene expression data were analysed using the comparative $2^{-\Delta\Delta Ct}$ method, using ama-1 mRNA as the internal control.

2.10. Statistical tools

One-way ANOVA was used to compare more than two groups, and when significant ($p < 0.05$), the Tukey HSD test was applied to test for differences between individual groups. $p < 0.05$ was considered statistically significant. All the data were analysed with Origin 8.0 (Northampton, MA). Standard error bars of the averages are shown in figures.

3. Results

3.1. TPM improves the stress resistance of C. elegans under stress conditions

To evaluate the potential longevity-extending effect of TPM on wild-type *C. elegans* N2 under oxidative stress, the worms that had just reached adulthood were pretreated with various concentration of TPM (5, 10, 20 mM) for 48 h and then exposed to juglone (500 μM). Juglone, a pro-oxidant that can be reduced by diaphorases in the presence of NAD(P)H, converts oxygen to superoxide anion and consequently increases intracellular oxidative stress. Our results showed that TPM pretreatment with 10 mM had a strong protective effect. As show by the statistical results, the mean survival rate was significantly increased by 27.6% in the TPM-treated groups compared with the control (Fig. 1A). The results indicated that TPM pretreatment improved the worms' resistance to oxidative stress induced by juglone.

In the thermo-tolerance assay, the worms that had just reached adulthood were pretreated with TPM (10 mM) for 48 h before being exposed to heat shock at 35 °C. The data showed that TPM treatment significantly increased the mean survival rate of the worms by 36.9% (Fig. 1B). The result indicated that TPM pretreatment enhanced the worms' resistance to heat stress, thus generating an increased survival rate under the heat shock.

3.2. TPM decreases the intracellular ROS level in C. elegans under oxidative stress

TPM may enhance the stress resistance of *C. elegans* by scavenging free radicals. To explore the mechanisms by which TPM enhanced the stress resistance of *C. elegans* under environmental stress, the free-radical scavenging abilities of TPM were evaluated in subsequent experiments. First, the total antioxidant ability of TPM was investigated in an *in vitro* chemical system containing $ABTS^+$. The data showed that TPM could effectively scavenge $ABTS^+$ and the IC_{50} (the concentration of antioxidant at which 50% of the reaction was inhibited) of TPM in this reaction system was 0.13 mM (Fig. 2A). The ability of TPM to remove superoxide anions was then investigated in a pyrogallol self-oxidation system and the data showed that TPM could effectively scavenge the free radicals produced by pyrogallol self-oxidation with an IC_{50} value of 0.0027 mM (Fig. 2B). Next, the ability of TPM to remove hydroxyl free radical was investigated in the Fenton's reaction system and the data showed that TPM could effectively scavenge the hydroxyl free radical with an IC_{50} value of 0.41 mM (Fig. 2C).

Furthermore, TPM was also demonstrated to have ROS-scavenging ability in *C. elegans*. Our data showed that the pretreatment with TPM at a concentration of 10 mM effectively reduced ROS accumulation in juglone (300 μM)-treated wild-type *C. elegans*. (compared with the control, $p < 0.05$; Fig. 2D).

These results showed that TPM is a versatile free radical scavenger both *in vitro* and *in vivo*.

3.3. TPM up-regulates SOD-3::GFP expression in transgenic C. elegans CF1553

To further study the mechanisms of the protective effects of TPM in *C. elegans*, SOD-3::GFP reporter gene expression in transgenic CF1553 treated with or without TPM treatment was investigated. The CF1553 worms were treated with or without TPM after stimulation with juglone (300 μM). Compared with the control group, the TPM-treated group demonstrated a higher SOD-3::GFP intensity as shown by confocal laser scanning microscopy (Fig. 3A and B). The intensity of the fluorescence was then quantified by a Thermo Labsystems Fluoroskan Ascent microplate reader and the result showed that TPM at 10 mM significantly up-regu-

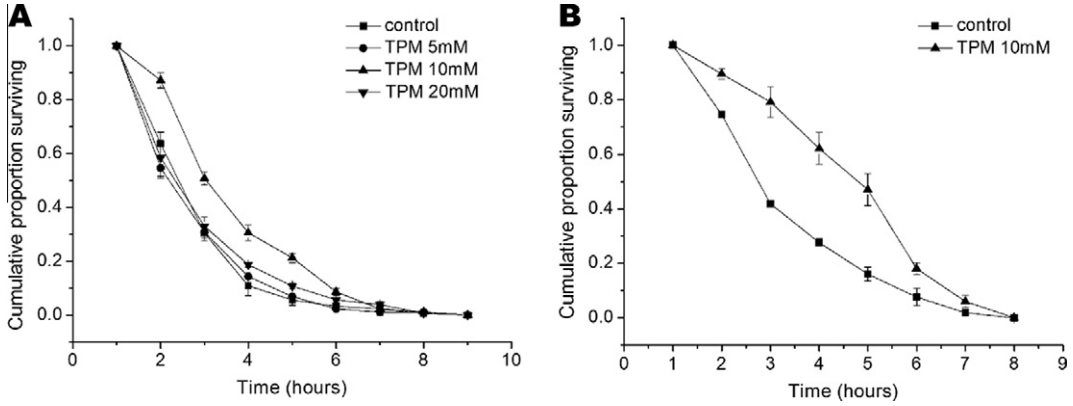

Fig. 1. TPM improves the stress resistance of *C. elegans* under stress conditions and up-regulates the stress-resistance-related proteins expression. Protective effects of TPM on wild-type *C. elegans* N2 under oxidative stress and heat stress. (A) Under oxidative stress, compared with the control, TPM extended the lifespan of *C. elegans* by 27.6% at 10 mM. (B) At 35 °C, compared with the control, TPM extended the lifespan of *C. elegans* by 36.9% at 10 mM. All survival curves are presented based on three individual experiments.

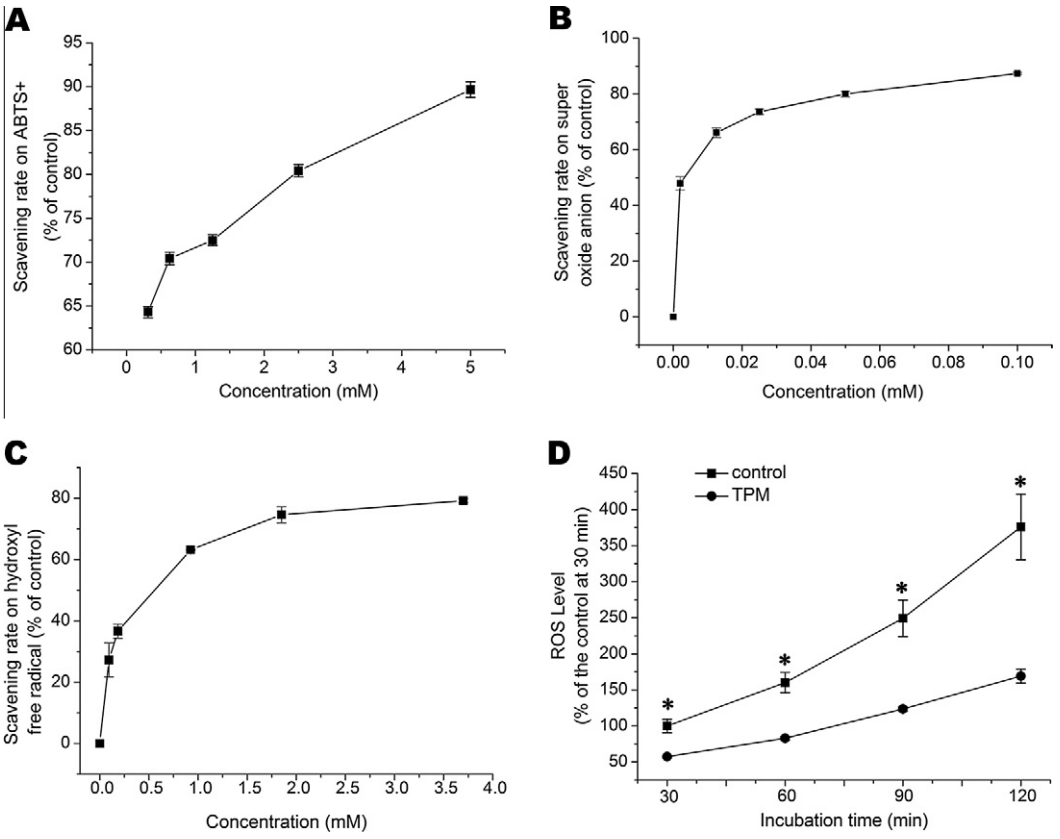

Fig. 2. Free radical-scavenging effect of TPM *in vitro* and *in vivo*. (A) TPM effectively scavenged $ABTS^{+}$. (B) TPM dramatically scavenged the free radicals generated by pyrogallol self-oxidation. (C) TPM effectively scavenged the hydroxyl free radical generated by Fenton's reaction. (D) TPM at 10 mM reduced ROS accumulation in *C. elegans* under juglone-generated oxidative stress, detected in a 2-h course. Error bars indicate SE; $^{*}p < 0.05$.

lated SOD-3::GFP expression by 47.2% in transgenic CF1553 (Fig. 3C), indicating that TPM could increase the expression of SOD-3::GFP expression under oxidative stress in *C. elegans*.

3.4. TPM up-regulates the expression of heat shock protein HSP-16.2 in transgenic C. elegans CL2070

HSP-16.2 can serve as a stress-sensitive reporter to predict longevity in *C. elegans* (Hsu, Murphy, & Kenyon, 2003; Rea et al., 2005). Higher levels of HSP-16.2::GFP predict longer remaining life expectancy of *C. elegans* (Hsu et al., 2003; Rea et al., 2005). The effect of TPM on the expression of HSP-16.2 was then investigated to provide further details on the protective effects of TPM on *C. elegans* under environmental stress. CL2070 worms containing the HSP-16.2::GFP reporter gene were treated with heat shock at 35 °C for 1 h and allowed to recover at 20 °C for 12 h. Compared with the control group, the 10 mM TPM-treated group showed higher HSP-16.2::GFP intensity, as shown by confocal laser scanning microscopy (Fig. 4A and B). The intensity of the fluorescence was then quantified and the data showed that TPM significantly up-regulated HSP-16.2::GFP expression by 55.2% in CL2070 (Fig. 4C, $p < 0.01$ compared with the control), indicating that TPM might increase the life expectancy of *C. elegans* under thermal stress by up-regulating the expression of HSP-16.2.

3.5. TPM regulates the mRNA expression of ageing-associated genes in wild-type C. elegans N2

A variety of genes are involved in the regulation of the life expectancy of *C. elegans*. Among them, DAF-16/forkhead transcription factor, the downstream target of the insulin-like signalling in *C. elegans*, is indispensable for both life span regulation and stress resistance (Lin, Hsin, Libina, & Kenyon, 2001). The down-regulation of DAF-16 can be caused by the activation of, the insulin/IGF-1 receptor DAF-2 that signals through a conservative PI3-kinase/AKT pathway (Dorman, Albinder, Shroyer, & Kenyon, 1995; Kenyon, Chang, Gensch, Rudner, & Tabtiang, 1993). One of the downstream effectors of DAF-16, SOD-3, also acts as an important regulator of life span and stress resistance in *C. elegans* (Braeckman & Vanfleteren, 2007; Dorman et al., 1995; Kenyon et al., 1993). SKN-1 is another transcription factor that can positively regulate the life span and stress resistance in *C. elegans* (An & Blackwell, 2003; An et al., 2005). CTL-1 and CTL-2 encode *C. elegans* catalases, which are predicted to function as antioxidant enzymes that protect *C. elegans* cells from reactive oxygen species. Quantitative real-time PCR experiments were performed to investigate whether TPM could regulate the expression of the ageing-associated genes daf-2, daf-16, sod-3, skn-1, hsp-16.2, ctl-1 and ctl-2. The results showed that TPM significantly down-regulated the expression of daf-2 ($p < 0.01$), and up-regulated the expression of daf-16 ($p < 0.01$), sod-3 ($p < 0.01$), hsp-16.2 ($p < 0.01$), ctl-1 ($p < 0.01$), ctl-2 ($p < 0.01$) and skn-1 ($p < 0.05$) (Fig. 5). The up-regulation of sod-3 expression by TPM is consistent with the effect of TPM on SOD-3::GFP expression. The results suggested that TPM might improve the stress resistance of *C. elegans* and extended life expectancy of the worms through regulating the above ageing-related genes.

4. Discussion

Traditionally, dietary protein is regarded as a source of energy and essential amino acids, which are needed for growth and main-

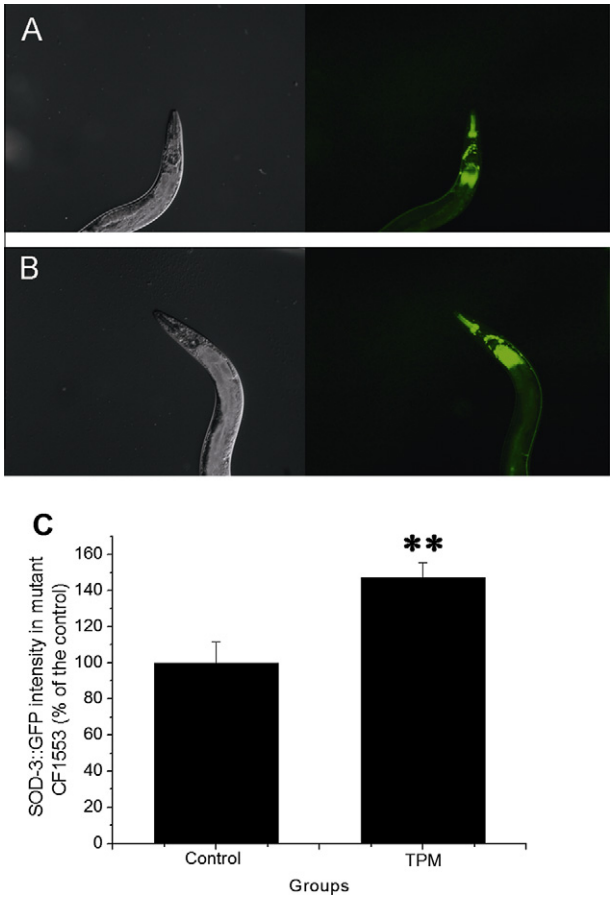

Fig. 3. TPM up-regulates SOD-3::GFP expression in transgenic *C. elegans* CF1553. (A) Image of SOD-3::GFP expression in control worms. (B) Image of SOD-3::GFP expression in 10 mM TPM-treated worms. The SOD-3::GFP expression in TPM-treated worms is higher than that in control worms. (C) Quantified GFP intensity (±SE) in CF1553 from three individual experiments with 30 worms in each treatment (**$p < 0.01$).

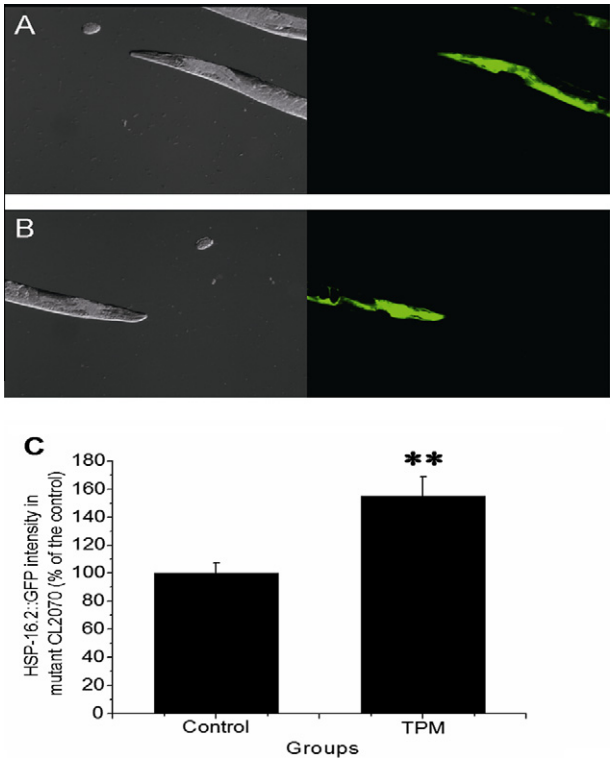

Fig. 4. Effects of TPM on the expression of heat shock protein HSP-16.2 in CL2070. (A) Image of HSP-16.2::GFP expression in control worms. (B) Image of HSP-16.2::GFP expression in the 10 mM TPM-treated group. The HSP-16.2::GFP expression in TPM-treated worms is higher than that in control worms. (C) Quantification of HSP-16.2::GFP intensity (±SE) in CL2070 from three individual experiments, with 30 worms in each treatment, was performed using a Thermo Labsystems Fluoroskan Ascent microplate reader (**$p < 0.01$).

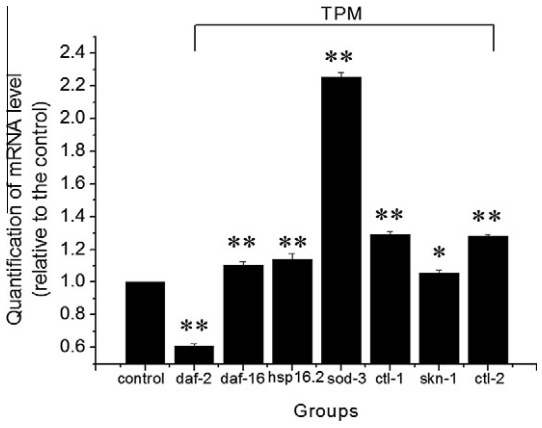

Fig. 5. Effects of TPM on the expression of ageing-associated genes in wild-type *C. elegans* N2. Quantitative real-time PCR was performed to investigate the effects of TPM on the expression of the ageing-associated genes daf-2, daf-16, hsp-16.2, sod-3, ctl-1, skn-1 and ctl-2 in *C. elegans*. The TPM group was treated with 10 mM TPM. Error bars indicate SE; *$p < 0.05$; **$p < 0.01$.

tenance of physiological functions. Recently, interest has been emerging to identify and characterize bioactive peptides from plant and animal sources. Based on their structural properties and their amino acid composition and sequences, these peptides may play various roles, such as opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidative, antithrombotic, hypocholesterolaemic and antihypertensive functions. Moreover, several peptides have been found to possess multifunctional properties (Sarmadi & Ismail, 2010). The present paper focuses on TPM significantly extending the survival of *C. elegans* under stress. However, we do not know whether TPM is released by enzymatic hydrolysis *in vivo* or not; this problem will need further investigation.

In this study, we found that TPM could significantly extend the life expectancy of *C. elegans* under heat stress or oxidative stress. Since the mortality in a population is usually closely associated with causes from environmental stress, these results demonstrated a great anti-ageing potential for TPM. Harman (1956) advanced the free radical theory of ageing and hypothesised that free radical species caused deterioration of an organism. Aerobic organisms have evolved to have a cellular metabolism that takes oxygen as an electron acceptor, but, as a consequence, they continuously generate ROS, namely hydroxyl radical, superoxide anion, and hydrogen peroxide; on the other hand, they possess antioxidant defence systems that can effectively remove these ROS (Harman, 1956). Does TPM enhance the stress resistance of *C. elegans* under environmental stress, prolonging its longevity by removing ROS? We received a positive answer from this study; TPM treatment significantly down-regulated ROS levels under oxidative stress (Fig. 2D).

Reductions in insulin/IGF-1 like signalling (IIS) have been associated with increased stress resistance and longevity in diverse species (Kenyon, 2005). In *C. elegans*, signalling through the IIS receptor DAF-2 ultimately directs the related kinases AKT-1, -2, and SGK-1 to phosphorylate the FOXO protein DAF-16, thereby

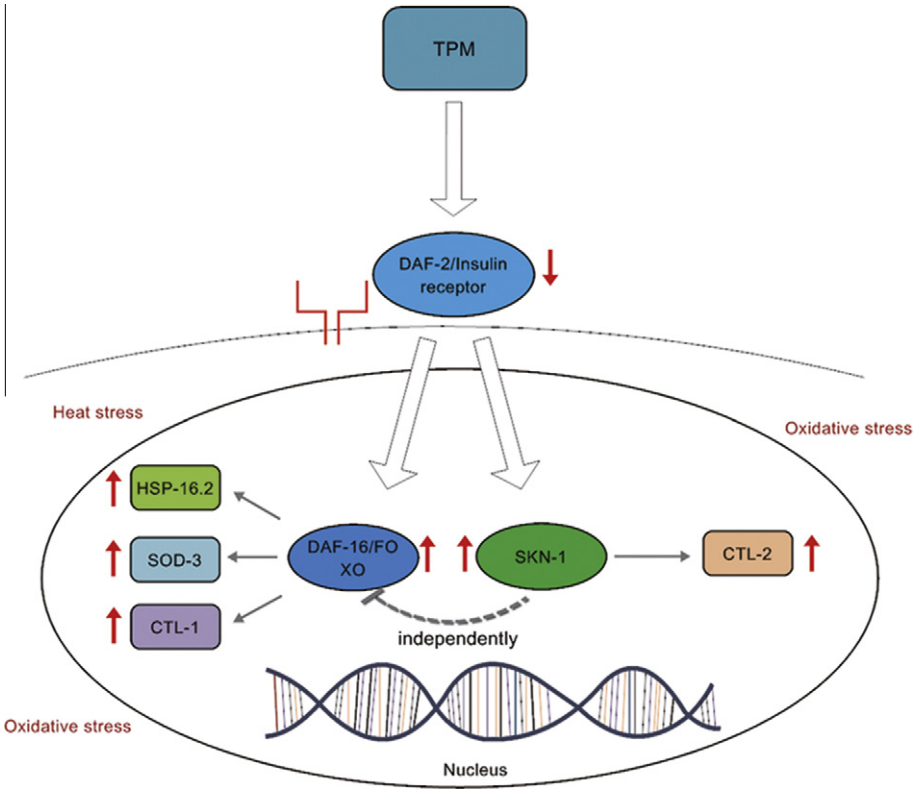

Fig. 6. TPM regulates the mRNA expression of ageing-associated genes in wild-type *C. elegans* N2. TPM affects insulin/IGF-1 like signalling (IIS) activation through acting with the insulin/IGF-1 receptor DAF-2 that signals through a conserved PI3-kinase/AKT pathway, ultimately causing the up-regulation of DAF-16. Overexpression of DAF-16 then leads to the regulation of its downstream target genes (SOD-3, HSP-16.2, CTL-1) up-regulation. Reduced DAF-2 signalling should also allow overexpression of SKN-1, which in turn induces the up-regulation of its downstream target genes including CTL-2.

inhibiting its accumulation in nuclei (Henderson & Johnson, 2001; Hertweck, Gobel, & Baumeister, 2004; Lin et al., 2001; Ogg et al., 1997). DAF-16 is required for phenotypes that are associated with decreased IIS, including increases in stress resistance, longevity, and the propensity to undergo diapause, the formation of long-lived dauer larvae that can survive adverse environmental conditions (Kenyon, 2005; Kenyon et al., 1993; Kimura, Tissenbaum, Liu, & Ruvkun, 1997). DAF-16 regulates genes that represent diverse processes, including resistance to oxidative and other stresses (Antebi, 2007; Kenyon, 2005). Overexpression of at least some genes up-regulated in the long-lived mutants, such as superoxide dismutase (Sun, Folk, Bradley, & Tower, 2002; Sun & Tower, 1999) and small heat-shock protein genes (Walker & Lithgow, 2003; Wang, Kazemi-Esfarjani, & Benzer, 2004), have been shown to extend lifespan.

The *C. elegans* transcription factor SKN-1 also defends against oxidative stress by mobilising the conserved phase 2 detoxification responses. SKN-1 that is constitutively active increases lifespan independently of DAF-16. A recent study indicates that the transcription network regulated by SKN-1 promotes longevity and is an important direct target of IIS (Tullet et al., 2008).

In our study, we found that TPM could affect IIS activation through the insulin/IGF-1 receptor DAF-2 that signals through a conserved PI3-kinase/AKT pathway, ultimately causing the up-regulation of DAF-16. SOD-3, HSP-16.2 and CTL-1, which are the downstream effectors of DAF-16 and can serve as stress-sensitive reporters to predict longevity in *C. elegans* (Hsu et al., 2003; Rea et al., 2005; Wolff et al., 2006). In addition, reduced insulin/IGF-1 signalling (IIS) causes nuclear accumulation of SKN-1 (Tullet et al., 2008). We found that TPM could up-regulate the expression of SKN-1 through inhibiting DAF-2 activation. Overexpression of SKN-1 induces CTL-2, a catalase that protects cells from ROS, while the up-regulation of SOD-3, HSP-16.2 and CTL-1 do not require the expression of SKN-1 (Back, Matthijssens, Vlaeminck, Braeckman, & Vanfleteren, 2010). (Fig. 6). Fluorescence quantitative real-time PCR results suggested that TPM could significantly up-regulate the expression of the ageing-associated genes DAF-16, SOD-3, HSP-16.2, SKN-1, CTL-1 and CTL-2, partly explaining how TPM could significantly extend the longevity of *C. elegans* under heat stress and oxidative stress.

In our study, we found that the longevity-improving effects of TPM in the nematode *C. elegans* under stress might be attributed to its direct ROS-scavenging activity and indirect free radical-scavenging activity, through regulating some anti-ageing associated genes, such as daf-2, daf-16, sod-3, hsp-16.2, skn-1, ctl-1 and ctl-2 in wild-type worms. These interesting findings highlight the potential of TPM to extend human average life expectancy by providing protection against environmental stress.

Acknowledgements

This work was supported by grants from the National High Technology Research and Development Program (“863” Program) of China (2008AA10Z321) and the National Natural Sciences Foundation of China (30970719, 30370361, 30930036), Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences.

.

References

An, J. H., & Blackwell, T. K. (2003). SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. *Genes and Development, 17*(15), 1882–1893.

An, J. H., Vranas, K., Lucke, M., Inoue, H., Hisamoto, N., & Matsumoto, K. (2005). Regulation of the Caenorhabditis elegans oxidative stress defense protein SKN-1 by glycogen synthase kinase-3. *Proceedings of the National Academy of Sciences of the USA, 102*(45), 16275–16280.

Antebi, A. (2007). Genetics of aging in Caenorhabditis elegans. *PLoS Genetics, 3*(9), 1565–1571.

Back, P., Matthijssens, F., Vlaeminck, C., Braeckman, B. P., & Vanfleteren, J. R. (2010). Effects of sod gene overexpression and deletion mutation on the expression profiles of reporter genes of major detoxification pathways in *Caenorhabditis elegans*. *Experimental Gerontology, 45*(7–8), 603–610.

Braeckman, B. P., & Vanfleteren, J. R. (2007). Genetic control of longevity in *C. elegans*. *Experimental Gerontology, 42*(1–2), 90–98.

Dorman, J. B., Albinder, B., Shroyer, T., & Kenyon, C. (1995). The age-1 and daf-2 genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. *Genetics, 141*(4), 1399–1406.

Hansen, M., Hsu, A. L., Dillin, A., & Kenyon, C. (2005). New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. *PLoS Genetics, 1*(1), 119–128.

Harman, D. (1956). Aging: A theory based on free radical and radiation chemistry. *Journal of Gerontology, 11*(3), 298–300.

Henderson, S. T., & Johnson, T. E. (2001). Daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Current Biology, 11*(24), 1975–1980.

Hertweck, M., Gobel, C., & Baumeister, R. (2004). *C. elegans* SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. *Developmental Cell, 6*(4), 577–588.

Hsu, A. L., Murphy, C. T., & Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science, 300*(5622), 1142–1145.

Kenyon, C. (2005). The plasticity of aging: Insights from long-lived mutants. *Cell, 120*(4), 449–460.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., & Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. *Nature, 366*(6454), 461–464.

Kimura, K. D., Tissenbaum, H. A., Liu, Y., & Ruvkun, G. (1997). Daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science, 277*(5328), 942–946.

Lin, K., Hsin, H., Libina, N., & Kenyon, C. (2001). Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germLine signaling. *Nature Genetics, 28*(2), 139–145.

Lithgow, G. J., White, T. M., Melov, S., & Johnson, T. E. (1995). Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proceedings of the National Academy of Sciences of the USA, 92*(16), 7540–7544.

Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A., & Van Remmen, H. (2007). Trends in oxidative aging theories. *Free Radical Biology and Medicine, 43*(4), 477–503.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., & Tissenbaum, H. A. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature, 389*(6654), 994–999.

Rea, S. L., Wu, D., Cypser, J. R., Vaupel, J. W., & Johnson, T. E. (2005). A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. *Nature Genetics, 37*(8), 894–898.

Sang-Kyu, P., Patricia, M. T., & Thomas, E. J. (2009). Oxidative stress and longevity in *C. elegans* as mediated by SKN-1. *Aging Cell, 8*(3), 258–269.

Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: A review. *Peptides, 31*(10), 1949–1956.

Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M., & Ristow, M. (2007). Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metabolism, 6*(4), 280–293.

Sun, J., Folk, D., Bradley, T. J., & Tower, J. (2002). Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult *Drosophila melanogaster*. *Genetics, 161*(2), 661–672.

Sun, J., & Tower, J. (1999). FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Molecular and Cellular Biology, 19*(1), 216–228.

Tanja, H., Kai, H., Hannelore, D., & Uwe, W. (2010). *Caenorhabditis elegans* lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. *Biogerontology, 11*, 183–195.

Tullet, J. M., Hertweck, M., An, J. H., Baker, J., Hwang, J. Y., & Liu, S. (2008). Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. *Cell, 132*(6), 1025–1038.

Walker, G. A., & Lithgow, G. J. (2003). Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. *Aging Cell, 2*(2), 131–139.

Wang, H. D., Kazemi-Esfarjani, P., & Benzer, S. (2004). Multiple-stress analysis for isolation of *Drosophila* longevity genes. *Proceedings of the National Academy of Sciences of the USA, 101*(34), 12610–12615.

Wolff, S., Ma, H., Burch, D., Maciel, G. A., Hunter, T., & Dillin, A. (2006). SMK-1, an essential regulator of DAF-16-mediated longevity. *Cell, 124*(5), 1039–1053.

Xu, L., Li, X. L., Wu, X. X., Wang, H., Hou, R. Z., & Huang, Y. B. (2004). Preparation and structural characterization of a new corn anti-oxidative peptide. *Chemical Journal of Chinese Universities-Chinese, 25*(3), 466–469.