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DHS-21, a dicarbonyl/L-xylulose reductase (DCXR) ortholog, regulates longevity and reproduction in *Caenorhabditis elegans*

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ABSTRACT

Dicarbonyl/L-xylulose reductase (DCXR) converts L-xylulose into xylitol, and reduces various α-dicarbonyl compounds, thus performing a dual role in carbohydrate metabolism and detoxification. In this study, we identified DHS-21 as the only DCXR ortholog in *Caenorhabditis elegans*. The *dhs-21* gene is expressed in various tissues including the intestine, gonadal sheath cells, uterine seam (utse) cells, the spermathecal-uterus (sp-ut) valve and on the plasma membrane of spermatids. Recombinant DHS-21 was shown to convert L-xylulose to xylitol using NADPH as a cofactor. *Dhs*-21 null mutants of *C. elegans* show defects in longevity, reproduction and egg-laying. Knock-down of *daf-16* and *elt-2* transcription factors affected *dhs-21* expression. These results suggest that DHS-21 is a bona fide DCXR of *C. elegans*, essential for normal life span and reproduction.

1. Introduction

Dicarbonyl/L-xylulose reductase (DCXR), which converts L-xylulose to xylitol, belongs to the short-chain dehydrogenase/reductase (SDR) and aldo–keto reductase superfamilies. In some fungi and yeasts, DCXR functions in the oxy-reductive L-arabinose pathway to catabolize L-arabinose, the second most abundant pentose sugar after L-xylose in plants [1]. In higher organisms, DCXR functions in the glucuronate pathway, an alternative route to glucose-6-phosphate oxidation, which accounts for up to 5% of total glucose catabolism in humans [2]. DCXR deficiency causes human essential pentosuria, which is often misdiagnosed as diabetes because the patient's urine displays enormous reducing power. This reflects the high urinary content of L-xylulose, a five-carbon reducing sugar, in this condition [3].

Carbonyl compounds are routinely generated in the course of various metabolic reactions and by oxidative stress in vivo [4]. Molecules bearing α-dicarbonyl groups are highly reactive and tend to combine with vital tissue components to produce advanced glycation end-products (AGEs). The AGE content of tissues is associated with kidney renal failure, and a constellation of age-related diseases. DCXR reduces certain α-dicarbonyl compounds, as well as aromatic aldehydes and aromatic ketones, from both endogenous and xenobiotic sources [5].

DCXR expression displays tissue-specific regulation in both normal and clinical conditions. Expression profiling and protein purification reveal high levels of DCXR expression in mammalian renal tubules and liver extracts [5]. The DCXR may also be essential for human sperm maturation and fertility, because P34H, a protein identical to DCXR, is highly expressed in epididymis and is absent on the surface of less motile sperm from infertile males [6]. Human

Abbreviations: DCXR, dicarbonyl/L-xylulose dehydrogenase; SDR, short-chain dehydrogenase/reductase; NGM, nematode growth medium; EM, electron microscopy

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prostate cancers and melanomas may up-regulate DCXR [7,8] and expression is markedly increased in T lymphoma cells induced to undergo apoptosis by toxic environmental substances [9]. However, DCXR gene regulation in normal physiological processes is not well understood.

Although properties of DCXRs from various species have been characterized biochemically, regulatory features of the gene have been little explored in genetically altered model organisms. *Caenorhabditis elegans*, a free-living soil nematode, provides a tractable model system for genetic studies of biological pathways that are evolutionarily conserved. In this study, we identified *dhs-21* as the only DCXR ortholog that reduces L-xylulose and diacetyl compounds in *C. elegans*. We found that *dhs-21* may also regulate lifespan and reproduction. *C. elegans* may therefore provide a useful model for research on and development of therapy for DCXR-related medical conditions, notably renal dysfunction and infertility.

2. Materials and methods

2.1. Genetic background and maintenance of C. elegans strains

The following *C. elegans* strains were obtained from the CGC (Caenorhabditis Genetics Center) at the University of Minnesota (St. Paul, MN, USA): Bristol type (N2), *daf-16(m26), daf-2(e1370),* and *him-8(e1489)*. Worms were grown on standard Nematode Growth Medium (NGM) seeded with *Escherichia coli* OP50 and handled according to the established methods.

2.2. Chemicals

All chemicals were purchased from Sigma–Aldrich Company (St. Louis, MO, USA), unless otherwise indicated.

2.3. Isolation of a dhs-21 deletion mutant

Screening for a *dhs-21* mutant from a mutational library generated by TMP (trimethylpsoralen)/UV mutagenesis was performed using nested PCR. A pair of outer primers (OutF: 5′- GGA ATG GCA TTG ATA ACA AAA ATA G-3′; OutB: 5′-AAC CTT TTT ACT GCC AGC TTA ATT TC-3′) was used for the first round of PCR using single-worm lysate as a template. The inner primer pair (InnerF: 5′-TCA AGG TAA GTC TTG AGA TGT TGA A-3′; InnerB: 5′-ATG GAA TGA TAA GAT CAC GAA AGA A-3′) was used for the second round of PCR using the first round of PCR products as a template. The primer located in the deletion region (DelB: 5′-CCA TCG GAA GTC TTC ATG AAC GAT-3′) was paired with the InnerF primer to confirm homozygous deletion mutants.

2.4. Constructs and transgenic animals

To construct pAN249 (His(6X)::DHS-21), *dhs-21* cDNA was amplified from a cDNA library with the following primers: dhs21 senseF4 (5′-CCGGAATTCCCCGCCAATTACGATTTCAC-3′), and dhs21 antisense R4 (5′-CCGCTCGAGCGGTTAGTTATTCGAAAATCCTC-3′). To construct the GFP fusion expression plasmid, the 2-kb upstream sequence of the *dhs-21* gene was amplified by PCR using R2 and R3 primers (R2: 5′-CCC AAG CTT GAT GAA ACG GTT AG-3′; R3: 5′-CTA GTC TAG ACA TTG TTT CGT CAG ATG ATA-3′). The *dhs-21* genomic DNA spanning the entire coding region was amplified by PCR using IF and IR primers (IF: 5′-AAA CTG CAG GAG ATT AAG AAA TG-3′; IR: 5′-TTC CCC GGG GTT ATT CGA AAA TC-3′). These PCR products were cloned into the pPD95.79 vector to obtain the GFP fusion pAN499 construct (p*dhs-21*::*DHS-21*::*gfp*). pAN499 (100 ng/μl) was co-injected with injection marker plasmid pRF4 (100 ng/μl) into the N2 wild type worms to obtain transgenic progeny designated as KJ 6063.

2.5. Feeding RNAi

Synchronized young adults (P_0) were placed on *daf-16* or *elt-2* Arhinger RNAi plates. DHS-21 GFP reporter expression or DHS-21 protein levels were measured in young adults of F1 generation.

2.6. Phenotypic analysis

The analyses of lifespan, brood size, and serotonin-or imipramine-mediated egg-laying behavior were described previously [10].

2.7. Recombinant DHS-21

The His(6x)-tagged recombinant DHS-21 protein was prepared according to the standard method. Western blotting was performed using polyclonal rabbit anti-calsequestrin antibodies, and anti-DHS-21 antibody that was raised against recombinant DHS-21.

2.8. DCXR assay

The DCXR assay was a modification of the previous method [5]. The standard reaction mixture for the DCXR activity consisted of 0.1 M potassium phosphate buffer (pH 7.0), 0.1 mM NADPH (NADH), at least five different concentrations between 0.02 and 8 mM of L-xylulose, and recombinant worm or rat DCXR in a total volume of 1.0 ml. We used this assay to obtain apparent K_m and V_{max} values. For the reverse reaction, xylitol and $NADP^+$ were used in place of substrate and NADPH, respectively.

2.9. Microscopy

Epi-fluorescence of GFP expression in transgenic worms was imaged using Axio Imager A1 microscope (Carl Zeiss, Germany), and AxioVision image analysis program, AxioVs40 (v.4.6.3.0) (Carl Zeiss Imaging Solution GmbH). For colocalization analysis, images were captured using Carl Zeiss confocal microscope equipped with the BD CARV II™ Confocal Imager, and manipulated with iVision v4.0.11 software (Biovision Technologies, Exton, PA).

3. Results

3.1. C. elegans DHS-21 is a DCXR homolog using NADPH as a cofactor

DCXRs reduce L-xylulose to xylitol in the presence of NADPH or NADH (Fig. 1A). A Blast search revealed that *dhs-21* is the only putative DCXRin *C. elegans*. A Clustal X analysis showed that the deduced amino acid sequence of *C. elegans* DHS-21 is approximately 47% identical to other mammalian DCXR orthologs, and 68% similar to human DCXR at the protein level (Fig. 1B).

All of the known DCXRs except ALX1 from *Ambrosiozyma monospora* use NADPH as an enzymatic cofactor [11]. Most residues engaged in binding NADPH in human DCXR were well conserved in DHS-21 (Table S1). To confirm that DHS-21 is a bonafide NADPH-dependent DCXR in the worm, we assayed the enzymatic activity of recombinant DHS-21in the presence of either NADPH or NADH. The recombinant DHS-21 reduced not only L-xylulose, but also various other sugars and diacetyl ketones in the presence of NADPH, but not NADH (Table 1). The DHS-21 activity was comparable to that of rat DCXR. Under these conditions, the reverse reaction, from xylitol to L-xylulose, was not observed using