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Authors

Sea, Kevin W
Sheng, Yuewei
Lelie, Herman L
et al.

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Yeast copper–zinc superoxide dismutase can be activated in the absence of its copper chaperone

Kevin W. Sea,
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA

Department of Chemistry, Pomona College, 645 N. College Ave., Claremont, CA 91711, USA

Yuewei Sheng,
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA

Herman L. Lelie,
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA

Bruin Biometrics LLC, 10960 Wilshire Blvd., Suite 950, Los Angeles, CA 90024, USA

Lindsay Kane Barnese,
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA

Concordia University, 1530 Concordia West, Irvine, CA 92612, USA

Armando Durazo,
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA

Department of Chemical and Environmental Engineering, University of Arizona, 1133 E. James E. Rogers Way, Room 108, Tucson, AZ 85721, USA

Joan Selverstone Valentine, and
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA

Department of Bioinspired Science, Ewha Womans University, Seoul 120-750, Republic of Korea

Edith Butler Gralla
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA

Kevin W. Sea: kevin.sea@pomona.edu

Abstract

Copper–zinc superoxide dismutase (Sod1) is an abundant intracellular enzyme that catalyzes the disproportionation of superoxide to give hydrogen peroxide and dioxygen. In most organisms, Sod1 acquires copper by a combination of two pathways, one dependent on the copper chaperone for Sod1 (CCS), and the other independent of CCS. Examples have been reported of two exceptions: *Saccharomyces cerevisiae*, in which Sod1 appeared to be fully dependent on CCS, and

Correspondence to: Kevin W. Sea, kevin.sea@pomona.edu.

Caenorhabditis elegans, in which Sod1 was completely independent of CCS. Here, however, using overexpressed Sod1, we show there is also a significant amount of CCS-independent activation of *S. cerevisiae* Sod1, even in low-copper medium. In addition, we show CCS-independent oxidation of the disulfide bond in *S. cerevisiae* Sod1. There appears to be a continuum between CCS-dependent and CCS-independent activation of Sod1,with yeast falling near but not at the CCS-dependent end.

Keywords

Disulfide bond; Sod1; Copper chaperone; CCS1; Copper transport

Introduction

Copper–zinc superoxide dismutase (Sod1) is a homodimeric enzyme in which each subunit contains one copper ion, one zinc ion, and an intrasubunit disulfide bond. The enzyme catalyzes the disproportionation of two superoxide molecules into dioxygen and hydrogen peroxide [1]. Copper is required for any activity, and full activity requires oxidation of the disulfide bond (disulfide-reduced Sod1 has only 10 % activity) [2].

As a redox-active compound, copper is normally sequestered from cellular components susceptible to damage [3]. Sequestration includes carefully controlled access of copper to the interior of the cell as well as chaperone transport of copper inside the cell. The copper chaperones for human Sod1 (hSod1) and yeast Sod1 (ySod1) were discovered in 1997 [4]. In 1998, overexpression of hSod in yeast cells showed some activation of Sod1 independent of the copper chaperone for Sod1 (CCS) [5]. In 2004, Carroll et al. [6] verified that a CCS-independent path for copper acquisition existed in hSod1. Experiments suggested, however, that no such CCS-independent pathway existed for Sod1 in *Saccharomyces cerevisiae* [4, 6–8]. *Caenorhabditis elegans* was found to have no CCS homolog, but its Sod1 was fully activated [9]. Thus, there appeared to be a spectrum of Sod1 activation, with full CCS dependence (in *S. cerevisiae*) at one end and full CCS independence (in *C. elegans*) at the other end.

The presence of a disulfide bond in a cytosolic protein such as Sod1 is relatively rare [10], yet it is conserved in Sod1 enzymes. Partly owing to the rigidity of Sod1 (wild-type, holo bovine Sod1 maintains its structure up to 92 °C [11]), most researchers originally believed the disulfide bond served a structural purpose. However, the backbone of the disulfide-reduced Sod1 is very similar to that of the disulfide-oxidized Sod1, leading some to hypothesize a functional role for the disulfide bond [11]. Formation of the disulfide bond in ySod1 was believed to be dependent on CCS [7] (in yeast, yCcs1) and to require the presence of O_2 [12].

Here, we report that ySod1 can be activated and the disulfide bond can be formed without yCcs1. The yCcs1-independent pathway does not provide enough activity to rescue the lysine auxotrophy (normally seen in *sod1*Δ cells) unless ySod1 is overexpressed. However, when it is overexpressed, the amount of activity is enough to rescue lysine auxotrophy, even in low-copper medium. In addition, we tested ySod1 for copper content in vivo in the absence of yCcs1 and found some copper in ySod1 both when ySod1 was overexpressed and when ySod1 was expressed at single-copy levels.

Materials and methods

Yeast strains and plasmids

The yeast strains and plasmids are given in Tables 1 and 2. Yeast strain EG335 was derived from yeast strain EG103.

Culture conditions

Yeast cells were grown at 30 °C, with 220-rpm shaking, in, as required, yeast extract-peptone, synthetic dropout, or synthetic complete medium, each with 2 % glucose [13]. For low-copper experiments, 100 or 250 μM bathocuproine disulfonate (BCS) was added to the medium.

Growth studies and Sod1 activity gels

Growth in media lacking lysine was assayed by streaking yeast on plates selective for the plasmid marker, as applicable. After growth from 3 to 5 days, depending on the vigor of the strain, overnight liquid cultures of synthetic dropout minus the appropriate plasmid-selectable marker were inoculated with colonies. For low-copper experiments, 100 or 250 μM BCS was added to the medium. After approximately 24 h, 10 mL of medium without lysine was inoculated with colonies from the overnight cultures to a cell density corresponding to an optical density at 600 nm of 0.05. Growth was assayed, normally after 24 h, by the cell density at 600 nm. For Sod1 activity gels, cells grown similarly were centrifuged, washed with ice-cold water, and lysed by shaking with glass beads in 50 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.5, containing 0.1 mM EDTA and protease inhibitor cocktail (Roche Complete EDTA-free). Lysates were separated by nondenaturing gel electrophoresis. Gels were stained with nitroblue tetrazolium [14], and the Sod1 activity appeared as a clear band in contrast to the purple background.

Immunoblotting

For assays of disulfide oxidation status, immunoblots (Western blots) were done using antibodies against ySod1 [8] and yCcs1 [7], both obtained from the Culotta laboratory at Johns Hopkins University. Secondary antibodies contained infrared dye (LI-COR goat anti-rabbit), with quantification done using an Odyssey imager (LI-COR Biosciences).

Oxidation state of the disulfide bond

The status of the disulfide bonds was assayed by labeling reduced thiols with 4-acetamido-4′-maleimidylstilbene-2,2′-disulfonate (AMS; Invitrogen) or iodoacetamide (IAM; Sigma-Aldrich). Disulfide-oxidized Sod1 runs faster in sodium dodecyl sulfide polyacryalamide gel electrophoresis (SDS-PAGE) than disulfide-reduced Sod1 in which the free thiols have been labeled with either AMS or IAM. Yeast cells were grown and lysed as indicated in “Growth studies and Sod1 activity gels,” except that lysis was conducted in an inert-atmosphere box. IAM was then added to the crude lysates, which were then incubated in an inert-atmosphere box for 1 h at room temperature, followed by centrifugation at 10,000*g* for 5 min. To verify which bands were oxidized and which were reduced, aliquots of the same lysates were incubated in 5 mM hydrogen peroxide for 30 min at room temperature, prior to labeling with IAM. Protein content was assayed by Bradford assay using Quick Start Bradford dye reagent (Bio-Rad) with bovine serum albumin as the standard. Lysates were separated by nonreducing SDS-PAGE and probed by immunoblot. The percentage of disulfide oxidation was determined using an Odyssey imager (LI-COR Biosciences).

Metal binding

Metals in Sod1 in yeast cell lysates were determined by a high-performance liquid chromatography (HPLC)–inductively coupled plasma mass spectrometry (ICP-MS) method [15]. Briefly, yeast cells were grown for 48–96 h, depending on the vigor of the strain, and were lysed with Y-PER yeast protein extraction reagent (Pierce). Lysates were separated on a G75 size-exclusion column in metal-free phosphate-buffered saline, pH 7.0, and fractions containing ySod1 were identified by activity assay [16] and by Western blot. Sod1-containing fractions were then run on an Agilent 1200 series HPLC instrument with a size-exclusion column (SW-2000, 7.8 mm × 300 mm, TOSOH Biosciences). The mobile phase was metal-free 25 mM potassium phosphate, pH 6.7, with 20 mM NaCl. Yeast Sod1 (ySod1) was eluted at approximately 15 min, and the concentration was determined using the area under the A_{214} absorbance curve relative to standards of known concentrations of ySod1. The eluent was immediately fed to an Agilent 7500ce ICP-MS instrument for determination of the metal content of the ySod1 peak.

Results

In the course of comparing the properties of ySod1 with hSod1, we put ySod1 on an overexpression plasmid. Because prior investigations into ySod1 activity appeared to show that ySod1 could only be activated in vivo in the presence of yCcs1 [4, 6–8], we put ySod1 on that overexpression plasmid into an *sod1*Δ *ccs1*Δ yeast strain, to use as a negative control. Surprisingly, we saw rescue of the *sod1*Δ lysine auxotrophy in the double-deletion (*sod1*Δ *ccs1*Δ) strain in addition to the *sod1*Δ strain. We noted that the first observation that hSod1 could be activated in yeast in the absence of CCS was made in an experiment in which hSod1 was similarly overexpressed [5]. Therefore, we went on to use overexpressed ySod1 to examine, in the presence and absence of yCcs1, Sod1 activity, the status of disulfide oxidation, and metal content in vivo.

Rescue of Sod1 lysine auxotrophy

Yeasts normally synthesize lysine, but yeasts lacking Sod1 activity are auxotrophic for lysine when grown aerobically [17]. Thus, a sensitive test for Sod1 activity is to grow yeast in medium lacking lysine [5]. Yeast Sod1 (ySod1) was cloned into the overexpression plasmid pRS424 and expressed in the EG118 (*sod1*Δ) and EG335 (*sod1*Δ *ccs1*Δ) yeast strains. In both cases, overexpression of ySod1 restored growth in medium lacking lysine, i.e., it rescued the *sod1*Δ lysine auxotrophy (Fig. 1), suggesting ySod1 was active, even in the absence of yCcs1.

Activity in low-copper medium

Leitch et al. [7] found ySod1 activity was reduced when cells were grown in low-copper medium, even when yCcs1 was present. Yeast Sod1 (ySod1) was expressed at normal levels (on a centromeric plasmid) in those experiments. To compare these results with the results for overexpressed ySod1, we tested rescue of lysine auxotrophy in the absence of yCcs1 in low-copper medium. EG118 (*sod1*Δ) and EG335 (*sod1*Δ *ccs1*Δ) cells overexpressing ySod1 were grown in medium lacking lysine but containing 100 or 250 μM BCS, an extracellular copper chelator. In this low-copper medium, overexpressed ySod1 was observed to rescue the *sod1*Δ lysine auxotrophy, both in the presence and in the absence of yCcs1 (Fig. 1).

Activity measured by native activity gel

Given the rescue of lysine auxotrophy by ySod1 in the absence of yCcs1, we investigated ySod1 activity in lysates directly using nondenaturing (native) electrophoresis activity gels. EG118 (*sod1*Δ) and EG335 (*sod1*Δ *ccs1*Δ) cells overexpressing ySod1 were grown for

approximately 24 h and lysed, and then 10 μg of total lysate protein was separated by native gel electrophoresis. Activity was assessed by the nitroblue tetrazolium assay. The colorless band in Fig. 2 (lane 8) shows that wild-type ySod1 was active in the absence of yCcs1. Note also that in the absence of yCcs1, normal expression levels of ySod1 (when 10 μg of total lysate protein was loaded) do not show up as active (strain JW101; lane 5), which is consistent with prior findings [7]. However, when we loaded 30–50 μg of total lysate protein from strain JW101 on a gel, ySod1 activity was apparent on the gel (data not shown).

In the native gel in Fig. 2, ySod1 overexpressed with or without yCcs1 (lanes 7 and 8) runs at slightly different rates. Our laboratory has observed for years that Sod1 on native activity gels such as in this study can separate into as many as three bands, and others have observed multiple bands with Sod1 from *C. elegans* [8] as well. The reason for the separation is unknown, and no correlation with the presence of yCcs1 has been noted. We recently purified yeast wild-type Sod1 and found both phosphorylated and unphosphorylated versions [18], and Leitch et al. [8] recently described phosphorylation of Sod1 at serine 38. The purified unphosphorylated and phosphorylated ySod1 are shown in lanes 1 and 2. The unphosphorylated ySod1 (lane 1) runs at the samerate as ySod1 in the presence ofyCcs1 (lanes 3 and 7). However, the phosphorylated ySod1 (lane 2) runs below the band for ySod1 in the absence of yCcs1 (lane 8). Thus, phosphorylation status alone does not appear to explain the difference in run rates with and without yCcs1.

Oxidation of the disulfide bond

Prior investigations showed oxidation of the disulfide bond in ySod1 to be dependent on yCcs1 [7]. However, the same investigations showed no activation of ySod1 without yCcs1. Since our lysine auxotrophy assays indicated that ySod1 could be activated independently of yCcs1, we investigated whether the disulfide bond could also be oxidized independently of yCcs1. To measure disulfide bond formation in vivo, strains with various combinations of yCcs1 and ySod1 were lysed anaerobically and were treated with IAM or AMS, to label reduced thiols, and the ySod1 was visualized by Western blot. Figure 3 shows a representative gel, in this case labeled with IAM. No disulfide-reduced ySod1 is detectable in yeast with wild-type levels of yCcs1 and ySod1 (lane 1). When ySod1 is overexpressed in the presence of yCcs1 (lane 3), most of the ySod1 is disulfide-oxidized and there is a small amount of disulfide-reduced ySod1. Notably, in the absence of yCcs1 (lane 4), although there is an increase in the amount of disulfide-reduced ySod1, a substantial fraction of the ySod1 (30 % as determined by the Odyssey imager) remains disulfide-oxidized. In some other experiments with AMS instead of IAM, we found up to 50 % of ySod1 had an oxidized disulfide bond (data not shown).

Metal binding in vivo

Activation of Sod1 requires the presence of copper in the active site of the enzyme. To determine which metal ions are bound in vivo by ySod1 in cells lacking yCcs1, we used a procedure to isolate ySod1 from cell lysates and to analyze it for metal content [15]. Cell lysates were prepared in metal-free buffer and separated by size-exclusion chromatography, and the ySod1 protein and metal content were identified by an HPLC and ICP-MS setup in tandem. Sod1 is eluted alone from the HPLC column, and the eluent can be directed immediately to an ICP-MS analyzer. Thus, this method provides a nearly simultaneous readout of the protein concentration and metal content of Sod1 as it is found in vivo. The results in Fig. 4 show that wild-type ySod1 expressed in the presence of yCcs1 (strain EG103) contained approximately 2 equiv of copper per ySod1 dimer, as expected for fully mature protein. On the other hand, ySod1 overexpressed in the presence of yCcs1 (*EG118/ySod1oe*) contained 0.81 equiv of copper, whereas in the absence of yCcs1 (*EG335/ySod1oe*), it contained 0.35 equiv of copper. The presence of a small amount of copper in

ySod1 expressed in the absence of yCcs1 is consistent with the enzymatic activity of ySod1 seen in this work. Surprisingly, normal levels of ySod1 expressed in the absence of yCcs1 (*EG335/ySod1*) contained 0.31 equiv of copper per ySod1 dimer, but did not appear active in other assays, probably because the total amount of active Sod1 is smaller in this case. Zinc exceeded 2 equiv per dimer in some cases. This is consistent with previous results in which Sod1 isolated from yeast expression systems contained no more than 4 equiv of metal per dimer, but the number of equivalents of zinc sometimes exceeded the number of equivalents of copper per dimer [19, 20].

Discussion

Sod1 activation involves both insertion of copper and oxidation of the intrasubunit disulfide bond. Insertion of copper is required for any enzymatic activity. Oxidation of the disulfide bond is required to increase the enzymatic activity from approximately 10 % in disulfide-reduced Sod1 to 100 % in disulfide-oxidized Sod1 [2].

There exist at least two pathways for both obtaining copper and oxidizing the disulfide bond. The first pathway depends on CCS both for delivery of copper and for oxidation of the disulfide bond. The second pathway is independent of CCS. Many species, including humans [21], mice [22], *Drosophila melanogaster* [23], *Arabidopsis thaliana* [24], and fission yeast [25], appear to use a combination of both a CCS-dependent and a CCS-independent pathway. At least one species, *C. elegans*, uses only the CCS-independent pathway since it has no CCS homolog. Prior to this work it was believed the budding yeast *S. cerevisiae* used only the yCcs1-dependent pathway and that its SOD1 was unable to be activated independently of yCcs1. However, we have shown here that *S. cerevisiae* Sod1 (ySod1) can be activated by the yCcs1-independent pathway as well. Thus, there are at present no known species in which the activation of Sod1 is 100 % dependent on CCS (Fig. 5). Sod1 of *C. elegans* still falls at the CCS-independent end, but for all the other species listed above, including *S. cerevisiae*, Sod1 falls somewhere in the middle of the range, having the ability to be activated both by CCS and by a pathway independent of CCS.

Before we discuss possible mechanisms for the CCS-independent pathway, we mention two items of note. First, although much of this work was done with overexpression levels of ySod1, we have shown that both overexpressed and normally expressed ySod1 do, in vivo, acquire copper in the absence of yCcs1 (Fig. 4). Given this finding, why was activity not detected in prior experiments? The likely explanation is that unless ySod1 is overexpressed, the total ySod1 activity in the cell does not cross the detection threshold of any readily available assay for Sod1 activity. In fact, when ySod1 expression levels were normal, our lysine auxotrophy (Fig. 1) and native activity gel (Fig. 2) experiments were in agreement with prior observations. The same experiments showed activity when yeast ySod1 was overexpressed.

The second item to note is the CCS-independent pathway was active even in low-copper medium (Fig. 1). This suggests copper acquisition by overexpressed ySod1 in the absence of yCcs1 is not due to simple mass action.

The CCS-independent pathway for copper

The nature of the CCS-independent pathway has been investigated and although some candidates for copper delivery have been ruled out, others have not. The first to receive attention were the copper chaperones other than CCS. In yeast, three copper chaperones are known: yCcs1, Atx1, and Cox17. Atx1 and Cox17 were unable to rescue the *ccs1*Δ phenotype in yeast, and thus appear to be ruled out as copper donors to Sod1 [6].

Also present in yeast are accessory proteins which have roles in delivery of copper and/or oxidative equivalents to cytochrome *c* oxidase, Cox17 and yCcs1 [26]. Most of these accessory proteins are secured in the intermembrane space of the mitochondrion by oxidation of their disulfide bond, with oxidizing equivalents being relayed by Mia40. To date there is no evidence of direct contact for delivery of copper or oxidizing equivalents between these accessory proteins and Sod1 [27].

Another possible copper donor is glutathione, which is known to bind copper [28]. When Carroll et al. [6] reduced the level of glutathione in yeast, activation of hSod1 was reduced significantly. In addition, Cu(I)–glutathione complexes were shown by Ciriolo et al. [29] to reconstitute apo-Sod1 rapidly in vitro. Thus, it appears glutathione may have some yet undefined role in CCS-independent copper delivery to Sod1.

Another possible copper donor is a low molecular weight, nonproteinaceous, anionic copper–ligand complex found in the mitochondrial matrix and described by Cobine et al. [30]. This complex has yet to be characterized, but it is conserved in mammals and in yeast, where it contains 85 % of mitochondrial copper [31]. The ligand appears to exist in the cytoplasm as well, where it is mostly in the apo form and uncharged, but a small amount is copper-bound. Thus, the complex may pick up copper in the cytoplasm for transport to the copper pool in the mitochondrial matrix, much as siderophores are used by bacteria to collect metals from outside the cell [31]. Copper is bound in the complex as Cu(I) and has a binding affinity approximating 10^{19}, but both hSod1 and cytochrome *c* oxidase can readily compete with the complex for copper [31]. There is no evidence that the complex delivers copper directly to Sod1, but a role for the complex in the CCS-independent pathway has not been ruled out.

The CCS-independent pathway for disulfide bond oxidation

In addition to copper insertion without yCcs1, we found oxidation of the disulfide bond in ySod1 can occurs independently of yCcs1 (Fig. 3). This contrasts with earlier experiments in which ySod1, not overexpressed, showed no oxidation of the disulfide bond in the absence of yCcs1 [7].

Like that for copper delivery, the mechanism of CCS-independent disulfide oxidation is unknown. In this CCS-independent pathway, the two electrons removed when the cysteine thiols are oxidized are presumably transferred to an electron acceptor via a relay similar to the CCS–Mia40–Erv1 relay. In the latter case, electrons are ultimately deposited by cytochrome *c* oxidase on dioxygen [32]. In the CCS-independent relay, the nature of the relay and the final electron acceptor are unknown. It is possible that Erv1, Mia40, or another disulfide-containing accessory protein in the intermembrane space could contact Sod1 directly, but to date no such contact between these accessory proteins and Sod1 has been detected [27].

Role of proline 144

Finally, it is worth discussing the role of proline 144 in both yCcs1-independent copper acquisition and disulfide bond formation. When proline 144 was removed from ySod1 in *ccs1*Δ yeast, ySod1 (not overexpressed) was able to acquire copper and its disulfide bond was oxidized [7]. Presumably, proline 144 confers increased dependence on yCcs1 but not absolute dependence, since we saw activation independent of yCcs1 even with proline 144 in place. Interestingly, proline 144 in Sod1 is limited to *Ascomycota* yeast [7], and confers on this phylum the increased dependence on CCS, possibly for purposes of regulation of Sod1 activity.

Conclusion

This work establishes that in yeast there is a yCcs1-independent mechanism of copper delivery and disulfide oxidation of ySod1. It appears there is a continuum, from almost complete dependence on CCS for activation of Sod1, to complete independence from CCS. Yeast Sod1 (ySod1), like Sod1 in humans and other species, falls between the extremes of this continuum, having some yet unknown mechanism of yCcs1-independent activation.

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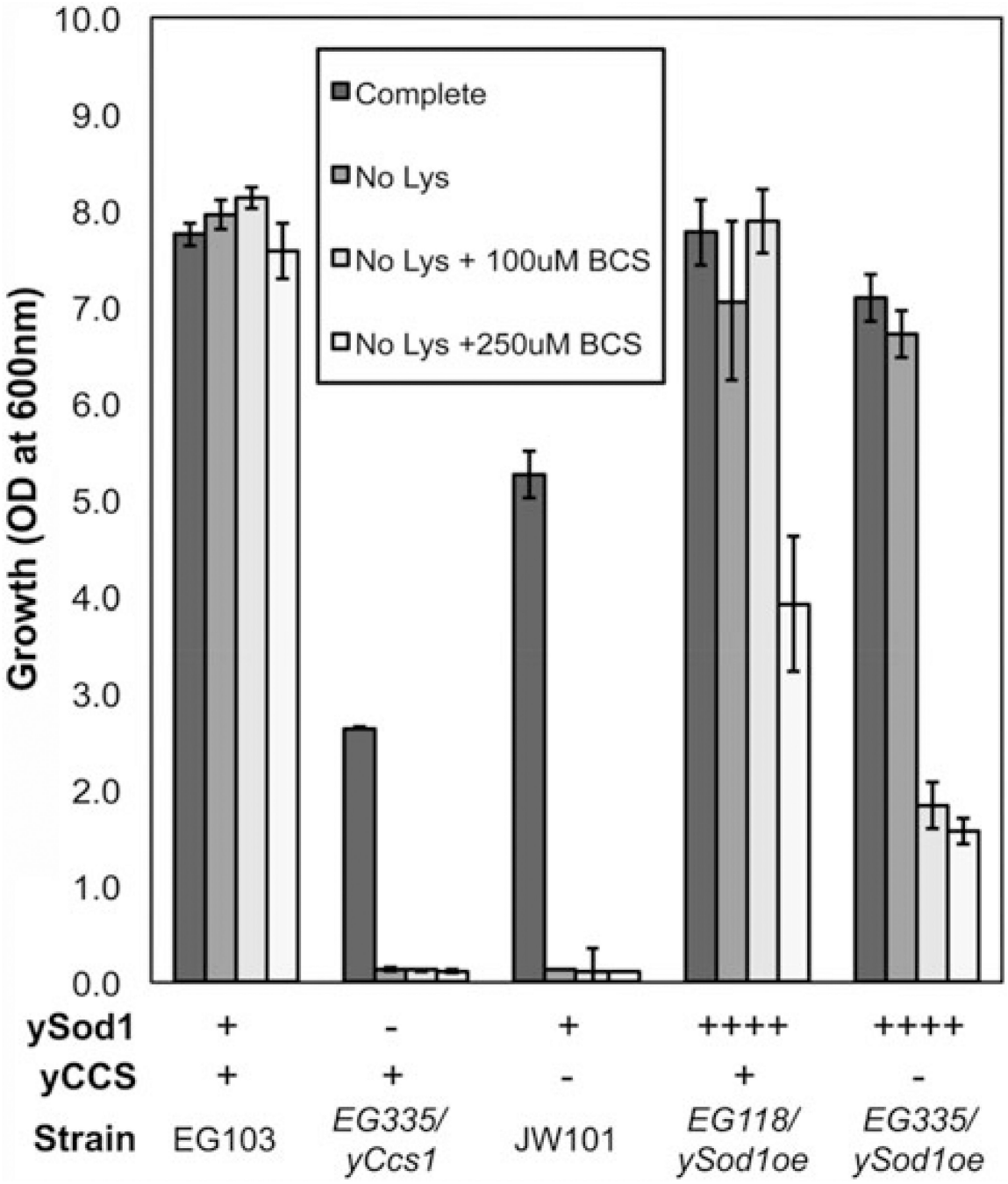

Fig. 1.

Overexpressed ySod1 rescues the lysine auxotrophy of *sod1*Δ yeast in the absence of yCcs1, even in low-copper medium. Low-copper medium was created by addition of bathocuproine disulfonate (*BCS*), an extracellular copper chelator. Cells were grown for 24 h and growth was monitored by the optical density (*OD*) at 600 nm. The yeast strains and plasmids were as follows: *group 1*, EG103; *group 2*, empty plasmid pRS424 in *EG335/yCcs1*; *group 3*, JW101; *group 4*, *EG118/ySod1oe*; *group 5*, *EG335/ySod1oe*. The growth media from *left to right* in each group were as follows: complete medium, no-lysine medium, no lysine plus

100 μM BCS, no lysine plus 250 μM BCS. Overexpressed ySod1 is indicated by *four plus signs*

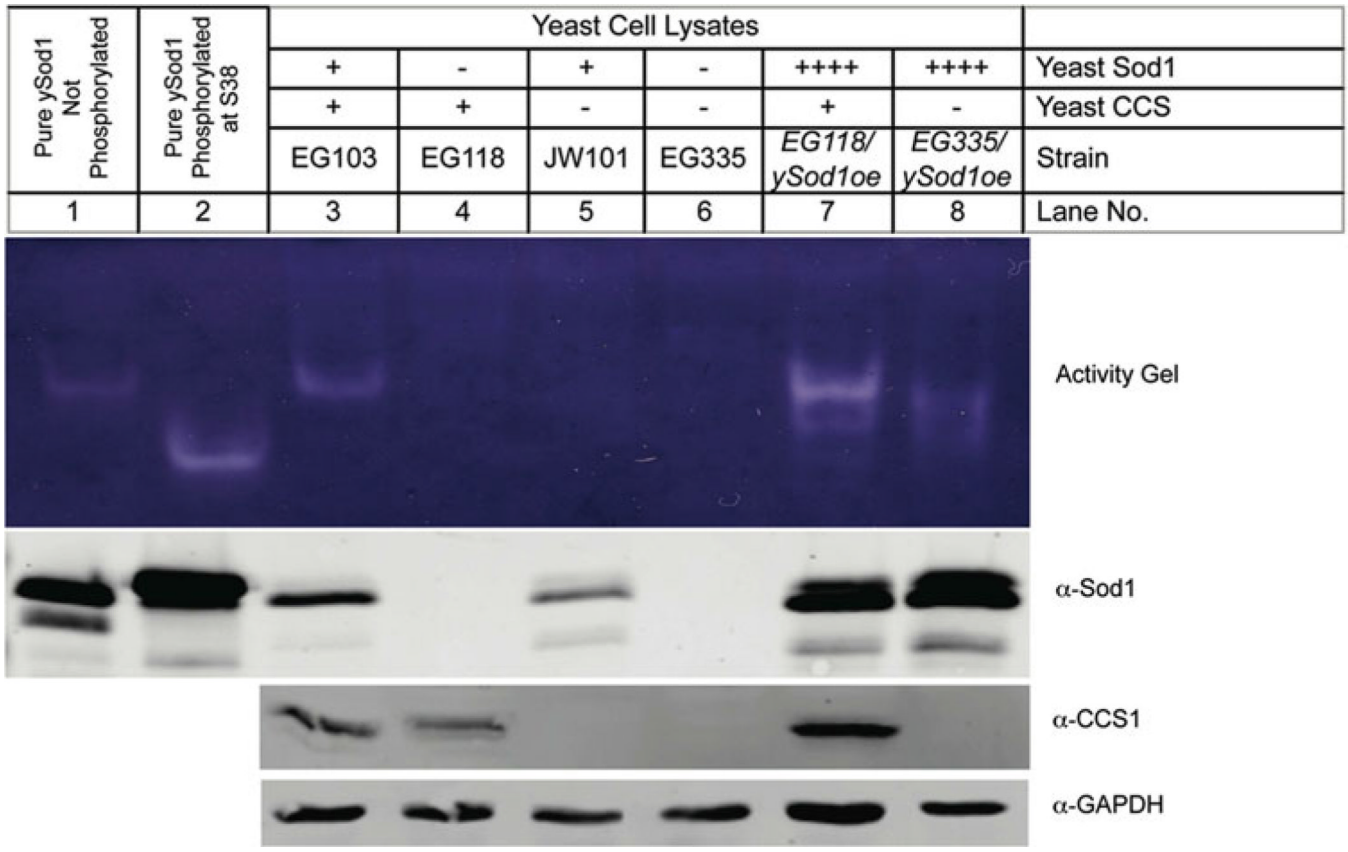

Fig. 2.

Overexpressed ySod1 shows activity in the absence of yCcs1. Activity was measured by nondenaturing (native) gel electrophoresis with nitroblue tetrazolium staining. All white bands are active ySod1; different ySod1 species run at different points on a native gel (see the text). In each of *lanes 3–8*, 10 μg of total lysate protein was added. *Lane 1* purified ySod1 protein, not phosphorylated; *lane 2* purified ySod1 protein, phosphorylated at serine 38; *lane 3* EG103; *lane 4* EG118; *lane 5* JW101; *lane 6* EG335; *lane 7 EG118/ySod1oe*; *lane 8 EG335/ySod1oe*. Overexpressed ySod1 is indicated by *four plus signs*. Below the activity gel are immunoblots using antibodies to ySod1, yCcs1, and yeast glyceraldehyde 3-phosphate dehydrogenase (*GADPH*; loading control). When we added excess (30–50 μg) total lysate protein to a gel, ySod1 activity was noted for strain JW101 (data not shown)

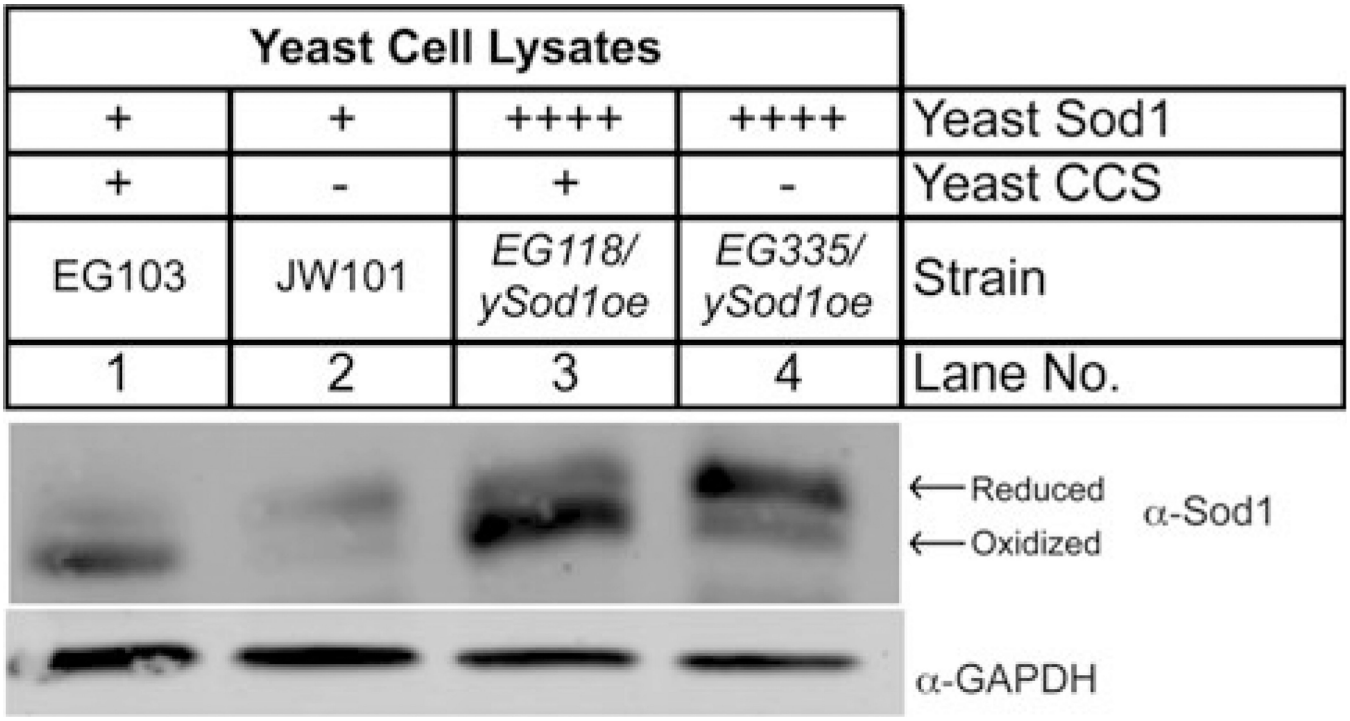

Fig. 3.

The ySod1 disulfide bond can be oxidized in the absence of yCcs1. Disulfide oxidation is identified by anaerobically lysing cells followed by addition of the thiol labeling agent iodoacetamide (IAM), running on nonreducing SDS-PAGE, and immunoblotting. IAM-labeled (reduced) Sod1 runs more slowly than nonlabeled (oxidized) Sod1, as indicated. All samples were treated with IAM. Yeast GADPH is shown as a loading control. *Lane 1* EG103; *lane 2* JW101; *lane 3 EG118/ySod1oe*; *lane 4 EG335/ySod1oe*. Overexpressed ySod1 is indicated by *four plus signs*. Verification of oxidized and reduced bands was done by running the same samples following treatment with hydrogen peroxide (data not shown)

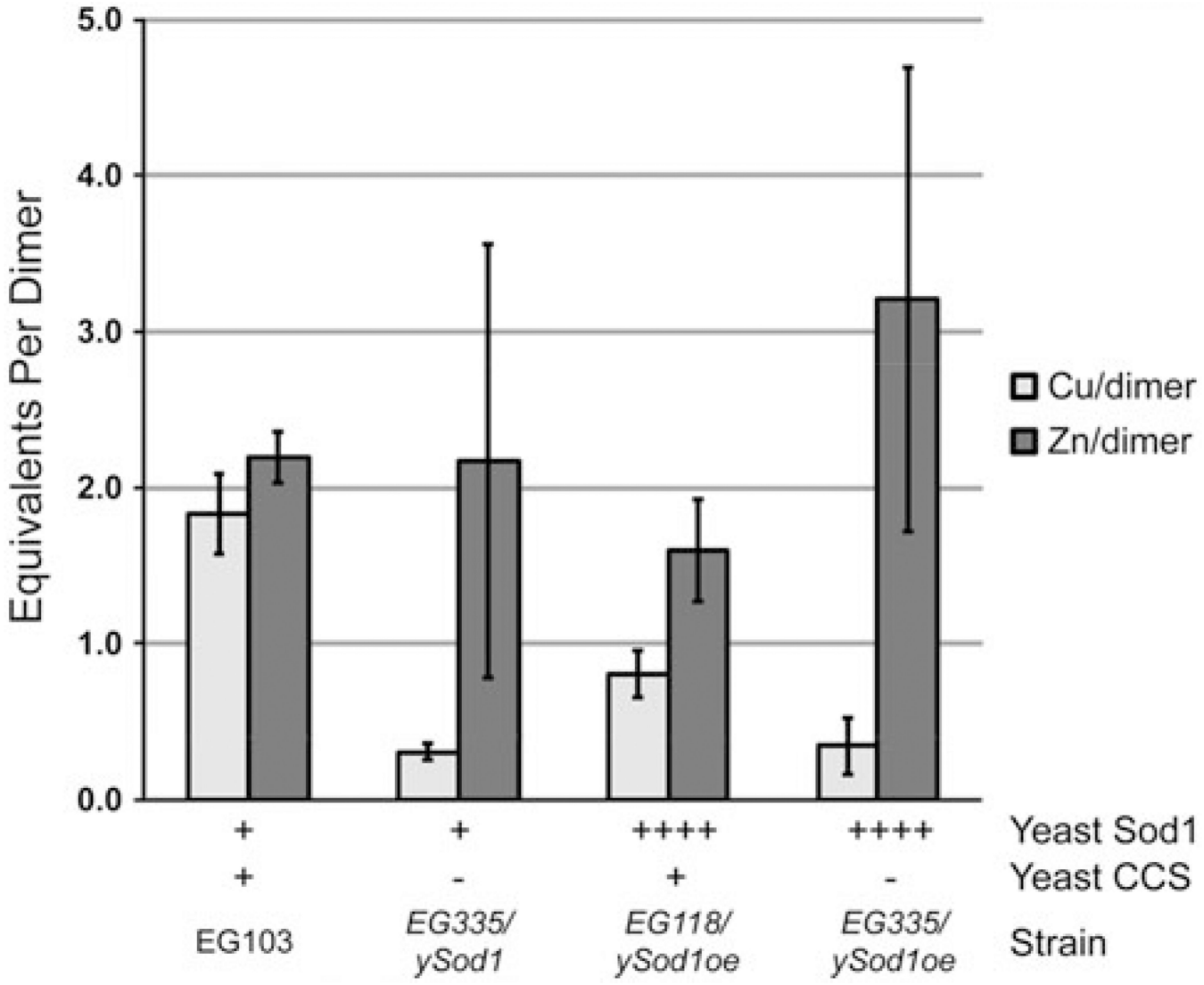

Fig. 4.

Copper is bound by ySod1 in vivo in the absence of yCcs1. Analysis of metal content was done by HPLC-ICPMS. The yeast strains were EG103, *EG335/ySod1*, *EG118/ySod1oe*, and *EG335/ySod1oe*. Overexpressed ySod1 is indicated by *four plus signs*

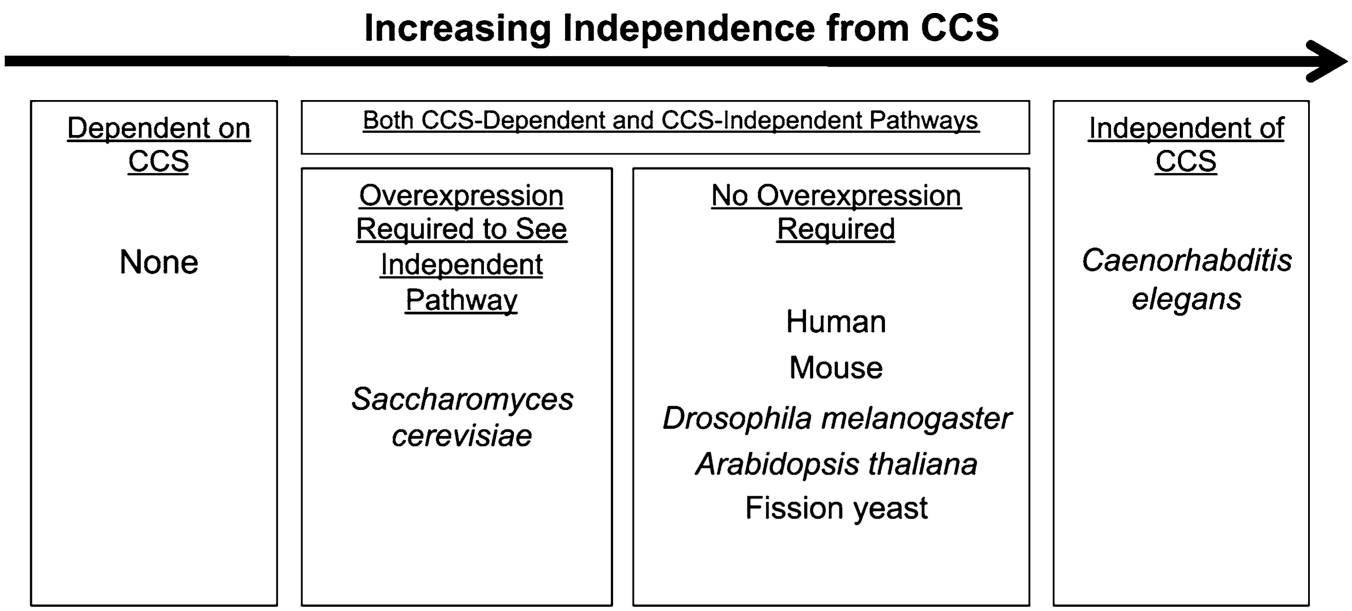

Fig. 5.

Relative independence of Sod1 from the copper chaperone for Sod1 (*CCS*) in various species. The copper required by Sod1 for enzymatic activity can be obtained by both CCS-dependent and CCS-independent pathways. At one end of the range is *Caenorhabditis elegans* Sod1, which has no CCS and thus obtains all copper independently of CCS. At the other end of the range, there are now no known Sod1 enzymes that are completely dependent on CCS. In the middle of the range are the species in which Sod1 can be activated by both CCS-dependent and CCS-independent pathways. In one species, *Saccharomyces cerevisiae*, the CCS-independent pathway is only apparent when Sod1 is overexpressed. See the text for references

Table 1

Yeast strains used in these experiments

Yeast strain	Relevant genotype	Complete genotype	References
EG103	Wild type	*MATα leu2 his3 trp1 ura3*	[33]
EG118	*sod1Δ*	*MATα leu2 his3 trp1 ura3 sod1Δ::URA3*	[33]
JW101	*ccs1Δ*	*MATα leu2 his3 trp1 ura3 ccs1Δ::LEU2*	[34]
EG335	*sod1Δ ccs1Δ*	*MATα his3 trp1 ura3 sod1Δ::KAN ccs1Δ::HIS3*	This work

Table 2

Yeast strain and plasmid combinations used in this study

Name	Yeast strain	Gene added on plasmid	Parent plasmid	Expression level	Relevant genotype
EG118/ySod1oe	EG118	Yeast *SOD1*	pRS424 (2 μ) (Trp)	Overexpressed	*sod1*Δ yeast strain with yWT *sod1* (overexpressed)
EG335/ySod1oe	EG335	Yeast *SOD1*	pRS424 (2 μ) (Trp)	Overexpressed	*sod1*Δ *ccs1*Δ yeast strain with yWT *sod1* (overexpressed)[a]
EG335/ySod1	EG335	Yeast *SOD1*	pRS304 (CEN) (Trp)	Single copy	*sod1*Δ *ccs1*Δ yeast strain with yWT *sod1*
EG335/yCcs1	EG335	Yeast *CCS1*	pRS306 (CEN) (Ura)	Single copy	*sod1*Δ *ccs1*Δ yeast strain with yWT *ccs1*[b]

All parent plasmids are from [35]

yWT yeast wild type

[a] Also contains empty vector pRS306

[b] Also contains empty vector pRS424