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Different Regulatory Mechanisms Modulate the Expression of a Dinoflagellate Iron-Superoxide Dismutase*

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Regulation of antioxidant enzymes is critical to control the levels of reactive oxygen species in cell compartments highly susceptible to oxidative stress. In this work, we studied the regulation of a chloroplastic iron superoxide dismutase (Fe-SOD) from *Lingulodinium polyedrum* (formerly *Gonyaulax polyedra*) under different physiological conditions. A cDNA-encoding Fe-SOD was isolated from this dinoflagellate, showing high sequence similarity to cyanobacterial, algal, and plant Fe-SODs. Under standard growth conditions, on a 12:12-h light-dark cycle, *Lingulodinium polyedrum* Fe-SOD exhibited a daily rhythm of activity and cellular abundance with the maximum occurring during the middle of the light phase. Northern analyses showed that this rhythmicity is not related to changes in Fe-SOD mRNA levels, indicative of translational regulation. By contrast, conditions of metal-induced oxidative stress resulted in higher levels of Fe-SOD transcripts, suggesting that transcriptional control is responsible for increased protein and activity levels. Daily (circadian) and metal-induced up-regulation of Fe-SOD expression in *L. polyedrum* are thus mediated by different regulatory pathways, allowing biochemically distinct changes appropriate to oxidative challenges.

Reactive oxygen species (ROS)[1] such as superoxide ($O_2^{\cdot-}$), and in some cases hydrogen peroxide (H_2O_2), are normal by-products of oxidative metabolism and have the potential to give rise to hydroxyl radicals ($HO^{\cdot}$). Although some ROS may function as important signaling molecules that alter gene expression and modulate the activity of specific defense proteins (1), all ROS may be harmful and pose a threat to aerobic organisms. Oxidative damage to DNA, proteins, and lipids can lead to mutagenesis, carcinogenesis, and alterations in cell structure (2).

Organisms combat toxic effects of oxygen with antioxidants, which include detoxifying enzymes and low molecular weight compounds. The enzyme superoxide dismutase (SOD) represents a first step in such ROS scavenging systems. SOD isoforms, including the copper/zinc-containing (CuZn-SOD), manganese-containing (Mn-SOD), and iron-containing (Fe-SOD) metalloenzymes, catalyze the dismutation of $O_2^{\cdot-}$ to H_2O_2 and oxygen. In photosynthetic eukaryotes, CuZn-SOD is usually located in the cytosol and extracellular space, although some plants also possess a chloroplastic CuZn-SOD isoform. Mn-SOD and Fe-SOD are found within the mitochondria and chloroplast, respectively (3).

Irradiation by visible light in the presence of a photosensitizer leads to the production of ROS, which in plants and algae is linked to photosynthesis (4). Because of the elevated oxygen concentration and intense electron flux within chloroplasts, electrons inevitably react with oxygen, thereby generating $O_2^{\cdot-}$, which dismutates to oxygen and H_2O_2, producing the highly reactive $HO^{\cdot}$ through the metal ion catalyzed Haber-Weiss reaction (5). Even under nonstress conditions, this ROS-generating mechanism can do harm and inactivate the photosystem II reaction center, resulting in photoinhibition (6, 7). Thus, tolerance of photosynthetic organisms to oxidative challenge is enhanced by defense responses that prevent oxidative damage to chloroplasts. Because $O_2^{\cdot-}$ is a precursor of several other reactive species, control over the steady-state $O_2^{\cdot-}$ levels by SOD is critical.

The presence of metal ions due to human activities is another important cause of oxidative stress in living systems (8). Such metals can promote oxidative damage both by directly increasing the cellular concentration of ROS (2, 9) and by reducing the cellular antioxidant capacity (10).

In contrast to higher plants, the antioxidant response to oxidative and environmental stress has not been investigated in dinoflagellates at the molecular level. These are a diverse group of unicellular eukaryotes containing bioluminescent, photosynthetic, heterotrophic, and symbiotic members having important ecological roles as primary producers and consumers in aquatic environments. Dinoflagellates are responsible for red tides, with those that are toxic having the potential for producing serious health and economic problems. They have unique genomic features, including large amounts of DNA (up to 200 pg/nucleus) packed in permanently condensed chromosomes (11) and an absence of classical histones (12), which make their mechanisms of genetic regulation of great interest.

In previous studies, we found that oxidative stress is an important mediator of metal toxicity in unicellular algae and that SOD is an essential component of the antioxidant defense system of dinoflagellates (13, 14). Here we show that the chloroplastic Fe-SOD isoform of *Lingulodinium polyedrum* is under the control of two different regulatory mechanisms: increases in enzyme activity caused by treatment with metal ions is attributable to higher Fe-SOD transcript levels, whereas a

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[1] The abbreviations used are: ROS, reactive oxygen species; LD, light-dark cycle; RT-PCR, reverse transcription/polymerase chain reaction; LBP, luciferin-binding protein; SOD, superoxide dismutase.

daily rhythm of Fe-SOD expression is clock-controlled at the translational level.

EXPERIMENTAL PROCEDURES

Culture Conditions—Cells of the dinoflagellate *L. polyedrum* (strain GP 70) were cultured at 20 ± 1 °C on a 12:12-h light-dark (LD) cycle with cool white fluorescent light at an irradiance of 150 $\mu E \cdot m^{-2} \cdot s^{-1}$. Typically, 1 liter of f/2 medium (15) was inoculated with 150 ml of a dense culture (10^4 cells·ml^{-1}) and used after 4–5 weeks of growth. For the metal stress experiments, cells were exposed for 6 h to either 0.04 μM Hg^{2+}, 4.8 μM Cd^{2+}, 18 μM Pb^{2+}, or 1.6 μM Cu^{2+}. Metal salts were added directly to the medium at the beginning of the light period (LD 0). Metal ion concentrations and exposure time used in the experimental model were chosen on the basis of previous toxicity bioassays and are known to induce oxidative stress in *L. polyedrum* (14, 16).

Cell Extracts and Chloroplast Purification—Cells were harvested by filtration, suspended in 0.1 M sodium phosphate buffer pH 7.8, and lysed in a nitrogen pressure apparatus. Following centrifugation at 12,000 × *g* for 10 min at 4 °C, the supernatant was used as crude extract for enzyme activity and protein assays.

Chloroplasts were purified from 1-liter cell cultures on a 90% Percoll, 0.25 M sucrose density gradient as previously described (17). The band containing chloroplasts (1.15 g·ml^{-1} density, monitored by absorbance at 670 nm) was suspended in 1 ml of ice-cold extraction buffer (150 mM Tris, pH 8.0, 2 mM EDTA, 0.3 M sucrose, 20 mM 2-mercaptoethanol) and lysed by high nitrogen pressure (150 atm, 5 min, at 4 °C). Excess debris was removed by centrifugation at 12,000 × *g* for 1 min, at 4 °C, and the supernatant was used immediately for the biochemical assays.

Enzyme Activity Assays—SOD activity was determined by SOD inhibition of superoxide-initiated ferricytochrome *c* reduction (18). Superoxide was generated by the xanthine/xanthine oxidase system, and assays were carried out using either crude extracts or chloroplast extracts. Ferricytochrome *c* reduction was followed at 550 nm for 1 min. Fe-SOD activity in chloroplast extracts was measured in the presence of 5 mM KCN. One unit of SOD is defined as the amount causing 50% inhibition of ferricytochrome *c* reduction at 25 °C.

Extracts of *L. polyedrum* were also electrophoresed on 12% nondenaturing polyacrylamide gels and stained directly for SOD activity, which appears as light bands against the deep blue gel background (19). The distinct SOD isoforms present in *L. polyedrum* extracts have been previously identified by their different sensitivities to CN^- and H_2O_2 (14). Thus, identification of the chloroplast SOD was inferred by comparison of the electrophoretic profile of SOD isoforms from chloroplast and crude extracts.

PCR Cloning and Sequence Analysis—Total RNA was isolated in denaturing buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% SDS, and 10 mM 2-mercaptoethanol) and precipitated with 2 M lithium chloride, as previously described (20). Poly(A)$^+$ RNA was purified from total RNA with the QuickPrep Micro mRNA purification kit (Amersham Pharmacia Biotech), according to the manufacturer's instructions.

cDNAs encoding Fe-SOD were isolated using reverse transcription-polymerase chain reaction (RT-PCR). First strand reactions contained 2 mM $MgCl_2$, 200 μM dNTPs, 10 pmol of oligo$(dT)_{16}$ primer, 500 ng of *L. polyedrum* mRNA, and 2 units of Superscript II reverse transcriptase (Life Technologies, Inc.). After RT for 1 h at 42 °C, the remaining mRNA was removed enzymatically, and the cDNA products were amplified using different combinations of degenerate Fe-SOD primers (FSD1, 5′ CAYTAYGGHAARCAYCAY 3′; FSD2r, 5′ GTRTGRTTCCAVACYTGBGC 3′; FSD2, 5′ GCVCARGTBTGGAAYCAYAC 3′; and FSD3r, 5′ GCRTGYTCCCAVACRTC 3′), with *Taq* DNA polymerase and the following PCR cycle: 3-min denaturation at 94 °C, 30 cycles of 30 s at 94 °C, 35 s at 55 °C, and 40 s at 72 °C, followed by a 10-min extension at 72 °C. The Fe-SOD 3′ and 5′ untranslated region sequences were obtained by rapid amplification of cDNA ends technique, using the same PCR conditions.

Amplified cDNA fragments were gel-purified, cloned into pGEM-T vector (Promega), and sequenced by the ABI dye-terminator method (PerkinElmer Applied Biosystems). Sequence analyses were performed with the SeqLab software[2] (Wisconsin Package 10.0, GCG, Madison, WI).

Quantification of Fe-SOD Proteins and Transcripts—Proteins (20 μg), electrophoresed on 12% polyacrylamide-SDS, were transferred to a nitrocellulose membrane for Western blotting analysis (21) using polyclonal antibodies against bacterial Fe-SOD (Sigma). After radioactive probing with ^{125}I-Protein A, membranes were autoradiographed for detection of bound antibody.

Total RNA (1–10 μg/lane) and poly(A)$^+$ RNA (1 μg/lane) were electrophoresed on a 1.2% agarose formaldehyde gel, blotted to a positively charged nylon membrane and probed with the cloned Fe-SOD cDNA. Northern hybridizations and washes were carried out under stringent conditions. Fe-SOD transcripts were also quantified by RT-PCR using Fe-SOD primers FSD2/FSD3r and PCR conditions described above. As a control for variations in RNA loading, expression of the *L. polyedrum* luciferin-binding protein (LBP) was also monitored on both Northern blots and RT-PCR analysis. Densitometry analyses were performed with the NIH Image v1.54.

Statistical Analysis—Statistically significant differences among treatments were determined by analysis of variance complemented by the Dunnet's test. All conclusions are based on at least a 5% level of significance ($p < 0.05$).

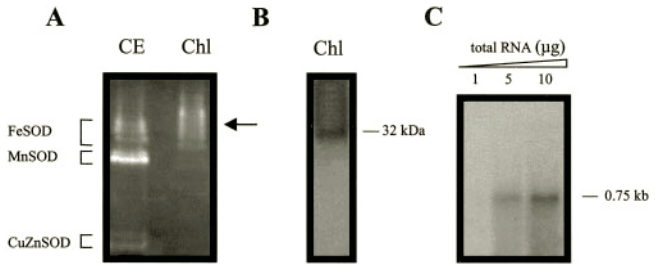

FIG. 1. **Identification of Fe-SOD in *L. polyedrum*.** *A*, banding patterns of three SOD isoforms in activity gels. Equal amounts of proteins (20 μg/lane) from crude extracts (*CE*) and chloroplast (*Chl*) extracts were electrophoresed on native polyacrylamide gels and stained for SOD activity. Chloroplast Fe-SOD is broadly distributed in the *light area*, confirmed by other tests (see text). *B*, immunodetection of Fe-SOD in chloroplast extracts by Western blot (20 μg/lane). *C*, Northern analysis of Fe-SOD transcripts using different amounts of total RNA and *L. polyedrum* Fe-SOD cDNA as a probe.

RESULTS

Fe-SOD in L. polyedrum—The isoforms Fe-SOD, Mn-SOD, and CuZn-SOD have been previously identified in crude extracts of *L. polyedrum* by inhibition assays with CN^- and H_2O_2. Fe-SOD is CN^--resistant and H_2O_2-sensitive, Mn-SOD is CN^-- and H_2O_2-resistant, whereas CuZn-SOD is sensitive to both substances. From the SOD banding pattern of crude extracts on native gels (14), several protein bands corresponding to Fe-SOD activity could be detected in extracts of isolated chloroplasts (Fig. 1*A*). Although the chloroplastic SOD bands are not resolved in the native gels, resistance to CN^- and inactivation after H_2O_2 treatment confirmed their Fe-SOD nature (not shown). Other evidence for the presence of the Fe-SOD isoform in chloroplast extracts was provided by immunodetection with heterologous Fe-SOD antibodies, which cross-reacted with an ~32-kDa chloroplastic protein (Fig. 1*B*).

Different sets of degenerate PCR primers, designed on the basis of amino acid sequences of conserved Fe-SOD domains, were used to amplify overlapping sequences of the coding region of this enzyme, using mRNA as starting template. Blast searches of the GenBank™ database indicated that the predicted amino acid sequence of the resulting 690-bp ORF has high sequence identity to cyanobacterial (*Synechocystis,* 59%; *Synechococcus,* 55%; *P. boryanum,* 53%), green algal (*C. reinhardtii,* 57%), and higher plant (*A. thaliana,* 52%; *N. plumbaginifolia*, 52%) Fe-SODs (Fig. 2). The presence of conserved residues known to be responsible for iron binding (Fig. 2, *bolded*) further confirms the identity of this nuclear-encoded *L. polyedrum* gene as the Fe-SOD isoform. In addition, its N-terminal region contains a hydrophobic stretch followed by several hydroxylated and basic residues, typical of transit peptide sequences (Fig. 2, *underlined*). When the cloned cDNA was

[2] The full-length cDNA sequence of Fe-SOD from *L. polyedrum* is available in the GenBank™ sequence data base under GenBank™ Accession Number AF 289824.

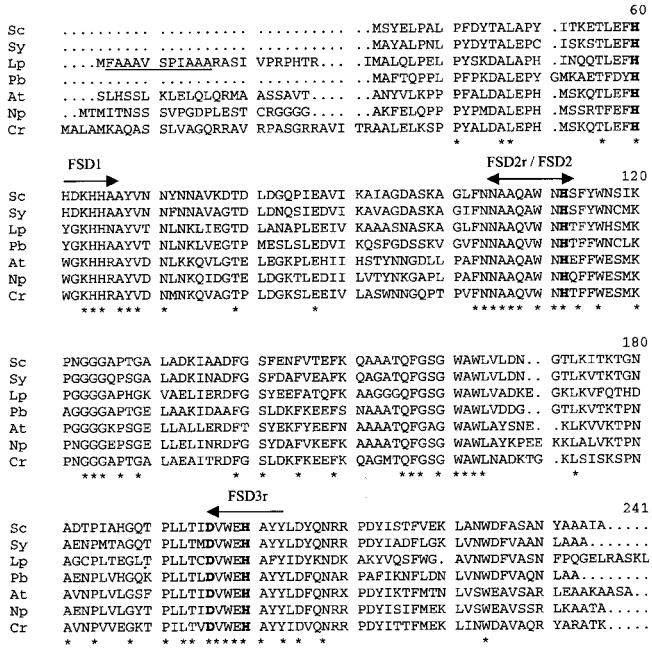

FIG. 2. **Alignment of the deduced amino acid sequence of *L. polyedrum* Fe-SOD (*Lp*; AF 289824) with those of *Synechocystis sp* (*Sy*; BAA 18027), *Synechococcus sp* (*Sc*; CAB 57855), *Plectonema boryanum* (*Pb*; AAA 69954.1), *Chlamydomonas reinhardtii* (*Cr*; AAB 04944.1), *Nicotiana plumbaginifolia* (*Np*; AAA 34074.1), and *Arabidopsis thaliana* (*At*; AAA 33960.1).** The N-terminal region rich in hydrophobic residues is *underlined*. Conserved amino acid domains used for primer design are indicated by *arrows*. Identical amino acid residues are marked with *asterisks*, and those related to iron binding are in *boldface*. *Dots* within sequences indicate gaps introduced for best alignment.

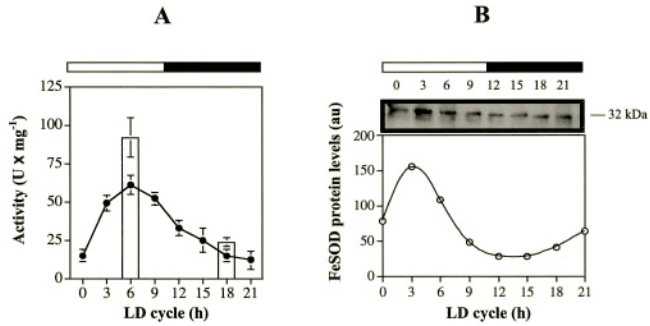

FIG. 3. **Daily rhythm of Fe-SOD from cells grown under 12:12-h LD cycle.** *A*, total SOD (*line*) and Fe-SOD (*vertical bars*) activities measured in crude extracts and chloroplast extracts, respectively. Each *data point* is the mean ± S.E. of 3 experiments. *B*, Western blots. Total proteins (20 μg/lane) were extracted every 3 h, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and probed with Fe-SOD antibodies. Densitometry data are plotted as arbitrary units (*au*) *versus* time. *White* and *black horizontal bars* represent light and dark phases, respectively.

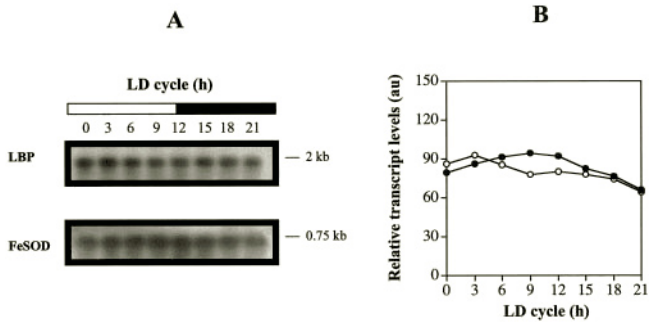

FIG. 4. **Daily levels of Fe-SOD transcripts are constant.** *A*, Northern analyses were performed with poly(A)$^+$RNA (1 μg/lane), extracted every 3 h from cells grown under 12:12 h LD cycle, and gene-specific probes. Expression of LBP was also monitored as a control for RNA loading. *B*, quantification of Fe-SOD (*solid circles*) and LBP (*open circles*) transcripts in the respective blots. Densitometry data are plotted as arbitrary units (*au*) *versus* time.

used as probe in Northern blots, a specific ~750-bp transcript was detected (Fig. 1*C*), which is in agreement with the average size of Fe-SOD transcripts. However, no hybridization signal could be detected if 1 μg or less of total RNA was blotted, indicating that the basal level of Fe-SOD transcripts is low compared with other *L. polyedrum* genes (LBP, luciferase, and glyceraldehyde-3-phosphate dehydrogenase).

Daily Expression of Fe-SOD Is Rhythmic and Controlled at the Translational Level—Organisms may be subject to oxygen toxicity under normal conditions because of physiological activity. Because production of ROS is associated with exposure to light and photosynthetic activity, the concentration of ROS within chloroplasts is expected to be greater during the day than at night. To determine whether there is a corresponding rhythmicity in Fe-SOD, its expression was monitored in *L. polyedrum* cells throughout a 24-h time interval, under a 12:12-h LD cycle. The activity of Fe-SOD was determined on the basis of selective tests, as described above, and found to be 4-fold higher in chloroplasts of *L. polyedrum* cells harvested during the light phase (LD 6) than during the dark phase (LD 18; Fig. 3*A*). In agreement with previous data (22), total SOD activity in crude extracts also displayed a diurnal rhythm, with a maximum occurring in the middle of the light phase (LD 6). Western blot analysis of crude extracts confirmed that the cellular levels of Fe-SOD also varied during the 12:12-h LD cycle with an amplitude similar to that of the enzyme activity rhythm (Fig. 3*B*).

However, these changes were not reflected in the levels of Fe-SOD mRNA; the abundance of Fe-SOD transcripts remained relatively constant over a daily cycle in cells harvested at different times, as shown by Northern blot analyses of mRNA extracted every 3 h (Fig. 4). LBP was included in the study first because this is a protein whose synthesis and cellular concentration is controlled by a circadian clock (23, 24), yet its activity is not directly involved in the metabolism of ROS. Second, the message level of LBP is established as being constant over the LD cycle, and the regulation of its translation is circadian-controlled (23), also shown for another rhythmic protein, glyceraldehyde-3-phosphate dehydrogenase (25). The parallel leads us to the postulate that Fe-SOD synthesis and its cellular abundance is regulated, not by ROS, but by the circadian system.

Fe-SOD Transcript Levels Are Higher after Metal Stress—Metal ions are known to cause oxidative stress and damage in aerobic organisms, including *L. polyedrum* (26). In previous studies, exposures of either 0.04 μM Hg^{2+}, 4.8 μM Cd^{2+}, 18 μM Pb^{2+}, or 1.6 μM Cu^{2+} were reported to result in increases in total SOD activity, maximal after 6 h (14). In the present work, the cellular levels of the Fe-SOD isoform were found to be 3- to 4-fold higher after similar exposures to such metal ions (Fig. 5*A*), and significant increases in Fe-SOD activity, measured in chloroplast extracts, were also found (Fig. 5*B*).

Unlike the diurnal regulation of Fe-SOD, these increases appear to be mediated by increased transcript levels, as shown by Northern analyses (Fig. 6*A*). When an alternate and more sensitive method for detecting transcripts was used (RT-PCR), similar results were obtained (Fig. 6*B*). As in the experiments of Fig. 5, cultures were exposed to each of the four metal ions individually, and in each case there was a substantial increase in the Fe-SOD transcript level, but not in that of the LBP transcripts. This provides direct evidence

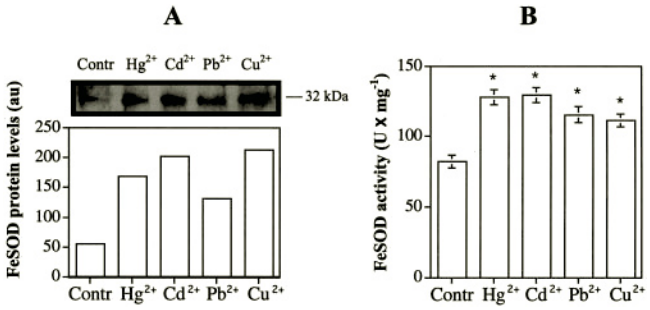

FIG. 5. **Increase of Fe-SOD following exposure to toxic metal ions.** *A*, cellular levels of Fe-SOD in extracts prepared from cells exposed to either 0.04 μM Hg^{2+}, 4.8 μM Cd^{2+}, 18 μM Pb^{2+}, or 1.6 μM Cu^{2+} for 6 h. Western blots were performed with total proteins (20 μg/lane) probed with Fe-SOD antibodies. Densitometry analysis in arbitrary units (*au*) is given by *bar graphs*. *B*, Fe-SOD activity in chloroplasts prepared from cells after treatment with metal ions as above. Control cells were grown in the absence of toxic metals. Each *data point* represents the mean ± S.E. of 3 experiments. *, significantly different from control group ($p < 0.05$).

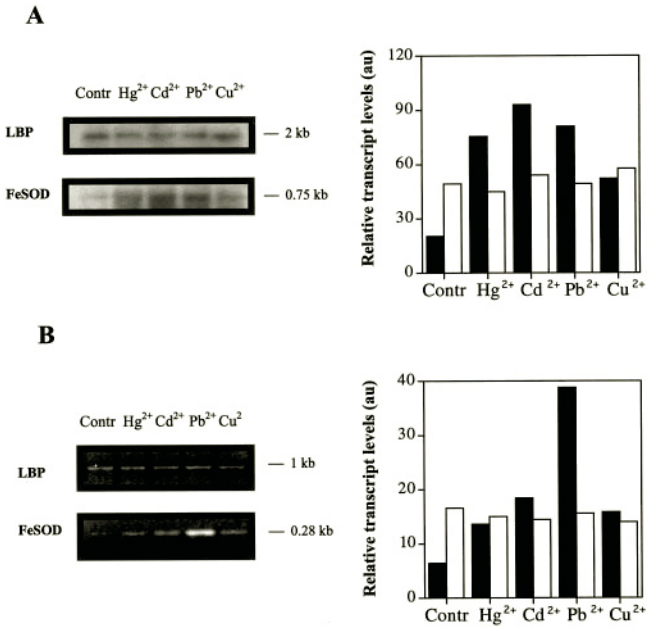

FIG. 6. **Increased Fe-SOD transcript levels after treatment with toxic metal ions.** Densitometry analysis in arbitrary units (*au*) is given by *bar graphs* (Fe-SOD, *solid bars*; LBP, *open bars*). *A*, Northern analyses of Fe-SOD and LBP transcript abundance in total RNA (10 μg/lane) extracted from cells exposed to either 0.04 μM Hg^{2+}, 4.8 μM Cd^{2+}, 18 μM Pb^{2+} or 1.6 μM Cu^{2+} for 6 h. *B*, measurement of Fe-SOD and LBP transcript levels by RT-PCR in cells treated as above. Poly(A)$^+$RNA (500 ng/reaction) was reverse-transcribed in the presence of gene-specific primers. PCR products were electrophoresed on 1.5% agarose gel and visualized by ethidium bromide staining.

that the regulatory pathways for daily and metal-induced up-regulation of Fe-SOD expression do not involve a common intermediate.

SOD isoforms are able to incorporate different metal ions in their active sites depending on metal availability in the medium (27). Although some SOD activity may be retained in such cambialistic (metal-exchangeable) SODs, the binding of metal ions without appropriate redox capacity may lead to enzymatically inactive isoforms (2, 28). One could speculate that, in our experiments, treatment with other metal ions displaces iron from the active site and affects enzyme activity. This could explain the lower enhancement of Fe-SOD activity compared with the increases in transcript and protein levels found in cells subjected to metal stress.

DISCUSSION

Whereas SOD genes have been isolated from many different species, the Fe-SOD isoform has been reported from only a few, and this is the first gene of any SOD isoform to be isolated from a dinoflagellate, from which fewer than a dozen genes altogether have been cloned. This Fe-SOD is nuclear-encoded; its deduced amino acid sequence is 52–59% identical to Fe-SOD isoforms from plants and cyanobacteria, respectively, and residues responsible for iron binding are fully conserved. Preliminary results indicate that, as for other *L. polyedrum* genes so far studied, there are no introns.

Although enhanced transcription of plant Fe-SODs has been reported as a consequence of environmental adversities in several cases (29, 30), this is the first demonstration of such a response after exposure to different metal ions. As in other species, SOD in *L. polyedrum* surely provides a mechanism for inactivation of ROS, but different mechanisms of regulation of Fe-SOD are operative. Exposure to metal ions results in an increased level of the Fe-SOD mRNA, whereas the daily variation in Fe-SOD protein and activity levels involves translational regulation. There is no evidence in either case for the direct regulation of Fe-SOD by ROS.

With cells grown on a light-dark cycle, Fe-SOD exhibits a robust daily rhythm in both protein level and enzyme activity, peaking in the light phase. However, this is not accompanied by changes in the cellular level of the mRNA, so it is concluded that translational regulation is responsible for the Fe-SOD rhythm. Antioxidants, such as SOD, glutathione *S*-transferase, and β-carotene have been reported to be controlled by the circadian clock (22, 31, 32), as are many *L. polyedrum* proteins (33, 34), several of which have been shown to be regulated at the level of translation (23, 25, 35). The results presented here show that even with cells exposed to a light-dark cycle, and thus expected to generate a rhythm in the amount of O_2^- (4), the regulation of Fe-SOD does not involve a change in the amount of its message. Thus, it is not the ROS that directly regulate the Fe-SOD rhythm but the circadian clock.

Translational regulation of SOD expression has been reported in rats, where Mn-SOD abundance is regulated post-transcriptionally by the binding of a redox-sensitive trans-acting protein to the 3′ untranslated region of its transcript, and binding enhances protein translation (36, 37). This is similar to the circadian control of LBP synthesis in *L. polyedrum*, but in the case of LBP, binding represses translation (24).

In contrast to the daily rhythm, induction of Fe-SOD by metal ions in *L. polyedrum* was accompanied by 3–4-fold increases in the amount of its cellular mRNA and protein, with a somewhat smaller increase in Fe-SOD activity. Whether this is caused by increased transcription or slower degradation of mRNA, or both, cannot be specified. Transcriptional control of the expression of other genes in dinoflagellates has been reported recently (38, 39), and in other eukaryotes transcription of SOD genes is activated by redox-sensitive transcription factors such as AP-1 and NF-κB (40, 41). Indeed, the promoter regions of many eukaryotic SOD genes contain multiple regulatory elements responsible for transcriptional control in response to a variety of stimuli (42–44). In bacteria, the expression of SOD occurring after shifts in the cellular oxidative balance is regulated by the transcription factors Sox R and Sox S (45).

Although little is known about the mechanisms of SOD regulation in plants and algae, it seems likely that metal-induced expression of Fe-SOD in *L. polyedrum* is under the control of protein that binds to a metal-responsive element, as in the case of the rat CuZn-SOD isoform (46). This is supported by the observations that Fe-SOD transcript levels in *L. polyedrum* are

increased by exposure to either a redox active metal ion such as the micronutrient Cu^{2+} or to nonessential metal ions without redox capacity such as Hg^{2+}, Cd^{2+}, and Pb^{2+}.

In summary, our findings show clearly that regulation of *L. polyedrum* Fe-SOD can take place at different steps of gene expression. Translation of Fe-SOD varies over the course of a 24-h light-dark cycle, whereas an increase in the Fe-SOD transcript pool occurs after exposure to toxic metal ions, a novel finding. In both cases, a prompt induction of Fe-SOD expression is critical for controlling the steady-state levels of $O_2^{\cdot-}$ and thus preventing oxidative damage within subcellular sites highly prone to oxidative stress such as chloroplasts.

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