Removal of hydrogen peroxide by the 29 kDa protein of *Entamoeba histolytica*

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The 29 kDa protein of *Entamoeba histolytica* (Eh29), as well as a truncated variant of this protein, which lacks a cysteine-rich N-terminal region of 40 amino acid residues (Eh29mut), were recombinantly expressed in *Escherichia coli* and purified to homogeneity. Both recombinant proteins (recEh29, recEh29mut) were found to have hydrogen peroxide (H₂O₂)-removing activity, but recEh29 was twice as active as recEh29mut. For the consumption of exogenous H₂O₂, activity was dependent on the presence of reducing equivalents, such as dithiothreitol (DTT), indicating that Eh29 constitutes a thiol-dependent peroxidase. DTT was not required to remove H₂O₂ by recEh29 or recEh29mut when H₂O₂ was generated enzymically by the *E. histolytica* NADPH:flavin oxidoreductase. This enzyme produces H₂O₂

under aerobic conditions and simultaneously serves as a hydrogen donor for Eh29. Peroxidase activity of the recombinant proteins was further supported by complementation of an *E. coli* strain that lacks the entire alkyl hydroperoxide reductase locus. The high sensitivity of these bacteria against cumene hydroperoxide was significantly reduced by the introduction of the genes encoding recEh29 or recEh29mut. Using antisera raised against the recombinant proteins, native Eh29 was localized within the cytoplasm of the amoebae. In addition, the antisera reacted with proteins of *E. histolytica* lysates with apparent molecular masses of 35 kDa and 160–300 kDa. All of them exhibited thiol-peroxidase activity.

INTRODUCTION

The enteric protozoan parasite *Entamoeba histolytica* is well known for its high potential for invading and destroying human tissues, leading to potentially life-threatening diseases such as haemorrhagic colitis and/or extraintestinal abscesses. The parasite usually lives and multiplies within the human gut, which constitutes an environment of reduced oxygen pressure. During tissue invasion *E. histolytica* is exposed to elevated amounts of exogenous reactive oxygen species (ROS) such as superoxide radical anions (O₂^{•−}) and hydrogen peroxide (H₂O₂), which are highly toxic, and therefore have to be inactivated [1–3]. In addition, *E. histolytica* has to inactivate ROS that are produced by endogenous enzymes. The activity of at least two *E. histolytica* enzymes, the iron-containing superoxide dismutase and the NADPH:flavin oxidoreductase (Eh34), result in the formation of H₂O₂ [4–8] and Bruchhaus, I., Richter, S. and Tannich, E., unpublished work). Interestingly, *E. histolytica* does not possess any detectable catalase or peroxidase activity. Therefore the mechanism that enables *E. histolytica* to inactivate H₂O₂ remains obscure.

Three laboratories reported on the identification of an *E. histolytica* gene encoding a 29 kDa protein (Eh29) [9–12]. This polypeptide was found to have a substantial degree of similarity to a new family of antioxidants identified in various prokaryotic and eukaryotic organisms [12,13]. The function of two members of these antioxidants has been studied in detail. One of them is the AhpC subunit of the alkyl hydroperoxide reductase (AhpR) system first described for *Salmonella typhimurium*. This enzyme, composed of a 21 kDa (AhpC) and a 57 kDa (AhpF) component, is capable of reducing H₂O₂ and organic hydroperoxides [14–18]. The second enzyme, the thiol-specific antioxidant enzyme (TSA) originally isolated from *Saccharomyces cerevisiae* has a molecular

mass of 25 kDa. This enzyme catalyses the reduction of H₂O₂ and organic hydroperoxides using reducing equivalents provided by the thioredoxin reductase/thioredoxin/NADPH system. In addition, TSA contains thiol peroxidase activity. This activity depends on thiols that are necessary to convert oxidized TSA back to the active thiol form [19–22]. Two cysteine residues, Cys-47 and Cys-170, are essential for the activity of this enzyme. These residues are conserved among most members of the TSA/AhpC family, including the 29 kDa protein of *E. histolytica* [23]. The main difference between the *E. histolytica* protein and the other TSA/AhpC members of the family is a cysteine-rich N-terminal region that is found in the *E. histolytica* 29 kDa molecule only (35 % cysteine residues over 25 amino acids) [12].

In this study, we report on recombinant expression and characterization of the 29 kDa *E. histolytica* protein (Eh29), as well as of a truncated form that lacks the first 40 amino acids of the cysteine-rich N-terminal region (Eh29mut).

MATERIALS AND METHODS

Recombinant expression and purification of the *E. histolytica* 29 kDa protein

Two forms of the 29 kDa protein were recombinantly expressed in *Escherichia coli*. The full-length protein was termed recEh29, whereas the protein missing the 40 N-terminal amino acid residues was termed recEh29mut. For amplification of the corresponding DNA sequences the following oligonucleotide primers were used: 5'-TCA ATC AGT CAT ATG TCT TGC AAT CAA C (Eh29-S28), 5'-TAA ACA CAT TTC ATA TGG CAC AAA TTG G (Eh29mut-S28) and 5'-TAA AGG ATC CTG TTT GTT TTA ATG TGC TG (Eh29-AS29). The primers

Abbreviations used: Eh29, *Entamoeba histolytica* 29 kDa protein; Eh29mut, truncated form of Eh29; Eh34, *Entamoeba histolytica* NADPH:flavin oxidoreductase; DTT, dithiothreitol; AhpR, alkyl hydroperoxide reductase; TSA, thiol-specific antioxidant enzyme; ROS, reactive oxygen species; ABTS, 2,2'-azinobis-(3-ethylbenzthiazolinesulphonic acid).

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contain *Nde*I and *Bam*HI restriction sites respectively, which allows rapid cloning of the amplified DNA in the predicted orientation into the prokaryotic expression plasmid pJC45, a derivative of pJC40 [24]. In this vector 10 histidine residues are added to the N-terminus of the respective gene product. Recombinant plasmids were transformed into BL21(DE3)[pAPlacIQ] and the bacteria were plated on Luria broth agar plates [ampicillin (100 µg/ml)/kanamycin (50 µg/ml)/2% (w/v) glucose]. Single colonies were inoculated into Luria broth medium and grown at 37 °C until A_{600} reached 0.3. Subsequently, isopropyl- β -D-thiogalactoside was added (final concentration 1 mM) and incubation was continued for an additional 3 h. Purification of the two recombinant proteins was performed using Ni-NTA resin following the manufacturer's recommendations for native purification of cytoplasmic proteins (Qiagen, Hilden, Germany).

Enzymic assays and determination of H₂O₂ concentration

Two different assays were performed to measure removal of H₂O₂ by recEh29 or recEh29mut. (i) The consumption of H₂O₂ that was directly added to the reaction was investigated using a reaction mixture containing 50 mM Tris/HCl, pH 8.0, 2 mM EDTA and 0.2 mM dithiothreitol (DTT), and various amounts of H₂O₂ (4–100 nmol), as well as of the recombinant proteins (0.125–2 µg). (ii) The consumption of H₂O₂ that was enzymically generated by recombinant Eh34 was measured using 1 µg of recombinant Eh34, 50 mM Tris/HCl buffer, pH 8.0, 2 mM EDTA, 0.25 mM NADPH and various amounts of recombinant Eh29 (0.125–2 µg). For both assays, after different incubation periods of 5–30 min, the resulting H₂O₂ concentration was determined as previously described [25], using 20 mM 2,2'-azino-bis-(3-ethylbenzthiazolinesulphonic acid) (ABTS) and 40 units of horseradish peroxidase. H₂O₂ was determined spectrophotometrically (414 nm; ϵ 36×10^3 M⁻¹·cm⁻¹) using known amounts of H₂O₂ (2–100 nmol) as the standard.

Determination of the molecular mass of the native Eh29

The molecular mass of native Eh29 was determined by gel filtration. The *E. histolytica* isolate HM-1:IMSS was cultured axenically in TYI-S-33 medium [26]. Approx. 3×10^8 cells at late-exponential growth phase were harvested by chilling on ice for 10 min and centrifuged at 430 g at 4 °C for 5 min. The pellet was washed twice in PBS, freeze-thawed 5 times in solid CO₂/ethanol and sedimented by centrifugation at 150 000 g at 4 °C for 40 min. The 150 000 g supernatant was passed over a Hi/Load Superdex 200 HR 16/60 FPLC column (Pharmacia, Uppsala, Sweden) with 50 mM Tris/HCl buffer, pH 8.0. The fractions were tested for thiol peroxidase activity by direct addition of H₂O₂ in the presence of DTT. The reaction mixture contained 50 mM Tris/HCl buffer, pH 8.0, 2 mM EDTA, 0.2 mM DTT, 100 nmol of H₂O₂ and 50 µl of each fraction in a final volume of 1 ml. After 10 min the consumption of H₂O₂ was measured using the ABTS method [25]. Active fractions were tested by Western blot analyses using the specific antiserum to recEh29.

Proteins were separated on SDS/12% PAGE and subsequent immunoblots were carried out as described [27].

Rabbit antisera

Antisera to recEh29 and recEh29mut respectively were generated by immunization of rabbits with 50 µg of purified proteins emulsified in complete Freund's adjuvant. After three booster immunizations at intervals of 14 days, animals were bled 5 days after the final injection.

Complementation assay using an *E. coli* *ahp*⁻ mutant

The genes encoding Eh29 and Eh29mut were amplified by PCR and cloned into the *E. coli* expression vector pHis8. For amplification the following oligonucleotide primers were used: Eh29-S30 (5'-AAA AGC TTA TGG CAC AAA TTG GAA AAG AAG) and Eh29-AS29 for Eh29, and Eh29mutS30 (5'-AAA AGC TTA TGT CTT GCA ATC AAC AAA AAG) and Eh29mut-AS29 for Eh29mut. The oligonucleotides contain *Hind*III and *Bam*HI restriction sites respectively which allowed rapid cloning of the amplified DNA in the predicted orientation. The recombinant plasmids were transformed into the *E. coli* strain TA4315. Strain TA4315, which lacks the entire *ahp* locus, was kindly provided by Gisela Storz (National Institute of Health, Bethesda, MD, U.S.A.). As controls TA4315/pAQ27 (encoding AhpC and AhpF from *S. typhimurium*) and TA4315/pGSO2 (encoding AhpC) were used [28]. These two plasmids were also kindly provided by G. Storz. Cells were grown overnight in Luria-Bertani liquid cultures at 37 °C, supplemented with 100 µg/ml ampicillin. Bacteria (200 µl of each overnight culture) were added to 3.5 ml of top agar (0.8%, w/v) and plated on Luria-Bertani agar plates. Bacteria transformed with the recombinant pHis8 plasmid were cultivated in the presence of isopropyl- β -D-thiogalactoside (final concentration 1 mM). Filter discs (diameter 5 mm) were placed on top of the agar. Samples (5 ml) of solution containing 5% (w/v) cumene hydroperoxide dissolved in DMSO were spotted on to the filters. The diameter of the zone of killing was measured after 24 h of incubation at 37 °C.

RESULTS

Recombinant expression and purification of Eh29 and Eh29mut

The 29 kDa *E. histolytica* protein (Eh29), as well as a truncated version (Eh29mut) that lacks the N-terminal cysteine-rich region, were recombinantly expressed in *E. coli*, resulting in a high yield of both proteins (approx. 5 mg/l cultured bacteria). Purification was achieved by metal chelate chromatography using Ni-NTA resin. Under reducing conditions, homogeneous recombinant proteins revealed apparent molecular masses of approx. 26 kDa (recEh29mut) and 30 kDa (recEh29), which is in agreement with the molecular masses predicted from the amino acid sequences including the histidine tail (Figure 1A). Under non-reducing conditions recEh29 formed a high-molecular-mass complex that could be reduced to multimers of 30 kDa under mild treatment with DTT (Figure 1B).

Removal of H₂O₂ by the recombinant Eh29

Both recEh29 and the truncated form, recEh29mut, were found to have the capacity to remove H₂O₂. This activity was dependent on the presence of DTT (0.2 mM) and could be increased by approx. 10-fold by preincubation of the recombinant proteins for 30 min at room temperature with DTT before the addition of H₂O₂. Table 1 shows the amount of H₂O₂ that was removed using different amounts of recEh29 or recEh29mut. recEh29 shows a higher activity than recEh29mut. For recEh29, the apparent K_m value for H₂O₂ was 100 µM and the apparent V_{max} was 25 µmol/min per mg of protein. A K_{cat} value of 750 min⁻¹ was determined. For recEh29mut, an apparent K_m value for H₂O₂ of 35 µM was determined. The apparent V_{max} and K_{cat} values are lower than for recEh29, with 13 µmol/min per mg of protein and 340 min⁻¹ respectively. These results indicate that Eh29 possesses thiol peroxidase activity. After freeze and thaw, or after heating at 95 °C for 5 min, the recombinant proteins lost their ability to remove H₂O₂, even after preincubation with DTT.

Figure 1 Purification of recombinant expressed Eh29 and Eh29mut from *E. histolytica*

(A) The different purification steps were monitored by SDS/12% PAGE under reducing conditions with 50 mM DTT (silver stain). Lane 1, lysate of *E. coli* transformed with pJC45 (40 μ g; control); lane 2, lysate of *E. coli* transformed with recombinant pJC45-Eh29mut (40 μ g); lane 3, recombinant expressed Eh29mut after purification over Ni-NTA agarose (0.4 μ g); lane 4, lysate of *E. coli* transformed with pJC45-Eh29 (40 μ g); lane 5, recombinant expressed Eh29 after purification over Ni-NTA agarose (0.7 μ g). (B) Detection of recombinant Eh29 (4 μ g) under reducing and non-reducing conditions (silver stain). Lane 1, recEh29 reduced in the presence of 50 mM DTT (5 min, 95 °C); lane 2, recEh29 reduced in the presence of 5 mM DTT (5 min, 95 °C); lane 3, recEh29 non-reduced (5 min, 95 °C); lane 4, recEh29 non-reduced (5 min, room temperature). Molecular mass markers are shown on the left.

Besides the ability to remove exogenous added H_2O_2 , both enzymes were able to remove H_2O_2 that was produced enzymically by Eh34. During the reaction of recombinant Eh34, oxygen was reduced in the presence of NADPH, which lead to the production of H_2O_2 . During a 10 min period, approx. 110 nmol of H_2O_2 were formed using 1 μ g of Eh34. In response to the addition of recEh29, less H_2O_2 was measured. Eh29 (0.13 nmol) was able to remove approx. 50 nmol of H_2O_2 . Similarly, a decrease in the amount of H_2O_2 was obtained using recEh29mut. However, it was only half as active as recEh29 since 0.3 nmol of Eh29mut were necessary to remove 50 nmol of H_2O_2 . In contrast with the H_2O_2 -removing activity measured by direct addition of H_2O_2 in the presence of DTT, the H_2O_2 -removing activity in the presence of Eh34 was approx. 10-fold less. In this

Figure 2 Molecular mass determination of Eh29

The molecular mass of Eh29 was estimated by gel filtration on a Superdex 200 column of trophozoite extract in 50 mM Tris/HCl, pH 8.0. Before the addition of H_2O_2 (100 μ M) samples (50 μ l of each fraction) were preincubated with 0.2 mM DTT. H_2O_2 consumption was determined using the ABTS method [25]. Arrows denote protein standards with molecular mass in kDa. Thiol-peroxidase activity was eluted at positions corresponding to 35 kDa and 160–300 kDa. Vo, void volume.

reaction, the addition of DTT did not enhance the peroxidase activity. After freeze/thawing and denaturation of the protein at 95 °C, a total loss of activity was also observed. In combination with recEh29, Eh34 has two functions. Firstly it produces H_2O_2 under aerobic conditions, and secondly it functions as a reductant to transfer recEh29 into the reduced, active state. The H_2O_2 -removing activity was stable at 4 °C for several weeks, but was lost after incubation at 37 °C overnight.

Identification of thiol peroxidase activity in *E. histolytica* lysates

To study whether thiol peroxidase activity is present in *E. histolytica* lysates, trophozoite extracts were separated by gel filtration, resulting in two peaks of activity. The main peak corresponded to a molecular mass of approx. 35 kDa. In addition, peroxidase activity was also present in fractions containing proteins with molecular masses ranging from 160 to 300 kDa (Figure 2). Under reducing conditions, a protein of approx.

Table 1 Hydrogen peroxide consumption by recEh29 and recEh29mut

Hydrogen peroxide (80 nmol) was added to different amounts of recEh29 and recEh29mut under several conditions (after 30 min preincubation of the proteins with 0.2 mM DTT before addition of H_2O_2 ; without addition of DTT; after 1 time freeze/thaw and preincubation with 0.2 mM DTT; after denaturation for 5 min at 95 °C and preincubation with 0.2 mM DTT). The amount of H_2O_2 that was removed was determined using the ABTS method. Values are means $\pm$ S.D. from six experiments. n.d., not done.

	H_2O_2 removed (nmol $\cdot$ min $^{-1}$)							
	0.2 mM DTT		Without DTT		Freeze/thaw		5 min/95 °C	
	Eh29	Eh29mut	Eh29	Eh29mut	Eh29	Eh29mut	Eh29	Eh29mut
recEh29/recEh29mut (μ g)								
0.125	3.2 $\pm$ 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.25	5.4 $\pm$ 0.3	1 $\pm$ 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	12 $\pm$ 1	7 $\pm$ 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1.0	26 $\pm$ 2.3	12 $\pm$ 2	1	0	0	1	0	0
2.0	50 $\pm$ 3.4	21 $\pm$ 2.1	2	1	0	0	0	0

Figure 3 Western blot analyses using antisera raised against Eh29 or Eh29mut

Trophozoites of *E. histolytica* were washed in PBS, freeze/thawed 5 times in solid CO₂/ethanol and sedimented by centrifugation at 4 °C for 40 min. The resulted pellet was washed twice in PBS. The membrane fraction (M) and the 150 000 g supernatant (S) were separated on SDS/12% PAGE under reducing conditions. The proteins were blotted on to nitrocellulose and the Western blots were developed with antisera to recEh29 (anti-recEh29) and recEh29mut (anti-recEh29mut). Molecular mass markers are shown on the left.

Table 2 Functional analysis of Eh29 and Eh29mut in *E. coli* *ahp*⁻-mutant TA4315

The plasmids were transformed into the *E. coli* *ahp*⁻-mutant TA4315. The susceptibility to cumene hydroperoxide was measured using the filter discs assay. The reduction (%) of susceptibility to cumene hydroperoxide was calculated in comparison with *E. coli* *ahp*⁻-mutant TA4315. Data are averages from 10 experiments $\pm$ S.E. The reduction of the susceptibility to cumene hydroperoxide is significant ($P < 0.01$) for pHis8-Eh29, pHis8-Eh29mut, pGS02 and pAQ27, using Student's *t* test.

Plasmid	Plasmid-encoded proteins	Reduction of susceptibility to cumene hydroperoxide (%)
pHis8	—	0 $\pm$ 5.3
pHis8-Eh29	Eh29	15.2 $\pm$ 2.2
pHis8-Eh29mut	Eh29mut	14 $\pm$ 2.2
pGS02	AhpC	28 $\pm$ 3.1
pAQ27	AhpC, AhpF	58.9 $\pm$ 0.6

29 kDa that was only present in the active fractions was found to react specifically with an antiserum raised against recEh29. The 29 kDa protein reacting with the antiserum was detected in soluble, but not in membrane, fractions of trophozoite extracts (Figure 3), which is in line with indirect immunofluorescence microscopy, indicating that the protein is localized in the cytoplasm of the amoebae (results not shown).

Complementation of *E. coli* *ahp*⁻-mutant by recombinant expression of Eh29

To analyse whether Eh29 and Eh29mut exhibit Ahp activity, the two proteins were recombinantly expressed using an *E. coli* mutant lacking the *ahp* locus (TA4315). As controls, the respective expression plasmid without the amoeba genes, as well as two expression plasmids containing the entire *E. coli* *ahp* locus (pAQ27) or the *E. coli* *ahpC* gene (pGS02), were introduced. The various *E. coli* transformants were studied for their susceptibility to cumene hydroperoxide. The results, as summarized in Table 2 indicated that both Eh29 and Eh29mut are able to significantly reduce sensitivity of TA4315 cells to cumene hydroperoxide by approx. 15%. This is approx. half the reduction observed by

expression of the similar *E. coli* gene (*ahpC*). Expression of the entire *E. coli* *ahp* locus reduced cumene hydroperoxide susceptibility by approx. 60%.

DISCUSSION

Amino acid sequence-similarity analysis showed that the 29 kDa protein of *E. histolytica* (Eh29) belongs to the AhpC/TSA protein family, and comprises more than 25 different proteins that have been identified so far [13]. The main difference between Eh29 and the other members of the TSA/AhpC family is a cysteine-rich region at the N-terminal end of Eh29. Similarity of this region to metallothioneins is obvious (approx. 35–45% identity over 25 amino acid residues). Metallothioneins are small, cysteine-rich proteins that are involved in the metal-ion metabolism of the cell and in the cellular response to oxidative stress. It remains to be studied if this N-terminal region of Eh29 is involved in the protection against oxidative stress, as is described for other metallothioneins [29–31].

AhpC as well as TSA have the ability to catalyse the destruction of H₂O₂ or organic hydrogen peroxides. For this reaction both proteins need reducing equivalents. For the reaction catalysed by AhpC the reducing equivalents are provided by the larger subunit of the Ahp reductase, AhpF [14–18]. TSA is a peroxidase that reduces H₂O₂ with the use of hydrogens (electrons) provided by thioredoxin, thioredoxin reductase and NADPH [21]. Furthermore, TSA has thiol peroxidase activity. For this reaction thiols, like DTT, are necessary to convert TSA from the oxidized form back to the reduced, active form [21,22]. Our data indicate that H₂O₂ is consumed by Eh29. Like TSA, Eh29 has a thiol peroxidase activity. It needs DTT to catalyse the consumption of H₂O₂. In comparison with TSA, recEh29 has a higher activity. It catalyses the consumption of 50 μ M $\cdot$ min⁻¹ using 0.07 μ M recombinant protein. In contrast, 6 μ M TSA is necessary to catalyse the removal of 60 μ M H₂O₂ within 20 min [22]. Therefore recEh29 is approx. 2000-fold more active than TSA.

On the other hand, the reducing equivalents used for the reduction of H₂O₂, could also be provided by Eh34. The amoebic NADPH:flavin oxidoreductase produces H₂O₂ in the presence of oxygen and NADPH [4,8] (Bruchhaus, I., Richter, S. and Tannich, E., unpublished work). The bound flavin within the NADPH:flavin oxidoreductase is responsible for its H₂O₂-forming activity (Bruchhaus, I., Richter, S. and Tannich, E., unpublished work). In the presence of recEh29 or recEh29mut less H₂O₂ was measured. Therefore Eh34 is able to transfer reducing equivalents to oxygen to form H₂O₂, as well as to Eh29 to convert the protein from the oxidized, back to the reduced, form. This postulated reaction may be the same as that proposed for oxygen reduction catalysed by *Amphibacillus xylanus* NADH oxidase in the presence of AhpC from *S. typhimurium*. During this reaction both enzymes catalyse the 4-electron reduction of oxygen to water [32]. The truncated form of Eh29, which lacks the cysteine-rich N-terminal region of the protein, has only 50% activity compared with the full-length enzyme. In which way the N-terminal region of the protein is involved in the peroxidase activity remains to be studied.

Complementation assays using an *ahp*⁻-mutant of *E. coli*, which is highly sensitive to cumene hydroperoxide, showed that the Eh29 can significantly reduce the susceptibility of this mutant to cumene hydroperoxide. These results indicate that Eh29 has alkyl hydroperoxide reductase activity, as was described for AhpC. No difference could be observed between the mutated and the wild-type enzyme, which shows that the N-terminal cysteine-rich region is not necessary for alkyl hydroperoxide reductase activity.

Active TSA exists predominantly as a dimer [23]. Determination of the molecular mass using gel filtration showed that Eh29 is active in the monomeric state. In addition, a less-active high-molecular-mass complex of aggregated Eh29 was found. It seems that Eh29 aggregates over time or after freezing. This aggregation correlates with the loss of thiol-peroxidase activity (results not shown). Using antisera against recEh29, as well as recEh29mut, we localized the protein in the cytoplasm of the amoebae. Using Western blot analyses, as well as immunofluorescence, the localization on the membrane, which was postulated in a previous report, could not be verified [33].

Eh29 acts enzymically in the detoxification of ROS. It works in concert with other amoebic enzymes, such as the *E. histolytica* iron-containing superoxide dismutase and Eh34, which both detoxify ROS by the formation of toxic H₂O₂. Since *E. histolytica* lacks any detectable catalase activity, Eh29 might be an essential part in the enzymic chain of ROS detoxification. Vast amounts of ROS are produced by activated phagocytes of the host during oxidative burst. Therefore Eh29 might be an important enzyme to protect amoebae against ROS after tissue invasion.

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