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Purification of a Catalase-Peroxidase from *Halobacterium halobium*: Characterization of Some Unique Properties of the Halophilic Enzyme†

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A hydroperoxidase purified from the halophilic archaeon *Halobacterium halobium* exhibited both catalase and peroxidase activities, which were greatly diminished in a low-salt environment. Therefore, the purification was carried out in 2 M NaCl. Purified protein exhibited catalase activity over the narrow pH range of 6.0 to 7.5 and exhibited peroxidase activity between pH 6.5 and 8.0. Peroxidase activity was maximal at NaCl concentrations above 1 M, although catalase activity required 2 M NaCl for optimal function. Catalase activity was greatest at 50°C; at 90°C, the enzymatic activity was 20% greater than at 25°C. Peroxidase activity decreased rapidly above its maximum at 40°C. An activation energy of 2.5 kcal (ca. 10 kJ)/mol was calculated for catalase, and an activation energy of 4.0 kcal (ca. 17 kJ)/mol was calculated for peroxidase. Catalase activity was not inhibited by 3-amino-1,2,4-triazole but was inhibited by KCN and NaN_3 (apparent K_i [K_i^{App}] of 50 and 67.5 μM, respectively). Peroxidative activity was inhibited equally by KCN and NaN_3 (K_i^{App} for both, ~30 μM). The absorption spectrum showed a Soret peak at 404 nm, and there was no apparent reduction by dithionite. A heme content of 1.43 per tetramer was determined. The protein has a pI of 3.8 and an M_r of 240,000 and consists of four subunits of 60,300 each.

Metabolism in an oxygen-enriched environment often results in the generation of reactive oxygen species such as superoxide, hydroxyl radical, and hydrogen peroxide (8, 22). Organisms have evolved specific enzyme systems to neutralize potentially lethal reactive oxygen species. Among these systems is the group of heme proteins designated hydroperoxidases. The group consists of two classes of proteins: catalases and peroxidases. The former is characterized by electron pair transitions in which H_2O_2 is decomposed to O_2 and H_2O, whereas the latter is characterized by single electron transfers resulting in the oxidation of various organic compounds by H_2O_2 (5, 6).

Catalases isolated from higher organisms are similar to each other in that they have molecular weights in the range of 225,000 to 270,000, contain four equally sized subunits each containing one ferric heme prosthetic group (protoporphyrin IX), show a broad pH range of 5 to 10.5, and are specifically inhibited by 3-amino-1,2,4-triazole (5, 21, 25). Peroxidases, like catalases, are heme enzymes (5), but they are monomeric proteins which show diversity in their molecular weights (6). Moreover, the heme iron of catalases is not reducible, whereas the heme iron of the peroxidases can be reduced by dithionite (2, 6).

The hydroperoxidase class of proteins has recently been shown to contain enzymes which exhibit both catalase and peroxidase activities. Like that of typical peroxidases, the heme component of these enzymes can be readily reduced by dithionite. They are also similar to typical catalases in that they exhibit a tetrameric molecular weight in the range of 240,000 with equally sized subunits. However, these enzymes show properties which are distinct from those of typical catalases in that they possess narrow pH ranges for maximal activity and increased sensitivity to temperature, are inactivated by H_2O_2, and are not inhibited by 3-amino-1,2,4-triazole (10, 12). Catalase-peroxidase proteins have been observed in the following bacteria: *Escherichia coli* (3), *Rhodopseudomonas capsulata* (14), *Klebsiella pneumoniae* (13), *Chromatium vinosum* (21), *Rhodobacter capsulatus* (12), *Salmonella typhimurium* (18), and the alkalophilic *Bacillus* strain YN-2000 (28). Therefore, it would appear that some microorganisms contain a novel group of hydroperoxidases which possess both catalase and peroxidase activities and which share characteristics with the typical catalases and peroxidases from higher organisms.

Despite the detection of catalase-peroxidase in diverse organisms, we do not know how universally distributed this new enzyme class may be. Halobacteria are members of the urkingdom *Archaea* and are characterized by requirements for high salt concentrations for growth. They are facultatively aerobic and under anaerobic conditions generate ATP by means of an ATP synthase coupled to a light-driven proton pump, bacteriorhodopsin (11, 15). Under aerobic conditions, they exhibit standard respiratory metabolism. The unique metabolism of these organisms as well as the facultative nature of their growth have led us to investigate halobacterial adaptations to aerobic environments. We have recently reported on the purification of a *Halobacterium halobium* superoxide dismutase and on the gene coding for its presence (23, 24). We examined how this organism might contend with H_2O_2, and in this paper we report on the purification and characterization of a hydroperoxidase from *H. halobium*. The protein is a catalase-peroxidase type of enzyme as seen in some other procaryotes. However, the protein exhibits additional unique characteristics not previously seen in the catalase-peroxidase group of hydroperoxidases.

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MATERIALS AND METHODS

Organism and culture conditions. *H. halobium* (strain NRL) was grown in 1-liter flasks in complex medium (25% [wt/vol] NaCl, 2% [wt/vol] $MgSO_4 \cdot 7H_2O$, 0.3% [wt/vol] citric acid, 0.2% [wt/vol] KCl, and 1% [wt/vol] peptone) adjusted to pH 7.0. Culture flasks were shaken at 160 rpm in a water bath maintained at 40°C.

Assays and other procedures. All enzyme assays were done at 25°C, unless stated otherwise, in a buffer of 50 mM potassium phosphate containing 2 M NaCl. The pH of the buffer was adjusted to 6.5 for catalase assays and to 7.5 for peroxidase assays. Catalase activity was determined spectrophotometrically at 240 nm (1) with 20 mM H_2O_2. Peroxidase activity was monitored by the spectrophotometric assay at 510 nm with 4-aminoantipyrine as a substrate (27). The H_2O_2 concentration was 2 mM, and the reaction was initiated by addition of enzyme. One unit of activity for both catalase and peroxidase is defined as the decomposition of 1 μmol of H_2O_2 per min at 25°C (27) at the specified pH. The peroxidation of NADH and NADPH was monitored at 340 nm, that of ascorbate was monitored at 265 nm, that of reduced cytochrome *c* was monitored at 550 nm, and that of *o*-dianisidine was monitored at 460 nm.

Nondenaturing polyacrylamide gel electrophoresis (PAGE) was performed in 7.5% (wt/vol) acrylamide tube gels according to the method of Davis (4). Salt was dialyzed out of the protein solution prior to electrophoresis. Protein bands were visualized after the gels were stained with Coomassie brilliant blue R. Subunit size was determined by sodium dodecyl sulfate (SDS)-PAGE on 10% (wt/vol) acrylamide according to the procedure of Laemmli (16). The molecular weight of the native enzyme was determined by size exclusion on a Sephadex G-200 column (0.75 by 84 cm) equilibrated in 50 mM potassium phosphate (pH 7.0) in 2 M NaCl. The isoelectric point was determined by focusing of the purified protein along with standards on Servalyte precotes (pH 3 to 10). Protein was determined by the method of Lowry et al. (19) for the first three steps of the purification procedure, whereas in the latter stages of purification it was determined by $E_{280}^{1\%} = 12$, where $E_{280}^{1\%}$ is the extinction coefficient at 280 nm of a 1% solution of the protein. The $E_{280}^{1\%}$ value was determined from a regression of several purified catalase-peroxidase concentrations, measured by the method of Lowry et al. (19), versus optical density at 280 nm. The protoheme content was determined by the pyridine ferrohemochrome method (8% [vol/vol] pyridine and 0.17 N NaOH in 50 mM potassium phosphate [pH 7.0] containing 2 M NaCl) and calculated by using $E_{409} = 16.3 \times 10^4$ M (7). Spectrophotometric determinations of enzyme activity and recording of absorbance spectra were performed with a Perkin-Elmer 552 spectrophotometer.

Purification of *H. halobium* catalase-peroxidase. Cultures at early to mid-log phase of growth (optical density at 600 nm of 0.400 to 0.500) were harvested by centrifugation at 7,500 × *g* for 10 min at 4°C and then washed twice with 50 mM potassium phosphate (pH 7.0) containing 2 M NaCl. The cells were resuspended in 50 ml of the wash buffer and then frozen (−70°C, 15 min) and thawed (37°C, 3 min) five times. To further ensure maximal cell disruption, cells were subjected to sonication at full power on a Branson sonifier. Sonic disruption at 4°C was performed for 20 s and was followed by 2 min of cooling in order to prevent excessive heating. At the end of 3 min (total) of sonication, 1 mg of DNase I was added to the homogenate and the suspension was stirred at room temperature for 1.5 h. The extract was centrifuged for 15 min at 10,000 × *g* at 4°C, and solid 6,000- to 8,000-molecular-weight polyethylene glycol (5% [wt/vol] final concentration) was added to the clarified supernatant. The mixture was stirred at room temperature for 15 min and then centrifuged at 7,500 × *g* for 15 min. Additional polyethylene glycol was added to the supernatant to bring the final concentration to 15% (wt/vol), and the mixture was recentrifuged after 15 min of stirring at room temperature. The resulting pellet was resuspended in 50 ml of 50 mM potassium phosphate (pH 7.0) containing 2 M NaCl and dialyzed overnight into the same buffer. Solid hydroxylapatite (2 g) was added to the solution, and after 15 min of stirring at 4°C, the protein was eluted in a batchwise fashion, first with 100 mM potassium phosphate containing 2 M NaCl and then with 300 mM potassium phosphate containing 2 M NaCl. The last eluent from the hydroxlapatite was assayed for catalase activity and then dialyzed overnight against 50 mM potassium phosphate (pH 7.0) containing 2 M NaCl. The preparation was then concentrated to 1.0 ml by Amicon ultrafiltration, and 500-μl aliquots were injected into a Macrosphere GPC 300 column (300 by 7.5 mm; Altech) attached to a Waters model 600E high-performance liquid chromatograph (HPLC). The mobile phase consisted of 50 mM potassium phosphate (pH 7.0) containing 2 M NaCl, and the flow rate was 0.25 ml/min. A_{280} was monitored, and protein peaks were collected and assayed for catalase activity. The elution time for the appearance of catalase activity varied from 23.5 to 25 min. Eluants containing activity were concentrated to approximately 500 μl and reinjected into the HPLC. The protein peak containing enzymatic activity was collected and loaded onto a Sephadex G-150 column (0.75 by 75 cm) equilibrated with 50 mM potassium phosphate (pH 7.0) containing 2 M NaCl, and the protein was eluted with the same buffer. Fractions were assayed for catalase activity, and all fractions containing activity were pooled, concentrated to approximately 1.5 ml, and stored at 4°C.

Chemicals. Sephadex G-200 was purchased from Pharmacia, Sephadex G-150 was purchased from Sigma, and hydroxylapatite was purchased from ICN Biochemicals. Isoelectric focusing standards, molecular weight standards for gel exclusion, and Servalyte precotes were purchased from Serva. Molecular weight standards for SDS gel electrophoresis were from Bio-Rad. DNase I was purchased from United States Biochemical Corporation. Acrylamide, H_2O_2, bisacrylamide, polyethylene glycol, 3-amino-1,2,4-triazole, and 4-aminoantipyrine were from Sigma. All other chemicals were reagent grade.

RESULTS

Purification of *H. halobium* catalase-peroxidase. Extracts obtained from *H. halobium* exhibited catalase and peroxidase activities which were stable only in buffers containing an excess of 1 M NaCl. Therefore, all steps in the purification were performed in buffers containing 2 M NaCl. A summary of the purification procedure can be seen in Table 1, in which data for catalase activity as well as peroxidase activity are presented. From 3.66 g of cell paste, approximately 1.48 mg of protein was obtained. Yields for catalase activity and peroxidase activity were 8.6 and 20%, respectively. On the basis of catalatic activity, the protein was purified nearly 13-fold, whereas on the basis of peroxidative activity, an approximately 30-fold purification was achieved. Polyethylene glycol precipitation removed some contaminating protein, although significant losses in both catalase and peroxidase activities occurred. The addition of dithiothreitol

TABLE 1. Purification of *H. halobium* catalase-peroxidase[a]

Step	Vol (ml)	Protein (mg/ml)	Total protein (mg)	Catalase				Peroxidase			
				Sp act (U/mg)	Total (U)	Yield (%)	Purification (fold)	Sp act (U/mg)	Total (U)	Yield (%)	Purification (fold)
Crude extract	47.0	4.64[b]	218.08	3.4	741.5	100.0	1.0	3.9	850.5	100.0	1.0
15% polyethylene glycol	47.0	3.27[b]	153.69	3.1	476.4	64.2	0.9	2.4	378.8	44.5	0.6
Hydroxylapatite	82.0	0.50[b]	41.00	7.9	323.9	43.7	2.3	14.8	606.8	71.3	3.8
HPLC	3.6	1.45[c]	5.22	27.2	142.0	19.2	8.0	97.5	509.0	59.8	25.0
Sephadex G-150	1.7	0.87[c]	1.48	43.2	63.9	8.6	12.7	104.3	154.4	20.1	29.6

[a] Purification was from 3.66 g of cell paste. Enzymatic activities were assayed as described in Materials and Methods.
[b] Determined by the method of Lowry et al. (19).
[c] Determined by $E_{280}^{1\%} = 12$.

and phenylmethylsulfonyl fluoride did not improve the retention of activity through any of the purification steps. Enzymatic activity was lost in each step of the purification procedure. Therefore, the specific activity may represent a lower value than might actually exist.

Figure 1A shows a nondenaturing polyacrylamide gel of the purified enzyme stained for protein. One band is seen, indicating that the protein was purified to apparent electrophoretic homogeneity. The elution profile shown in Fig. 1B indicates that catalase and peroxidase activities coeluted off the Sephadex G-150 column.

Molecular weight and isoelectric point. The native molecular weight of the purified protein was estimated to be approximately 240,000 by gel filtration through a Sephadex G-200 column in the presence of 2 M NaCl. Dissociation of the protein into monomers was achieved in the presence of 5% (vol/vol) 2-mercaptoethanol in either the presence or absence of 2 M NaCl. The subunit molecular weight of the protein estimated by SDS-PAGE was 60,300 (data not shown). The results indicate that the *H. halobium* catalase-peroxidase is a tetramer composed of four equally sized subunits.

Purified protein was electrophoresed through a gradient of pH 3 to 10 in agarose gels. The isoelectric point of the enzyme was determined to be 3.8. Dialysis of the protein into buffer without NaCl did not alter the pI value obtained.

Spectroscopic properties of catalase-peroxidase. The absorption spectrum of the purified catalase-peroxidase showed a Soret peak at 404 nm and an additional minor peak at 597 nm (Fig. 2). Reduction with dithionite did not alter the spectral shape, although it slightly blue-shifted the Soret peak to 403 nm. Additionally, KCN did not result in an

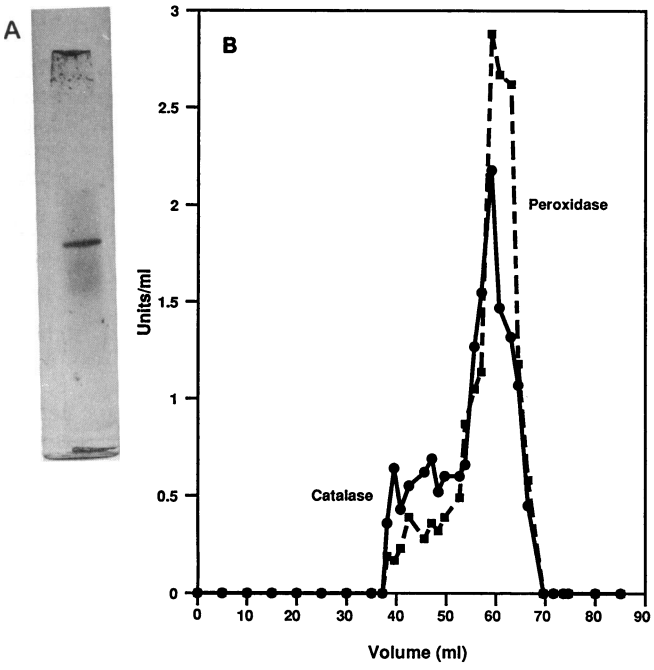

FIG. 1. Copurification of catalase and peroxidase activities of *H. halobium*. (A) PAGE of *H. halobium* catalase-peroxidase purified from a Sephadex G-150 column. Protein (25 μg) was electrophoresed on 7.5% (wt/vol) nondenaturing gels after removal of NaCl by dialysis and stained with Coomassie brilliant blue. (B) Elution profile from a Sephadex G-150 column.

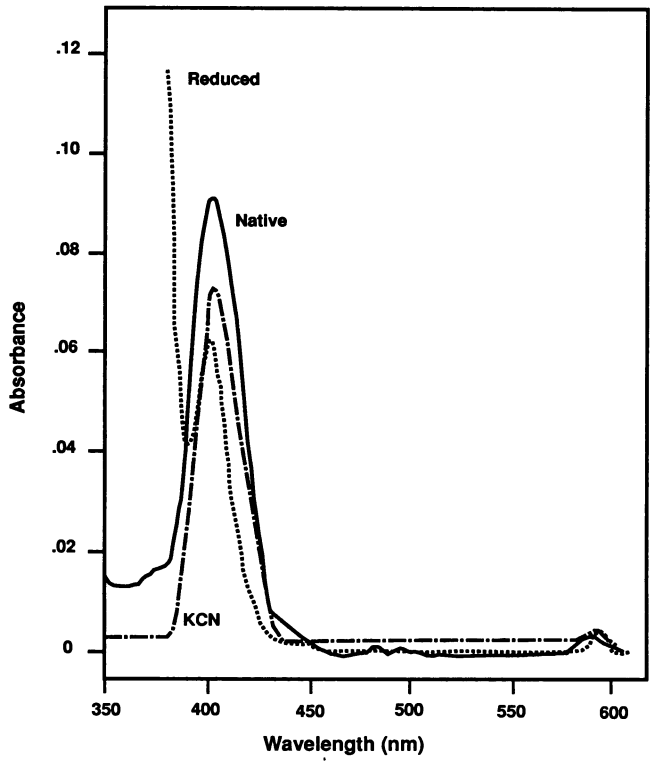

FIG. 2. Absorption spectra of *H. halobium* catalase-peroxidase. ——, native enzyme; — · — · —, with 10 mM KCN; · · · · · · ·, with 1 mM sodium dithionite. Protein content, 170 μg.

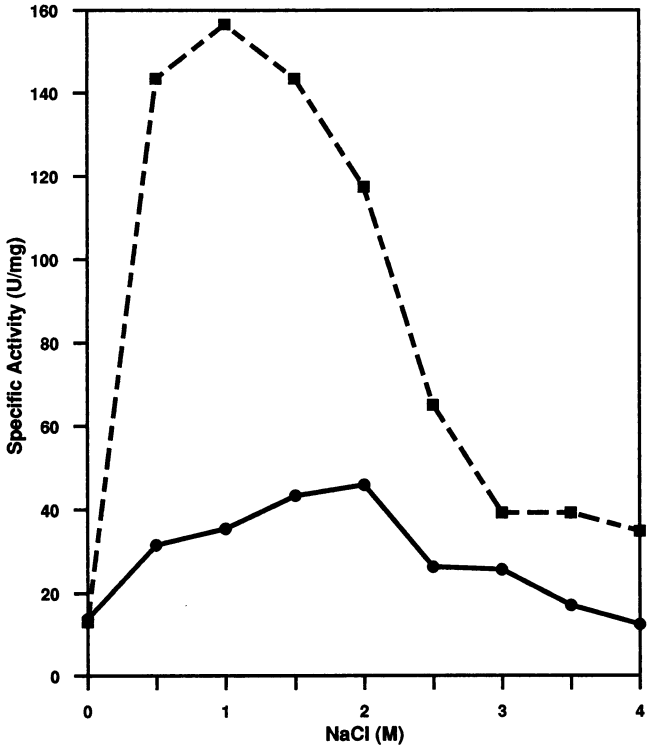

FIG. 3. Salt dependence of *H. halobium* catalase-peroxidase. Activities were measured at 25°C in 50 mM potassium phosphate containing the indicated NaCl concentrations. Assay procedures were as described in Materials and Methods. ——, catalase activity; - - - - - - -, peroxidase activity.

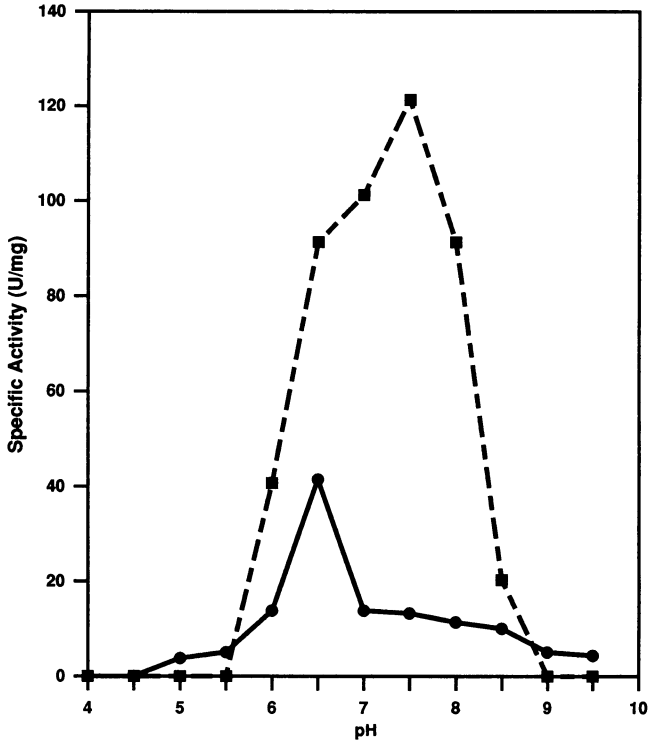

FIG. 4. Effect of pH on *H. halobium* catalase-peroxidase activity. Catalase and peroxidase activities were monitored at 25°C in 2 M NaCl–50 mM potassium phosphate adjusted to the indicated pHs. At the extreme pH ranges, the final pH was determined in the cuvette prior to the onset of the reaction. Assay procedures were as described in Materials and Methods. ——, catalase activity; - - - - - -, peroxidase activity.

altered spectrum. Analogous spectra were obtained from four separate preparations of the protein.

The protoheme content of the catalase-peroxidase, estimated from the pyridine hemochromogen peak at 403 nm, was 1.43 hemes per tetramer and was reproducible in all enzyme preparations. The low heme content is in agreement with the relatively low value (0.44) of A_{404}/A_{280}.

Salt and pH dependence of the *H. halobium* catalase-peroxidase. The salt dependence of catalase and peroxidase activities of the hydroperoxidase can be seen in Fig. 3. Assay of enzymatic activity in the absence of NaCl resulted in only minimal enzymatic function. Increasing salt concentrations above 0.5 M NaCl yielded dramatic increases in enzymatic activity. For catalase the peak was reached at a concentration of 2 M NaCl, whereas peroxidase activity was maximal at 1 M NaCl. Removal of NaCl by dialysis resulted in a complete loss of enzymatic function which could not be reversed by the readdition of salt.

The effect of determining hydroperoxidase activity at various pH values is seen in Fig. 4. Catalatic activity showed a narrow range with a maximum at pH 6.5. Peroxidative function was slightly broader in range with a maximum at pH 7.5.

Temperature dependence. Purified enzyme was incubated at various temperatures for 5 min and then immediately assayed for catalase and peroxidase activities (Fig. 5). Peroxidase function was maximal at 40°C. Activity declined after incubation at higher temperatures, although at 70°C nearly 50% of the activity at 25°C was retained. Catalatic activity of the hydroperoxidase was surprisingly resistant to heat. Maximal function was observed at 50°C, but the enzyme retained activity after incubation at temperatures as high as 90°C. The heat resistance was not due to salt per se, since bovine catalase and horseradish root peroxidase were completely inactivated at temperatures above 50°C in the presence of 2 M NaCl (data not shown).

An Arrhenius plot for catalase and peroxidase yielded activation energies of 2.5 and 4.0 kcal (ca. 10 and 17 kJ)/mol, respectively. Pseudo-first-order decay constants (K_{dnat}) were calculated for the catalatic activity at 90°C and for the peroxidative activity at 50°C. The K_{dnat} for catalase was 0.132 min^{-1}, and the K_{dnat} for peroxidase was 0.11 min^{-1}.

Catalytic properties of catalase-peroxidase. Catalatic activity of the purified *H. halobium* catalase-peroxidase was not inhibited by incubation for 2.5 h with 20 mM 3 amino-1,2,4-triazole (data not shown). However, CN^- and N_3^- were effective inhibitors. Apparent inhibition constants (K_i^{App}) for these two compounds for catalase and peroxidase activities are shown in Table 2. Also shown in Table 2 are the K_m and 50% inhibition values of H_2O_2 for catalase and peroxidase activities. Hydrogen peroxide in concentrations above 35 mM resulted in a decrease in catalase function; therefore, the K_m value for catalase was determined at H_2O_2 concentrations below 14 mM. Although we routinely assayed the peroxidase with 4-aminoantipyrine as a substrate, we observed that *o*-dianisidine, 3,3'-diaminobenzidine, ascorbate, NADH, NADPH, and reduced cytochrome *c* could all be used as substrates for the peroxidase.

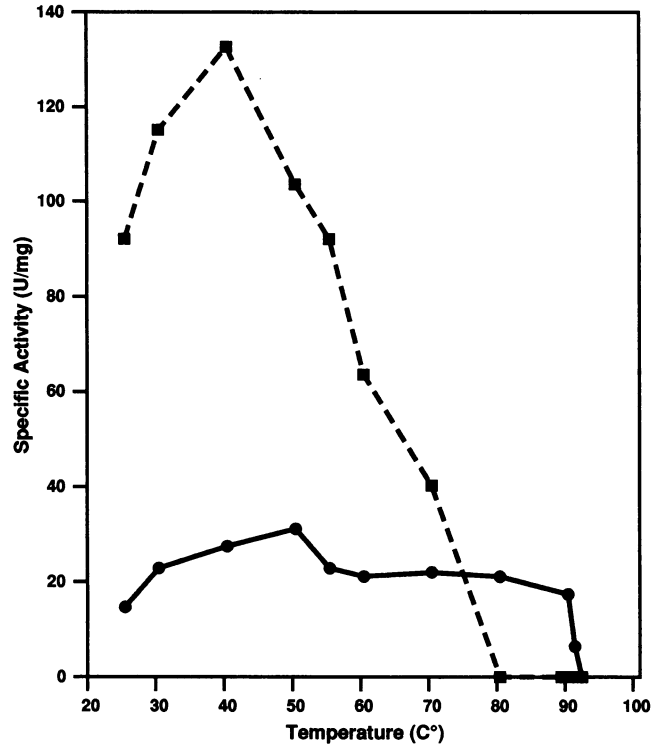

FIG. 5. Effect of temperature on *H. halobium* catalase-peroxidase activity. Catalase and peroxidase activities were assayed as described in Materials and Methods. Enzyme and buffer were incubated for 5 min at the indicated temperatures prior to initiation of the reaction. ——, catalase activity; – – – – –, peroxidase activity.

DISCUSSION

The *H. halobium* catalase-peroxidase described here, like most other proteins purified from halophilic organisms (15, 17, 24), requires salt in excess of 1 M for maximal activity. In the absence of salt, irreversible inactivation takes place, and therefore all purification procedures required sufficient salt in order to maintain a stable protein. Despite the presence of 2 M NaCl, losses of both catalase and peroxidase activities persisted in many of the purification steps. It is not clear what the source of inactivation might be, although the presence of a reducing agent as well as a protease inhibitor did not alter the apparent inactivation. Other investigators have experienced similar losses in catalase and peroxidase activities during purification procedures (3, 13, 14). It is also noteworthy that the observed differences in yields and purification enrichment between catalase and peroxidase activities for *H. halobium* (Table 1) appear to be a common phenomenon when a single protein with dual enzymatic functions is purified (3, 13, 14). This does not necessarily represent the presence of multiple proteins but could be a function of distinct active sites and distinct substrates.

TABLE 2. Kinetic properties of *H. halobium* catalase-peroxidase[a]

Enzyme activity	H_2O_2 K_m (mM)	H_2O_2 50% inhibition (mM)	K_i^{App} (μM)	
			KCN	NaN_3
Catalase	3.7	4.5	50	67.5
Peroxidase	0.14	0.4	30	32.5

[a] The enzymatic activities were assayed as described in Materials and Methods.

The catalase-peroxidase from *H. halobium* shares characteristics with eubacterial catalase-peroxidases. Among the similarities is the narrow pH range for the enzyme, in marked contrast to the apparent insensitivity to H^+ concentrations for typical catalases (5). A narrow peak of catalase activity was observed at pH 6.5. This is in agreement with the values observed for catalase-peroxidases from *R. capsulata* (pH 6 to 6.5) (21) and *Bacillus* strain YN-2000 (pH 6.0) (28) and for the catalase from *E. coli* (pH 6 to 6.5) (21). A narrow pH range for activity is not a general characteristic of bacterial catalases (13, 21). The H^+ stability of typical catalases and the relatively narrow pH range of the catalase-peroxidase group would indicate distinct protein classes.

Additional characteristics shared with other bacterial catalase-peroxidases include the inhibition by CN^- and N_3^- and the insensitivity to inhibition by 3-amino-1,2,4-triazole. The lack of inhibition of the catalase portion of the *H. halobium* hydroperoxidase by 3-amino-1,2,4-triazole can be taken as evidence that the preparation was not contaminated with a typical catalase, which would have been inhibited by the aminotriazole. Moreover, the *H. halobium* catalase-peroxidase is inhibited by H_2O_2 in excess of 30 mM. Substrate inhibition is characteristic of the other catalase-peroxidase proteins (3, 13, 28) but not of typical catalases (21), and this further shows the diversity within the hydroperoxidase class. The *H. halobium* catalase-peroxidase is acidic, with a pI of 3.8. This is characteristic of catalases (5) as well as of the catalase-peroxidase from *R. capsulata* (14). However, many halophilic proteins are acidic as well (15, 17, 24). Therefore, the acidic nature of the catalase-peroxidase protein might not be solely a function of its membership in the hydroperoxidase group.

The value for *H. halobium* catalase activity is lower than that reported for catalytic activity in other catalase-peroxidases (3, 13, 14, 28). However, the peroxidase activity value for *H. halobium* is higher (3, 14), although it should be noted that different peroxidase substrates were used. The relatively greater amount of peroxidative activity in relation to catalase activity is unique to this enzyme. Additionally, the *H. halobium* catalase-peroxidase contained 1.43 hemes per tetramer, whereas many other bacterial catalase-peroxidases contain 2 hemes per tetramer (28). The diminished heme content could be responsible for the relatively low A_{404}/A_{280} ratio of 0.44 observed for the enzyme and might represent heme lost or degraded during the purification process.

The *H. halobium* catalase-peroxidase can be distinguished from other catalase-peroxidases by its inability to be reduced by dithionite, its salt requirement, and its temperature stability. Although typical catalases are not reduced or are only slightly reduced by dithionite (5), the catalase-peroxidases so far described are readily reduced (14, 28). The red shift in spectra reported for other catalase-peroxidases in the presence of dithionite is not apparent for the halobacterial enzyme. This observation was consistent in all *H. halobium* enzyme preparations. Stearic factors may play a role in the resistance of typical catalases as well as the *H. halobium* catalase-peroxidase to reduction by dithionite.

The hydroperoxidase described here is unique in that it has a rigid salt requirement. Insight into the salt requirement would be gained by determination of the primary structure of the protein. Attempts at obtaining an N-terminal sequence were unsuccessful, perhaps because of blockage or a cyclized glutamyl residue at the N terminus. However, we have generated fragments of the internal regions of the

protein which can be used as a basis for oligonucleotide synthesis in an attempt to clone the gene.

The stability of the *H. halobium* catalase-peroxidase at elevated temperatures is noteworthy. The enzymatic activities of catalase-peroxidases from other organisms were completely inhibited at temperatures above 50°C (13, 14, 21). Moreover, the apparent thermal stability of the halobacterial enzyme cannot be explained entirely as due to high salt concentrations, since bovine catalase and horseradish root peroxidase were not protected from thermal denaturation by the presence of high salt concentrations. The molecular nature of the inherent thermal stability is not known at this point.

While a single catalase-peroxidase enzyme has been observed in an increasing number of microorganisms (3, 13, 14, 18, 21, 28), there has been little discussion concerning the function of a dual-activity enzyme. In the case of *H. halobium*, alterations in environment could dictate which of the activities of the catalase-peroxidase will function maximally. At elevated temperatures, catalase activity would be enhanced. Under acidic conditions, catalase activity would be greatest, whereas peroxidase activity would be elevated in a shift to a basic intracellular environment.

It should be noted that *H. halobium* contains another hydroperoxidase (9). This protein, like the hydroperoxidase reported here, has both peroxidase and catalase activities and is salt dependent. However, the two enzymes appear to be different in size, salt requirement, and the ability to be reduced by dithionite. Therefore, it would appear that *H. halobium* contains two distinct enzymes that perform the same function. This apparent redundancy might be accounted for by unique physiological substrates and therefore specific functions for the two proteins. Clearly, little is known about how archaea respond to oxidative stress. With the cloning of the hydroperoxidase genes as well as the superoxide dismutase genes (20, 23, 26), we would be in a better position to initiate studies at the molecular level.

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