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Distribution of Superoxide Dismutase, Catalase, and Peroxidase Activities Among *Treponema pallidum* and Other Spirochetes

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Representative members of *Spirochaetales* were surveyed for their content of superoxide dismutase (SOD), catalase, and peroxidase activities. Only *Leptospira* exhibited peroxidase activity. Obligately anaerobic cultivable *Treponema* and *Spirochaeta* possessed no SOD or peroxidative capabilities. Upon polyacrylamide gel electrophoresis, *Spirochaeta aurantia*, *Borrelia hermsi*, and five *Leptospira biflexa* serovars showed SOD activity associated with one electrophoretic band which was inhibited by H_2O_2, suggesting that they were iron-containing dismutases. These spirochetes could be distinguished by differences in relative mobilities of their SODs. SOD activity, but not catalase activity, was induced aerobically in *S. aurantia*. All *Leptospira interrogans* serovars and two *L. biflexa* serovars lacked significant SOD activity. These SOD-deficient strains of *Leptospira*, with one exception, possessed high levels of catalase activity. The Nichols strain of virulent *Treponema pallidum* possessed SOD and catalase activities, but lacked peroxidase activity. The SOD in *T. pallidum* exhibited two electrophoretic bands containing copper and zinc, and its relative mobility was identical to that of purified rabbit SOD. Immunization of sheep with purified rabbit SOD resulted in antiserum which inhibited both rabbit SOD and *T. pallidum* SOD assayed by spectrophotometric analysis or activity staining following polyacrylamide gel electrophoresis. In agarose gel diffusion, precipitin lines of identity were observed between purified rabbit SOD and cell extracts of *T. pallidum*. These data indicated that the SOD activity detected in *T. pallidum* was host derived.

Reduction of O_2 to water by microorganisms may result in the production of toxic oxidized intermediates such as the superoxide radical and H_2O_2. Enzymes which scavenge these such as superoxide dismutase (SOD), catalase, and peroxidase, may be essential defenses against such toxic radicals and compounds. Similarly, O_2 metabolism is of prime importance in the physiology and classification of microorganisms. The spirochetes are excellent examples of microbial diversity in O_2 utilization. Obligately aerobic *Leptospira* utilize primarily long-chain fatty acids for energy, whereas the anaerobic and facultatively anaerobic, free-living *Spirochaeta* are saccharolytic (11). *Borrelia hermsi* has been cultivated recently and appears to require O_2 for growth (26). Included in the genus *Treponema* are host-associated cultivable anaerobic species (11) as well as pathogenic species, such as *Treponema pallidum*, which have not been cultivated in vitro.

Previous investigations in our laboratory indicated that *T. pallidum* was capable of aerobic respiration (16, 29, 30). These studies revealed that H_2O_2 was produced during the oxidation of both pyruvate (3) and reduced nicotinamide adenine dinucleotide (30) and that incubation of whole cells at high dissolved O_2 concentrations resulted in a loss of pyruvate decarboxylase activity (4). In addition, reduced flavoproteins and cyanide-resistant respiration, which are known to be potential sites for superoxide radical generation in several electron transport systems (21, 32), have been demonstrated in *T. pallidum* (29, 30). Some means of protection against O_2 toxicity seemed likely to be required by *T. pallidum* in vitro, and therefore we have analyzed the distribution of dismutative and peroxidative enzymes in this treponeme in comparison with other spirochetes.

MATERIALS AND METHODS

Organisms. Propagation in rabbits, extraction, pu-

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rification, and counting of the Nichols strain of virulent *T. pallidum* have been described previously (16, 29, 39). Extraction of treponemes from infected testicles was carried out aerobically with shaking for 1 h at room temperature in phosphate-buffered saline containing 2 mM reduced glutathione (pH 7.4). After sequential filtration through Nuclepore filters to a final pore size of 0.8 μm, treponeme suspensions were centrifuged at 17,000 × *g* for 20 min. High-speed pellets were rinsed, but not disrupted, in fresh phosphate-buffered saline containing 2 mM reduced glutathione and recentrifuged at 17,000 × *g* for 20 min. Pellets were suspended in a minimal volume of sterile distilled water, and the suspensions were either used immediately or stored in liquid nitrogen. Cultivable *Treponema* and *Spirochaeta* were provided by E. Canale-Parola of this department. *Treponema denticola* strain 10 was cultivated at 35°C in GM-1 medium as described by Blakemore and Canale-Parola (7). *Treponema succinifaciens* strain 6091 and *Treponema bryantii* strain RUS-1 were cultivated in RFC medium containing 0.4% glucose instead of cellobiose (41). *Spirochaeta litoralis* strain R1 was cultivated at 30°C in a medium containing 0.4% yeast extract (Difco Laboratories, Detroit, Mich.), 0.2% glucose, 0.05% L-cysteine hydrochloride, 0.2% Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 0.2% peptone (Difco), 50 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.5), and 50% artificial seawater (5) at a final pH of 7.5. *Spirochaeta aurantia* strain J1 was cultivated at 30°C in GTY broth (10) both aerobically on a rotary shaker and anaerobically by methods based on those of Hungate (25). *B. hermsi* was obtained from R. Kelly and was cultivated as described by him (26). The B and H strains of *Leptospira biflexa* were isolated in this laboratory from surface water samples. *L. biflexa* serovars *patoc* 1, *semaranga*, *andamana*, and *illini* 3055 and *Leptospira interrogans* serovars *canicola* Hond Utrecht, *pomona* Pomona, *pomona* Riggs, *hardjo* Hardjo, *grippotyphosa* 1540, and *copenhageni* M-20 were obtained originally from various laboratories (23). Cells were grown in SM-7 medium (14) containing 0.02% Tween 80 and 0.2% bovine serum albumin fraction V (Miles Laboratories, Inc., Elkhart, Ind.). Cultivation was static at 30°C, and the cells were harvested when the growth achieved a turbidity of 60 to 80 nepholometer units. The turbidity was measured with a Coleman model 9 nephocolorimeter adjusted to 20 nepholometer units with a Coleman 81 nepholos standard. Cells were enumerated with a Petroff-Hausser chamber, and a turbidity of 60 corresponded to approximately 1×10^8 to 1.5×10^8 cells per ml. *Escherichia coli* B was provided by C. B. Thorne of this department and was grown at 37°C on a rotary shaker as described by Hassan and Fridovich (22). The medium contained 1% tryptose (Difco), 0.5% peptone (Difco), and 0.1% NaCl at a final pH of 7.0.

Preparation of CFE. Cells were harvested by centrifugation, washed in 20 mM potassium phosphate buffer, (pH 7.4) and suspended in a minimal volume of this buffer before sonic disruption. Cellular debris was removed by centrifugation at 20,000 × *g* for 15 min, and the supernatant fraction or cell-free extract (CFE) was analyzed. *T. pallidum* CFE was concentrated by lyophilization for use in the Ouchterlony procedure.

Enzyme assays. SOD (EC 1.15.1.1) was assayed as described by Marklund and Marklund (31) in 300-μl reaction mixtures with the rate of autoxidation of pyrogallol adjusted to 0.01 absorbance unit per min to increase sensitivity. In addition, SOD was assayed by the xanthine oxidase-cytochrome *c* method of McCord and Fridovich (33) using 300-μl reaction mixtures. One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of autoxidation of pyrogallol or the rate of reduction of cytochrome *c* by 50%. Specific activities were expressed as units per milligram of CFE protein. When less than 50% inhibition of the rate of autoxidation of pyrogallol was observed, the specific activity was calculated from the highest concentration of CFE protein assayed, which ranged from 0.15 mg of protein for *L. interrogans* serovar *pomona* Riggs to 0.42 mg of protein for *T. succinifaciens*. SOD was separated by electrophoresis of samples in 7.0% polyacrylamide gels (PAGE) with 10 mM tris(hydroxymethyl)aminomethane-hydrochloride–50 mM glycine buffer (pH 8.3) as described by Davis (17), stained for enzyme activity (6), and quantitated by linear scanning densitometry. Iron-, manganese-, and copper-zinc-containing enzymes were distinguished in gels by including 0.1 mM ethylenediamine-tetraacetate plus either 5.0 mM H_2O_2 (9) or 1.0 mM NaCN (45) in the staining reagents.

Catalase (EC 1.11.1.6) was assayed polarographically as described by Rorth and Jensen (38) with few modifications. Reactions were conducted at 30°C in 3.0 ml of 50 mM potassium phosphate buffer (pH 7.0) saturated with air by sparging. The absolute oxygen concentration in the buffer was 0.31 μmol of O_2 per ml as determined by the method of Robinson and Cooper (37). Reactions were initiated by the addition of 10 μl of H_2O_2 resulting in a final concentration of 15 mM. One unit of catalase activity was defined as the amount of enzyme catalyzing the decomposition of 1.0 μmol of H_2O_2 per min at 30°C. Specific activities were expressed as units per milligram of CFE protein. The highest concentration of CFE protein assayed was used in calculations of specific activities of less than 0.1 U/mg and ranged from 0.22 mg of protein for *B. hermsi* to 2.86 mg of protein for *T. denticola*.

Peroxidase (EC 1.11.1.7) was assayed as described previously (46) in 325-μl reaction mixtures with *o*-dianisidine as the hydrogen donor. One unit of peroxidase activity was defined as the amount of enzyme catalyzing the decomposition of 1.0 μmol of H_2O_2 per min at 25°C. Specific activities were expressed as units per 100 mg of CFE protein. The highest concentration of CFE protein assayed was used in calculations of specific activities of less than 0.1 U/100 mg and ranged from 0.05 mg of protein for *T. pallidum* to 0.66 mg of protein for aerobically grown *S. aurantia*.

Purification of rabbit SOD. SOD was purified from noninfected rabbit testicles and rabbit erythrocytes (Pel-Freez, Rogers, Ark.) as described by McCord and Fridovich (33). Briefly, testicles were ground with sand and distilled water, or erythrocytes were lysed with distilled water. Hemoglobin was precipitated by the addition of cold chloroform and ethanol followed by centrifugation. The addition of solid

K_2HPO_4 to the supernatant fluid resulted in a liquid phase separation. Cold acetone was added to the upper phase containing most of the enzyme activity, and after centrifugation the precipitate was dissolved in 0.05 M potassium phosphate buffer (pH 7.8). The SOD-containing fraction was purified further by precipitation with $(NH_4)_2SO_4$ to 90% saturation. After centrifugation the precipitate was dissolved in and dialyzed against 1.0 mM potassium phosphate buffer (pH 7.8) overnight at 4°C. This partially purified testicular or erythrocyte fraction represented rabbit SOD. Rabbit SOD contained at least four electrophoretically distinct bands of activity, which are referred to as SOD-1, -2, -3, and -4, in order of increasing mobility. There was no difference in electrophoretic properties of rabbit testicular SOD and rabbit erythrocyte SOD in this system; this finding was in agreement with results obtained in previous studies on SOD purified from different tissues (12). The specific activity of rabbit erythrocyte SOD was 3,946 U/mg.

Preparation of rabbit SOD antiserum and its use. Preimmune serum was obtained from a sheep before starting the immunization procedures. Antibody to rabbit SOD was produced by intramuscularly injecting a sheep with 1.3 mg of rabbit SOD-1 sliced out of polyacrylamide gels. After 3 weeks whole serum was assayed for antibody by the ring precipitin reaction with a clarified crude extract of noninfected rabbit testicles as antigen and decimal dilutions of serum. No titer of antibody was obtained with undiluted or diluted test or preimmune sera. Subsequently, a total of 7.5 mg of rabbit SOD-1 emulsified in complete Freund adjuvant was administered over a 3-week period. A ring precipitin titer of 1:1,000 was obtained 4 weeks after the final injection. The immunoglobulin fraction was prepared by precipitation of serum with 14% Na_2SO_4 and used in studying its interaction with both rabbit SOD and *T. pallidum* CFE by spectrophotometric enzyme assays, PAGE, and the Ouchterlony method of double diffusion (36). A solution of 0.8% agarose (Miles Laboratories, Inc.), 0.5% NaCl, and 0.002% NaN_3 was used in the double-diffusion tests. Photographs were taken after at least 20 h of incubation at room temperature. Protein was determined by the method of Lowry et al. (27) with bovine serum albumin as a standard.

RESULTS

Table 1 summarizes the distribution of SOD, catalase, and peroxidase activities among several spirochetes. *E. coli* B was grown under aerobic conditions and served as a reference in the study. Good agreement in specific activities was observed when *E. coli* CFE was assayed for SOD activity by both the pyrogallol and xanthine oxidase-cytochrome *c* methods. *E. coli* catalase and peroxidase activities were similar to those observed in a previous study (22).

Although *B. hermsi* contained significant amounts of SOD activity, catalase and peroxidase activities were not detected. *S. litoralis* and the cultivable treponemes *T. denticola*, *T. succinifaciens*, and *T. bryantii* did not contain appreciable levels of SOD activity, and these levels measured by spectrophotometric assays were too low for detection in polyacrylamide gels. None of these obligately anaerobic spirochetes contained catalase or peroxidase activities, and similar negative results had been obtained when *T. bryantii* CFE was assayed for catalase and peroxidase activities by a procedure involving the oxidation of 3,3'-diaminobenzidine (42). In contrast, *S. aurantia* possessed SOD activity when cultivated in both aerobic and anaerobic conditions, with a sevenfold increase in activity in the aerobic cultures. The specific activity of SOD in aerobically grown *S. aurantia* was comparable to that observed in aerobically grown *E. coli*. Although *S. aurantia* contained no peroxidase activity, catalase was present in aerobic and anaerobic cultures at similar specific activities. These results quantitatively confirm the weakly catalase-positive reaction exhibited by aerobically grown cell pellets when flooded with a solution of H_2O_2 (8).

A total of 13 serovars of *Leptospira*, comprising pathogens and water isolates, were included in this study. All serovars of the pathogenic *L. interrogans* and B-7 and H-23 serovars of the presumably nonpathogenic *L. biflexa* possessed insignificant SOD activity when measured by two spectrophotometric assays and PAGE. However, five other serovars of *L. biflexa* showed significant levels of SOD activity. Further attempts were made to demonstrate SOD activity in the *L. interrogans* serovars and B-7 and H-23 serovars of *L. biflexa*. Cytochrome *c* reductase activity, which might mask SOD activity in the xanthine oxidase-cytochrome *c* assay, was not detected in any of these extracts. To test the possibility that extracts of *L. interrogans* contained an inhibitor of SOD, various amounts of CFE of several of the *L. interrogans* serovars were added to CFE of *L. biflexa* serovar B-16 or *semaranga*; no inhibition of SOD activity was observed.

Catalase and peroxidase activities were distributed among the *Leptospira* serovars in a pattern which had been observed previously in semiquantitative assays performed in this laboratory (14). Peroxidase activities fluctuated approximately 30-fold within the genus, with the pathogenic *L. interrogans* serovars possessing lower amounts than the *L. biflexa* serovars. A wide range of catalase values was observed, with the *L. interrogans* serovars possessing higher activities than the *L. biflexa* serovars. There were only two exceptions, *L. biflexa* serovars H-23 and *illini*, which contained relatively high levels of catalase.

B. hermsi, *S. aurantia*, and certain *L. biflexa* serovars (Table 1) exhibited a single SOD activ-

TABLE 1. *Distribution of SOD, catalase, and peroxidase activities among spirochetes*

Organism	SOD				Catalase (U/mg)[b]	Peroxidase (U/100 mg)[c]
	U/mg[a]	Electrophoretic bands				
		Relative mobility	Inhibition by:			
			H_2O_2	NaCN		
T. pallidum	20 (17)	0.33	+	+	1.66	<0.11
		0.38	+	+		
T. denticola	<3	0[d]			<0.01	<0.02
T. succinifaciens	<3 (2)	0			<0.01	<0.02
T. bryantii	<3	0			<0.02	<0.02
B. hermsi	35	0.54	+	−	<0.09	<0.06
S. litoralis	<3 (2)	0			<0.01	<0.02
S. aurantia (anaerobic)	30	0.24	+	−	0.22	<0.02
S. aurantia (aerobic)	220	0.24	+	−	0.21	<0.01
L. biflexa serovars						
B-16	75 (51)	0.40	+	−	0.19	10.2
patoc	54 (42)	0.39	+	−	0.34	10.2
semaranga	56 (61)	0.39	+	−	0.34	6.5
andamana	70 (72)	0.40	+	−	0.52	19.7
illini	60 (62)	0.40	+	−	37.20	3.6
H-23	5 (6)	0			6.42	2.9
B-7	3 (7)	0			0.42	6.3
L. interrogans serovars						
canicola	<5 (3)	0			245.4	0.6
pomona Pomona	<6 (1)	0			103.8	1.2
pomona Riggs	<6 (4)	0			488.2	0.9
hardjo	<4 (3)	0			60.8	1.4
grippotyphosa	<5 (4)	0			14.2	1.0
copenhageni	<6 (3)	0			70.2	1.4
E. coli B (aerobic)	245 (250)	0.31	−	−	49.0	5.2
		0.44	+	−		
		0.60	+	−		

[a] Units per milligram of CFE protein measured by the pyrogallol method. Values in parentheses represent units per milligram of CFE protein measured by the xanthine oxidase-cytochrome *c* method. One unit is the amount of enzyme required to inhibit the rate of autoxidation of pyrogallol or the rate of reduction of cytochrome *c* by 50%.

[b] Units per milligram of CFE protein. One unit is the amount of enzyme catalyzing the decomposition of 1.0 μmol of H_2O_2 per min at 30°C.

[c] Units per 100 mg of CFE protein. One unit is the amount of enzyme catalyzing the decomposition of 1.0 μmol of H_2O_2 per min at 25°C.

[d] No activity band observed.

ity band which was sensitive to only H_2O_2, indicating that they were iron-containing dismutases (9); these genera could be distinguished on the basis of differences in the relative mobilities of their SODs. PAGE of *E. coli* CFE resulted in three bands of SOD activity, with the two faster-migrating bands inactivated by H_2O_2. The slowest migrating band in *E. coli* was insensitive to H_2O_2 and NaCN, suggesting that it was a manganese-containing dismutase (9).

In contrast, virulent *T. pallidum* possessed SOD and catalase activities but lacked peroxidase activity at the highest protein concentration tested. This treponeme had at least two electrophoretic bands of SOD activity that were inactivated by both H_2O_2 and NaCN, a characteristic of copper-zinc-containing dismutases (45). The indication that *T. pallidum* possessed a copper-zinc-containing SOD appeared to be unique since that enzyme is associated primarily with eucaryotic cells (45). However, the treponemes used in this study had been extracted from rabbit testicles, and Alderete and Baseman (1) had demonstrated the presence of a number of host proteins on the surface of *T. pallidum*. Based upon these observations, the possibility that the copper-zinc-containing SOD in *T. pallidum* originated in host tissue was further explored.

Partially purified rabbit SOD was subjected to PAGE and displayed four distinct bands of activity. These multiple bands are characteristic of purified eucaryotic SOD and are considered to be either products of the purification process

(40) or isozymes (45). Relative mobilities of rabbit SOD-1, -2, -3, and -4 were 0.33, 0.38, 0.44, and 0.48, respectively, and all bands were sensitive to H_2O_2 and NaCN. Rabbit SOD-1 and -2 were identical to the two SOD bands detected in *T. pallidum* CFE in terms of relative mobility and sensitivity to both H_2O_2 and NaCN.

The reactivity of sheep anti-rabbit SOD-1 serum with homologous rabbit SOD was determined spectrophotometrically and, after resolution, by PAGE by the inhibition of SOD activity. A diluted rabbit SOD fraction containing approximately 1 U of SOD per mg of protein was incubated with equal volumes of twofold serial dilutions of antiserum overnight at 4°C. After incubation immune complexes were detected as precipitates in the mixtures containing lower dilutions of antiserum and were removed by centrifugation. Analysis of the supernatant fractions by the pyrogallol method revealed that dilutions of 1:8 or less of antiserum completely inhibited the SOD activity. Upon PAGE of supernatant fractions followed by activity staining for SOD, no enzymatic activity was observed with a 1:2 dilution of antiserum in the area where four bands appeared using nonabsorbed rabbit SOD. These results indicated that antibody raised against rabbit SOD-1 reacted with all four rabbit SOD PAGE bands, thereby eliminating enzymatic activity and electrophoretic bands.

Similarly, sheep anti-rabbit SOD-1 serum also reacted with *T. pallidum* SOD, resulting in the disappearance of the two PAGE bands of treponeme SOD. Additional support for the presence of rabbit SOD in *T. pallidum* CFE was obtained by agarose gel double-diffusion with sheep anti-rabbit SOD-1 serum (Fig. 1). Rabbit SOD yielded three precipitin arcs a, b, and c, with arc b being very sharp and arcs a and c being diffuse. *T. pallidum* CFE exhibited two precipitin arcs which showed complete identity with the corresponding arcs b and c of rabbit SOD. The absence of arc a in *T. pallidum* CFE may have been due to the sensitivity of the assay and the limited amounts of treponeme protein currently available. The agarose gel was stained for SOD activity by the method employed for staining polyacrylamide gels (6), and the results are shown in Fig. 2. Achromatic zones, indicative of SOD activity, extended from the antigen wells in all directions. However, the precipitin arcs of both rabbit SOD and *T. pallidum* CFE appeared blue, which indicated the absence of enzyme activity in the arcs and thus confirmed the ability of the antibody to inhibit rabbit SOD activity.

DISCUSSION

SOD has been regarded in recent years as an enzyme ubiquitous among O_2-metabolizing organisms and absent in obligately anaerobic bacteria. From these observations McCord et al. (34) proposed an enzyme-based theory of obligate anaerobiosis which implicated SOD in protection against O_2 toxicity. Indeed, the absence of SOD, as well as catalase and peroxidase, in *T. denticola*, *T. succinifaciens*, *T. bryantii*, and *S. litoralis* in the present study would seem to support that concept. However, subsequent studies have shown that various anaerobic bacteria contain SOD activity (19, 24, 43), and recently it has been reported that some strains of the obligately aerobic *Neisseria gonorrhoeae* (35) and *Mycoplasma pneumoniae* (28), as well as the facultative *Lactobacillus plantarum* (2), lack detectable levels of SOD activity. Our find-

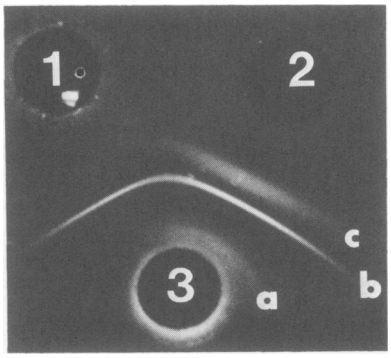

FIG. 1. *Agarose gel double-diffusion with T. pallidum CFE and purified rabbit SOD. Well 1, 180 μg of T. pallidum CFE protein containing 3.0 U of SOD activity; well 2, 30 μg of purified rabbit SOD containing 117 U of enzyme activity; well 3, 10 μl of sheep anti-rabbit SOD-1 serum.*

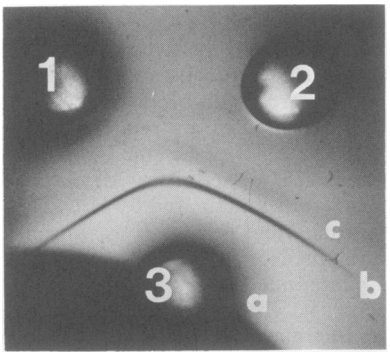

FIG. 2. *Agarose gel double-diffusion with T. pallidum CFE and purified rabbit SOD. Well contents are the same as in Fig. 1. The agarose gel was stained for SOD activity as described by Beauchamp and Fridovich (6).*

ing that B-7 and H-23 serovars of *L. biflexa* and all serovars of *L. interrogans* tested lacked detectable activities of SOD is another exception to the presumed requirement of SOD for aerobic life.

The SOD-deficient strains of *Leptospira*, with the exception of *L. biflexa* B-7, resemble the SOD-deficient strains of *N. gonorrhoeae* (35) in that they possess extremely high levels of catalase activity. *L. biflexa* B-7 and other serovars of *L. biflexa* possess peroxidase activity. These findings generally support a previous semiquantitative survey of peroxidative activities in *Leptospira* (14). The presence of catalase or peroxidase activities (or both) may alleviate the need for SOD in these spirochetes. Kinetic data have indicated that the peroxidase of *L. biflexa* functions optimally at low concentrations of H_2O_2, whereas the catalase of *L. interrogans* functions optimally at higher concentrations of H_2O_2 (15). Therefore, H_2O_2 may be scavenged efficiently by these enzymes so that it cannot react with metals reduced by the superoxide radical to form the more reactive hydroxyl radical (20). The presence of SOD in some serovars of *L. biflexa* may be indicative of excessive formation of such toxic oxidized intermediates.

Diversity in the ability of spirochetes to scavenge oxidized intermediates is exemplified further by the distribution of peroxidative enzymes in *B. hermsi* and *S. aurantia*. *B. hermsi* requires the presence of O_2 for growth in vitro (26). However, the amount of O_2 must be reduced, which suggests that this spirochete possesses only limited defenses against toxic oxidized intermediates. This is consistent with our findings that *B. hermsi* possessed no detectable amount of catalase or peroxidase, but did contain an iron SOD. *S. aurantia* is a free-living, facultatively anaerobic spirochete which was cultivated under both aerobic and anaerobic conditions in this study. The level of catalase in this spirochete was relatively low and appeared to be noninducible by O_2. However, the SOD in *S. aurantia* was induced by O_2 with about a sevenfold increase in activity during aerobic growth. An increased rate of SOD synthesis in response to O_2 has been observed in *E. coli* and *Streptococcus faecalis* (18), but *S. aurantia* differs from these bacteria in that its inducible SOD contained iron rather than manganese.

The evidence permits us to conclude that SOD from *T. pallidum* originated in rabbit tissue, which is consistent with the finding of Alderete and Baseman (1) that a surface coat of host proteins exists on the treponeme. The observed catalase activity may represent either an additional host-derived protein or an intrinsic treponeme enzyme. Turner and Hollander (44) suggested that the development of syphilitic infection may not depend on the animal species but rather on host conditions of temperature, O_2 levels, hormonal influences, metals, etc. Perhaps a dependence on host SOD, and possibly host catalase, by *T. pallidum* for growth in oxygenated tissues could explain the reported differences among animals in susceptibility to infection by this particular strain. It is attractive to speculate that human or rabbit SOD and catalase would function better for *T. pallidum* freshly extracted from humans or rabbits than enzymes derived from more resistant animals.

In our assay conditions *T. pallidum* resembles the host-associated cultivable treponemes in lacking their own dismutative and peroxidative enzymes (Table 1). Although the rabbit SOD associated with *T. pallidum* is enzymatically active, we cannot rule out the possibility that its presence in *T. pallidum* may be fortuitous. However, one must consider the possibility that rabbit SOD, as well as catalase, does function for the benefit of *T. pallidum*. Resolution of the cellular location of rabbit SOD would be essential in evaluating the functional role of this enzyme. It has been proposed that the surface coat of host proteins may protect *T. pallidum* by masking immunogens or promoting membrane integrity (1, 13). Thus, rabbit SOD associated with the outermost surface of the treponeme might prevent cellular damage by catalyzing the dismutation of superoxide radicals generated extracellularly or by shielding key outer membrane components from oxidation or by both. The enzyme might also catalyze the dismutation of any intracellular superoxide radicals which may have diffused to the exterior of the cell. It is difficult to envision such diffusion occurring without concomitant cellular damage; however, one must remember that this treponeme has not yet been cultivated in vitro.

The numerous host proteins that have been detected previously were either loosely or avidly associated with the treponeme surface, and the presence of noncompetitive binding sites was suggested (1). The mechanism of binding of rabbit SOD to *T. pallidum* is as yet unknown. If rabbit SOD were loosely adsorbed to the treponeme surface, then it might readily dissociate upon extraction of cells from tissue or during maintenance in vitro. The level of rabbit SOD detected may only be a fraction of that required by the treponeme for protection against oxidized intermediates of O_2 when removed from tissue. The inhibiting antibody prepared in this study may be useful in monitoring the association of rabbit SOD with the treponeme and further elucidating its role in the aerobic metabolism of *T. pallidum*.

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