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Characterization of Catalase by Micro-Immunoprecipitation in Tissue-Derived Cells of *Mycobacterium lepraemurium* TMC 1701

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Cell-free extracts of tissue-derived cells of *Mycobacterium lepraemurium* TMC 1701 have been found to have mycobacterial catalase which is of the T type. Immunological distance measurements of this catalase against three reference mycobacterial systems, as determined by a micro-immunoprecipitation technique, showed that catalase from *M. lepraemurium* TMC 1701 is most closely related to that of *Mycobacterium avium* but is still distinct from it. In this respect, this strain is uniquely positioned between *Mycobacterium tuberculosis* and *M. avium*.

Mycobacterial catalases have been broadly grouped into two major classes, T and M (10), depending upon their physical properties, susceptibility to various inhibitors, and kinetics of activity (1, 2, 10). In earlier studies (8–10), measurement of the differences in their serological specifications has proven to be a useful tool for taxonomic or (indirectly) evolutionary studies of mycobacteria. Immunological distance correlates well with the structural divergence of proteins having substitutions of up to about 40% of the component amino acids (4). However, studies on tissue-derived mycobacteria are difficult because of low yields of organisms as well as contamination with host enzymes (11). Previous studies (3, 5) have described the presence of peroxidase in the Antwerp strain of *Mycobacterium lepraemurium* grown in vivo and of catalase in Keishicho-strain cells grown in vitro. This study was undertaken to confirm the mycobacterial origin of the catalase present in tissue-derived *M. lepraemurium* TMC 1701 suspensions and also to evaluate its taxonomic importance.

MATERIALS AND METHODS

The strain of *M. lepraemurium* used in this study was the Hawaiian strain (TMC 1701) grown in ABG6 hybrid mice (supplied from the Trudeau Institute Inc., Saranac Lake, N.Y.). Cells were harvested and purified by the method of P. Draper (personal communication). The two-polymer aqueous-phase system consisted of 0.50 g of 1% polyethylene glycol palmitate (supplied by the National Institute of Medical Research, London), 0.245 g of polyethylene glycol 6000 (Union Carbide Corp., New York, N.Y.; recently named 8000), 0.35 g of Dextran T500 (Pharmacia Fine Chemicals, Piscataway, N.J.; lot no. 11648), 0.025 ml of 2 M NaCl, 0.10 ml of 0.05 M phosphate buffer (pH 6.9), and 3.28 ml of distilled water; this was sufficient to purify 0.2 ml of packed bacterial cells in the final stages of purification. The cells were suspended in low-salt buffer and were sonicated by a method described previously (2). Sonic extracts were added to a suspension of DEAE-cellulose (Sigma Chemical Co., St. Louis, Mo.) and were washed in low-salt buffer; the product was then eluted in 0.5 M NaCl for use in this study (9).

The catalase preparation was first assayed both in the presence and the absence of 3-amino-1,2,4-triazole (AT). Equal volumes of catalase preparation and AT (final concentration, 10 mg/ml) were incubated at 37°C for 2 h and then were analyzed for catalase activity by the zero-order $TiCl_4$ assay of residual H_2O_2 as described previously (10).

Untreated cell-free extracts were then incubated with serial dilutions of reference sera specific for mycobacterial catalases from different mycobacterial species, and the unreacted catalase was assayed by a microenzyme titration technique which is a modification of the method described earlier (9). Briefly, 10 μl of catalase preparation (0.01 U) was mixed with 10 μl of each of the serum dilutions in polyethylene centrifuge tubes and was incubated at 37°C for 1 h, followed by overnight incubation at 5°C. The contents were then drawn into 20-μl microcapillary tubes (Corning Glass Works, Corning, N.Y.; catalog no. 70995-20). The lower ends of these capillary tubes were sealed with putty and the tubes were then centrifuged at 900 × *g* for 20 min. The capillary tubes were cut at a distance from the meniscus corresponding to 15 μl of contents, and this supernatant was discharged and analyzed for catalase as described earlier (9), except that the volumes of H_2O_2 and titanium tetrachloride-sulfuric acid used were reduced proportionately. The first-order calculations of the T catalase activity and immunological distance were based upon earlier publications (8–10). The reference antisera to T catalases were prepared as also described previously (8–10); the

sera included in the study were RS 75-32726 (anti-*Mycobacterium avium* TMC 716 catalase), RS 114-30180 (anti-*Mycobacterium kansasii* TMC 1201 catalase), and RS 120-10381 (anti-*Mycobacterium tuberculosis* $H_{37}Rv$ catalase). The micro method was compared with the standard method (9) and was found to give comparable immunological distances for several mycobacterial catalase titration systems.

RESULTS

It was found that AT had no significant effect on catalase activity in the cell-free preparation of *M. lepraemurium* sonicates; the mean changes in absorbance at 410 nm observed in the assays were 0.200 and 0.204 with and without AT treatment, respectively. This resistance to AT indicated that the catalase in the preparation was of the T type and that there was no significant M-type catalase activity, either of host or bacterial origin, in these sonicates.

The immunological distance experiments (Table 1) revealed that the catalase from *M. lepraemurium* TMC 1701 preparations was most closely related to *M. avium* catalase. The standard and micro methods showed a good correlation, the immunological distance between *M. avium* and *M. lepraemurium* TMC 1701 being 24 ± 4 by the standard method and 24 ± 6 by the micro method. It was found that the *M. lepraemurium* TMC 1701 catalase was positioned between those of *M. avium* and *M. tuberculosis* and was quite distant from that of *M. kansasii*. That is, the sum of the distances from *M. lepraemurium* TMC 1701 to *M. tuberculosis* and to *M. avium* is almost identical to the reciprocal distances between *M. avium* and *M. tuberculosis* reported previously (9). Furthermore, the relative distances seen among three reference systems exhibited a high order of complementarity to the relative values previously reported between these three reference systems (8–10). This zero-order micro technique required only 0.01 U per test as compared with 0.05 U per test by the zero-order standard method (8) or 0.5 U per test by the first-order macro method used in the earliest studies (2).

TABLE 1. Immunological distances of T catalase of *M. lepraemurium* TMC 1701 from three standard T catalase reference systems

Strain tested	Immunological distances from:[a]		
	M. tuberculosis $H_{37}Rv$	*M. avium* TMC 716	*M. kansasii* TMC 1201
M. tuberculosis $H_{37}Rv$		(97 ± 5)	(145 ± 9)
M. lepraemurium TMC 1701	68 ± 8	24 ± 6	70 ± 6
M. avium TMC 716	(98 ± 6)		(15 ± 7)
M. kansasii TMC 1201	(143 ± 16)	(29 ± 3)	

[a] Values show immunological distance ± standard deviation (3 replications). Numbers in parentheses are data from previously published studies (8–10).

DISCUSSION

Stanford (6) noted a marked similarity between *M. avium* and *M. lepraemurium* strains in terms of numbers of shared antigens as determined by immunodiffusion, and Stanford and Grange later (7) suggested that *M. lepraemurium* should be recognized as a subspecies of *M. avium*. The present study, with a different parameter, supports the findings of Stanford (6) that *M. lepraemurium* is more closely related to *M. avium* than to any other species tested, but there does appear to be a distinct immunological distance between catalases of the two species. In a previous study, Wayne and Diaz suggested a correlation between the position of species on a diagram of divergence of their T catalases and the evolution of these species in terms of their saprophytic and pathogenic natures (10). In the present study, catalase from *M. lepraemurium* TMC 1701 was found to be uniquely positioned between those of *M. avium* and *M. tuberculosis*, providing further support for a correlation between the evolution of species and their pathogenicity. So far, no other mycobacterial species have been described to be positioned like this on the scale of divergence.

It can be concluded from the present study that the catalase detected in *M. lepraemurium* TMC 1701 preparations is a mycobacterial T catalase rather than a host enzyme and that it has a position between the catalases of *M. avium* and *M. tuberculosis*. Furthermore, the facts that this micro technique needs 5 times less enzyme than the semimicro standard technique (8) and 50 times less enzyme than the macro method (2) and is equally sensitive and specific make it highly suitable where a very small amount of material, e.g., tissue-derived bacteria, is available. Thus, in this study five mice yielded about 0.5 ml of packed cells of *M. lepraemurium* TMC 1701 and enough mycobacterial catalase for about 50 serum titrations. This kind of serological approach may be useful for studying other enzyme systems as well.

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REPRINT REQUESTS

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