Catalases, Peroxidases, and Superoxide Dismutases in *Mycobacterium leprae* and Other Mycobacteria Studied by Crossed Immunoelectrophoresis and Polyacrylamide Gel Electrophoresis

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Received 3 March 1986/Accepted 19 August 1986

The five mycobacteria *Mycobacterium leprae*, *M. lepra*, *M. bovis* BCG, *M. smegmatis*, and *M. intracellulare* were studied. Catalase and peroxidase activities were demonstrated in polyacrylamide and crossed immunoelectrophoresis gels for *M. leprae*, *M. intracellulare*, and BCG, but not for *M. lepra*.

Peroxidase and catalase activities were associated with the same precipitate line in crossed immunoelectrophoresis for *M. leprae*, *M. intracellulare*, and BCG, showing that in these mycobacteria the two enzyme activities resided in the same molecule. *M. smegmatis* peroxidase and catalase activities were closely associated on polyacrylamide gel electrophoresis, but on the crossed immunoelectrophoresis catalase and peroxidase activities were associated with two different precipitate lines. Catalases without peroxidase activity were demonstrated in crossed immunoelectrophoresis and polyacrylamide gel electrophoresis in *M. intracellulare* and *M. smegmatis*. The catalase without peroxidase activity in *M. intracellulare* was heat resistant and therefore classified as an m-catalase. In *M. smegmatis* the catalase without peroxidase activity was only partially heat resistant. All of the catalases with peroxidase activity were heat-sensitive t-catalases. Superoxide dismutase activity in the crossed immunoelectrophoresis was associated with the *M. lepra* antigen no. 4 and with cross-reacting antigens in the other mycobacteria studied. Several superoxide dismutases were demonstrated in *Mycobacterium duelles*. They were antigenically different from the other superoxide dismutases in this study, as shown by lack of reactivity with a monoclonal antibody to *M. leprae* superoxide dismutase. Molecular weights were estimated for all the enzymes in this study by sodium dodecyl sulfate-polyacrylamide gels.

The antigenic relationship of *Mycobacterium leprae* to other mycobacteria may contribute to a further clarification of its taxonomic position. Furthermore, identification of the species most closely related antigenically to the leprosy bacillus could provide information relevant to the understanding of the epidemiology of leprosy.

Previous immunological studies have given conflicting results. Based on immunodiffusion analysis Stanford and co-workers (24) found a closer relationship with *Mycobacterium vaccae* than with other mycobacteria. Widebæk et al. (28) studied the cross-reactions with antigen no. 21 of *Mycobacterium smegmatis* and found that among 14 mycobacterial species *M. leprae* was most closely related to *Mycobacterium tuberculosis* and *Mycobacterium avium*. When *M. leprae* antigen was run in crossed immunoelectrophoresis (CIE) against polyvalent rabbit antiserum to seven other mycobacteria *Nisseria asteroides*, antiserum to *Mycobacterium bovis* BCG, *M. avium*, and *Mycobacterium leprae* identified the highest number of cross-reacting components (7).

Because the structure of proteins is directly coded for by genes, the structural relationship between proteins of different species would be assumed to reflect their phylogenetic relationship. Enzymes are particularly useful in this kind of analysis because many of them are widely distributed, and their enzymatic activity provides a functional marker which is useful in combination with immunological criteria.

The immunological cross-reactions of catalase have been used by Wayne and his group for extensive taxonomic studies among mycobacteria (26). However, attempts to detect catalase in *M. leprae* have so far been unsuccessful (16, 27). In the present study, renewed attempts to detect catalase, peroxidase, and superoxide dismutase in *M. leprae* and other mycobacteria was undertaken. We wanted to use the technique of CIE because reference systems are worked out for several mycobacteria (4–6, 17), and cross-reactions between certain antigenic components have been extensively studied (5, 13, 18, 19, 28).

**MATERIALS AND METHODS**

**Mycobacterial antigens.** *M. smegmatis* (NCTC 333) from J. Stanford, London, and *Mycobacterium duelles* (NCTC 358) from M. Magnusson, Copenhagen, were grown on Sauton medium. The bacteria were harvested, washed three times with phosphate-buffered saline (0.15 M NaCl, 0.03 M phosphate), and then suspended in 1.7 ml of phosphate-buffered saline per g of wet weight. *Mycobacterium intracellulare* (Battey-Boone) TMC 1403 antigen was obtained from M. F. Thorel, Maisons-Alfort, France. *M. leprae*, grown in armadillo, was provided in the World Health Organization through its Immunology of Leprosy Program. *M. leprae* (Douglas strain) was cultivated in vivo in mice. Bacilli were purified from mouse liver tissue (5).

Ultrasonic treatment of the above-mentioned antigens was performed in a rosette cooling cell for 10 min by using a Branson sonifier B12 (Branson Sonic Power Co., Danbury, Conn.) with an effect of 90 W. The sonicates were centri-

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fuged at 20,000 × g for 20 min to remove insoluble material. The protein concentrations varied between 7 mg/ml (M. intracellulare) and 1.3 mg/ml (M. leprae).

A culture filtrate of M. bovis BCG (strain 1331) was obtained from Statens Seruminstitut, Copenhagen, Denmark. It was prepared as described before (4). The protein concentration of the concentrated filtrate was 3 mg/ml.

The antigens were stored at −20°C. Protein determinations were performed by the modified Folin method (22).

**Antisera.** Concentrated anti-BCG immunoglobulin (3.5 mg/ml) was obtained from Dakopatts A/S, Copenhagen, Denmark (code B124, lot 116). Rabbits were immunized with M. smegmatis and M. lepraeurium by a schedule described previously (5).

Anti-M. intracellulare TMC 1403 immunoglobulin (4.0 mg/ml) was prepared from burro sera by S. Chaparas, Bethesda, Md. Rabbit antiserum to M. leprae were prepared by immunizing rabbits with concentrated M. leprae antigen as described before (6). Monospecific antiserum to the no. 13 antigen of M. lepraeurium was prepared by immunization of rabbits with immune precipitates cut out of gels after CIE as described previously (12).

**CIE.** Gels contained 1% agarose (Litex type HSA with moderate electrodosmotic flow; Litex, Glostrup, Denmark) in Tris-barbital buffer (pH 8.6) with an ionic strength of 0.02. The first-dimension gels were run with 10 µl of antigen. The intermediate gel contained 15 µl of antisera per cm² or the same amount of 0.9% NaCl. The top gel contained 8 µl of antisera per cm² or 25 µg of immunoglobulin per cm². Washing, pressing, and staining of the gels was carried out as described in detail elsewhere (14).

**Fused rocket immunoelectrophoresis.** Several mycobacterial suspensions were added to separate circular wells and run against a monospecific antiserum to M. lepraeurium antigen no. 13. The technique was described in detail by others (14).

**Polyacrylamide gel electrophoresis (PAGE).** Slabs of 1.5-mm thickness were run in an electrophoresis apparatus from LKB, Bromma, Sweden. Gels and buffers were prepared by the method of Laemmli (23), except for the preparation of samples. Sucrose grains were added to the Mycobacterium sp. suspensions to increase the density, and 3 µl of 0.1% (wt/vol) aqueous bromophenol blue was added as a marker.

The antigen suspensions added to each well varied from 10 to 40 µl, which corresponded to a protein amount of 40 to 120 µg. Gels of 7, 10, and 12% acrylamide were run.

Staining for protein was done with Coomassie brilliant blue R in 12.5% trichloroacetic acid. Destaining required immersion in several changes of 10% trichloroacetic acid.

**Enzyme staining.** A slightly different washing procedure was used for agarose and acrylamide gels. Agarose gels were washed for 20 min with one change, acrylamide gels without sodium dodecyl sulfate (SDS) were washed for 20 min with four changes, and acrylamide gels with SDS were washed for 45 min with six changes of distilled water. The last washing procedure minimized yellow discoloration of the gels from a reaction between SDS and the nitro blue tetrazolium.

The assay procedure followed was that of Beauchamp et al. (3). After initial washing the gel was soaked for 20 min in 0.2 mg of nitro blue tetrazolium (Sigma grade III lot 22F-5026; Sigma Chemical Company, St. Louis, Mo.) per ml. The gel was transferred to a solution containing 0.028M Temed (Bio-Rad Laboratories, Richmond, Calif.), 0.28 µM riboflavin (Sigma), and 0.036 M potassium phosphate buffer (pH 7.4) for 15 min. Most of the solution was poured off, and the gel was illuminated with a 2×22W UV-lamp (LKB) for about 20 min. Superoxide dismutase (SOD)-active areas appeared as clear zones on a blue-violet background.

**Peroxidase.** The gel was soaked in a solution of 2 mM O-dianisidine (Sigma) and 10 mM potassium phosphate (pH 7.2) for 45 min. The gel was then transferred to a solution of 0.006% H₂O₂ prepared from a stock solution of 3% H₂O₂ in distilled water. Bands usually appeared within 15 min and were dark brown, whereas the gel remained unstained. Gels were incubated for 24 h at 22°C before the reaction was considered negative.

**Catalase.** The staining was performed as described by Woodbury et al. (29). The gel was soaked in a solution of 0.006% H₂O₂ prepared as described for the same solution for peroxidase staining. From stock solutions of 3% potassium ferricyanide pro analysis (E. Merck AG, Darmstadt, Federal Republic of Germany), and 2% FeCl₃, 6H₂O (Merck) was prepared a fresh 0.1% solution of both. The gel was transferred to this, and yellow catalase bands appeared on a blue-green background. Gels stained for enzymes were stored in distilled water at 4°C until photographed.

**Photographing of gels.** Gels with positive stains (protein stains and peroxidase) were best photographed on a white plate with two photographic lamps as the light source. Negatively stained gels (catalase, SOD) were photographed with incident illumination on a glass plate. The film used was Agfapan (25 ASA, 15 Din). A yellow filter was used for gels stained with Coomassie blue.

**Determination of molecular weights.** Standard proteins with known molecular weights (Pharmacia Fine Chemicals AB, Uppsala, Sweden) were included in each run. The gels were cut longitudinally, processed individually for either enzyme or protein staining, and then photographed.

The distance from the top of the gel to the middle of the enzyme or protein band (A) and to the marker (B) was measured on the photograph, and the Rₜ (A/B) was computed and then plotted on a standard curve from the same gel. The slope of the standard curve was steeper for the molecular weights above 90 kilodaltons (kDa).

**RESULTS**

Staining of CIE gels for catalase activity revealed enzyme activity in four out of five mycobacteria tested. In M. leprae CIE gels no catalase activity was detected. In M. intracellulare catalase activity was associated with two different precipitate lines, indicating the presence of two immunologically distinct catalases in this mycobacterium.

In M. smegmatis, M. lepraeurium, and BCG, catalase activity was associated with one precipitate line. In BCG and M. lepraeurium the enzyme active precipitate lines were identified in the CIE reference systems as lines no. 56 (Fig. 1) and 26, respectively (Table 1).

Lack of catalase activity in the precipitate pattern of M. leprae could be due to a lack of antibodies to catalase in the immunoglobulin preparation used in the top gel. To account for this possibility we looked for catalase activity in PAGE. This method revealed one band with catalase activity in M. lepraeurium. BCG showed one strong and one weak band. In M. smegmatis two equally strong bands with catalase activity were present. M. intracellulare exhibited several bands with catalase activity; two of them were stronger than the others. Despite the fact that the technique was sensitive enough to detect the quite weak additional bands, no catalase activity could be demonstrated in M. leprae. Empty gels...
showed no color changes that could be confused with enzyme activity.

The molecular weights of the catalases as determined from their SDS-PAGE mobilities are given in Table 1.

Catalases in mycobacteria are separated into two groups: heat-labile catalases (t-catalases), and heat-stable catalases (m-catalases). The t-catalases were inactivated by heating the mycobacterial suspensions for 1 min at 68°C immediately before PAGE. The catalases of 246, 290, and 348 kDa of *M.

### TABLE 1. Molecular masses and numbers in CIE reference systems for catalases and peroxidases in four mycobacteria

<table>
<thead>
<tr>
<th>Enzyme</th>
<th><em>R</em>&lt;sub&gt;a&lt;/sub&gt; (SD)</th>
<th>Mol mass, kDa (SD)</th>
<th>No.</th>
<th>CIE no.&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M. lepraemurium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxidase</td>
<td>0.26 (0.02)</td>
<td>110 (3.3)</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Catalase</td>
<td>0.255 (0.02)</td>
<td>115 (4.9)</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td><strong>BCG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxidase (strong)</td>
<td>0.30 (0.02)</td>
<td>96 (3.5)</td>
<td>8</td>
<td>56</td>
</tr>
<tr>
<td>Peroxidase (weak)</td>
<td>0.22</td>
<td>148</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Catalase (strong)</td>
<td>0.28 (0.02)</td>
<td>102 (4.7)</td>
<td>15</td>
<td>56</td>
</tr>
<tr>
<td>Catalase (weak)</td>
<td>0.22</td>
<td>150</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>M. smegmatis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxidase (strong)</td>
<td>0.27 (0.02)</td>
<td>104 (4.6)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Peroxidase (weak)</td>
<td>0.31</td>
<td>94</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Catalase (strong)</td>
<td>0.26 (0.02)</td>
<td>109 (4.3)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Catalase (weak)</td>
<td>0.22</td>
<td>148 (6.5)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>M. intracellulare</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxidase</td>
<td>0.27 (0.024)</td>
<td>106 (5.8)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Catalase (strong)</td>
<td>0.27 (0.04)</td>
<td>106 (5.6)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Catalase (weak)</td>
<td>0.21 (0.025)</td>
<td>156 (7.5)</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Catalase (weak)</td>
<td>0.083 (0.04)</td>
<td>246 (8.9)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Catalase (weak)</td>
<td>0.063 (0.03)</td>
<td>290 (6.2)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Catalase (strong)</td>
<td>0.048 (out of range)</td>
<td>348 (15.0)</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> *R*<sub>a</sub> are given for 10% polyacrylamide gels with SDS.

<sup>b</sup> Number of determinations of molecular mass.

<sup>c</sup> Antigen number in the respective CIE reference system (when available).
CATALASES, PEROXIDASES, AND SODs IN MYCOBACTERIA

FIG. 2. A 10% polyacrylamide gel with SDS run vertically from top to bottom was cut into two parts and stained for catalase (A) and peroxidase (B). Lanes: 1, M. leprae; 2, M. leprae (no activity); 3, BCG; 4, M. smegmatis; 5, M. intracellulare. Note that the positions of the peroxidase activities correspond to the fastest-moving catalases. The positions of the standard proteins included in the same run are shown to the right. Their molecular masses are given in kDa.

suggested that antigens no. 13 of M. lepraemurium, no. 62 of BCG, and no. 4 of M. leprae have common antigenic determinants.

Fused rocket immunoelectrophoresis of various mycobacterial antigens against a monospecific rabbit antiserum to M. lepraemurium antigen no. 13 had previously shown that a similar antigen was present in a number of mycobacterial species and N. asteroides, showing various degrees of antigenic similarity with the M. lepraemurium antigen (Fig. 4). The M. avium and M. smegmatis antigens showed reactions of identity, whereas the M. leprae antigen showed the reaction of partial identity with spur formation. M. duvalii, however, failed to give a precipitate line with this antiserum. Combined with the present result that identified

FIG. 3. (A) CIE gel of M. leprae stained for SOD activity. The activity is located at precipitate line no. 4 (arrow). (B) Protein staining of a corresponding CIE gel. The arrow points to line no. 4.

TABLE 2. Molecular masses and numbers in CIE reference systems for SODs in six mycobacteria

<table>
<thead>
<tr>
<th>SOD source</th>
<th>$R_f$ (SD)</th>
<th>Mol mass, kDa (SD)</th>
<th>Nb</th>
<th>CIE no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. lepraemurium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>0.55 (0.06)</td>
<td>47 (1.2)</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Weak</td>
<td>0.40</td>
<td>67</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>0.32</td>
<td>86</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>M. leprae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>0.53 (0.05)</td>
<td>50 (2.5)</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Weak</td>
<td>0.38</td>
<td>75</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>BCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>0.71 (0.06)</td>
<td>34 (2.7)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>0.54 (0.06)</td>
<td>44 (2.6)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>0.40 (0.04)</td>
<td>68 (1.5)</td>
<td>12</td>
<td>62</td>
</tr>
<tr>
<td>Weak</td>
<td>0.32 (0.06)</td>
<td>96 (2.7)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>M. smegmatis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>0.53 (0.07)</td>
<td>47 (1.0)</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>0.50 (0.07)</td>
<td>50 (2.2)</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

$R_f$s are given for 12% polyacrylamide gels with SDS.

See footnote b of Table 1.

See footnote c of Table 1.
M. lepraemurium antigen no. 13 as SOD, this meant that either the M. duvalii strain was lacking SOD or the enzyme was antigenically so different from that of the other mycobacteria and that of N. asteroides that it did not react with the antiserum. This could now be further analyzed in the SDS-PAGE.

Staining of PAGE gels for SOD activity revealed one or several bands with enzyme activity in all six mycobacteria studied (Fig. 5). Gels without antigen showed no color changes that could be mistaken for enzyme activity. Some of the bands showed only very weak activity, and the weakest activities were not always reproducible. The molecular masses were calculated from the relative electrophoretic mobilities (Table 2). The molecular masses of the bands with the strongest SOD activity were similar for M. intracellulare, M. smegmatis, M. leprae, and M. lepraemurium (47 to 50 kDa). Notably, M. duvalii was found to contain several bands with SOD activity.

By comparing gels that were cut into two parts and stained for either enzyme activity or protein, we were able to find which of the bands in the protein pattern were associated with the strongest enzyme activities (Fig. 6).

**DISCUSSION**

Catalase was chosen initially for this study because Wayne and co-workers have used the antigenic structure of this enzyme as a basis for taxonomic studies of mycobacteria (26). The molecular mass determined in the present study for the heaviest peroxidase-catalase enzyme in BCG (150 kDa) resembles that of the purified t-catalase from M. tuberculosis (160 kDa) (10). The heaviest catalase found in this study corresponds in molecular mass to the catalase found by Gruft and Gaafar in M. intracellulare (11).

Peroxidase activity is reported to occur in association with catalase activity in mycobacteria (2, 8). Purified catalase of M. tuberculosis was found to also have peroxidase activity.
In the present study combined peroxidase-catalase activities were demonstrated on CIE gels of BCG, *M. intracellulare*, and *M. lepraemurium*, showing that in these mycobacteria the two enzyme activities reside in the same molecule. In the SDS-PAGE gels the distributions of the two enzyme activities were slightly different from each other, and consequently molecular mass determinations based on the respective localization of catalase and peroxidase activity gave slightly different values even when the two activities were associated with the same protein band. For *M. smegmatis* there was an apparent discrepancy between the results obtained with CIE and SDS-PAGE. This could arise from a difference in sensitivity of the two techniques, since the enzyme is more concentrated in the PAGE. Alternatively, the rabbit antiserum used in the CIE might have inactivated the catalase activity but not the peroxidase activity of the combined enzyme.

Although the methods for detecting peroxidase and catalase activity functioned adequately, we were unable to detect even a trace of the enzymes in *M. leprae*. The failure to detect catalase or peroxidase activity in the *M. leprae* CIE gels could result if the enzyme were not sufficiently antigenic for rabbits to give rise to a precipitate line, or if the antiserum contained neutralizing antibodies. In the SDS-PAGE it is unlikely that we have used too little material, since the amount of *M. leprae* used for the SDS-PAGE analysis was twice the amount which was sufficient to demonstrate strong peroxidase and catalase activity in the other mycobacterial extracts. Unlike the other extracts, the *M. leprae* preparation had been exposed to irradiation with 2.5 megarads to inactivate the bacilli. It is possible that this treatment may have inactivated a peroxidase-catalase activity. Our negative finding is in accord with Katoch et al. (17), who found no t-catalase activity in *M. leprae* but weak m-catalase activity, which was believed to be of host tissue (armadillo) origin. Wheeler and Gregory (27) found weak peroxidase activity in PAGE gels, which they loaded with as much as 750 μg of *M. leprae* protein, but no catalase activity. The presence of a peroxidase in *M. leprae* is supported by the indirect evidence provided by Stavr et al. (25), who demonstrated antibodies reacting with the peroxidases of BCG and *M. smegmatis* in relatively high concentrations in pooled sera from patients with lepromatous leprosy. However, the prevalence of tuberculosis is high in the countries where leprosy is found, and the antibodies to peroxidase in the pooled sera may reflect concurrent tuberculosis in some of the patients.

Several workers have previously demonstrated SOD activity in mycobacteria, including *M. leprae* (20, 21, 27). Molecular mass determinations of SOD with PAGE are complicated by the fact that SDS inhibits the enzyme activity strongly, although not completely. Our estimated molecular mass for *M. lepraemurium* SOD was 47 kDa, which corresponds closely to the value (45 kDa) found for the purified enzyme (13). The SODs of *M. lepraemurium*, *M. leprae*, and *M. smegmatis* have all been shown to be manganese-containing SODs (Mn-SODs) (21). Although no information was available for *M. intracellulare*, its similar molecular mass and reaction of identity in fused rocket electrophoresis between the SODs of *M. avium*, *M. smegmatis*, and *M. lepraemurium* as demonstrated in the present study, suggest that this enzyme is an Mn-SOD. The SOD of BCG, on the other hand, has a different molecular mass (68 kDa) and is likely to be similar to the SOD of *M. tuberculosis*, which is an iron-containing SOD (Fe-SOD) (21). The nature of the SODs of *M. duchovii* is less certain since they appeared to be structurally less closely related to the SODs of the other mycobacteria studied, and since the main activity resided in a 25-kDa protein, possibly a monomer.

Even if there are several bands of enzyme activity in SDS-PAGE, there may be one or two main molecular species of a given enzyme. Oligomers and breakdown products may account for the different molecular masses observed. A possibility also exists that the weak enzyme bands could represent background (unspecific staining reactions). However, the activity was located only to one or two distinct precipitate lines in CIE (CIE and PAGE gels were stained by essentially the same methods). Empty gels showed no enzyme activity.

The successful staining of CIE gels for SOD activity enabled us to localize enzyme activity to certain antigens in all of the mycobacteria studied, except in *M. duchovii*, for which we lacked a suitable antiserum. This identified SOD as antigen no. 21 of *M. smegmatis*, which was shown by Kronvall et al. (18) to cross-react with an antigen of *M. leprae* that contains an *M. leprae*-specific epitope.

From the data already available it appears that there is enough structural heterogeneity in the SOD of mycobacteria to make it a useful marker for taxonomic studies in addition to catalase, its main advantage being that it is also present in *M. leprae*. The fused rocket immunoelectrophoresis experiment showed clearly that a structurally related SOD is also present in *N. asteroides*. On this background the finding of several immunologically unrelated SODs in *M. duchovii* is surprising. *M. duchovii* is either more distantly related to other mycobacteria than the three mycobacteria we have studied, or it is *N. asteroides*, or the structural relationship of functionally similar proteins is not always a valid indicator of the phylogenetic relationship between bacteria. The present study illustrates the importance of using a functional marker in addition to immunological markers when proteins are used as a taxonomic tool and carries promise for a future, more precise definition of the taxonomic position of *M. leprae*.

ACKNOWLEDGMENTS

This work was supported by the Immunology of Leprosy component of the World Health Organization-United Nations Development Program-World Bank Special Programme for Research and Training in Tropical Disease and the Laurine Maarschalk Fund. We are grateful to Ellen S. Karlström and Gro Lindberg for excellent technical assistance and to Elisabeth Wedge for valuable discussions and technical advice.

LITERATURE CITED


