Superoxide Dismutase from Streptococcus mutans

ISOLATION AND CHARACTERIZATION OF TWO FORMS OF THE ENZYME*

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SUMMARY

Superoxide dismutase, which catalyzes the dismutation of univalently reduced oxygen \( \text{O}_2^- + \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{H}_2\text{O}_2 \) has been shown in mammalian cells to contain copper and zinc. The only reported isolation of the enzyme from a procaryotic organism, Escherichia coli, showed the enzyme to contain only manganese and to differ from the mammalian enzyme in other physicochemical characteristics. We have now purified and characterized superoxide dismutase from another procaryotic organism, Streptococcus mutans. S. mutans was found to contain what appear to be two isoenzymes based on chromatographic patterns and disc gel electrophoresis. The major fraction (superoxide dismutase I) was found to contain 2 atoms of manganese per mole of enzyme as determined by integration of the electron paramagnetic resonance spectrum of the metal liberated from the protein. Disc gel electrophoresis in the presence of sodium dodecyl sulfate revealed two subunits of equal size. Molecular weight determinations by sedimentation equilibrium gave a value of 40,250 ± 2,000 for S. mutans dismutase, as compared to 39,500 ± 2,000 for the E. coli enzyme. Chemical analyses of the purified protein revealed the absence of copper and zinc. The ultraviolet and visible absorption spectra are identical with those of the E. coli enzyme. Amino acid analyses are reported with a large variation from the analyses of the E. coli enzyme. Superoxide dismutase II was not fully characterized, but was found to contain manganese and not to contain any copper or zinc. The specific activities of superoxide dismutase I and II are 5,500 and 5,800 while the specific activity of the E. coli enzyme is 3,800.

MATERIALS AND METHODS

Cytochrome c, type III, and xanthine were obtained from Sigma. Microgranular diethylaminoethylcellulose (DE-32) and microgranular carboxymethylcellulose (CM-32) were obtained from Reeve Angel Co., New York, N.Y. Ammonium sulfate, enzyme grade, was a product of Mann Biochemical Co., New York, N.Y. Xanthine oxidase was purified from raw cream by a procedure which avoided exposure to proteolytic agents (9). All other materials were obtained from commercial sources at the highest available states of purity.

S. mutans 6715 (obtained from Dr. Charles Wittenberger, National Institute of Dental Research, Bethesda, Maryland) was grown in 250-liter batches in a commercial fermentor in the laboratory of Dr. Earl Stadtman, National Institutes of Health, Bethesda, Maryland. The cells were grown at 37° under an atmosphere of air at a flow rate of 100 liters per min. The pH was maintained at 6.8 by the constant addition of sodium hydroxide. The cells were harvested at 4° by centrifugation at early stationary growth phase.

Superoxide dismutase was assayed by a slight modification of an indirect method which depends upon the ability of the enzyme

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to compete with ferricytochrome c for superoxide anions generated by the aerobic xanthine oxidase system and thus to inhibit the reduction of cytochrome c by this system (1). The standard assay (1) was performed in 3.0 ml of a solution containing 1 × 10⁻⁴ M ferricytochrome c, 5 × 10⁻⁴ M xanthine, 1 × 10⁻⁴ M EDTA, and 0.05 M potassium phosphate (pH 7.8) at 30°. Sufficient xanthine oxidase was added to produce a rate of reduction of cytochrome c corresponding at an increase of 0.025 A per min at 550 nm. Under the specified conditions 1 unit of superoxide dismutase is that amount which gave a 50% decrease in the rate of reduction of cytochrome c.

Previously the assay for superoxide dismutase had been done at 25°. During this procedure, for convenience, we chose to use 30°. Even though there is an increase in the rate of the xanthine oxidase reaction it is difficult to assess the effect of this 5° temperature differential on the specific activity of the superoxide dismutase. When the purified S. mutans superoxide dismutase was assayed at 25° there was only about a 5% decrease in the specific activity which could be attributed to the accuracy of the assay procedure.

All spectrophotometric assays were performed with a Gilford model 2000 absorbance indicator equipped with a thermostatted sample compartment. Absorption spectra were obtained with a Cary model 14 recording spectrophotometer. Electron paramagnetic resonance spectroscopy was performed with a Varian model E-9 HF spectrometer (Varian Associates, Palo Alto, California). Measurements of sedimentation equilibrium were performed at pH 7.0 in 0.1 M sodium chloride utilizing a Beckman model E analytical ultracentrifuge by the method of Yphantis (10). Disc gel electrophoresis was performed essentially as described by Davis (11). The molecular weight of the subunits of the enzyme was estimated after electrophoresis in the presence of sodium dodecyl sulfate on 10% acrylamide gels by the method of Weber and Osborn (12). Molecular weight standards used to calibrate the gels were phosphorylase a, 94,000; bovine serum albumin, 68,000; catalase, 60,000; ovalbumin, 48,000; pepsin, 35,000; chymotrypsinogen A, 26,000; and ribonuclease, 13,600.

Amino acid analyses were performed with a Beckman model 121 amino acid analyzer. Samples were prepared by dialysis against glass distilled water. Aliquots were then sealed under vacuum in 1 ml of 6 M hydrochloric acid. The samples were hydrolyzed at 110° for periods of 24, 48, and 72 hours. Single samples of duplicate preparations of the enzyme were used for calculations of amino acid content. Essentially identical values were obtained from the 24-, 48- and 72-hour samples.

Zinc was quantitated by the method of Malmstrom (13) and copper by the method of Flesisch (14).

RESULTS

Purification of Enzyme—Frozen cells of S. mutans, 500 g, were suspended in 2,000 ml of 0.05 M potassium phosphate containing 1 × 10⁻⁴ M EDTA, at pH 7.8. The suspended cells were disrupted by sonication at 4° in a Rosett cell in 300-ml batches. A Branson sonifier was used at a power setting of 125 watts. Sonication was done for two 15-min intervals with a 5-min pause for recoupling. The suspension was clarified by centrifugation for 60 min at 13,700 × g. After the addition of KCl to a final concentration of 0.1 M, the supernatant solution was heated in 100-ml batches to 60° for 5 min and then cooled to 4°. The precipitated denatured proteins were removed by centrifugation. Streptomycin sulfate was added to the supernatant to a final concentra-

![Fig. 1 Chromatography on CM-32](http://www.jbc.org/) The **Streptococcus mutans** preparation after dialysis for 84 hours, as described in the text, was applied to a column (2.5 × 38 cm) of CM-32 equilibrated at 4° with 2 mM potassium phosphate, pH 5.5, and was then eluted with this buffer up to 60 fractions (8 ml) had been collected. At this point, 200 ml of a gradient (0.002 to 0.2 M) of the same buffer was applied. Fractions (5.4 ml) were collected until this gradient had been exhausted. Then the column was eluted with 2 mM potassium phosphate, pH 7.8, until protein was no longer being eluted. The tubes containing
TABLE I

**Purification of S. mutans superoxide dismutase**

<table>
<thead>
<tr>
<th>Purification stage</th>
<th>Purification stage description</th>
<th>mg/ml</th>
<th>ml</th>
<th>mg</th>
<th>units/mg</th>
<th>Total units</th>
<th>Yield</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant from sonicate</td>
<td>...........................................</td>
<td>68</td>
<td>1,850</td>
<td>125,000</td>
<td>2.3</td>
<td>284,000</td>
<td>100</td>
<td>-fold</td>
</tr>
<tr>
<td>Heat step</td>
<td>...........................................</td>
<td>44</td>
<td>1,750</td>
<td>78,800</td>
<td>2.8</td>
<td>218,800</td>
<td>77</td>
<td>1.2</td>
</tr>
<tr>
<td>Streptomycin sulfate</td>
<td>...........................................</td>
<td>42</td>
<td>1,750</td>
<td>72,000</td>
<td>2.8</td>
<td>208,000</td>
<td>73</td>
<td>1.2</td>
</tr>
<tr>
<td>50% (NH₄)₂SO₄ precipitate redissolved</td>
<td>...........................................</td>
<td>32</td>
<td>2,050</td>
<td>65,800</td>
<td>5.2</td>
<td>341,700</td>
<td>85</td>
<td>46</td>
</tr>
<tr>
<td>Dialysis and centrifugation</td>
<td>...........................................</td>
<td>11</td>
<td>100</td>
<td>2,200</td>
<td>2%</td>
<td>222,200</td>
<td>78</td>
<td>87</td>
</tr>
<tr>
<td>After chromatography on CM-32</td>
<td>...........................................</td>
<td>Fraction I</td>
<td>3.4</td>
<td>152</td>
<td>547</td>
<td>500</td>
<td>158,000</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>...........................................</td>
<td>Fraction II</td>
<td>2.7</td>
<td>56</td>
<td>151</td>
<td>250</td>
<td>32,000</td>
<td>11</td>
</tr>
<tr>
<td>After chromatography on DE-32</td>
<td>...........................................</td>
<td>Fraction I</td>
<td>0.2</td>
<td>127</td>
<td>25.0</td>
<td>3,700</td>
<td>88,000</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>...........................................</td>
<td>Fraction II</td>
<td>0.1</td>
<td>60</td>
<td>6.0</td>
<td>2,500</td>
<td>15,000</td>
<td>5</td>
</tr>
<tr>
<td>After chromatography on Sephadex G-75</td>
<td>...........................................</td>
<td>Fraction I</td>
<td>0.3</td>
<td>50</td>
<td>15.0</td>
<td>5,500</td>
<td>78,000</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>...........................................</td>
<td>Fraction II</td>
<td>0.018</td>
<td>98</td>
<td>1.8</td>
<td>5,800</td>
<td>20,000</td>
<td>7</td>
</tr>
</tbody>
</table>

* Protein concentration on all fractions, other than those obtained as column effluents was measured in terms of absorbance at 280 nm assuming that E₁ₐ₅₃₄ = 100. The concentration of protein in column effluents was determined by the method of Murphy and Kies (15).

* Fraction I is the fraction which did not adsorb to CM-32 according to the described procedure under "Materials and Methods."

* Fraction II is the fraction which did adsorb to CM-32 according to the described procedures under "Materials and Methods."

Superoxide dismutase activity which was located between tubes 272 and 279 was pooled and concentrated for chromatography on G-75 Sephadex.

The second DE 32 column containing Fraction II was eluted with 225 ml of 5 mm potassium phosphate, pH 7.8, with 5.4-m1 fractions being collected. Fraction II which was eluted in tubes 9 through 20 were pooled and concentrated for chromatography on G-75 Sephadex.

Each fraction (I and II) was individually dialyzed at 4º for 48 hours against 0.05 M Tris-HCl, pH 7.8, containing 0.1 M potassium chloride. The buffer was changed every 12 hours during this dialysis. Fractions I and II were then chromatographed on individual columns of G-75 Sephadex (2.5 x 80 cm) equilibrated against the dialysis buffer. Both columns were eluted with the same buffer as was used for dialysis and equilibration of the column. Fractions of 4.9 ml were collected from each of the G-75 Sephadex columns.

Tubes (36 to 44) from the chromatography of Fraction I were pooled, concentrated, and saved for further analytical analyses. In the case of chromatography of Fraction II, tubes 37 to 52 were pooled, concentrated, and retained for analyses.

The results of this purification procedure of the two superoxide dismutase enzymes are summarized in Table I. The yield was 15 mg of Fraction I and 1.8 mg of Fraction II with respective specific activities of 5500 units per mg and 5800 units per mg. This compares with the specific activity of 3800 units per mg observed with the enzyme from E. coli (8).

**Molecular Weight—**Superoxide dismutase I (Fraction I) at a concentration of 0.26 mg per ml in 0.1 M NaCl was equilibrated in a centrifugal field, and the resultant distribution of protein was analyzed by the method of Yphantis (10). When ln fringe displacement was plotted as a function of the square of the distance from the center of rotation, a straight line was obtained. The slope of this line and an assumed partial specific volume of the protein concentration and volume are shown in Table I.

**Fig. 2 (left).** Disc gel electrophoresis of Fraction I (left) and Fraction II (right). The gels were run in 0.25 M sodium phosphate, pH 7.2, for 4 hours at 2.5 mivolts at room temperature. One hundred micrograms of protein were placed on each gel.

**Fig. 3 (right).** Disc gel electrophoresis of Fraction I (left) and Fraction II (right) by the method of Weber and Osborne (12) for the determination of molecular weight of subunits. The gels were calibrated in terms of molecular weights by using the protein standards listed in the text, under identical experimental conditions. β-Mercaptoethanol was not necessary for dissociation into subunits. The molecular weight of Fraction I was 18,500 ± 1,000 and the molecular weight of Fraction II was 19,500 ± 100. One hundred fifty micrograms of protein were placed on each gel.
gel electrophoresis at pH 7.2 in 0.25 M Tris-HCl at pH 7.8 with 0.1 M potassium chloride present.

The spectra in the ultraviolet and visible regions were obtained with solutions containing 0.24 mg per ml and 4.8 mg per ml, respectively, of the enzyme in 0.05 M Tris-HCl at pH 7.8.

The results of this electrophoresis are seen in Fig. 3. Since \( \beta \)-mercaptoethanol was not necessary for dissociation into subunits it may be concluded that *S. mutans* superoxide dismutases I and II are composed of subunits of equal size which appear to be not bound together by disulfide bridges. The smearing of the gel can possibly be attributed to some overloading of the gel since 150 \( \mu \)g was placed on each gel.

The absorption spectrum of *S. mutans* superoxide dismutase I after heating to 100°C for 2 min in 0.2 M HCl was kept at 100°C for 2 min. After cooling, the extract was centrifuged and the clear supernatant solution was used for electron paramagnetic resonance spectroscopy at room temperature. The instrument settings were: Microwave frequency, 9.369 GHz; microwave power, 24 milliwatts; modulation frequency, 100 KHz; modulation amplitude, 8 gauss; receiver gain, 2,500 (top tracing) or 25,000 (bottom tracing); time constant, 1.0 s; and scanning rate, 500 gauss per min. The top spectrum was obtained from 1.95 mg per ml of *S. mutans* superoxide dismutase I after heating to 100°C for 2 min in 0.2 M HCl. The lower tracing was obtained with native enzyme at a concentration of 4.9 mg per ml in water at a 10-fold higher sensitivity of the instrument.

The amino acid composition of *S. mutans* superoxide dismutase is presented in Table II. When this is compared to the amino acid composition of the *E. coli* enzyme (8), the molar extinction coefficient of the *S. mutans* superoxide dismutase I at 473 nm was 560. The absorption spectrum of *E. coli* superoxide dismutase II was not determined because of insufficiency of the sample.

The instrument settings were: Microwave frequency, 9.369 GHz; microwave power, 24 milliwatts; modulation frequency, 100 KHz; modulation amplitude, 8 gauss; receiver gain, 2,500 (top tracing) or 25,000 (bottom tracing); time constant, 1.0 s; and scanning rate, 500 gauss per min. The top spectrum was obtained from 1.95 mg per ml of *S. mutans* superoxide dismutase I after heating to 100°C for 2 min in 0.2 M HCl. The lower tracing was obtained with native enzyme at a concentration of 4.9 mg per ml in water at a 10-fold higher sensitivity of the instrument.

**Amino Acid Analyses**—The amino acid composition of *S. mutans* superoxide dismutase I is presented in Table II. When this is compared to the amino acid composition of the *E. coli* enzyme (8), the molar extinction coefficient of the *S. mutans* superoxide dismutase I at 473 nm was 560. The absorption spectrum of superoxide dismutase II was not determined because of insufficiency of the sample.

**Manganese**—As in the case of *E. coli* superoxide dismutase, no electron paramagnetic resonance signal was observed with the native enzyme. However, upon denaturation by heating at 100°C...

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**TABLE II**

Comparison of amino acid composition of *S. mutans* and *E. coli* superoxide dismutase

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid content</th>
<th>Residues per mole of enzyme (nearest integer)</th>
<th>Residues per mole of enzyme from <em>E. coli</em> (nearest integer)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mole/mole enzyme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>19.5</td>
<td>20</td>
<td>29</td>
</tr>
<tr>
<td>Histidine</td>
<td>11.3</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>42.0</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Threonine</td>
<td>19.3</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Serine</td>
<td>7.6</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>43.4</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>Proline</td>
<td>13.5</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Glycine</td>
<td>23.6</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>Alanine</td>
<td>57.6</td>
<td>58</td>
<td>47</td>
</tr>
<tr>
<td>Valine</td>
<td>24.5</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Methionine</td>
<td>4.2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>24.5</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>Leucine</td>
<td>37.5</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>15.9</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>14.7</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Total residues</td>
<td>368</td>
<td>364</td>
<td></td>
</tr>
</tbody>
</table>

---

* Single samples of duplicate preparations were hydrolyzed for 24 hours and the mean obtained.

* All calculations were based on a molecular weight of 40,250.

* No differences were observed when 24-, 48-, and 72-hour samples were extrapolated to zero.

* No differences were observed when 72-hour samples were used.

0.730 indicated a molecular weight of 40,250 ± 2,000. The linearity of the points along this line indicated a high degree of homogeneity.

**Polyacrylamide Disc Gel Electrophoresis**—Polyacrylamide disc gel electrophoresis at pH 7.2 in 0.25 M sodium phosphate buffer indicated that the two fractions (I and II) were homogenous, yet different with respect to their electrophoretic mobilities (Fig. 2).

*S. mutans* superoxide dismutases I and II were dissociated with sodium dodecyl sulfate without \( \beta \)-mercaptoethanol and the molecular weights determined by the method of Weber and Osborn (12). Proteins of known subunits weight were used as standards as described under "Materials and Methods." Superoxide dismutase I was found to be composed of subunits having a molecular weight of 18,500 ± 1,000 while superoxide dismutase II had a subunit molecular weight of 19,500 ± 100. The results of this electrophoresis are seen in Fig. 3. Since \( \beta \)-mercaptoethanol was not necessary for dissociation into subunits it may be concluded that *S. mutans* superoxide dismutases I and II are composed of subunits of equal size which appear to be not bound together by disulfide bridges. The smearing of the gel can possibly be attributed to some overloading of the gel.

**Spectral Properties**—Concentrated *S. mutans* superoxide dismutase I was seen to be wine red, the same color of the *E. coli* enzyme (8). Therefore, as shown in Fig. 4, the *S. mutans* enzyme exhibits an absorption spectrum, of *E. coli* superoxide dismutase (8). The molar extinction coefficient of the *S. mutans* superoxide dismutase I at 473 nm was 560. The absorption spectrum of superoxide dismutase II was not determined because of insufficiency of the sample.

**Amino Acid Analyses**—The amino acid composition of *S. mutans* superoxide dismutase I is presented in Table II. When this is compared to the amino acid composition of the *E. coli* enzyme (8), the molar extinction coefficient of the *S. mutans* superoxide dismutase I at 473 nm was 560. The absorption spectrum of superoxide dismutase II was not determined because of insufficiency of the sample.

**Manganese**—As in the case of *E. coli* superoxide dismutase, no electron paramagnetic resonance signal was observed with the native enzyme. However, upon denaturation by heating at 100°C...
for 3 min in 0.2 M HCl, a characteristic manganese (II) signal was observed as shown in Fig. 5. The top spectrum was obtained from 1.96 mg per ml of S. mutans superoxide dismutase I after heating to 100°C for 2 min in 0.2 M HCl. The lower tracing was obtained with native enzyme at a concentration of 4.9 mg per ml in water at a 10-fold higher sensitivity of the instrument. These spectra were obtained with a flat cell at room temperature and were repeated three times. Each repetition was preceded by careful checking of the alignment of the flat cell in the magnetic field. Comparison of the signal shown in Fig. 5 with a standard solution of MnSO₄ indicated that S. mutans superoxide dismutase I had 1.85 atoms of manganese per mole of enzyme, suggesting that the dimeric enzyme molecule contains 2 atoms of manganese.

Copper and Zinc—When either native or denatured superoxide dismutase was assayed for the presence of copper or zinc no trace above the blank value was found.

DISCUSSION

The report by McCord and Fridovich (1) that the copper-and zinc-containing protein erythrocytoperoxin has a very important enzymatic function, i.e., the dismutation of the highly reactive superoxide free radical, generated a great deal of interest in the isolation and characterization of this enzyme from other sources. An interesting example was the isolation of superoxide dismutase from E. coli by Keele et al. (8) where it was found that aside from the specificity of this enzyme for the superoxide radical, the enzyme from E. coli bore little resemblance to those previously isolated from mammalian tissue (1, 2). The existence of two totally different proteins with identical enzyme activity generated the desire to isolate this enzyme from other sources, both procaryotic and eucaryotic, in order to gain some insight into the possible evolutionary significance of the enzyme. It was also very important to isolate the enzyme from other sources following the report by McCord et al. (16) that superoxide dismutase function appeared to be vital for the aerobic survival of an organism. Because of our interest in the oral streptococcus S. mutans we decided to isolate the enzyme from this species for several reasons. (a) S. mutans is potentially one of the most damaging organisms as far as dental caries is concerned. (b) S. mutans is a fastidious facultative anaerobic organism and therefore is quite different from E. coli in its growth characteristics. (c) S. mutans is a gram-positive microorganism while E. coli is a gram-negative bacterium.

From the chromatographic data presented in Fig. 1 and summarized in the text it is obvious that there are two distinct superoxide dismutase enzymes in S. mutans. Confirmation of this can be seen in Fig. 2 where disc gel electrophoresis shows the two enzymes moving with different electrophoretic mobilities. This was quite significant since this is the first report of the isolation of two superoxide dismutase enzymes from the same organism. Since there was not enough material of superoxide dismutase II it was not possible to completely characterize this enzyme and compare it to superoxide dismutase I.

However when enzyme I from S. mutans is compared to the previously isolated E. coli superoxide dismutase, some differences are observed. The molecular weight of the S. mutans enzyme compares favorably with that of the E. coli superoxide dismutase, 40,250 versus 39,500. The subunit composition of the enzyme is also very similar between the two species of bacteria, two identical subunits not held together by disulfide bridges. The metal cofactor content of the enzymes from the two species is also the same where both contain 2 atoms of manganese per mole of enzyme with no copper being present. The spectral properties, both ultraviolet and visible are essentially identical for both bacterial enzymes and different from the mammalian enzyme spectrum (1, 2).

The most striking difference between the enzyme from S. mutans and that from E. coli is seen in the amino acid composition (Table II).

From the data presented here and in the reports on isolation of the enzyme from E. coli and from mammalian sources it is known that there are at least two classes of superoxide dismutase, (a) the mammalian or eucaryotic type which has 2 atoms of copper and 2 atoms of zinc per mole of enzyme, little or no aromatic amino acids, with two subunits presumably held together by disulfide bridges, and (b) the bacterial or procaryotic type which has 2 atoms of manganese per mole of enzyme, contains aromatic residues, and has two subunits not held together by disulfide bridges.

The significance of two apparent isoenzymes of superoxide dismutase in S. mutans remains to be established. This does not appear to be an isolated example, since apparent isoenzymic patterns have been observed in other systems as well (17).

It should be very interesting to isolate superoxide dismutase from other procaryotic and eucaryotic organisms and examine whether the two species of the enzyme (procaryotic and eucaryotic) evolved independently or whether they represent rather drastic divergent products from a common ancestral gene.

Acknowledgments—The sedimentation equilibrium was kindly performed by Mr. James Huston. The assistance of Dr. Howard Steinman and Mr. Dennis Winge in the amino acid analyses is gratefully acknowledged. We would like to express sincere thanks to Dr. Irwin Fridovich for his support and suggestions during this investigation.

REFERENCES

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