Oxidation of Elemental Sulfur to Thiosulfate by Streptomyces

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Streptomyces sioyaensis, which produces the antibiotic siomycin, oxidizes elemental sulfur when added to the culture medium and accumulates thiosulfate in the fermented broth. The accumulated thiosulfate was isolated as the ammonium salt and was identified by melting point, IR spectrum, and paper chromatography. A variety of other streptomycetes also oxidized elemental sulfur and accumulated thiosulfate.

Although the participation of heterotrophic microorganisms in the oxidation of elemental sulfur was suggested by Guittoneau (1) and Guittoneau and Keilling (2) about 40 years ago, the generally accepted concept concerning the geochemical cycle of elemental sulfur is that the bacteria that oxidize elemental sulfur are limited to chemolithotrophic Thio Bacilli (Beggiatoaceae and Achromaciaceae) and to phototrophs (Thiorhodaceae and Chlorobacteriaceae).

During the course of our investigations on sulfur metabolism in Streptomyces sioyaensis, which produces the chromopeptide antibiotic siomycin (3), we found that this streptomycete oxidized elemental sulfur. The oxidized product was accumulated as thiosulfate.

Further studies with various streptomycetes showed that the oxidation of elemental sulfur is a generally observable phenomenon in microorganisms belonging to this genus.

MATERIALS AND METHODS

Microorganisms and cultivation. S. sioyaensis and other streptomycetes were obtained from M. Mayama of our laboratory. For the cultivation of S. sioyaensis, spores from a slant were inoculated into a seed flask containing 100 ml of sterile Bennett's medium composed of 1% glucose, 0.2% NZ-Amine A (Sheffield Chemical), 0.1% beef extract (Difco), and 0.1% yeast extract (Difco). After incubation for 36 to 42 hr at 28°C on a reciprocal shaker (140 strokes/min with 5-cm amplitude), 5-ml portions of the culture were inoculated into 100 ml of a fermentation medium consisting of 6% sucrose, 1% yeast extract, and 1% polypeptone (Daigo) with or without elemental sulfur. The pH was not adjusted.

Other streptomycetes were precultured in test tubes (2.5 by 20 cm) containing 10 ml of Bennett's medium under shaking (28°C, 40 hr). One milliliter of culture was inoculated into 10 ml of the same medium with or without elemental sulfur and incubated for 144 hr.

Thiosulfate analysis. Thiosulfate was determined by the method of Sörbo (6), after removal of protein by Cd²⁺ ions.

Elemental sulfur analysis. At a suitable time, the culture of S. sioyaensis was filtered through a Buchner funnel, and the mycelia and elemental sulfur obtained were dried under vacuum. The solids were extracted with three portions of benzene. The extract was diluted with benzene to an appropriate volume, and the elemental sulfur was determined spectrophotometrically by the method of Ozawa (5) with a Hitachi-Perkin Elmer spectrophotometer model 139.

Paper chromatography. Samples were applied to Töyo Roshi no. 50 paper; if necessary, after deproteinization by the addition of an equal volume of ethanol. The chromatograms were developed by ascending chromatography at room temperature by using methanol-1-butanol-water (3:1:1; solvent A; reference 7) and ethanol-pyridine-water-28% NH₄OH (30:30:40:2.5; solvent B; reference 4). Thiosulfate and polythionates were located by spraying with 5% (w/v) AgNO₃, 5% (w/v) HgNO₃, or 3% (w/v) NaN₃ in 0.1 N I₂ solution (7).

RESULTS

Time course of the oxidation of elemental sulfur to thiosulfate. The course of a typical oxidation of elemental sulfur to thiosulfate is shown in Fig. 1. The sum of both the remaining elemental sulfur and the sulfur in the formed thiosulfate (both are expressed as milligrams of sulfur/100 ml) was constantly maintained at approximately 150 mg per flask throughout the incubation period. In the culture without added elemental sulfur, the accumulation of thiosulfate was very small or none.

These results suggest that the thiosulfate found in the culture fluid of S. sioyaensis grown in medium with elemental sulfur added was derived from the elemental sulfur.

Polythionates were barely detected in depro-
teinized fermentation medium at any time, either colorimetrically or paper chromatographically.

**Isolation of oxidation product.** *S. sioyaensis* was grown for 195 hr in a medium containing 6% sucrose, 1% yeast extract, 1% polypeptone, and 0.4% elemental sulfur under the conditions described above. After fermentation, the mycelia and elemental sulfur were removed by centrifugation. To 4 liters of clear supernatant fluid, 20 g of charcoal was added, and the suspension was stirred for 30 min at room temperature. After removal of charcoal by filtration, the clear filtrate was adjusted to pH 3.0 with 10% HCl. The acidified filtrate (3,900 ml) was applied to a column of IR-45 resin (OH type, 4 by 38 cm), the column was washed with 1,000 ml of deionized water, and thiosulfate was eluted with 1,000 ml of 0.01 M NH₄OH. The eluted solution was concentrated to dryness in vacuo. The crystals obtained were recrystallized from a minimum amount of hot water. The yield was 440 mg.

**Identification of oxidized product.** The recrystallized specimen, which decomposed at 143 to 146 °C, showed 98% pure ammonium thiosulfate as determined colorimetrically. The infrared spectrum of the isolated crystal was indistinguishable from that of authentic ammonium thiosulfate (Fig. 2). The specimen also migrated the same distance as the authentic ammonium thiosulfate on paper chromatography solvent systems A and B. From these results, it was concluded that the accumulated compound was thiosulfate.

**Effect of the amount of elemental sulfur added on cell growth and accumulation of thiosulfate.** Figure 3 shows the effect of the added elemental sulfur on cell growth and on the thiosulfate concentration. The addition of a small amount of elemental sulfur (0.05 g to 0.2 g/100 ml) caused a slight stimulation of cell growth as determined by dry weight per 100 ml of culture. Larger amounts of elemental sulfur resulted in a retardation of cell growth. The amount of thiosulfate accumulated increased with the amount of added sulfur. Figure 3 also indicates the percentage of thiosulfate formation for the various amounts of elemental sulfur. The highest oxidation ratio (80%) was obtained when 0.15 g of elemental sulfur was added to 100 ml of medium.
Oxidation of elemental sulfur by various streptomycetes. The above results clearly indicate that *S. sioyaeensis* oxidizes a considerable amount of elemental sulfur and accumulates thiosulfate. To observe whether the oxidation of elemental sulfur is a unique property for *S. sioyaeensis* or a general property among streptomycetes, 36 species belonging to this genus were cultured in the presence or absence of elemental sulfur. With the exception of *S. virginiae*, *S. viridans*, and *S. erythreus*, all species tested oxidized elemental sulfur and accumulated appreciable amounts of thiosulfate (Table 1).

**DISCUSSION**

The present study clearly demonstrates that *S. sioyaeensis* oxidizes elemental sulfur. Most, if not all, of the oxidized product accumulated as thiosulfate under the existing culture conditions. However, as indicated in the accompanying paper (8), accumulated thiosulfate can be metabolized further, and this metabolism appears important for the stimulation of siomycin production.

The present study also indicates that a variety of streptomycetes oxidize elemental sulfur. Since the species belonging to this genus are widely distributed in soil, it is probable that streptomycetes play important ecological roles in the oxidation of elemental sulfur.

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### Table 1. Oxidation of elemental sulfur to thiosulfate by various Streptomyces

| Organism                        | Growth | Thiosulfate formed (µg/ml) |
|---------------------------------|--------|---------------------------|
| *Streptomyces aburaviensis*     | +   | 129                       |
| *S. acidomyceticus*             | +   | 164                       |
| *S. albireticuli*               | +   | 47                        |
| *S. albus*                      | +   | 24                        |
| *S. albus*                      | +   | 74                        |
| *S. antibioticus*               | +   | 127                       |
| *S. aureus*                     | +   | 92                        |
| *S. chartreusis*                | +   | 74                        |
| *S. cinnamoneus*                | +   | 21                        |
| *S. cinereus*                   | +   | 38                        |
| *S. coelicolor*                 | +   | 33                        |
| *S. flavogriseus*               | +   | 16                        |
| *S. flavus*                     | +   | 24                        |
| *S. galbus*                     | +   | 47                        |
| *S. gelaticus*                  | +   | 158                       |
| *S. lavendulae*                 | +   | 7                         |
| *S. lavendulae*                 | +   | 8                         |
| *S. minoensis*                  | +   | 169                       |
| *S. mirabilis*                  | +   | 100                       |
| *S. orchidaceus*                | +   | 57                        |
| *S. reticuli*                   | +   | 63                        |
| *S. rimosus*                    | +   | 187                       |
| *S. roseochromogenes*            | +   | 170                       |
| *S. sioyaeensis*                | +   | 230                       |
| *S. sulphureus*                 | +   | 154                       |
| *S. thioluteus*                 | +   | 41                        |
| *S. toyokaensis*                | +   | 314                       |
| *S. venezuelae*                 | +   | 10                        |
| *S. verne*                      | +   | 187                       |
| *S. virginiae*                  | +   | 0                         |
| *S. viridans*                   | +   | 0                         |
| *S. griseus*                    | +   | 172                       |
| *S. griseus*                    | +   | 122                       |
| *S. erythreus*                  | -   | 0                         |