Sulfur Regulation of Heparinase and Sulfatases in *Flavobacterium heparinum*

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Sulfur regulation of heparinase synthesis and sulfatase synthesis was studied in *Flavobacterium heparinum*. Heparinase synthesis was strongly repressed by sulfate and L-cysteine, while the activity of this enzyme showed little or no inhibition by these compounds. Heparinase was synthesized in the absence of heparin when L-methionine was used as the sole sulfur source. The sulfatases produced by *F. heparinum*, which include the sulfatases involved in heparin catabolism, were also studied. At least some of the sulfatase activity was regulated by sulfur compounds in a manner similar to heparinase regulation. L-Cysteic acid and taurine were not suitable sulfur sources to support the growth of *F. heparinum*.

Heparinase or heparin lyase (EC 4.2.2.7) is an inducible cell-associated enzyme that is produced by *Flavobacterium heparinum* as the first enzyme in the catabolic pathway of heparin degradation. The properties of heparinase and its mode of action have been studied by Linker and Hovingh (28, 29), Dietrich et al. (9), and Langer et al. (23). Recently, Galliher et al. (14) suggested a modified catabolic pathway for heparin degradation in *F. heparinum* in which several enzymes are involved, including heparinase, sulfoesterase I (4,5-uronate sulfatase), glycuronidase, sulfoesterase II (disulfoglucosamine 6-sulfatase; EC 3.1.6.11), and sulfamidase.

The increasing interest in the use of heparinase as a means of controlling heparin activity during heparin therapy (21-26) led us to try to improve production methods for this enzyme. The kinetics of growth and heparinase production were studied in complex and defined media supplemented with the inducer heparin (14). The defined medium, which we called "high-sulfate medium," improved the growth rate, heparinase production, and stability compared with the complex medium and allowed volumetric enzyme production that was 640 times higher than the production achieved by previous workers (29). However, heparin is required in this medium to induce heparinase; this makes large-scale fermentation for heparinase preparation expensive. The effects of various carbon, nitrogen, and sulfur sources on heparinase synthesis in the presence or absence of the inducer heparin were studied, and it appears that the most important method of regulation is repression by sulfate (15). In a sulfate-free defined medium (which we called "low-sulfate medium") containing L-methionine as the sole sulfur source, heparinase is produced without the inducer heparin (15). Fermentation cost calculations showed that there was a 45-fold decrease in medium cost per unit of heparinase in the low-sulfate medium compared with the high-sulfate medium containing heparin as an inducer. This substantial reduction in fermentation costs led us to investigate the sulfur regulation of heparinase more closely.

Similar examples of sulfur-regulated enzymes have been reported previously. However, only arylsulfatases in bacte-

ria and fungi have been studied in detail (1, 3, 10, 12, 18, 19, 23, 32, 34, 38). A comprehensive review of microbial sulfatases was done by Dodgson et al. (11). We also studied the sulfatases produced by *F. heparinum* that are involved in heparin degradation to determine whether there are similar mechanisms of sulfur regulation for heparinase and these sulfatases.

MATERIALS AND METHODS

Chemicals. Amino acids (L-histidine, L-methionine, and L-cysteine), L-cysteic acid, and taurine were purchased from Sigma Chemical Co., St. Louis, Mo. L-Cysteine hydrochloride · H₂O was obtained from MCB Manufacturing Chemist. Azure A dye was obtained from Fisher Scientific Co., Pittsburgh, Pa. (catalog no. A-970; certified biological stain; total dye content, 70%). All other organic and inorganic chemicals were reagent grade. The dye reagent concentrate for the protein assay was purchased from Bio-Rad Laboratories, Richmond, Calif.

Analytical determinations. Samples of *F. heparinum* to be assayed for heparinase and sulfatase activities were centrifuged at 4°C for 10 min at 12,800 × g. The resulting cell pellets were immediately sonicated at a cell protein concentration of 8 g/liter as previously described (14) and were frozen for later assays. In experiments in which only heparinase activities were measured, the cell pellets were suspended in 5 ml of 0.15 M potassium phosphate buffer (pH 7.0); in experiments in which both heparinase and sulfatase activities were determined, the pellets were suspended in 5 ml of distilled water to avoid phosphate inhibition of the sulfatases (7). Heparinase activity was measured by using an Azure A dye assay (14); 1 U of heparinase activity was defined as the amount of enzyme that degraded 1 mg of heparin per h (international units were not used due to the difficulty in relating the metachromatic shift of the dye to the number of moles of bonds broken [15]). Sulfatase activity, which was expressed in picokatal, was determined by using the nonspecific synthetic substrate *p*-nitrocatechol sulfate (25). The protein concentrations of cell sonic extracts were measured by using the microassay procedure of Bradford (2). Glucose was measured by the dinitrosalicylic acid method (31). Assays were performed by using a model 3723 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The amino acid concentrations in the super-

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natants of culture broth were measured by using the method of Hill et al. (20); this method consisted of reverse-phase separation and fluorescence detection of the amino acids as their *O*-phthalaldehyde-ethanethiol derivatives. A high-pressure liquid chromatograph (Waters Associates, Inc., Milford, Mass.) equipped with two model 600A pumps, a model 660 solvent programmer, and a model U6K injector was used. A model FS-9670 liquid chromatographic fluorometer was obtained from Schoeffel Instrumental Corp. The reverse-phase column was a μ Bondapak C₁₈ column (Waters Associates).

Microorganism. *F. heparinum* is a gram-negative, rod-shaped (1.0 by 0.3 μ m), nonmotile, nonsporeforming soil bacterium. We used a strain that was obtained from Alfred Linker, Veterans Administration Hospital, Salt Lake City, Utah. The microorganism was grown at 23°C or at room temperature. The cultures were stored on agar slants of defined medium as previously described (14). Two culture preparations were used; one was stored on agar slants of high-sulfate medium, and the other was stored on agar slants of low-sulfate medium. The high- and low-sulfate media used for culture storage were the same media used for shake flask cultivation (see below), except that the glucose concentration was 4.0 g/liter.

Shake flask cultivation. Experiments were conducted in 0.5-liter sidearm baffled shake flasks containing 50 ml of medium. The high-sulfate medium contained 10^{-4} M trace salts ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$), 8.0 g of glucose per liter, 2.0 g of $(\text{NH}_4)_2\text{SO}_4$ per liter, 2.5 g of K_2HPO_4 per liter, 2.5 g of Na_2HPO_4 per liter, 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (sterilized separately) per liter, 0.2 g of L-histidine per liter, 0.2 g of L-methionine per liter, 1.0 g of heparin (sodium salt) per liter, and 4 drops of type P-2000 antifoaming agent per liter. The low-sulfate medium contained 10^{-4} M trace salts ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot \text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), 8.0 g of glucose per liter, 2.0 g of NH_4Cl per liter, 2.5 g of K_2HPO_4 per liter, 0.2 g of L-histidine per liter, 0.2 g of L-methionine per liter, and 4 drops of type P-2000 antifoaming agent per liter. The low-sulfate medium did not contain any heparin. Glucose and magnesium salts were autoclaved separately and added to the sterilized medium; L-histidine and L-methionine were added after sterilization by filtration through a 0.22- μ m membrane syringe filter (Millipore Corp., Bedford, Mass.). When L-cysteine was added to the medium, it was also filter sterilized. Trace salts were dissolved and stored at 25-fold concentrations at pH 2.0 in solutions containing 0.5 ml of concentrated H_2SO_4 or HCl per liter. After sterilization, the pHs of both media were 7.4 ± 0.2 . Inocula (4 to 6%, vol/vol) of cells in exponential growth were used. Cultures were incubated at room temperature on a shaker with a 2.5-cm stroke at 200 rpm. Growth was measured by determining turbidity with a Klett-Summerson colorimeter equipped with a no. 66 red filter; the conversion factor used was 250 Klett units per g (dry cell weight) per liter. Growth continued until a reading of 400 to 500 Klett units was achieved, during which time the pH fell to 5.0 ± 0.2 . Cells from the flasks were then harvested for enzyme assays or used to inoculate other flasks or a fermentor.

For heparinase and sulfatase assays, 75 to 100% of a 50-ml flask culture was used in order to get a cell protein concentration of about 8 g/liter in the 5-ml crude sonic extract. In all low-sulfate medium experiments, deionized distilled water was used, and inocula were transferred several times in

low-sulfate medium to avoid sulfate contamination from the agar. A trace analysis of the low-sulfate medium done by Galbraith Laboratories, Inc., Knoxville, Tenn., indicated that the level of sulfur contamination of the medium (based on sulfate and sulfite) was 4.5×10^{-5} M sulfur.

Fermentor cultivation. A 2-liter fermentor with a 1.5-liter working volume was equipped so that we could control agitation, aeration, pH, temperature, and dissolved oxygen. The fermentor media used were the same as the media described above, except that KH_2PO_4 (1.0 g/liter) and NaH_2PO_4 (1.0 g/liter) were substituted for K_2HPO_4 and Na_2HPO_4 . The fully equipped fermentor was sterilized at 121°C. Glucose and magnesium salts were autoclaved separately and added to the fermentor after it had cooled to 23°C. Amino acids were sterilized by filtration as described above. Aeration and agitation were controlled to maintain a dissolved oxygen level of about 50% of air saturation. The temperature was controlled at $23 \pm 1^\circ\text{C}$, and the pH was controlled at 7.0 ± 0.2 by adding 12% NH_4OH .

RESULTS

Effect of sulfate on heparinase and sulfatase synthesis and activity. The effect of adding sulfate (as Na_2SO_4) to an actively growing culture that was synthesizing heparinase in low-sulfate medium was studied by using a 2-liter fermentor. The inoculum was prepared in low-sulfate medium. The growth and heparinase-specific and volumetric activities are shown in Figure 1A. Arrow 1 indicates the time at which glucose (5 g/liter), L-histidine (0.1 g/liter), and L-methionine (0.1 g/liter) were added (at 33.5 h), and arrow 2 indicates the time at which Na_2SO_4 (2.5 g/liter; 14 mM) was added (at 37.25 h). Glucose depletion, L-histidine depletion, and L-methionine depletion, as well as the production of alanine during fermentation, are shown in Fig. 1B. Growth was exponential at a specific growth rate of 0.1 h^{-1} until the cell concentration reached 1.9 g (dry cell weight) per liter, at which time the specific growth rate began to decrease. During the rapid exponential phase, both specific and volumetric heparinase activities initially increased, and then at about 22 h these activities decreased abruptly; heparinase synthesis resumed at 26 h. When sulfate was added at 37.25 h, the heparinase specific activity decreased from 3.7 to 0.7 U/mg protein. No heparinase was found in the supernatant of the culture broth.

Heparinase production in low-sulfate medium under similar experimental conditions has been described previously by Galliher et al. (15). The results of these authors provide a control for the experiment shown in Fig. 1A. A plot of the differential rate of synthesis expressed as dry cell weight during the two fermentations is shown in Fig. 2. The results clearly show that there was sulfate repression of heparinase synthesis.

The effects of initial sulfate concentrations between 0 and 75 mM on growth, heparinase synthesis, and sulfatase synthesis when these concentrations were added to low-sulfate medium were reported previously by Galliher et al. (15). Cultures grown in the presence of up to 7.5×10^{-3} mM sulfate had an initial specific growth rate of 0.11 h^{-1} , the heparinase specific activity averaged 4.6 U/mg of protein, and the sulfatase specific activity averaged 19 U/mg of protein. When the initial sulfate concentration was increased to 7.5×10^{-2} mM, the specific growth rate increased to 0.18 h^{-1} , the heparinase specific activity dropped to almost zero, and the sulfatase specific activity dropped to 6 U/mg of protein. At 75 mM sulfate, growth was inhibited, showing a 24-h lag phase. Figure 3 shows the heparinase and sulfatase

FIG. 1. (A) Effect of sulfate addition during growth of *F. heparinum* in low-sulfate medium. The arrows indicate the times of addition of glucose, L-histidine, and L-methionine (arrow 1) and Na₂SO₄ (arrow 2). (B) Alanine production and glucose, L-histidine, and L-methionine depletion in low-sulfate medium fermentation of *F. heparinum* with Na₂SO₄ added at 37.25 h.

specific activities as a function of the initial sulfate concentration.

The level of sulfate inhibition of heparinase was reported by Galliher et al. to be less than 10% with 0.2 M sulfate (15). Sulfate inhibition of sulfatases was studied (data not shown) with a crude cell extract that was prepared by sonication of *F. heparinum* cells grown in a low-sulfate medium. This cell

FIG. 2. Heparinase specific activity as a function of cell concentration in low-sulfate medium (O) and in low-sulfate medium containing Na₂SO₄ (□).

extract was incubated with 7.5×10^{-4} , 7.5×10^{-2} , and 7.5 mM Na₂SO₄. A slight inhibition (15 to 20%) was observed at an Na₂SO₄ concentration of 7.5 nM. While results obtained with crude extracts need to be interpreted with caution, such results support the suggestion that the observed effect of sulfate addition is not primarily attributable to enzyme inhibition.

Cell yields on sulfate, cysteine, and methionine. The overall cell yields (calculated from the data in Fig. 1A and B) in the presence of L-histidine and L-methionine in low-sulfur medium were 10.5 g of cells per g of L-histidine and 17.5 g of cells per g of L-methionine. Robert et al. (35) found that in *Escherichia coli* about one-half the sulfur in the cells is in the form of methionine, while the other half is in the form of cysteine. Thus, in high-sulfate medium, which contained both sulfate and L-methionine as sulfur sources, L-methionine appeared to provide the methionine sulfur for cell protein, while sulfate provided the cysteine sulfur. In low-sulfate medium, the low yield with L-methionine is consistent with the fact that L-methionine provided all of the sulfur for cell protein. The low cell yield in the presence of L-histidine is unexplained. The low cell yield in the presence of glucose (0.18 g of cells per g of glucose consumed) may be explained in part by the formation of by-products, such as alanine, which was produced at a maximum specific yield of 0.08 g of alanine per g of cells, but only when glucose was present in excess.

Effect of L-cysteine on heparinase and sulfatase synthesis and activity. *F. heparinum* was grown in a low-sulfate medium in which L-cysteine hydrochloride (0.2 g/liter) was substituted for L-methionine. Five cultures were grown in shake flasks and harvested at different times during expo-

nential phase and stationary phase. The inoculum was prepared in regular low-sulfate medium containing L-methionine (0.2 g/liter) as the sole sulfur source. The specific growth rate in the presence of L-cysteine was 0.12 h^{-1} , compared with 0.09 h^{-1} for the control grown in low-sulfate medium containing L-methionine. No heparinase activity was found in any of the cultures grown in the presence of L-cysteine.

Heparinase synthesis was studied in the presence of a growth-limiting concentration of L-cysteine as the sole sulfur source. Flasks were inoculated with an exponentially growing culture grown in low-sulfate medium containing L-methionine (0.2 g/liter). Cultures were grown in shake flasks in low-sulfate medium without L-methionine but containing 0.04 g of L-cysteine hydrochloride per liter (1.23 mM), and they were harvested at different times in exponential and stationary phases and assayed for heparinase activity. A control culture was grown in the same medium, except that it contained 0.2 g of L-cysteine hydrochloride per liter, and was harvested in the stationary phase at 52 h. Figure 4 shows the kinetics of growth and heparinase synthesis. The growth was initially exponential with a specific growth rate of 0.14 h^{-1} ; then at a concentration of 0.8 g of dry cell weight per liter the specific growth rate fell to 0.05 h^{-1} . Heparinase synthesis started at the transition point between the two growth phases. The specific activity increased during the second, slower exponential growth phase, reaching 0.9 U/mg of protein. At the onset of the stationary phase, the pH of the medium was 6.24 and thus was not growth limiting; the final cell density was 1.6 g/liter. The culture in the control flask contained excess L-cysteine and grew to a density of 2.6 g/liter with a specific growth rate of 0.17 h^{-1} ; the final pH was 5.3. There was no heparinase activity in the control.

The effect of the initial L-cysteine concentration in low-sulfate medium on growth, heparinase synthesis, and sulfatase synthesis was also studied. Six cultures were

FIG. 3. Effect of initial sulfate concentration added to low-sulfate medium on heparinase and sulfatase specific activities. The flask containing no added sulfate had a heparinase specific activity of 5.5 U/mg of protein and a sulfatase specific activity of 22 U/mg of protein.

FIG. 4. Growth and heparinase production in shake flasks during L-cysteine-starved fermentation of *F. heparinum*.

grown in shake flasks containing low-sulfate media supplemented with L-methionine or L-cysteine hydrochloride at initial concentrations of 0, 8.3×10^{-4} , 8.3×10^{-3} , 8.3×10^{-2} , 8.3×10^{-1} , and 8.3 mM. The cells from the flasks were harvested in early stationary phase and were assayed for heparinase and sulfatase activities. Cultures grown in the presence of 0, 8.3×10^{-4} , and 8.3×10^{-3} mM L-cysteine had growth rates of 0.11, 0.12, and 0.12 h^{-1} , respectively. In these cultures, the average heparinase specific activity was 8 U/mg. In the presence of 8.3×10^{-3} to 8.3×10^{-2} mM, the specific growth rate increased to 0.21 h^{-1} , the heparinase specific activity decreased to 0.5 U/mg of protein, and the sulfatase specific activity fell to 4 U/mg of protein. In the presence of 8.3 mM L-cysteine, growth was strongly inhibited, showing a 2-day lag phase. Figure 5 shows both enzyme activities as a function of L-cysteine initial concentration.

Heparinase inhibition by L-cysteine was studied by using a crude cell sonic extract prepared from a *F. heparinum* culture grown in high-sulfate medium containing heparin as the inducer. A slight inhibition (15%) was observed at an L-cysteine hydrochloride concentration of 57 mM. Sulfatase inhibition by L-cysteine was studied by using a crude cell sonic extract prepared from a *F. heparinum* culture that was grown in low-sulfate medium, and no inhibition was observed at an L-cysteine concentration of 8.3 mM. These results support the idea that the observations made for heparinase and sulfatase were due not to inhibition but to regression.

Growth of *F. heparinum* on different sulfur sources. Different sulfur sources, including sulfate, sulfite, L-cysteine, L-methionine L-cysteric acid, and taurine, were tested for their ability to support the growth of *F. heparinum*. Each

FIG. 5. Effect of initial L-cysteine concentration added to low-sulfate medium on heparinase and sulfatase specific activities. The flask containing no added L-cysteine had a heparinase specific activity of 8.9 U/mg of protein and a sulfatase specific activity of 10 U/mg of protein.

sulfur source was added to low-sulfate medium without L-methionine at a concentration equivalent to 0.045 g of sulfur per liter and was sterilized by filtration. *F. heparinum* had a high growth rate with sulfate, sulfite, L-cysteine, or L-methionine as the sole sulfur source. The highest growth rate occurred with L-cysteine, followed by sulfate, sulfite, and L-methionine. The presence of both sulfate (or L-cysteine) and L-methionine improved the growth rate further. *F. heparinum* grew extremely poorly with L-cysteic acid as the sole sulfur source and had a specific growth rate of $2 \times 10^{-3} \text{ h}^{-1}$. Taurine did not support growth.

DISCUSSION

The experiments described above indicate that there was full repression of heparinase by sulfate and L-cysteine when the initial concentrations of these compounds in low-sulfate medium were more than 7.5×10^{-3} and 8.3×10^{-3} mM, respectively. The sulfatases appeared to be only partially repressed; e.g., these enzymes were repressed about 60% by sulfate and L-cysteine at similar concentrations. The decrease in sulfatase activity cannot be explained by inhibition alone. It is important to remember that there are at least two and possibly more sulfatases, whose levels of inhibition and repression may differ.

When the sulfate concentration was increased from 7.5×10^{-3} to 7.2×10^{-2} mM and the L-cysteine concentration was increased from 8.3×10^{-3} to 8.3×10^{-2} mM and there was full repression of heparinase and partial repression of the sulfatases, there was a clear increase in the *F. heparinum* specific growth rate. Heparinase and, to a lesser extent, sulfatases appear to be synthesized in response to a growth-limiting concentration of sulfur; low initial levels of sulfate (7.5×10^{-2} mM) and L-cysteine (8.3×10^{-2} mM) added to L-methionine allow maximum growth rates to occur and repress both heparinase synthesis and sulfatase synthesis. The heparinase production profile in the presence of L-methionine as the sole sulfur source (Fig. 2) supports this hypothesis. During the initial exponential phase, the specific

growth rate (0.18 h^{-1}) approached the specific growth rate found by Galliher et al. (14) in high-sulfate medium which contained both sulfate and L-methionine. We believe that during rapid growth the cells utilize sulfate and sulfite that contaminate the medium or part of their internal sulfur pool or both, together with exogenous L-methionine; this allows a higher growth rate than when cells are grown on L-methionine alone. During rapid growth, sulfur does not limit growth; as a consequence, there was synthesis of heparin in the early part of the experiment shown in Fig. 1A. We speculate that when sulfur becomes limiting in the presence of methionine and in the absence of sulfate, heparin is repressed and its specific activity falls. This may explain the decreases in heparinase specific and volumetric activities shown in Fig. 1A. When the specific growth rate decreases, heparinase synthesis begins and continues during the second, slower growth phase; this may correspond to the period of utilization of L-methionine as the sole sulfur source. A comparison of the *F. heparinum* specific growth rates in the presence of L-cysteine, sulfate, and L-methionine as sole sulfur sources supports this hypothesis since the growth rates with both L-cysteine and sulfate are higher than the growth rates with L-methionine.

Heparinase production in a medium containing a growth-limiting concentration of L-cysteine as the sole sulfur source also clearly indicates that the enzyme is synthesized in response to a need for sulfur. During the first exponential phase, the added L-cysteine is probably used for growth. During this period, sulfur is not limiting, and heparinase is repressed. We hypothesize that the second, slower phase of growth corresponds to the utilization by cells of their internal sulfur pools. Heparinase synthesis begins when sulfur becomes growth limiting.

As shown in Fig. 1A, heparinase synthesis began when the specific growth rate decreased. Upon sulfate addition, both heparinase specific activity and heparinase volumetric activity dropped abruptly. No heparinase was released into the medium. As heparinase is not significantly inhibited by sulfate, we believe that the enzyme could have been degraded by proteases, which have been found in several fungi to be derepressed in sulfur-deficient media (4, 5, 17, 33, 37). An alternative explanation is that heparinase has a high endogenous turnover rate. The pathways of sulfate assimilation (6, 36), cysteine biosynthesis (16), and methionine biosynthesis (13) have been studied extensively. The major difference in the sulfur metabolism of enterobacteria and yeasts seems to reside in the interconversion of cysteine and methionine (35). Sulfate is assimilated via a series of activation reactions and is then reduced to the oxidation level of sulfide. In enterobacteria, cysteine is formed from sulfide and O-acetylserine. Cysteine sulfur can then be converted to methionine sulfur via cystathionine and homocysteine formation. In these organisms, methionine cannot give rise to cysteine, so that cysteine appears to be the most suitable compound for regulation of the sulfate assimilation pathway. In *Salmonella typhimurium* and *E. coli*, all of the enzymes concerned with cysteine biosynthesis except serine acetyltransferase are subject to derepression by sulfur starvation and to repression by growth on cysteine as a sulfur source. The metabolic relationship among sulfide, cysteine, and methionine in yeasts is more complex than the relationship in enterobacteria. Like bacteria, yeasts can convert sulfide and O-acetylserine to cysteine and cysteine to methionine. They can also convert methionine to cysteine. It seems that methionine can also be formed directly from sulfide and O-acetylhomoserine. In fact, methionine was found to be a

better sulfur source for growth of yeast cells than sulfate and cysteine. Thus, in yeasts the uptake of sulfate and its conversion to sulfide is under the control of methionine rather than cysteine. The sulfur regulation of arylsulfatases in bacteria and fungi seems to reflect these differences. In fungi, arylsulfatases are generally repressed by sulfate, cysteine, and methionine (3, 18). In bacteria, repression is relieved by growth on methionine as a sulfur source (1, 12, 19, 30, 32, 34, 38). This presumably reflects the poor ability of the cells to convert methionine to cysteine, both of which are required for cell protein synthesis. The sulfur regulation of heparinase and sulfatases in *F. heparinum* appears to be similar to the sulfur regulation of arylsulfatases in most of the bacteria studied. Heparinase synthesis and sulfatase synthesis seem to be closely related to sulfur availability for the cells. When the sulfur concentration is suboptimal for growth because the concentration of sulfate or L-cysteine is too low or the source of sulfur cannot be readily utilized, heparinase and sulfatases are synthesized after depletion of exogenous traces of sulfur or internal pools of sulfur or both. When sulfur is readily available in a sufficient quantity for growth, heparinase and, to a lesser extent, sulfatases are repressed.

Previous studies (15) indicated that sulfatases produced by *F. heparinum* are induced by heparin. Dietrich (8) also found that sulfamidase is induced by heparin and tetra- and hexasaccharides derived from heparin. These results, together with our results, suggest that the heparinase and sulfatases in *F. heparinum* are expressed and induced by similar mechanisms.

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