PN 1213

Hydrolysis of choline-O-sulfate by cell-free extracts from Penicillium

Choline-*O*-sulfate (sulfurylcholine) occurs widely in fungi[1]. A possible function for the compound is that of sulfate storage. However, attempts[2,3] to demonstrate hydrolysis of choline-*O*-sulfate by mold extracts have been unsuccessful. A sensitive test for choline-*O*-sulfate hydrolysis is the use as substrate of choline-*O*-[^{35}S]sulfate of high specific activity. The usual method of synthesis[4], heating choline chloride with conc. $H_2{}^{35}SO_4$, gives a specific activity very low compared to that of the original "carrier-free" $^{35}SO_4{}^{2-}$. A method was therefore devised for the preparation of "carrier-free" choline-*O*-sulfate.

To 50 mg choline chloride in a 1.0 ml beaker were added 0.2 ml 12 N HCl and 0.5 ml (53 mC) of "carrier-free" $^{35}SO_4{}^{2-}$ (Oak Ridge National Laboratory, Cat. No. S-35-P-1, specific activity 84 mC/μmole, 1.25 mM $SO_4{}^{2-}$). The resulting solution was placed in a 96° oven. The reaction mixture evaporated to a translucent semi-solid after 3 h of heating, but the formation of choline-*O*-[^{35}S]sulfate continued for 12 h. After 24 h the reaction mixture was cooled and dissolved in 1 ml of water. The solution was then applied to a Dowex-1 X8 ($HCO_3{}^-$ form) column (1.1 × 15 cm) and washed through with water at a flow rate of 0.3 ml/min. 1-ml fractions were collected. The unchanged excess choline (bicarbonate) was found in Fractions 5 and 6. More than 97 % of the choline-*O*-[^{35}S]sulfate was in Fractions 8–11 (75 % in Fractions 9 and 10). The recovered yield was approx. 90 % based on initial $^{35}SO_4{}^{2-}$. The product was characterized as choline-*O*-[^{35}S]sulfate by comparison with an authentic standard by paper chromatography (5 different solvent systems), electrophoretic mobility (zero at pH 3.1 and pH 8.1), hydrolysis rate in 1 N HCl at 100° (half hydrolysis in 30 min), and by behavior in cationic and anionic ion-exchange columns. Also, addition of the "carrier-free" choline-*O*-[^{35}S]sulfate to known amounts of unlabeled choline-*O*-sulfate, followed by recrystallization, yielded a product with the calculated specific activity.

Solutions of "carrier-free" choline-*O*-[^{35}S]sulfate very slowly hydrolyze, even at −20°. The small amount of $^{35}SO_4{}^{2-}$ may be easily removed by passing the solution through a short Dowex-1 ($HCO_3{}^-$) column.

Mycelium of *Penicillium chrysogenum* was grown for 24 h on a synthetic medium[5],

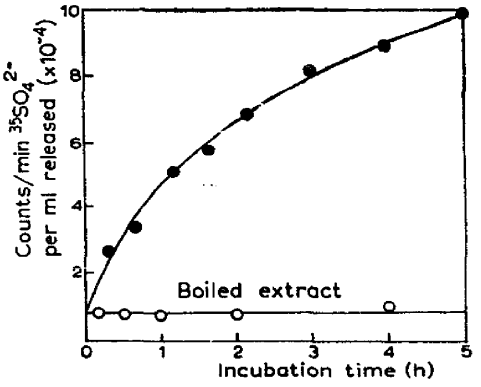

Fig. 1. Time course of choline-*O*-sulfate hydrolysis. Cell extract, 1 ml (pH 7.2), plus 0.02 ml "carrier-free" choline-*O*-sulfate; incubated at 28°. Samples (15 μl), withdrawn periodically, were chromatographed on paper with a solvent system (phenol–water, 8:2, w/w) in which the R_F of choline-*O*-sulfate is 0.92 and that of $SO_4{}^{2-}$ is 0.04.

filtered and washed. 6 g of wet mycelium were ground at 0° with 6 g sand and 6 ml Tris buffer. The suspension was centrifuged at low speed to remove sand and debris. As shown in Fig. 1, cell extracts prepared as described hydrolyzed choline-*O*-sulfate. The hydrolysis rate was low; after 5 h only about 22% of the added choline-*O*-[^{35}S]sulfate had been hydrolyzed. Fig. 2 shows the effect of extract concentration on apparent

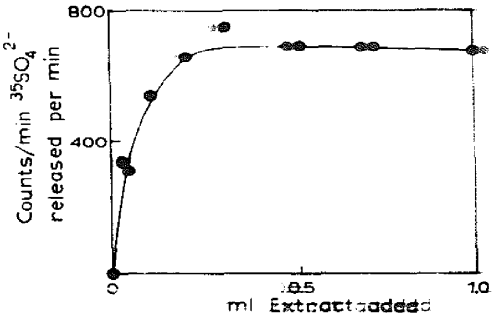

Fig. 2. Effect of extract volume on hydrolysis. The total volume was 1.03 ml, 0.2 M Tris buffer (pH 7.2) was present. Temperature was 28°. Samples were taken at 45 and 75 min. Hydrolysis was determined as in Fig. 1. The apparent hydrolysis rates calculated from the 45-min and 75-min samples were averaged and plotted against extract concentration.

hydrolysis rate. The extract contained unlabeled choline-*O*-sulfate (roughly 10% of the soluble sulfur in mycelium extracts is choline-*O*-sulfate). At the higher extract concentrations, this choline sulfate greatly reduced the specific activity of the small amount of "carrier-free" choline sulfate added as substrate. As seen from the figure, the rate of $^{35}SO_4^{2-}$ liberation was constant at the higher extract levels. This is the result expected if the amount of choline-*O*-sulfate added with the extract was large compared to the amount added as substrate, and if the total choline-*O*-sulfate concentration was large compared to the Michaelis constant of the enzyme.

A preliminary determination of the effect of pH on hydrolysis rate showed that hydrolysis was most rapid at pH 8; the rates at pH 7 and pH 9 being only about half the rate at pH 8. It is not as yet known whether the decrease in activity at high pH was due to enzyme instability. Enzyme activity was inhibited (0.1 M inhibitor) by cyanide (to 5% of the control) and by sulfite (to 0% of the control). Sulfate and fluoride gave little or no inhibition.

Choline-*O*-sulfate-hydrolyzing activity was found in two strains of *P. chrysogenum* (X-1612, a high penicillin-producing strain, and PS-5, a "wild-type" strain) and in *Aspergillus sydowi*.

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