Short Communications

SC 2218

The effect of NaF on the riboflavin production by *Eremothecium ashbyi*

In our previous paper[1] on the physiology of riboflavin over-production by *Eremothecium ashbyi* evidence is presented suggesting that riboflavin biosynthesis occurs after the growth of the yeast ceased, and that the physiological and biochemical properties of growing and producing cells differ considerably. In an attempt to elucidate these differences the sensitivity of both types of cells to various inhibitors was tested. A specific action of inhibitor on one of both types of cells might indicate the importance of an inhibited metabolic reaction for growth and for riboflavin production. Of various inhibitors assayed only NaF showed a different effect on growing cells and on producing mycelium of *E. ashbyi*.

Eremothecium ashbyi strain ZA[1] was maintained on Medium A (10 g glucose, 10 g peptone, 1 g yeast extract, distilled water to 1000 ml (pH 6.8) before sterilization for 20 min at 127°)[2]. A 500-ml flask with 50 ml Medium A was inoculated from an agar slope. After incubation at 28–30° for 40–45 h on a reciprocal shaker (105-mm stroke, 96 strokes/min), 10 ml of culture were inoculated into a 5-l flask containing 1000 ml Medium C (10 g glucose, 10 g Bacto vitamin-free casamino acids, 2 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g NaCl, 0.004 mg biotin, 0.4 mg thiamin, 40 mg inositol, distilled water to 1000 ml (pH 6.8) before sterilization for 20 min at 127°; glucose was sterilized separately). This flask was incubated on a reciprocal shaker (50-mm stroke, 84 strokes/min) at 28–30°. The dry weight of the mycelium and the total riboflavin

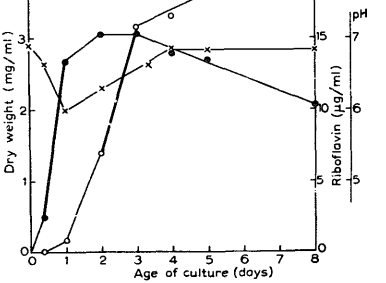

Fig. 1. Growth, riboflavin production and pH changes during the cultivation of *Eremothecium ashbyi* in Medium C. ●—●, growth; O—O, total riboflavin; ×—×, pH.

content was determined as earlier described[1]. Fig. 1 shows the growth and production of riboflavin on Medium C. After 8 h of cultivation the contents of this flask were aseptically distributed into 100-ml Erlenmeyer flasks, so that these flasks contained then 25 ml of the culture and 5 ml of the inhibitor studied. After cultivation at 28–30° for 16 h on reciprocal shaker (105-mm stroke, 96 strokes/min) the growth rate was estimated. Another parallel 5-l flask with 1000 ml of Medium C was cultivated for 48 h and then the contents were also distributed into 100-ml Erlenmeyer flasks and supplemented with inhibitor. After 24 h of cultivation under the same conditions as described above the rate of riboflavin production by non-growing mycelium was measured. In the control experiments it was found that this change in incubation conditions for limited periods had no influence on rates of growth and of riboflavin production in comparison with incubation in a 5-l flask.

From Fig. 2 it is clear that at concentrations lower than 0.3 mM NaF does not influence the rate of growth or riboflavin production. At higher concentrations the growth rate is inhibited whereas the rate of riboflavin production is stimulated. It is well-known that NaF inhibits the oxidation of glucose *via* the glycolytic pathway but that the oxidation of lactate or pyruvate remains unaltered. In our previous experiments[1] it was found that the riboflavin production is accompanied by a decreased rate of glucose oxidation and an enhanced rate of pyruvate oxidation. The opinion was expressed that riboflavin over-production is caused by a shift from the initial cytochrome type of terminal respiration (oxidizing glucose) to the flavoprotein type (oxidizing accumulated pyruvate *via* acetaldehyde) which is accompanied by

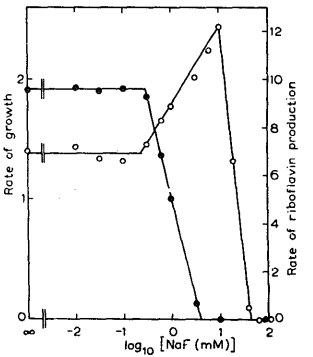

Fig. 2. The effect of NaF on growth and riboflavin production. ●—●, rate of growth (mg dry wt./ml/16 h); O—O, rate of riboflavin production (μg/ml/24 h).

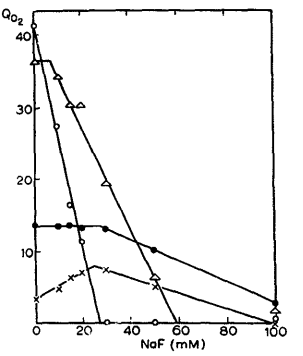

Fig. 3. The effect of NaF on the oxidation of glucose, acetate and pyruvate by 24-h-old washed mycelium grown on Medium A. ●—●, endogenous respiration; O—O, glucose; Δ—Δ, acetate; ×—×, pyruvate. Warburg vessels contained: 1 ml 0.06 M solution of substrate; 1 ml of washed mycelium (6.54 mg dry wt.) suspended in 0.8% (w/v) solution of NaCl; 0.5 ml 0.6 M phosphate buffer (pH 6.5); 0.5 ml of 0–600 mM NaF. Air as gas phase. For CO_2 absorption 0.2 ml of 20% (w/v) KOH was added to the central well. The respective rates of O_2 uptake are expressed as Q_{O_2}(μl O_2/mg mycelial dry wt./h) and are corrected for endogenous respiration.

a failure of regulation of FAD synthesis. It seems probable that in *Eremothecium ashbyi* NaF blocks the oxidation of the remaining glucose and therefore the flavin system oxidizing the accumulated pyruvate is strengthened. This is manifested by enhanced riboflavin production. In fact, manometric measurements performed by the direct method of Warburg have shown that 24-h-old washed mycelium is inhibited by NaF in its ability to oxidize glucose and acetate at concentrations of inhibitor which stimulate pyruvate oxidation (Fig. 3). NaF seems to be, therefore, an important factor shifting the metabolism of *E. ashbyi*, and its application in the riboflavin production process based on use of this microorganism should be considered.

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The effect of sulphonamides on ferricyanide reduction by illuminated spinach chloroplasts

The enzyme, carbonic anhydrase (EC 4.2.1.1), is present in considerable quantities in leaves[1]. This enzyme catalyzes a rapid exchange of oxygen between carbon dioxide and water. The occurrence of such exchange[2] sets a practical limit to the usefulness of ^{18}O as a tracer to determine the source of the O_2 evolved in photosynthesis. In the course of investigating the source of the O_2 in the Hill reaction[3], using carbonate labeled with ^{18}O, it was noted that spinach chloroplasts catalyzed a rapid exchange of $K_2C^{18}O_3$ with H_2O under experimental conditions where the non-enzymatic rate was very slow[2]. Diamox (5-acetylamino-1,3,4-thiadiazole-2-sulfonamide[4]), a potent inhibitor of animal carbonic anhydrase, was added to the chloroplast, buffer, ferricyanide mixture in hopes of avoiding this difficulty. This compound effected a marked inhibition of the Hill reaction with ferricyanide as the electron acceptor. Other sulfonamides were then tested for their effect on the photoreduction of ferricyanide by spinach chloroplasts and the results are reported here. The sulfonamides which inhibited the Hill reaction are potent inhibitors of animal carbonic anhydrase. The inhibition by the sulfonamides appeared particularly interesting in view of the catalytic requirement for CO_2 in the Hill reaction demonstrated by WARBURG AND KRIPPAHL[5,6] and confirmed by STERN AND VENNESLAND[7,8] and ABELES, BROWN AND MAYNE[9].

MANN AND KEILIN[10] originally observed that sulfanilamide and certain related sulfonamides that contained an unsubstituted sulfamyl group would inhibit animal carbonic anhydrase *in vitro*. MILLER, DESSERT AND ROBLIN[4] found that a number of heterocyclic unsubstituted sulfonamides, in which the sulfur atom of the sulfonamide group is joined to a carbon atom of the heterocyclic ring were particularly effective