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Deficient energy metabolism in streptomycin-dependent *Escherichia coli*

We have reported[1] that the yield of cells from the aerobic metabolism of glucose was about one-third lower for streptomycin-dependent *Escherichia coli* than for the wild-type parent strain. This result indicated an impairment in energy production in the streptomycin-dependent mutant which in turn provided an explanation for the observed relaxation of catabolite repression[1] in streptomycin-dependent strains of *E. coli*. We wish to report here results which indicate that the energy derived from oxidative phosphorylation is deficient in streptomycin-dependent *E. coli*.

Cultures were grown on minimal salts medium supplemented with glucose and dihydrostreptomycin (100 μg/ml) as required for the streptomycin-dependent strain. Although different yields from glucose were obtained aerobically (ref. 1 and Table I), the cell yields were the same when *E. coli* B (ATCC 11303) and the streptomycin-dependent mutant were grown anaerobically (Table I). This indicated that the energy deficiency of the streptomycin-dependent mutant was in an aerobic process.

TABLE I

CELL YIELDS FROM GLUCOSE OF STRAINS OF *E. coli* B

Strain	*Culture conditions*	*Cell yield**
Wild-type	Aerobic	0.53
	Anaerobic**	0.15
Streptomycin-dependent	Aerobic	0.32
	Anaerobic	0.15
Nondependent revertant***	Aerobic	0.33

* Cell yields are expressed in μg dry cells/μg glucose consumed.
** Anaerobic conditions were maintained by a constant flow of nitrogen.
*** The revertant was a streptomycin-sensitive strain obtained from streptomycin-dependent *E. coli* B.

For the estimation of ATP, the modified luciferase method of COLE *et al.*[2] was employed, using perchloric acid extracts of exponentially growing, aerobic cultures. Luminescence was estimated in a Nuclear-Chicago Mark I scintillation counter programmed to the most sensitive setting. Cell suspensions to be assayed for ATP were treated in the following manner. A culture in the exponential phase of growth on salts–glucose medium was harvested by centrifugation and resuspended in salts medium. The suspension was incubated without shaking at 37° for 30 min during which time the ATP pool (nmoles of ATP/mg dry cell wt.) decreases by 50–75%. Glucose was then added and shaking of the suspension initiated. Samples were taken during exponential growth for the determination of ATP. The rate of production of ATP in the wild-type culture was greater than the growth rate (Fig. 1A), whereas for the streptomycin-dependent mutant the increase in ATP during exponential growth was parallel with growth (Fig. 1B). These results indicated that the wild-type strain was capable of producing an excess of ATP on glucose–salts medium (confirming

observations of COLE *et al.*[2]), while the streptomycin-dependent strain appeared to be limited in its capability for ATP formation.

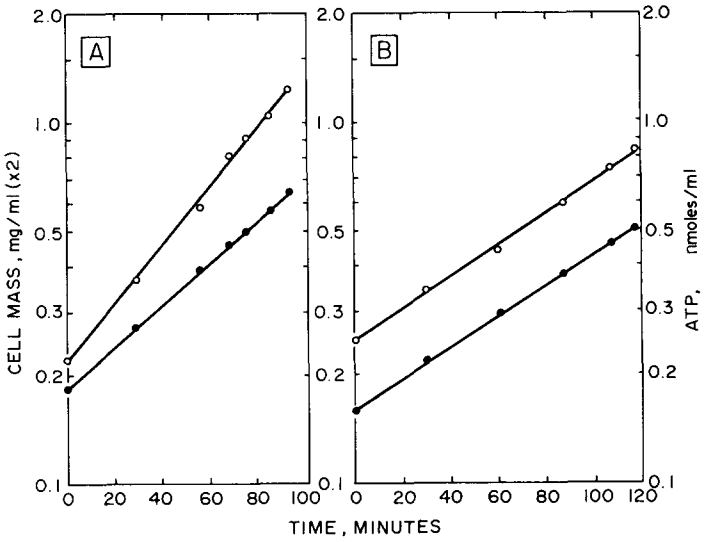

Fig. 1. Growth (●) and ATP concentration (○) of cultures of streptomycin-sensitive (A) and streptomycin-dependent (B) *E. coli* B growing exponentially in air on minimal salts–glucose medium. The medium was supplemented with 100 μg of dihydrostreptomycin per ml for the dependent culture.

The cell yield from glucose was the same for streptomycin-dependent *E. coli* B whether grown with a limiting concentration or with an excess of antibiotic although at a low concentration of antibiotic (10 μg/ml), the ATP pool was higher (6.6 nmoles/mg dry cell wt.) than after addition of an excess (1000 μg/ml) of antibiotic (ATP concentration: 4.6 nmoles/mg dry cell wt.). Previous work has shown that limitation of antibiotic decreases protein synthesis[3] in streptomycin-dependent cells and extracts and increases catabolite repression in growing cultures of streptomycin-dependent *E. coli*[4]. Thus, the differences in ATP levels observed at high and low antibiotic concentration probably are secondary to an effect of streptomycin on protein biosynthesis. The cell yield from glucose of a nondependent (streptomycin-sensitive) revertant was impaired to the same extent as that of the streptomycin-dependent *E. coli* strain from which it was derived (Table I). According to genetic studies by HASHIMOTO[5], revertants from streptomycin dependence to nondependence are double mutants, possessing both the original streptomycin-dependent mutation and a unique suppressor mutation mapping close to the streptomycin-dependence locus.

Excluding the possibility of two or more independent mutations leading, respectively, to streptomycin dependence and to energy impairment, these results may be construed as additional evidence supporting the proposal[6] that macromolecular synthesis (ribosome function) and electron transport (membrane function) are coordinated in the bacterial cytoplasmic membrane. BROCK[7] has suggested that the observed effects of streptomycin on both ribosomes and cell membranes could be due to the presence of a single species of streptomycin-sensitive protein at both of these cellular sites.

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Fluorescence lifetime of bacteriochlorophyll and reaction center photooxidation in a photosynthetic bacterium

It has been shown (see, for example, refs. 1 and 2) that the reciprocal of bacteriochlorophyll fluorescence yield, φ_{fl}, in photosynthetic bacteria is proportional to the concentration of active reaction centers. These data are in good agreement with a model, firstly advanced by VREDENBERG AND DUYSENS[1], in which several traps compete for a common pool of excitation quanta. The model predicts the $1/(1-\varphi_{ph})$-fold increase in bacteriochlorophyll fluorescence under conditions of fully inhibited photosynthesis (φ_{ph} is the quantum yield for the photooxidation of reaction centers). The latter value for purple bacteria is about 1.0 (see, for example, ref. 3). One should expect that the ratio of maximum to minimum fluorescence corresponding to closed and open traps should exceed 10. However, only 2- to 3-fold increases have been observed in experiments[1]. Three possible explanations of such a discrepancy may be considered:

1. The observed emission consists to a great extent of delayed-type fluorescence.

2. Reaction centers in the oxidized state are still functioning as traps for excitation quanta but somewhat less efficiently than in the reduced state.

3. There is some background fluorescence, *e.g.* emission with intensity which is not affected by the reaction centers' redox state.

In order to choose between the above possibilities, light-intensity dependences of φ_{fl} and fluorescence lifetime, τ_{fl}, as well as the portion of oxidized traps, $P^+/(P^+ + P)$, have been investigated (P^+ and P are reaction center concentrations in oxidized and reduced state, respectively).