Uncoupling in Bacterial Growth: Effect of Pantothenate Starvation on Growth of *Zymomonas mobilis*

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SUMMARY

Pantothenate starvation of *Zymomonas mobilis* resulted in a simultaneous decrease of both the molecular growth yield and of the specific growth rate, when the organism was grown on glucose. In all treatments the rate of cellular catabolic activity, i.e. the rate of glucose fermentation per unit of dry weight of organism, remained constant. These experiments showed that for *Z. mobilis*, as for numerous other organisms, anabolic processes do not control catabolic activity.

INTRODUCTION

Biosynthetic processes are well regulated in bacteria, essentially by end-product inhibition and by repression. Surprisingly, it seems that the catabolic reactions which supply the organisms with energy are much less controlled. Uncoupling in growth was simultaneously reported by Le Gall & Senez (1960) and Pichinoty (1960), who studied the growth in batch culture of *Desulfovibrio desulfuricans* and of *Aerobacter aerogenes*, respectively, and also by Rosenberger & Elsdén (1960) who studied the growth of *Streptococcus faecalis* in continuous culture. These authors found that the rate of substrate dissimilation per unit weight of organism was independent of the growth rate.

However, Neidhart (1963) in studying the continuous growth of *Aerobacter aerogenes* showed that catabolic and anabolic processes could be partially regulated.

Some years ago it was demonstrated that pantothenic acid was a growth factor for *Zymomonas mobilis* (Belaich & Senez, 1965*a*), and a preliminary report on the uncoupling of growth by pantothenate starvation has been made (Belaich, Simonpiétri & Belaich, 1969). The purpose of this paper is to describe growth-uncoupling experiments carried out with *Zymomonas mobilis*.

METHODS

Strain and media. The strain used in these experiments was *Zymomonas mobilis*, NCIB 8938. It was routinely maintained on slants on a complex agar medium containing per litre: 2 g of yeast extract (Difco), 2 g of Bacto-peptone (Difco) and 2 g of glucose (R.A.L.), buffered by 0.025 M-tris buffer at a pH of 7.

Defined medium: basal medium was prepared by dissolving constituents in 1 litre of tris-maleate buffer 0.025 M, pH 6.8. The salts were at the following final concentrations (mg/l): $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 237.7; CaCl_2 , 1.1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 7.2; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 4.2; $\text{CuSO}_4 \cdot \text{H}_2\text{O}$, 1.4; $\text{CoSO}_4 \cdot 5\text{H}_2\text{O}$, 1.4; KCl, 50.0; NaCl, 50.0. To this basal medium were added 20 amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, hydroxyproline, phenylalanine,

Fig. 1. Effect of different concentrations of buffer on the molar glucose growth yield (Y_g) of *Zymomonas mobilis* (pH 6.8). ○, Phosphate buffer, complex medium; ●, tris buffer, complex medium; △, tris buffer, defined medium.

proline, serine, threonine, tryptophan, tyrosine, and valine, each at final concentration of 60 mg/l), plus calcium pantothenate, 10 mg of KH_2PO_4 , 1.0 g of NH_4Cl , and 2.0 g of glucose. Glucose was dissolved in distilled water and sterilized separately.

Minimal medium: this was identical with the defined medium except that the amino acids were omitted and the NH_4Cl concentration was doubled.

All the experiments reported have been done in anaerobiosis under an Argon or pure Nitrogen gas phase. The temperature was 30°.

Analytical methods. Optical densities were measured with a Jean and Constant (Prolabo, Paris, France) spectrophotometer at 475 nm. This apparatus is graduated in arbitrary optical density units. The readings were proportional to the concentrations of dry weight of organisms in the samples. Molar growth yields (Y_g), expressed in grams dry weight of bacteria produced per mole of glucose consumed, were determined by comparing the arbitrary optical densities of the cultures with a previously determined curve relating the optical density to the dry weight of organism.

Glucose was estimated with identical results by the Glucostat technique (Worthington Biochemical Corp., Freehold, New Jersey, U.S.A.) and by the Folin-Malmros method (Umbreit, Burris & Stauffer, 1964). Ethyl alcohol was determined by a microdiffusion method (Conway, 1950).

The growth kinetics were obtained with a Bonet Maury Biophotometer (Jouan, France) and the growth thermograms by the technique described by Belaich (1963, 1967) and Belaich & Senez (1965b).

RESULTS

Zymomonas mobilis growing anaerobically in a complex medium containing high concentrations of yeast extract and Bacto-peptone has a molar glucose growth yield (Y_g) of 8.3 (Bauchop & Elsdén, 1960; Belaich, 1963). In the complex and defined media utilized here the cultures were buffered by tris-maleate, and Fig. 1 shows that tris-maleate depressed Y_g , the value of which fell to 6.5 for 0.025 M-tris. Phosphate buffer did not have the same effect. Nevertheless, because tris was suitable to allow the results of the pantothenate-starvation study to be compared with those obtained with experiments on phosphate starvation

(Senez & Belaich, 1963; Belaich, 1967), it was chosen for all experiments concerning the growth of *Z. mobilis*.

Figure 2 (*a, b*) shows total growth, i.e. final bacterial density versus initial glucose concentration, in two series of experiments using defined and minimal medium, in which the pantothenate concentration was varied between 5×10^{-3} and 5×10^{-8} mg/ml. For each pantothenate concentration the bacterial density was proportional to the initial glucose concentration. The slope of each straight line obtained is equal to the molar glucose growth yield for each pantothenate concentration studied. The values of Y_g obtained under these conditions are reported in Table 1. Y_g increased as a function of increasing pantothenate concentration to give the appearance of a saturation type curve (Fig. 3).

Growth in a medium containing starvation concentrations of pantothenate, i.e. pantothenate concentrations lower than 10^{-4} mg/ml with glucose as energy-limiting substrate gave sigmoid growth curves (characteristic of bacterial growth limited by energy). No abnormal growth phase such as a linear growth phase was observed even for the lowest pantothenate concentrations studied. Figure 4 shows two growth thermograms obtained by the technique of Belaich (1963, 1967), Belaich & Senez (1965*a*) and Murgier & Belaich (1971). These experiments were performed in defined medium with a glucose concentration of 1 mg/ml and pantothenate concentrations of 5×10^{-3} mg/ml (curve A) and 2×10^{-6} mg/ml (curve B). Both curves are characteristic of energy-limited growth thermograms as defined by Belaich (1963, 1967), Belaich & Senez (1965*a*) and Murgier & Belaich (1971), although the log phase is broader in curve B than in curve A. Figure 5 shows the optical densities corresponding to the log phases obtained in a series of experiments performed with defined medium and pantothenate starvation conditions. The specific growth rate, μ , like Y_g , increased when pantothenate concentration was increased (Table 1).

The cellular rate of catabolic activity A_c , as defined by the relationship (1)

$$A_c = \frac{ds_t}{dt} \frac{1}{m}, \quad (1)$$

where $\frac{ds_t}{dt}$ is the rate of energy substrate dissimilation of the culture and m the biomass can be easily calculated in the log phase of growth, from the specific growth rate μ and the molar glucose growth yield Y_g :

$$\text{Thus} \quad \frac{dm}{dt} = \mu m = Y_g \frac{ds_t}{dt} \quad \text{and} \quad A_c = \frac{\mu}{Y_g}. \quad (2)$$

The values of A_c , expressed in mmole of glucose metabolized/min/g dry wt have been calculated from the relationship (2) and reported on Table 1. A_c was constant and independent of the growth rate and of the nature of the culture media utilized.

DISCUSSION

The effect of pantothenate starvation on the growth rate of *Zymomonas mobilis* was similar to its effect described by Toennies, Das & Feng (1966) in a mutant of *Escherichia coli* auxotrophic for pantothenate. In both cases the limitation by pantothenate involves a decrease of the growth rate. Moreover, in *Z. mobilis*, the decrease of the growth rate was followed by a decrease of the molar glucose growth yield. In all instances cellular rate of catabolic activity was constant. These results are in good agreement with the experiments of Rosenberger & Elsdén (1960) and Le Gall & Senez (1960) and strengthen the concept of

Fig. 2. Final bacterial density of cultures of *Zymomonas mobilis* in media containing limiting pantothenate concentration versus initial glucose concentration. (a) Experiments on defined medium. (b) Results on minimal medium. The figures in the graphs refer to the pantothenate concentration.

Fig. 3. Molar glucose growth yield (Y_g) versus pantothenate concentration. S, synthetic medium; M, minimal medium.

Fig. 4. Thermograms of *Zymomonas mobilis* growing in defined medium. (Initial glucose concentration: 1 mg/ml in both cases). A, Pantothenate concentration 5×10^{-3} mg/ml; B, pantothenate concentration 2×10^{-6} mg/ml.

energy uncoupling. As demonstrated by Neidhart (1963) catabolite repression may mediate a control of the catabolism by anabolism in *Aerobacter aerogenes*. Yet this control is not total and a third of catabolic activity is not regulated, as in the lowest growth rate examined by this author. Our experiments with *Z. mobilis* in defined and minimal medium demonstrated that catabolite repression did not act on uncoupling pantothenate starved culture conditions.

The fact that the cellular rate of catabolic activity is constant for any culture conditions raises the problem of the dissipation of energy in excess. Indeed if we take the value of 8.3 for the molar glucose growth yield the percentage of lost energy in the uncoupling condition is

$$U = \frac{Y_g - Y_{gu}}{Y_g} \times 100,$$

Fig. 5. Log phases obtained in different degrees of pantothenate-starved growth of *Zymomonas mobilis* in synthetic medium. Figures in the graph refer to pantothenate concentration.

where Y_{gu} is the glucose yield in uncoupling culture condition. From the values in Table 1 we have calculated that this percentage can reach 70% in defined and minimal media.

The means by which the excess energy produced in uncoupling growth conditions may be dissipated have been extensively discussed by Gunsalus & Shuster (1961). *Zymomonas*

Table 1. Molar glucose growth yield (Y_g), specific growth rate (μ) and cellular rate of catabolic activity (A_c) of *Zymomonas mobilis*

Culture conditions	Pantothenate concentration (mg/ml)	Y_g	μ (H^{-1})	A_c mmole min^{-1} g^{-1}
Complex medium without buffer	—	8.3	0.400	0.803
Complex medium with tris buffer	—	6.5	0.345	0.884
Synthetic medium	5×10^{-3}	6.5	0.391	1.002
	4×10^{-6}	5.2	0.353	1.131
	2×10^{-6}	4.5	0.281	1.040
	1×10^{-6}	4.2	0.261	1.035
	1×10^{-7}	3	0.203	1.127
	5×10^{-8}	2.5	0.157	1.046
Minimal medium	5×10^{-3}	4.7	0.300	1.063
	5×10^{-5}	4.2	0.275	1.090
	4×10^{-6}	3.9	0.182	0.777
	1×10^{-6}	2.9	0.151	0.867
	5×10^{-7}	2.5	0.153	1.020
	5×10^{-8}	1.7	—	—

mobilis seems to be an organism of choice to study energetic uncoupling. Indeed, this organism is able to grow in all conditions of culture with only glucose as the energy source (Belaich & Senez, 1965*b*). The glucose fermentation balance is independent of the culture conditions. We have determined ethyl alcohol produced per mole of glucose dissimilated in the most drastic uncoupling condition, i.e. in minimal medium with a pantothenate concentration of 2×10^{-6} mg/ml. In these conditions 1.73 mole of ethyl alcohol are produced per mole of glucose fermented. This value is very close to the corresponding value observed when the organism was grown in complex medium (Belaich & Senez, 1965*a*). These results show that glucose is dissimilated in the same manner whatever the growth condition. Moreover, this bacterium is unable to synthesize glycogen as reserve material (Belaich & Senez, 1965*b*; Belaich, 1967).

That the catabolism of glucose is carried out in the same manner in all the conditions of growth and that no storage products are produced lead us to think that the excess energy is wasted by direct or indirect ATPase activity, in the sense defined by Gunsalus & Shuster (1961).

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