THE EFFECT OF TEMPERATURE ON THE CYTOCHROME PATTERN AND RESPIRATION OF *PSEUDOMONAS AERUGINOSA*

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1. Introduction

P. aeruginosa synthesizes an inducible periplasmic alkaline phosphatase when grown at 37°C in a phosphate deficient medium [1]; the enzyme is also excreted into the growth medium. Both the periplasmic and surface bound enzyme have been localized by electron microscopy [2]. In a recent study it was observed that when this strain forms long filaments at 46°C it contains very low levels of the enzyme. Other periplasmic enzymes followed this pattern, while cytoplasmic enzyme showed only a slight reduction in specific activity. These effects have been demonstrated to be reversible temperature dependent changes [3]. The aim of this study is to investigate the effect of temperature on membrane-bound enzymes, ie, the cytochrome system and to relate how energy metabolism is affected.

2. Materials and methods

Pseudomonas aeruginosa (ATCC, 9027) was grown in a phosphate deficient medium [1] at 37°C and 46°C as described by Bhatti and Ingram [3].

Cells were washed and disrupted in 50 mM Tris–HCl (pH 7.0) at 10–20 mg protein/ml in a French Press at 20 000 lb/sq. in. Particulate and supernatant fractions were obtained as outlined by Knowles et al. [4]. Quantitation of the cytochromes of the organism was determined from $Na_2S_2O_4$ reduced and $K_3Fe(CN)_6$ oxidized difference spectra as described by Weston and Knowles [5,6] and Knowles et al. [4]. Oxidase activities were determined as described by Weston et al. [7] at 37°C and 46°C with a variety of substrates all at 30 mM except NADH at 2.5 mM and ascorbate at 1.5 mM plus *N,N,N*^{1},*N*^{1}, tetramethyl phenylenediamine at 1.0 mM (A + T). Protein was determined by a modified Biuret method [8].

3. Results and discussion

This strain of *Pseudomonas aeruginosa* forms small, motile, rods at 37°C when grown in inorganic phosphate dificient medium but at 46°C it forms long non-motile filaments. Growth at 46°C affects the synthesis of periplasmic enzymes but not of cytoplasmic enzymes. These morphological and enzymological changes observed in this organism are only temperature dependent changes [3].

When cells of this organism are disrupted and then fractionated into particulate and supernatant fractions all cytochromes are found in the particulate fraction and are purified when compared with the cell-free extract. The soluble fractions contain no cytochromes.

Fig.1 shows the low temperature (77°K) and room temperature cytochrome patterns of particles of *Pseudomonas aeruginosa* when grown to late log phase at 37°C. Room temperature difference spectra show peaks at 598, 559 and 550 nm corresponding to the a_1, *b*, and *c* types respectively. Resolution into the components is not possible at room temperature. Clearly visible in the low temperature spectrum are an *a*-type peak at 594 nm, two *b*-types, at 555 and 557 nm and three *c*-types at 550.5, 547.5, and 546 nm. Table 1 shows the concentration of cytochrome present and

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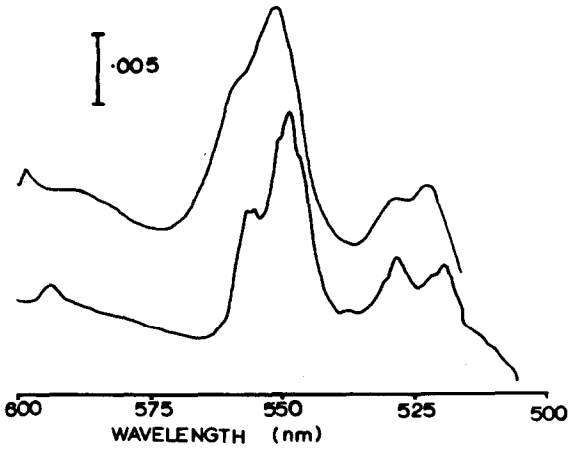

Fig.1. Difference spectra of particles of *Pseudomonas aeruginosa* grown at 37°C. $Na_2S_2O_4$ reduced minus $K_3Fe(CN)_6$ oxidised difference spectra of particles derived from log phase cells of *Pseudomonas aeruginosa* grown at 37°C. The difference spectra were recorded at 77°K in 0.8 M sucrose in 3 mm cuvettes (lower curve) and at room temperature in 1 cm cuvettes (upper curve). The protein concentration was 9 and 18 mg/ml respectively.

their mole ratios. $Na_2S_2O_4$ reduced plus CO and $Na_2S_2O_4$ reduced spectra (not shown) indicate that 85% of the a_1, and 42% of the *b* cytochromes are capable of binding CO, indicating the presence of an a_1 cytochrome oxidase and an '*O*'-type cytochrome oxidase (corresponding to one-half of the *b*-type cytochrome). This is compatible with the two *b*-type peaks seen in fig.1. The heights of the shoulders of the *c*-type cytochrome peaks indicate that the three *c*-types are most probably in equal proportions. The pattern for cells grown at 46°C is identical but the concentrations of the cytochromes are different for the two growth temperatures. The patterns for this pseudomonad are similar to that reported by other workers [9,10].

When the organism is grown at 46°C it forms filaments [3] and contains low levels of cytochromes (fig.2). On transfer to 37°C the filaments display a sudden burst in synthesis of all cytochromes over the next hour. After 30 min at 37°C the filaments begin to fragment into individual cells. This fragmentation is complete at two hours after which time the rate of cytochrome synthesis becomes constant. After 4 hr at 37°C, the cytochrome levels are identical to the levels found in cells grown for many generations at 37°C. It is worthy to note that the synthesis of all three types of cytochromes seems to be coordinated, ie, all three cytochromes show a burst of synthesis after transfer from 46°C to 37°C and when the rate of synthesis becomes constant, it is identical for all three types. It should be noted that the *b*- and *c*-types show a two-fold increase whereas the *a* shows a fourfold increase within the first hour of transfer to 37°C from 46°C. The levels of cytochromes *a*, *b*, and *c*, are 8-, 6- and 6.8-fold higher in 37°C than 46°C grown cells, respectively.

Since cells grown at 37°C and 46°C show similar cytochrome patterns, although they differ in the absolute concentration of the cytochromes, it seemed appropriate to test whether respiration of the organism correlated with cytochrome level or whether temperature had a much more drastic affect on the cells. To test these possibilities the cells were grown at both temperatures and assayed for oxidase activity at both temperatures.

Table 2 illustrates the effect of both these variables on respiration of the organism. For cells grown and assayed at 37°C, respiration of a variety of natural and

Table 1
Cytochrome properties of *Pseudomonas aeruginosa* grown at 37°C

Cytochrome type	CO binding (% of total)	Peak position at RT.	Peak position at 77°K	Shoulder size (% of peak)	Conc. of cytochrome (nmol/mg protein)	Molar ratio
a_1	85	598	594	100	0.06	1
b	42	559	555	100		
					0.25	4
			557	100		
c	0	550	550.5	80		
			547.5	100	0.37	6
			546	75		

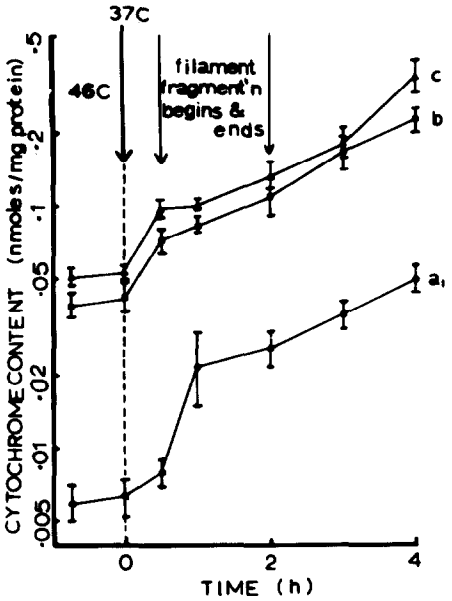

Fig.2. The relationship between cytochrome concentration and time of incubation at 37°C for *Pseudomonas aeruginosa*. Overnight cultures of *Pseudomonas aeruginosa* grown at 46°C were transferred to 37°C at time 0 and incubation was continued. At intervals, samples were removed and cytochrome concentration was determined on particles as described in the text. Concentrations of cytochromes a_1 (•), b (■) and c (▲) were expressed in nmol/mg protein. Also shown in the figure are photomicrographs of filaments and cells which are found at the two temperatures 46 and 37°C. Arrows indicate when the first filaments are observed to fragment and also when all filaments have fragmented to cells.

artificial substrates is active. If one compares respiration for these subtrates at both 37°C and 46°C assay temperatures then the substrates fall into two distinct groups. One group, which contains endogenous, NADH, A + T, glucose, lactate and succinate, shows an increase in activity at 46°C as compared to 37°C (8–48%). This increase is not as great as the almost two-fold, one would expect by the temperature increase (Q_{10}) indicating that while temperature increases activity another factor, which is unknown, decreases it. It should be noted that electrons from substrates such as NADH, lactate, succinate, glucose and A+ T are coupled into the respiratory chain directly and that the endogenous substrate is situated in the cell. The other group of substrates show a decrease in activity of 8% for pyruvate to 44% decrease for glumate. All these substrates require transport of the molecules into the cell before metabolism with final transfer of electrons to the respiratory chain presumably via NADH. Since periplasmic enzymes are inactivated in this organism by a temperature shift from 37°C to 46°C [3], it is possible that during the assay at 46°C the transport of certain substrates and hence their ability to supply electrons might be reduced by the loss of periplasmic binding proteins. This possibility has not been tested. Krebs cycle and other cytoplasmic enzymes are not drastically altered in specific activity at 46°C when compared to 37°C grown cells and hence the metabolism of these substrates should not be affected once they have pentrated the cell membrane [11].

Cells grown at 46°C and assayed at 37°C and 46°C show the same trend as for 37°C grown cells (table 2). Respiration of substrates such as endogenous, NADH, A + T, glucose, lactate, and succinate was higher at 46°C than 37°C, while respiration of the other substrates was markedly reduced at 46°C as compared to 37°C. It should be noted that stimulation at 46°C as compared to 37°C for the first group of substrates is not great whereas the inhibition of the other substrate-stimulated respiration at 46°C is more drastic when compared to the 37°C grown cells.

When one compares respiration of 37°C and 46°C grown cells, the respiratory activity is reduced for all substrates in the latter case. Endogenous reserves, or availability of these reserves, is drastically reduced in 46°C grown cells with respiration reduced to 4%. Respiration of NADH, A+ T, glucose, lactate and succinate is reduced by 56, 81, 80, 84, and 18%, respectively, while that of the other substrates is drastically reduced by over 95% in all cases. One can deduce, therefore, that if the absolute concentration of cytochrome was the only contributing factor to respiratory rate then respiration of all substrates should drop by 86%. While A + T, glucose, and lactate seem to agree with this others, namely, NADH and succinate are not so extensively decreased. Clearly, the components involved in the respiration of these latter compounds are not greatly affected by the temperature of growth. However, the ability to accumulate, metabolise and use certain other substrates as electron donors is greatly affected by growth temperature. At this stage it is not clear whether the uptake of these substrates or their further metabolism is affected by temperature. Based upon the argu-

Table 2
The effect of growth temperature and assay temperature on respiratory activities of *Pseudomonas aeruginosa*

Growth temperature	37°C		46°C	
Oxidase assay temperature	37°C	46°C	37°C	46°C
Substrates				
Endogenous	74[a]	109	3.3	4.25
NADH	65	70	29	38
A + T[b]	238	298	45	48
Glucose	150	195	30	33
Lactate	102	124	17	19
Succinate	56	62	46	51
Acetate	89	66	2.0	1.4
Pyruvate	79	73	1.3	0.9
Ethanol	79	55	2.0	0.9
Alanine	123	86	1.3	0.9
Glutamate	101	57	1.3	0.9
Glycine	63	40	1.3	0.9
Galactose	143	116	9.9	0.9

[a] n atoms O_2/min/mg protein.
[b] Ascorbate + N N N^1 N^1-Tetramethyl-P-phenylenediamine.

ments first presented, however, we favour the hypothesis that periplasmic or cytoplasmic membrane-bound binding proteins become inactivated or are not synthesized during growth at 46°C.

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