BBA 45803

THE EFFECT OF GLUCOSE ON THE ELECTRON-TRANSPORT SYSTEMS OF THE AEROBIC BACTERIA *AZOTOBACTER VINELANDII* AND *ACETOBACTER SUBOXYDANS*

R. M. DANIEL AND S. K. ERICKSON

Department of Biochemistry, University of Leicester, Leicester (Great Britain)

(Received January 27th, 1969)

SUMMARY

1. The effect of glucose on the formation of the electron-transport systems of the aerobic bacteria *Azotobacter vinelandii* (NCID 8660) and *Acetobacter suboxydans* (ATCC 621) was studied.

2. In *A. vinelandii*, growth on glucose repressed components of the respiratory chain, especially the terminal oxidase, cytochrome a_2, in both large and small electron-transport particles. The activity of the NADH oxidase decreased by approx. 50% in small particles.

3. In *A. suboxydans*, growth on glucose did not markedly affect cytochrome concentrations. The activity of the NADH oxidase system decreased by approx. 50% in small particles, but glucose and ethanol oxidase systems were not affected. In large particles, all oxidase activities decreased by over 50%.

4. The distribution of cytochromes and oxidase activities between large and small electron-transport particles from both bacteria changed when cells were grown on glucose. This may possibly reflect a change in fractionation characteristics of the membranes.

INTRODUCTION

Studies on the effect of environmental conditions on the composition and activity of bacterial electron-transport systems have revealed no simple relationships. The effects found vary widely in their nature and extent, and according to the type of bacterium.

WHITE[1], on the basis of the effect of culture conditions on electron-transport component formation, has proposed the division of bacteria into two classes. In the first group[2–6], O_2 induces and glucose represses cytochrome formation; in the second group[7–9], O_2 represses cytochrome formation and glucose has little effect, although it may influence membrane-bound dehydrogenases. In *Staphylococcus aureus* and *Escherichia coli*, growth on glucose represses the specific activity of the respiratory pigments as well as the activity of tricarboxylic acid cycle enzymes[10–12]. COX *et al.*[13] found variations in the distribution of quinones between large and small respiratory particles in *E. coli* grown on glucose compared with those grown on other substrates.

Since according to WHITE[1], there may be some correlation between the observed effects exerted by O_2 and that by glucose on respiratory chain formation, it is of interest to investigate some of these effects in the obligate aerobic bacteria such as *Azotobacter vinelandii* and *Acetobacter suboxydans*.

A. vinelandii is a nitrogen-fixing bacterium containing a branched electron-transport system with three terminal oxidases, cytochromes a_1, and a_2 and *o*. O_2 shows a variable effect, the concentration of cytochromes *b* and *c* decreasing above and below an optimal O_2 concentration[14]. However, KNOWLES[15] found no difference in cytochrome levels grown at high or low aeration. *A. suboxydans* lacks tricarboxylic acid cycle enzymes and has one terminal oxidase, cytochrome *o*. As in *A. vinelandii*, the concentration of respiratory pigments is a function of O_2 concentration, reaching a maximum at medium aeration[16].

MATERIALS AND METHODS

Chemicals

NADH was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. CO gas was obtained from Air Products, Darlaston, Staffs., England. All other chemicals were from British Drug Houses, Poole, England and, were the finest grade available. Glass-distilled water was used throughout.

Growth and maintenance

Azotobacter vinelandii (NCIB 8660) was grown and maintained as described by JONES AND REDFEARN[17]. The desired carbon source was added in 1.5% conc. Cells grown on mannitol in parallel with those grown on other carbon sources were used as controls in each experiment.

Acetobacter suboxydans (ATCC 621) was maintained on slopes containing a medium consisting of 1% dry yeast extract, 1% peptone, 0.1% KH_2PO_4 and 1% (w/v) glycerol or 5% glucose. The organisms were subcultured at intervals of 4–6 weeks, stored at 4° and grown at 30°. They were grown in 2-l flasks containing 700 ml of the above medium on a gyrotatory incubator shaker.

Preparation of subcellular fractions

Bacteria of both species were harvested in late log phase. Large and small particles containing respiratory chain components were prepared by the method of JONES AND REDFEARN[17]. Oxidase activities and quinone, cytochrome and flavoprotein concentrations were measured as described in JONES AND REDFEARN[17]. Cytochrome concentrations in *A. vinelandii* were calculated as by these authors. The concentration of cytochrome a_1 is omitted since the molar absorbance coefficient is not known. Concentrations of cytochrome *o* are not included as its CO band appears as a shoulder on the a_1-CO band and so is difficult to measure accurately. For the calculation of cytochrome concentrations in *A. suboxydans*, a molar absorbance coefficient of $\varepsilon = 19\,000$ (ref. 18) for $A_{550\,m\mu} - A_{540\,m\mu}$ was used for the *c*-type cytochrome and $\varepsilon = 80\,000$ (ref. 19) for $A_{418\,m\mu} - A_{428\,m\mu}$ for cytochrome *o* as measured by a CO–dithionite-reduced *minus* dithionite-reduced difference spectrum.

RESULTS AND DISCUSSION

A. vinelandii were grown on glucose. The effect of this growth on the respiratory components is shown in Table I. There seems to be a general repressive effect of glucose on the respiratory chain components. The decrease in content of cytochrome a_2 is marked. This cytochrome seems especially sensitive to growth conditions. KNOWLES AND REDFEARN[20] found a decrease in cytochome a_2 in cells grown on urea–mannitol medium. The concentration of this cytochrome in other bacteria is dependent on O_2 deficiency which stimulates its formation[1,21,22].

A comparison of large and small particles from cells grown on glucose indicates a more even distribution of cytochromes than in those grown on mannitol. The mannitol cells show a marked enrichment in the small particles.

For mannitol cells: $a_2 = 0.5$; $b_1 = 0.7$; $c_4 + c_5 = 0.6$

For glucose cells: $a_2 = 1.5$; $b_1 = 1.2$; $c_4 + c_5 = 1.2$

Growth on glucose affected the respiratory chain components of *A. suboxydans* as shown in Table II. The total cytochrome content of particles derived from both glucose- and glycerol-grown cells was similar. However, some change in the distribu-

TABLE I

CYTOCHROME, QUINONE AND FLAVOPROTEIN CONTENT AND OXIDASE ACTIVITIES OF ELECTRON-TRANSPORT PARTICLES FROM *A. vinelandii* GROWN ON MANNITOL AND ON GLUCOSE.

All concentrations are in μmoles/g protein. Oxidase activities are in μgatoms O_2/min per mg protein; they were determined as described in MATERIALS AND METHODS. The values are representative of at least 6 different preparations.

Particle size	*Medium*	*Cytochrome*			*Flavo-protein*	*Quinone*	*Oxidase activity*		
		a_2	b_1	c_4+c_5			*Succinate*	*Malate*	*NADH*
Large	Mannitol	0.23	0.88	0.91	—	6.7	0.11	0.10	1.41
	Glucose	0.12	0.78	0.92	—	5.7	0.13	0.16	1.04
Small	Mannitol	0.47	1.24	1.48	1.65	9.8	0.21	0.72	4.10
	Glucose	0.18	0.89	1.06	1.30	7.2	0.26	0.73	1.87

TABLE II

CYTOCHROME AND FLAVOPROTEIN CONTENT AND OXIDASE ACTIVITIES OF ELECTRON-TRANSPORT PARTICLES FROM *A. suboxydans* GROWN ON GLYCEROL AND ON GLUCOSE

All concentrations are in μmoles/g protein. Oxidase activities are in μgatoms O_2/min per mg protein; they were determined as described in MATERIALS AND METHODS. The values are representative of at least 6 preparations.

Particle size	*Medium*	*Cytochrome*		*Flavoprotein*	*Oxidase activity*		
		type c	*o*		*Glucose*	*Ethanol*	*NADH*
Large	Glycerol	0.80	0.46	1.05	4.10	2.09	2.06
	Glucose	0.76	0.37	0.37	1.37	1.02	0.66
Small	Glycerol	0.48	0.23	0.77	1.09	0.91	1.39
	Glucose	0.62	0.29	0.57	0.92	0.77	0.57

tion of cytochromes between large and small particles has occurred. The ratios of cytochrome concentrations in the large/small particles are as follows.

For glycerol cells: *c*-type = 1.6; *o* = 2.0

For glucose cells: *c*-type = 1.2; *o* = 1.3

Oxidase activities varied differently in the two bacteria. In *A. vinelandii* the succinate and malate oxidase activities were not affected; however, the NADH oxidase activity in small particles decreased by about 50% as shown in Table I. The decrease in NADH oxidase activity may reflect a glucose effect on the primary dehydrogenase and/or the decreased amount of cytochrome a_2 which could be rate-limiting, since it has been suggested that the majority of the NADH is oxidized *via* the following pathway[23,24]: cytochrome $b_1 \rightarrow$ cytochrome $a_2 \rightarrow O_2$. Dual-wavelength studies showed no difference in aerobic steady states of cytochrome *b* reached by glucose or mannitol particles oxidizing NADH which indicated that either of the effects mentioned could be operational. The 2,6-dichlorophenolindophenol–ascorbate oxidase activity (not shown) qualitatively increased or remained constant in glucose particles. This is in agreement with the branched chain hypothesis of JONES AND REDFEARN[24] for the pathway cytochrome c_4 + cytochrome $c_5 \rightarrow$ cytochrome a_1.

The distribution of NADH oxidase activity between glucose-grown large and small particles was 1:1.8, and in mannitol-grown, 1:2.9. There was no effect on the distribution of other oxidase activities.

Particles from cells grown on other carbon sources, sucrose, fructose, succinate and acetate, showed no marked differences from those grown on mannitol.

The oxidase activities of *A. suboxydans* grown on glucose and on glycerol are given in Table II. Oxidase activities in particles from cells grown on glucose were repressed relative to those derived from cells grown on glycerol. The NADH oxidase activity was between 50 and 60% of that in glycerol particles. The distribution of this oxidase activity between the large and small particles was 1:0.9 for glucose particles and 1:0.7 for glycerol particles. The distribution of the other oxidases in *A. suboxydans* particles changed. The ratios of distribution of glucose and of ethanol oxidases for large/small particles in glucose cells were 1:0.7 and 1:0.8, respectively; and in glycerol cells, 1:0.3 and 1:0.4, respectively.

Since growth on glucose appears to alter the fractionation characteristics of the membranes as reflected by distribution of oxidase activities and cytochromes in *A. suboxydans*, a preliminary investigation by electron microscopy was undertaken[16]. Results so far indicate that in this bacterium, a significant difference exists between respiratory particles from glycerol-grown cells and from glucose-grown cells. The latter were considerably smaller and more comminuted. This effect was found for both the large and small particles. Results with *A. vinelandii* were inconclusive.

It seems unlikely that cytochromes are normally involved in the rate-limiting steps in substrate oxidation[25], and therefore decreased cytochrome concentrations would not necessarily account for the low oxidase activities observed in growth on glucose. The decrease in activities may possibly reflect changes in membrane-bound flavoproteins or dehydrogenases. If this be so, then the electron microscope studies on *A. suboxydans* may indicate that flavoproteins also play a structural role in this bacterium.

The effect of glucose on the concentration of the terminal oxidases of *A. vinelandii* and *A. suboxydans* as well as on the distribution of cytochromes between large and small particles is difficult to explain at present. This may be a repression by glucose of some stage in cytochrome synthesis or may reflect a response of the electron-transport chain to different energy requirements.

ACKNOWLEDGEMENTS

We are indebted to the Science Research Council for financial support, and to the Medical Research Council for a grant to R.M.D. We wish to thank Dr. A. J. Rowe for guidance in the use of the electron microscope and for helpful discussion. The able technical assistance of Miss G. Parker is gratefully acknowledged.

DEDICATION

This paper is respectfully dedicated to the memory of the late Professor E. R. REDFEARN who was killed in a car accident on 6 March 1968. His help and guidance were a continual source of encouragement to us.

REFERENCES

1 D. C. WHITE, *J. Bacteriol.*, 93 (1967) 567.
2 P. SCHAEFFER, *Biochim. Biophys. Acta*, 9 (1952) 261.
3 M. H. RICHMOND AND O. MAALOE, *J. Gen. Microbiol.*, 27 (1962) 285.
4 E. ENGELSBERG, A. GIBOR AND J. B. LEVY, *J. Bacteriol.*, 68 (1954) 146.
5 E. ENGELSBERG, A. GIBOR AND J. B. LEVY, *J. Bacteriol.*, 68 (1954) 178.
6 E. AZOULAY, *Biochim. Biophys. Acta*, 92 (1964) 458.
7 P. CHAIX AND J. F. PETIT, *Biochim. Biophys. Acta*, 22 (1956) 66.
8 P. CHAIX AND J. F. PETIT, *Biochim. Biophys. Acta*, 25 (1957) 481.
9 D. C. WHITE, *J. Bacteriol.*, 89 (1965) 299.
10 K. C. STRASTERS AND K. C. WINKLER, *J. Gen. Microbiol.*, 33 (1963) 213.
11 C. T. GRAY, J. W. T. WIMPENNY AND M. R. MOSSMAN, *Biochim. Biophys. Acta*, 117 (1966) 33.
12 J. LASCELLES AND F. M. COLLINS, *J. Gen. Microbiol.*, 29 (1962) 531.
13 G. B. COX, A. M. SNOSWELL AND F. GIBSON, *Biochim. Biophys. Acta*, 153 (1968) 1.
14 L. L. LYSENKOVA AND I. A. KHMEL, *Mikrobiology, USSR, English Transl.*, 36 (1967) 905.
15 C. J. KNOWLES, Ph. D. Thesis, University of Leicester, 1967.
16 R. M. DANIEL, Ph. D. Thesis, University of Leicester, 1968.
17 C. W. JONES AND E. R. REDFEARN, *Biochim. Biophys. Acta*, 113 (1966) 467.
18 B. CHANCE AND G. R. WILLIAMS, *Biochim. Biophys. Acta*, 215 (1955) 395.
19 H. W. TABER AND M. MORRISON, *Arch. Biochem. Biophys.*, 105 (1964) 367.
20 C. J. KNOWLES AND E. R. REDFEARN, *Biochim. Biophys. Acta*, 162 (1968) 348.
21 F. MOSS, *Australian J. Exptl. Biol. Med. Sci.*, 30 (1952) 53.
22 F. MOSS, *Australian J. Exptl. Biol. Med. Sci.*, 30 (1956) 395.
23 R. REPASKE AND J. JOSTEN, *J. Biol. Chem.*, 233 (1958) 466.
24 C. W. JONES AND E. R. REDFEARN, *Biochim. Biophys. Acta*, 143 (1967) 340.
25 P. SCHOLES AND L. SMITH, *Biochim. Biophys. Acta*, 153 (1968) 363.