ACETATE UTILISATION BY *RHODOPSEUDOMONAS SPHEROIDES*

J.PAYNE and J.G.MORRIS

Department of Biochemistry, School of Biology, University of Leicester, Leicester, England

Received 29 May 1969

1. Introduction

During photosynthetic growth of *Rhodospirillum rubrum* on H_2 plus CO_2, some carbon dioxide is "fixed" via the reductive carboxylation cycle [1] which may also be the route whereby key amino acids are produced by this organism during its photosynthetic growth on acetate plus CO_2 [2]. In *R. rubrum*, as in *Chlorobium thiosulphatophilum* [1,2] the initial reactions of the full reductive carboxylation cycle are concerned with the synthesis of first pyruvate, and then oxaloacetate, from acetate plus CO_2; the process is dependent on a supply of reduced ferredoxin and ATP, and is accomplished by the sequential action of (A) reduced ferredoxin-dependent pyruvate synthase [3], (B) phosphoenolpyruvate synthase [4], and (C) phosphoenolpyruvate carboxylase (E.C. 4.1.1.31). *Rhodopseudomonas spheroides* lacks enzymes (B) and (C) and instead employs an apparently acetyl CoA-dependent pyruvate carboxylase (E.C. 6.4.1.1.) to synthesise oxaloacetate from pyruvate [5]. Thus, pyruvate carboxylase would be a key enzyme of the reductive carboxylation cycle in *Rps. spheroides* and would be essential for the photosynthetic growth of this organism on acetate plus CO_2 if oxaloacetate (and thence aspartate and glutamate) were produced from acetate by this route.

This communication reports that a mutant strain of *Rps. spheroides* which is devoid of pyruvate carboxylase activity and is consequently unable to grow on pyruvate or on glucose, is still able to grow both photosynthetically, and aerobically in the dark, on acetate plus CO_2. We conclude that *Rps. spheroides* can synthesise C_4-dicarboxylic acids from acetate plus CO_2 by a novel pathway which does not involve carboxylation of pyruvate.

2. Experimental procedures

The wild-type strain of *Rps. spheroides* was obtained from the National Collection of Industrial Bacteria (NICB No. 8287), and the pyruvate carboxylase-less mutant strain *Rps. spheroides* pc^- was derived from it by ultraviolet irradiation [5]. Cultures were grown in a minimal salts plus vitamins, basal, liquid medium containing NH_4Cl and specified carbon source(s); this medium, together with the growth conditions employed, have been described elsewhere [5]. Growth was measured turbidimetrically at 680 nm and the relation between D_{680} and dry weight was determined and found to be linear up to an extinction of approximately 0.8.

3. Results

Fig. 1a shows that wild-type *Rps. spheroides* when incubated anaerobically in the light in an atmosphere of 95% N_2 plus 5% CO_2 (v/v) grew photosynthetically when acetate or glucose or pyruvate were supplied as sole organic source of carbon. In contrast, *Rps. spheroides* pc^- was unable to grow photosynthetically on pyruvate or glucose plus CO_2, but on acetate plus CO_2 grew as well as did its wild-type parent (fig. 1b).

The same result was obtained when cultures of these two strains were incubated aerobically in the dark (fig. 2). At present we attach no significance to the longer lag period demonstrated in fig. 2b by the mutant strain growing aerobically in the dark on acetate plus CO_2, since the length of this lag seems to vary, and occasionally has been no greater than that displayed by the wild-type organism under the same growth conditions.

North-Holland Publishing Company – Amsterdam

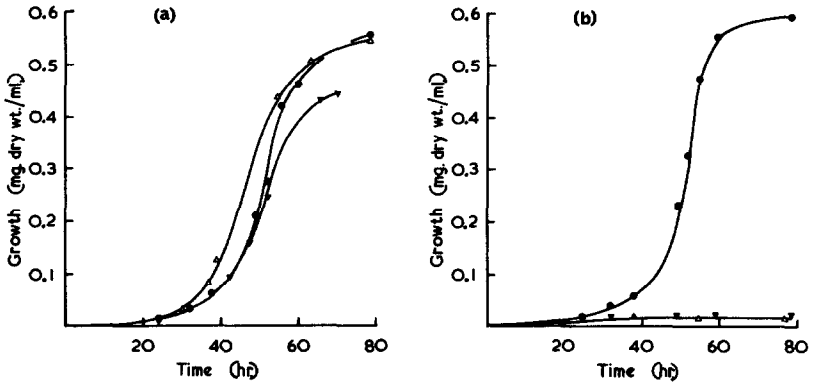

Fig. 1. Anaerobic/light growth of (a) wild-type *Rps. spheroides* and (b) pyruvate carboxylase-less mutant *Rps. spheroides pc*$^-$ (under 95% N_2: 5% CO_2, v/v; 700 ft candles illumination; 32°), in minimal salts plus vitamins, liquid medium [5] containing the following as sole organic sources of carbon: 25 mM acetate (•), or 20 mM pyruvate (△), or 12 mM glucose (▼).

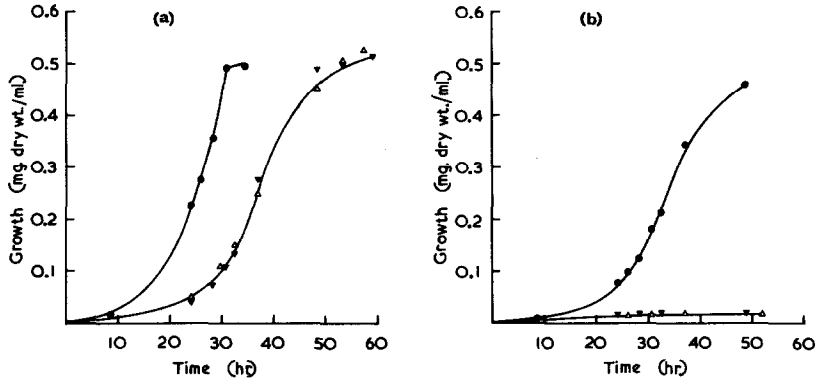

Fig. 2. Aerobic/dark growth of (a) wild-type *Rps. spheroides*, and (b) pyruvate carboxylase-less mutant *Rps. spheroides pc*$^-$ (flask cultures shaken aerobically in the dark at 30°), in minimal salts plus vitamins liquid medium [5] containing the following as sole sources of carbon: 25 mM acetate plus 6 mM bicarbonate (•), or 20 mM pyruvate (△), or 12 mM glucose (▼).

4. Discussion

Rps. spheroides pc$^-$ cannot grow on media containing glucose or pyruvate with CO_2 as the only sources of carbon, since it lacks the means to carboxylate pyruvate, and possesses no alternative phosphoenolpyruvate carboxylase activity. The mutant will therefore grown on these media only when they are supplemented with a readily available source of C_4-dicarboxylic acids e.g. 5 mM malate [5]. It follows that the ability of this mutant to grow on acetate plus CO_2 is evidence of its being able to synthesise C_4-dicarboxylic acids from these substances by some route which does not involve carboxylation of pyruvate or phosphoenolpyruvate, and which is therefore not the reductive carboxylation pathway [1].

Since like *R. rubrum, Rps. spheroides* produces no isocitrate lyase (E.C. 4.1.3.1) during its growth on acetate [6], it cannot operate the usual glyoxylate cycle. It was reported that during anaerobic/light incubation of *Rps. spheroides* which had previously been grown photosynthetically on malate plus glutamate, radioactivity from [2-^{14}C] acetate was at earliest times incorporated into glyoxylate, glycollate and glycine [7]. This could imply that the organism (which possesses malate synthase, E.C. 4.1.3.2.), can

circumvent the usual requirement for isocitrate lyase by directly oxidising acetate to yield glyoxylate [7]. Yet in short-term labelling experiments with cultures of *Rps. spheroides* growing aerobically in the dark on acetate plus CO_2 and supplied with [2-^{14}C] acetate, we have not obtained any evidence of early labelling of glyoxylate, glycollate or glycine [8]. It is therefore possible that this organism employs a novel anaplerotic route of the utilisation of acetate.

References

[1] M.C.W.Evans, B.B.Buchanan and D.I.Arnon, Proc. Natl. Acad. Sci. U.S., 55 (1966) 928.
[2] B.B.Buchanan, M.C.W.Evans and D.I.Arnon, Arch. für Mikrobiol. 59 (1967) 32.
[3] R.Bachofen, B.B.Buchanan and D.I.Arnon, Proc. Natl. Acad. Sci., U.S. 51 (1964) 690.
[4] R.A.Cooper and H.L.Kornberg, Proc. Roy. Soc., B. 168 (1967) 263.
[5] J.Payne and J.G.Morris, submitted to J. gen. Microbiol.
[6] J.Lascelles and H.L.Kornberg, J. gen. Microbiol. 23 (1960) 511.
[7] G.Kikuchi, S.Tsuiki, A.Muto and H.Yamada, Studies on Microalgae and Photosynthetic Bacteria (University of Tokyo Press, Tokyo, 1963) p. 547.
[8] J.Payne and J.G.Morris, to be published.