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Polyglucose synthesis in *Chloroflexus aurantiacus* studied by ^{13}C-NMR

Evidence for acetate metabolism by a new metabolic pathway in autotrophically grown cells

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Abstract. *Chloroflexus aurantiacus* OK-70 fl was grown photoautotrophically with hydrogen as electron source. The cultures were subjected to long term labelling experiments with ^{13}C-labelled acetate or alanine in the presence of sodium fluoroacetate. The presence of fluoroacetate caused the cells to accumulate large amounts of polyglucose which was hydrolysed and analysed by NMR. The labelling patterns of glucose were symmetric and in agreement with carbohydrate synthesis from acetate and CO_2 via pyruvate synthase. The content of carbon derived from added acetate was highest in C2 and C5 of glucose, at least 20% higher than in C1 and C6. About one third of the glucose carbon was derived from added acetate, the rest being from CO_2. Contrary to expectations, in glucose formed in the presence of C1-labelled acetate C1 and C6 contained more label than C2 and C5, and with C2-labelled acetate as the tracer glucose was mainly labelled in C2 and C5. Labelled CO_2 was formed from acetate labelled at either position. The labelling data indicate a new metabolic pathway in *C. aurantiacus*. It is suggested that the cells form C1-labelled acetyl-CoA from C2-labelled acetyl-CoA and vice versa by a cyclic mechanism involving concomitant CO_2 fixation and that this cycle is the part of the autotrophic CO_2 fixation pathway in *C. aurantiacus* in which acetyl-CoA is formed from CO_2.

The polyglucose of *C. aurantiacus* appears to have predominantly $\alpha(1-4)$-linked structure with about 10% $\alpha(1-6)$-linkages as revealed by ^{13}C-NMR.

Key words: Acetate assimilation – CO_2 fixation pathway – Acetyl-CoA – ^{13}C-NMR – *Chloroflexus aurantiacus*

Most phototrophs can grow with CO_2 as sole source of carbon. For many years it was believed that the reductive pentose phosphate cycle was the universal mechanism for autotrophic CO_2 fixation (Quayle and Ferenci 1978), but during later years it has been shown that alternative mechanisms exist among autotrophic bacteria. The green bacteria are the only phototrophs known that do not utilize the reductive pentose phosphate cycle for autotrophic CO_2 fixation. The green sulfur bacteria lack ribulose-1,5-bisphosphate carboxylase and fix CO_2 by the reductive tricarboxylic acid cycle (Evans et al. 1966; Sirevåg 1974; Fuchs et al. 1980b). Carbon metabolism in *Chloroflexus aurantiacus*, a green nonsulfur bacterium, has been less extensively studied. In a recent study we found that this organism does not use the reductive pentose phosphate cycle for autotrophic CO_2 fixation (Holo and Sirevåg 1986), and this is supported by Shively et al. (1986) who were unable to demonstrate hybridization of *C. aurantiacus* DNA with ribulose bisphosphate carboxylase gene probes from various phototrophs.

In addition to the reductive pentose phosphate cycle, two alternative CO_2 fixation pathways are known. These are the reductive tricarboxylic acid cycle and the acetyl-CoA pathway, the latter found in a number of anaerobic chemoautotrophs (for a review see Fuchs and Stupperich 1985). In both these pathways pyruvate synthase plays a central role in CO_2 fixation. Although *C. aurantiacus* does not seem to use either of these mechanisms for autotrophic CO_2 fixation, there is evidence that pyruvate synthase is involved (Holo and Sirevåg 1986). This enzyme forms pyruvate from acetyl-CoA and CO_2 and thus acetyl-CoA will be a central intermediate in the CO_2 fixation pathway of *C. aurantiacus*. We therefore undertook a long term labelling study with ^{13}C-acetate to learn more about carbon metabolism in autotrophically grown *C. aurantiacus*. The study was conducted in the presence of fluoroacetate. This compound was employed for two reasons (Holo and Sirevåg 1986); firstly, it inhibits growth but not autotrophic CO_2 fixation by *C. aurantiacus*. Furthermore, it causes an accumulation of citrate, suggesting that aconitase is blocked (Peters et al. 1953). Thus acetate metabolism by the tricarboxylic acid cycle and the glyoxylate cycle is inhibited. Secondly, the metabolism of fluoroacetate-treated cells is restricted and they make large amounts of polyglucose. This permits labelling patterns to be studied by ^{13}C-NMR which, though an insensitive method, has the advantage that the extent of labelling of all the individual carbon atoms in a molecule can be determined.

In principle there are two possible mechanisms by which acetyl-CoA can be formed from CO_2: a $C_1 + C_1$ mechanism or a cyclic pathway. In the latter case externally fed labelled acetate may give rise to acetyl-CoA with a different labelling pattern by passage through the cycle. The results of this report indicate that such labelling rearrangement does take place, suggesting that acetyl-CoA is formed from CO_2 by a novel metabolic cycle in *C. aurantiacus*.

Materials and methods

Organism

Chloroflexus aurantiacus strain OK-70 fl. was grown photoautotrophically with hydrogen as the electron donor as described by Holo and Sirevåg (1986).

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Long term labelling experiments

Sodium monofluoroacetate was added to exponentially growing 200 ml cultures containing 75 – 150 µg protein/ml. Following 2 h incubation in the presence of 0.2 mM fluoroacetate, the experiments were started by adding ^{13}C-labelled sodium acetate or alanine and the cultures were further incubated in 1 l rubber-stoppered infusion bottles with H_2/CO_2 (95:5) in the gas phase. All incubations were carried out at 55° C with a 25 or 40 W light bulb at a distance of about 50 cm as the light source. Where indicated, the cultures were stirred by a magnetic stirring bar at 300 r.p.m. and continuously gassed with H_2/CO_2 (95:5) at a rate of 100 ml/min. At the end of incubation the cells were harvested by centrifugation and washed twice with 0.5 M H_2SO_4.

Analytical methods

The cell pellets were hydrolysed in 0.5 M H_2SO_4 followed by neutralisation, then glucose was determined according to Sirevåg (1975). Samples containing 5 mg glucose were freeze dried, redissolved in 0.6 ml D_2O, mixed with 2.4 µl dioxane as an internal chemical shift and concentration standard and transferred to 5 mm diameter tubes for NMR. Polyglucose was isolated according to Paalme et al. (1982). 10 – 30 mg samples were prepared for NMR as above, but 6 mg sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used as the internal chemical shift standard. All NMR spectra were acquired at a frequency of 75 MHz for carbon and a temperature of 37° C on a Varian XL-300 spectrometer using a standard ^{1}H-broadband switchable 5 mm probe. The pulse angle was 26 degrees, acquisition time 0.75 s and the waiting time between transients was 3 s. Continuous broadband proton decoupling was used. Enrichment of ^{13}C in individual carbon atoms was determined from the NMR spectra as follows (Cohen et al. 1979 b); peak area ratios were measured for each carbon atom with respect to the dioxane reference. These ratios were divided by corresponding ratios obtained from a solution containing 5 mg of unenriched glucose. Errors due to differential nuclear Overhauser enhancements and saturation effects were eliminated by the use of identical concentrations and acquisition parameters for all samples.

The peaks used for enrichment determinations were (with respect to the dioxane reference at 67.4 ppm): 96.8 ppm (C1β), 75.0 ppm (C2β), 73.7 ppm (C3α), 70.6 to 70.5 ppm (C4α + C4β), 61.5 to 61.7 ppm (C6α + C6β). The enrichment of C5 of glucose was determined from the overlapping C2α and C5α peaks at 72.3 to 72.4 ppm by using the C2 enrichment value estimated from the C2β peak.

The formation of $^{14}CO_2$ from ^{14}C-acetate was measured in cultures treated with fluoroacetate as described above. At the end of incubation 0.5 ml supernatant aliquots were mixed with 0.5 ml 0.1 M $Ba(OH)_2$. The precipitates were washed with H_2O and the radioactivity determined by liquid scintillation counting. The radioactivity remaining when the precipitates were dissolved in 1 ml 0.1 M HCl and flushed with CO_2 was subtracted for $^{14}CO_2$ estimation.

Total cell protein was determined on methanol extracted samples with the method of Lowry et al. (1951) using bovine serum albumin as standard.

Chemicals. ^{13}C1-Na-acetate (90% ^{13}C) was from Stohler Isotope Chemicals (Rutherford, NJ, USA). ^{13}C3-alanine (98% ^{13}C) and ^{13}C2-Na-acetate (98% ^{13}C) were from Cambridge Isotope Laboratories (Woburn, MA, USA).

Results

Long term labelling experiments

Photoautotrophically grown cultures of *Chloroflexus aurantiacus* were incubated in the presence of fluoroacetate and ^{13}C-labelled alanine or acetate. Figure 1 shows ^{13}C-NMR spectra of glucose obtained from the cells at the end of incubation. The enrichment levels of the individual carbon atoms are presented in Table 1. In all cases the enrichment level of C3 exceeds that of C4. This may be due to impurity peaks in the 73 ppm area since a lower enrichment level was always found if the overlapping C3β – C5β peaks were used to calculate the enrichment of C3. Taking this into account, the labelling patterns in Table 1 can be considered symmetric. This shows that *C. aurantiacus* forms hexose by condensation of two C_3 units, as in a reversed Embden-Meyerhof pathway.

The cultures contained variable amounts of polyglucose at the beginning of the experiments, and the ^{13}C content was not the same in all the tracers used. These factors have been corrected for in the calculation of labelling of the various carbon atoms of glucose formed during the experiments (Table 1). The amount of carbon derived from a tracer molecule can be estimated from the sum of labelling of all the six carbon atoms of the glucose formed. From Table 1 it can thus be calculated that about one third of the carbon of glucose formed in the presence of labelled acetate has been derived from the acetate. This was also found with ^{14}C-acetate (Holo and Sirevåg 1986). The rest of the carbon is from CO_2 since polyglucose synthesis from fluoroacetate is negligible (Holo and Sirevåg 1986). The low degree of labelling of C3 and C4 in glucose formed in the presence of ^{13}C-acetate shows that these carbon atoms are derived almost entirely from CO_2 which supports the claim of pyruvate synthase operation in CO_2 fixation and acetate metabolism in *C. aurantiacus*. The observed labelling of C3 and C4 can probably be ascribed to fixation of $^{13}CO_2$ formed during the experiments (Table 2) since this labelling was reduced in cultures continuously gassed with H_2/CO_2.

Glucose formed from C1-labelled acetate via pyruvate synthase and the reversed Embden-Meyerhof pathway would be labelled in carbon atoms 2 and 5, whereas C2-labelled acetate would give rise to C1 and C6 labelled glucose. However, when ^{13}C1-acetate was used as the tracer, glucose became more labelled in C1 and C6 than in C2 and C5. Similarly, in the presence of ^{13}C2-acetate the glucose became more labelled in C2 and C5 than in C1 and C6. The declared labelling of the acetate tracers used was confirmed by NMR, showing that the unexpected results were not due to impure chemicals.

The ratio of labelling between C2 and C1 of glucose formed in the presence of C2-labelled acetate is greater than the inverse ratio of glucose formed in the presence of C1-labelled acetate. Thus by adding together enrichment levels of individual carbon atoms of glucose formed in the presence of the two acetate tracers, it can be seen that more carbon derived from the added acetate is found in C2 and C5 than in C1 and C6. The data from comparable experiments in Table 1 indicate that the content of carbon derived from added acetate is at least 20% higher in C2 of the glucose formed than in C1.

The C1 peaks in the spectra of glucose formed in the presence of C2-labelled acetate clearly show ^{13}C satellites (Fig. 1 C) indicating the presence of individual glucose

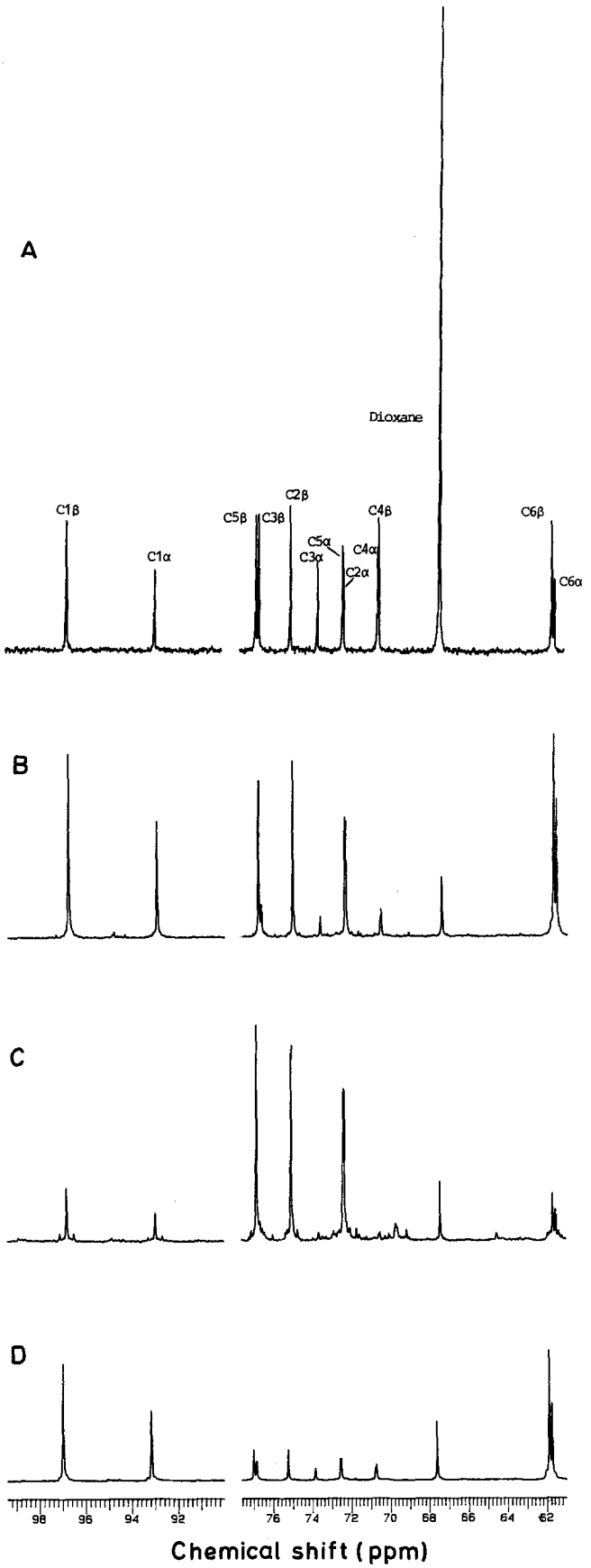

Fig. 1A–D. ^{13}C-NMR spectra of glucose. **A** unenriched glucose. **B–D** Glucose from *Chloroflexus aurantiacus* incubated in the presence of H_2, CO_2, fluoroacetate and 1-^{13}C acetate (**B**), 2-^{13}C acetate (**C**) and 3-^{13}C alanine (**D**)

molecules that are labelled in both C1 and C2. From the doublet peak areas it is calculated that about 10% of the glucose molecules labelled in C1 are also labelled in C2. Statistically, from the enrichment levels alone about 30% of them would be labelled this way. Thus labelled C1 and C2 carbon atoms are not randomly distributed between the glucose molecules. At the same time the doublets at C1 are much too big to be ascribed to $^{13}CO_2$ fixation and natural abundance ^{13}C. The doublets of the C1 peaks in the spectrum of glucose formed in the presence of C1-labelled acetate (Fig. 1 B) are too small to judge whether the content of glucose molecules labelled in C1 and C2 is any higher than expected from $^{13}CO_2$ fixation and natural abundance ^{13}C. In all the spectra glucose labelled in both C5 and C6 appeared to be as frequent as glucose labelled in both C1 and C2 as judged from the appearance of the C6 doublets.

The synthesis of polyglucose from CO_2 and H_2 by *C. aurantiacus* treated with fluoroacetate was inhibited by 0.2 mM KCN, about 50% inhibition being observed after 18 h of incubation. However, when acetate was added no KCN inhibition of polyglucose synthesis was observed. Since acetyl-CoA is considered to be an intermediate of the CO_2 fixation pathway in *C. aurantiacus*, these results suggest that acetyl-CoA synthesis from CO_2 is inhibited by KCN. Therefore the labelling experiments were also conducted in the presence of this compound. As is shown in Table 1 the glucose labelling patterns were altered by the presence of KCN, suggesting that there is a connection between the label rearrangements observed and acetyl-CoA synthesis from CO_2. However, the extent of labelling of glucose as a whole was not much affected by KCN. About two thirds of the glucose carbon was still derived from CO_2. Furthermore, in overall terms the apparent label rearrangement of acetyl-CoA seems to increase in the presence of KCN.

In our previous work we concluded that pyruvate is a central intermediate in the CO_2 fixation pathway in *C. aurantiacus* (Holo and Sirevåg 1986). Alanine, which is converted to pyruvate by the cells, was chosen as a tracer in this study because pyruvate itself is unstable. The extent of labelling of glucose formed in the presence of ^{13}C3-alanine indicates that only about 20% of the glucose carbon was derived from the added alanine, the rest being derived from CO_2. Furthermore, the majority of label is found in C1 and C6 of glucose, as expected by a reversed Embden-Meyerhof pathway.

The structure of polyglucose

The glycosidic linkages can be identified by comparing the ^{13}C NMR spectrum of polyglucose with that of glucose. The peak of a carbon atom in a glucosidic linkage is shifted 6–8 ppm downfield compared to that in glucose, whereas the chemical shift of a non-linked carbon atom differs from that in glucose by only 0.3–0.5 ppm (Usui et al. 1973). The peaks in the spectrum of polyglucose from *C. aurantiacus* were identified by comparison with published ppm values and by the spectra of polyglucose in which the carbon atoms were differentially labelled (Fig. 2). The two peaks at 100.8 and 99.6 ppm in the spectra shown in Fig. 2 are ascribed to C1 of α-glucose in glycosidic linkages. The absence of peaks in the 93 and 97 ppm regions suggests that negligible free C1 of glucose is to be found in the polymer. The peak at 78 ppm corresponds to C4 of glucose in glycosidic linkages,

Table 1. ^{13}C-labelling of individual carbon atoms in glucose from *Chloroflexus aurantiacus* incubated in the presence of H_2, CO_2, fluoroacetate and ^{13}C-labelled compounds

Tracer	Culture	Incubation time (h)	Polyglucose content (μmol glucose/ml culture)		^{13}C enrichment factor in glucose[a]						Labelling of glucose formed (% of tracer)[b]					
			initial	final	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
1-^{13}C Acetate, 3 mM	1	40	0.22	0.75	18	13	5	3	15	21	30	21	7	4	24	34
	2[c]	24	0.09	0.68	19	15	3	2	16	22	25	20	3	2	22	29
	3[d]	50	0.08	0.79	25	15	7	5	15	26	33	19	8	6	19	34
2-^{13}C Acetate, 3 mM	4	43	0.08	0.56	11	25	6	3	29	12	13	32	7	2	37	14
	5[c]	24	0.10	0.80	8	26	2	1	25	8	9	32	2	0	31	9
	6[d]	51	0.07	1.08	6	36	4	2	40	6	6	42	4	1	47	6
3-^{13}C Alanine, 1 mM	7	51	0.07	0.90	14	3	2	1	3	15	16	3	2	0	3	17

[a] Presented as ^{13}C content relative to ^{13}C content of unenriched carbon (1.1% ^{13}C)

[b] $\frac{\text{Polyglucose (final)}}{\text{Polyglucose (final)} - \text{Polyglucose (initial)}} \cdot \frac{[\%\,^{13}C\,\text{(sample)} - 1.1\%] \cdot 100}{[\%\,^{13}C\,\text{(tracer)} - 1.1\%]}$

[c] Continuously stirred and gassed with $H_2:CO_2$ (95:5)

[d] 0.2 mM KCN present

Table 2. Polyglucose and $^{14}CO_2$ formation by *Chloroflexus aurantiacus* incubated in the presence of H_2, CO_2, fluoroacetate and ^{14}C-labelled acetate

Tracer	Incubation time (h)	Polyglucose formed (μmol glucose/ml culture)	$^{14}CO_2$ formed (μmol/ml culture)
1-^{14}C Acetate, 2 mM	16	0.22	0.18
2-^{14}C Acetate, 2 mM	16	0.33	0.13

but the peak at 71.4 ppm shows that non-linked C4 is also present.

Polyglucose formed by *C. aurantiacus* appears therefore to have a predominantly α(1−4) linked structure. The spectrum of polyglucose labelled predominantly at C1 and C6 of glucose discloses a peak at 68.5 ppm (Fig. 2A); this peak is just discernable in the spectrum of unlabelled polyglucose and is hence assigned to be linked C6. From the relative areas of this peak and the peak due to unlinked C6 at 61.6 ppm, the amount of α(1−6) glycosidic linkage is estimated to be about 10%. The peak at 99.6 ppm corresponds to C1 in α(1−6) linkages and that at 100.8 ppm to C1 in α(1−4) linkages (Usui et al. 1973). Whether there are other minor glycosidic linkages in the polyglucose of *C. aurantiacus* is not known.

Several features of the spectra shown in Fig. 2 have not yet been explained; the resonance at 68.5 ppm ascribed to be linked C6 is extremely broad, and the resonance assigned to C1 at 100.8 ppm and to C4 at about 78 ppm are both split into two resonances of approximately equal intensity. These observations suggest additional heterogeneity of the structure and will be studied further.

Discussion

The symmetric labelling patterns of glucose suggest that *Chloroflexus aurantiacus* forms hexose by the reversed Embden-Meyerhof pathway. This is supported by the work of Kondratieva et al. (1985) who found the enzymes of the Embden-Meyerhof pathway in other *Chloroflexus* strains. The three-carbon intermediates of this pathway can be formed from acetate by two different pathways in autotrophically grown *C. aurantiacus* (Holo and Sirevåg 1986); via pyruvate synthase or via the glyoxylate cycle. The latter pathway would give glucose labelled in C3 and C4 from C1-labelled acetate. However, the results show that most and perhaps all of C3 and C4 are derived from CO_2, which is in accordance with the operation of pyruvate synthase and excludes the glyoxylate cycle as a major route under these conditions. This supports our conclusion that fluoroacetate treatment causes aconitase inhibition in *C. aurantiacus*.

Several of the results from this study suggest that *C. aurantiacus* differs from other organisms in its carbon metabolism. One question that remains to be answered is how labelled CO_2 can be formed from acetate labelled in C2 or C1 in these experiments. The preseence of fluoroacetate makes operation of the tricarboxylic acid cycle unlikely. Neither can all the CO_2 formation be ascribed to the oxidative pentose phosphate cycle; this cycle would cause extensive labelling of C3 of glucose and the labelling patterns would be highly asymmetric (Cohen et al. 1979a). The CO_2 formation is probably not due to acetate oxidation either, since this would produce reducing equivalents that could replace hydrogen in polyglucose production. No polyglucose synthesis was observed in fluoroacetate-treated cells

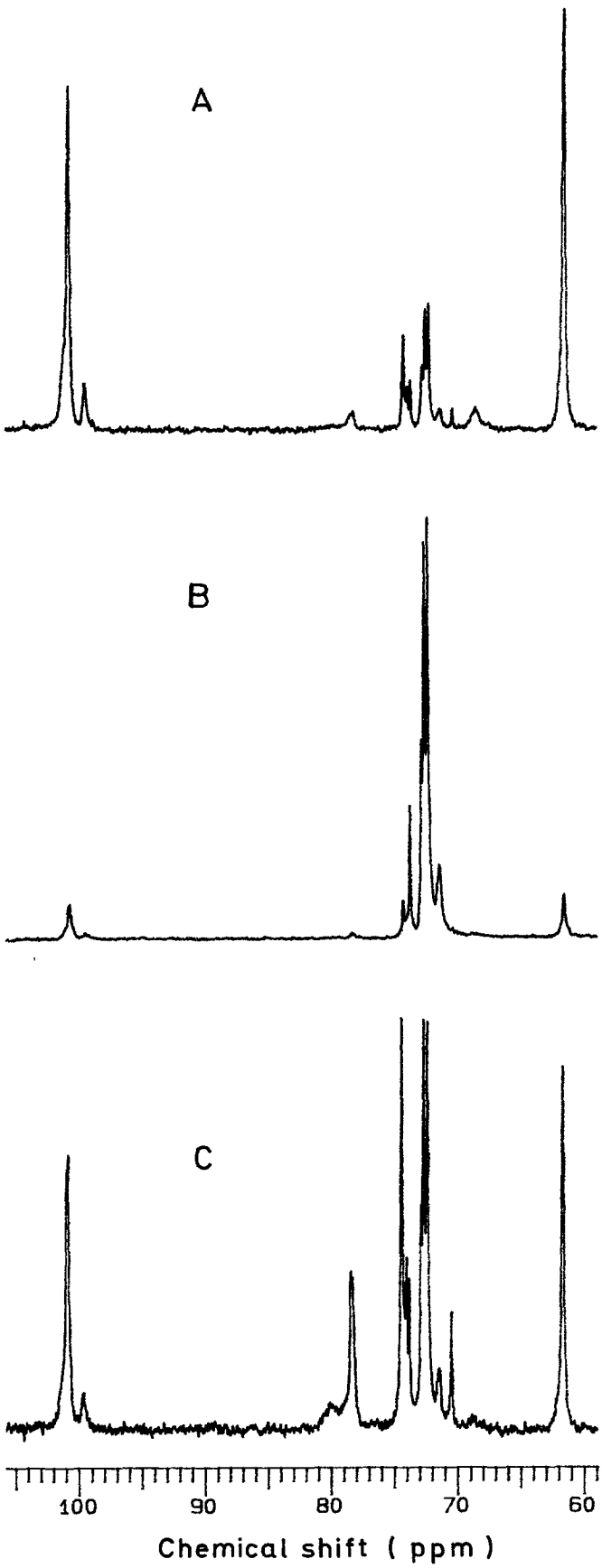

Fig. 2A–C. ^{13}C-NMR spectra of polyglucose from *Chloroflexus aurantiacus* incubated in the presence of H_2, CO_2, fluoroacetate and 3-^{13}C alanine (**A**), 2-^{13}C acetate plus 0,2 mM KCN (**B**) and unenriched acetate (**C**)

in the presence of acetate when hydrogen was replaced by argon.

This work supports our earlier findings that *C. aurantiacus* uses pyruvate synthase in CO_2 fixation (Holo and Sirevåg 1986). However, the labelling patterns of glucose do not fit in with a direct conversion of added acetate to carbohydrate via acetyl-CoA and pyruvate synthase. Scrambling of label in glucose formed from C_3 compounds can be caused by a futile metabolic loop like the multistep shuttling of carbon between phosphoenolpyruvate and fumarate which is symmetric (Cohen et al. 1979b). Phosphoenolpyruvate can be formed directly from pyruvate by pyruvate phosphate dikinase in *C. aurantiacus* (Holo and Sirevåg 1986). This cycle can explain the observation that C2 and C5 in glucose formed in the presence of ^{13}C3-alanine is somewhat labelled. However, such a loop can only cause the ratio of labelling between two carbon atoms to approach unity, the ratio can not be inverted. Thus the labelling patterns observed cannot be ascribed to acetate metabolism by any metabolic pathway known to us. On the other hand the labelling pattern of glucose formed from added alanine fits in with the common gluconeogenesis pathway. Thus the exceptional labelling patterns of glucose formed in the presence of acetate are caused by reactions prior to pyruvate and acetyl-CoA on the route leading from acetate to polyglucose.

C. aurantiacus can form acetyl-CoA from acetate directly by acetyl-CoA synthetase (Holo and Sirevåg 1986). The labelling patterns of glucose formed in the presence of labelled acetate therefore probably reflect a metabolic cycle in which C1-labelled acetyl-CoA is formed from C2-labelled acetyl-CoA and vice versa. The C1 and C6 doublets seen in the spectra of glucose formed in the presence of ^{13}C2-acetate may even indicate the formation of acetyl-CoA labelled in both C1 and C2. However, the doublets may also reflect a small contribution of the glyoxylate cycle in polyglucose synthesis from added acetate. Fuchs et al. (1980a) found that C2-labelled acetyl-CoA is formed from C1-labelled acetyl-CoA by the reductive tricarboxylic acid cycle in *Chlorobium,* but this cycle cannot carry out the opposite label rearrangement.

Even during acetate assimilation most of the polyglucose carbon was derived from CO_2. Thus the label rearrangements observed may not necessarily be caused by a futile mechanism but may also reflect a metabolic pathway in which CO_2 is converted to carbohydrate. Some of our results support this idea: The finding that the glucose appears to be formed mainly from acetyl-CoA labelled in the position opposite to that of the acetate tracer used indicates that the proposed cycle causing labelling rearrangements of acetyl-CoA operates at a higher rate than does polyglucose synthesis from added acetate. A high rate of turnover of such a cycle is only likely to cause label scrambling unless acetyl-CoA becomes less labelled by passing through the cycle. This could be brought about by introducing carbon derived from CO_2 into the cycle. In our previous work we concluded that acetyl-CoA is an intermediate in the autotrophic CO_2 fixation pathway in *C. aurantiacus* (Holo and Sirevåg 1986). The labelling data of glucose thus indicate that during the acetate labelling experiments only about half of the carbon in the acetyl-CoA pool of the cells was derived from added acetate and the rest being from CO_2. This suggests a close connection between the label rearrangement reactions of acetyl-CoA and the

formation of this compound from CO_2. This view is supported by the findings that both processes appear to be affected by KCN.

The finding that carbon derived from added acetate is found with some preference in C2 and C5 as opposed to C1 and C6 of glucose indicates that acetyl-CoA has been formed in which the carboxyl group is derived from acetate and the methyl group is derived from CO_2. By combining data from comparable experiments with different acetate tracers the minimal amount of acetyl-CoA with such a composition was estimated to be 10–26% of all the acetyl-CoA used for carbohydrate synthesis. The highest number was calculated for the cultures incubated in the presence of KCN. These numbers represent minimal values and may be much higher if the cells also form acetyl-CoA in which only C2 is derived from added acetate.

The presence of glucose molecules in which C2 is derived from added acetate and C1 is from CO_2 shows that the carbon-carbon bond in acetate becomes broken and gives rise to an intermediate in carbohydrate synthesis. Furthermore it indicates that a compound derived from acetate acts as a receptor in the CO_2 fixation pathway leading to acetyl-CoA. Thus altogether the results support a new cyclic mechanism for acetyl-CoA formation from CO_2. The details of this pathway are still not known and cannot be established by the methods used in this study. Photoautotrophic CO_2 fixation is an essential part of the carbon and energy cycles in nature, and clarification of the mechanism in *C. aurantiacus* is especially interesting in light of the distant phylogenetic relationship of this organism to any other phototroph (Woese et al. 1985).

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