Purification and properties of acetyl-CoA synthetase from *Bradyrhizobium japonicum* bacteroids

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Acetyl-CoA synthetase was purified 800-fold from *Bradyrhizobium japonicum* bacteroids. A specific activity of 16 $\mu\text{mol/min}$ per mg of protein was achieved, with a 30–40% yield. The purification scheme consisted of only three consecutive chromatography steps. The enzyme has a native M_r of 150 000, estimated by gel-permeation chromatography, and a subunit M_r of 72 000, determined by SDS/polyacrylamide-gel electrophoresis. The optimum pH and temperature are 8.5 and 50 °C respectively. The K_m values for acetate, CoA and ATP were 146, 202 and 275 μM respectively. The reaction was specific for acetate, as propionate and oleate were used very poorly. Likewise, the enzyme used only ATP, ADP or dATP; AMP, GTP, XTP and UTP could not replace ATP. Acetyl-CoA synthetase showed a broad specificity for metals; MnCl_2 could replace MgCl_2 . In addition, CaCl_2 and CoCl_2 were approx. 50% as effective as MgCl_2 , but FeCl_3 , NiCl_2 or ZnCl_2 could not effectively substitute for MgCl_2 . The enzyme may be regulated by NADP^+ and pyruvate; no effect was seen of amino acids, glucose catabolites, reduced nicotinamide nucleotides or acetyl-CoA. Inhibition was seen with AMP, PP_i , FMN and pyridoxal phosphate, with K_i values of 720, 222, 397 and 1050 μM respectively.

INTRODUCTION

Acetate performs multiple functions in the metabolism of prokaryotic and eukaryotic organisms. In bacteria, the primary route of activation of acetate to acetyl-CoA is via the sequential reactions of acetate kinase and phosphotransacetylase (Stadtman, 1952; Rose, 1955; Brown *et al.*, 1977). In the enteric bacteria these enzymes participate primarily in anaerobic energy production (Brown *et al.*, 1972, 1977), and it is acetyl-CoA synthetase that is primarily involved in biosynthetic functions.

Acetyl-CoA synthetase catalyses the reversible reaction:

The enzyme has been purified and characterized from a number of mammalian (Chou & Lipmann, 1952; Jones & Lipmann, 1955; Webster, 1965a,b; Dixon & Webb, 1979) and plant tissues (Hiatt & Evans, 1960; Huang & Stumpf, 1970) and from yeast (Berg, 1956; Patel & Walt, 1987), but the only bacterial sources from which acetyl-CoA synthetase has been partially purified are *Acetobacter aceti* (O'Sullivan & Ettinger, 1976) and *Rhodospirillum rubrum* (Eisenberg, 1955). The enzyme has not been obtained in highly purified form, nor has it been isolated from any bacterium capable of differentiation or symbiotic association where marked metabolic shifts may occur. In this report, we describe the first isolation of highly purified acetyl-CoA synthetase from a symbiotic nitrogen-fixing bacterium.

MATERIALS AND METHODS

Soybean seeds (*Glycine max* L. cv. Williams) were inoculated with *Bradyrhizobium japonicum* 311b-143 (obtained from H. J. Evans, Oregon State University, Corvallis, OR, U.S.A.) and planted in modified Leonard jars with Perlite as the growth support (Karr *et al.*, 1984). Plants were grown in a greenhouse with supplemental lighting and watered, as needed, with a nitrogen-limited nutrient solution (Ahmed & Evans, 1960). After 4–6 weeks, the plants were harvested, and the nodules were removed, cleaned and stored at -70°C .

Purification of acetyl-CoA synthetase

Extract preparation. Nodules were disrupted in a Waring blender in MEP buffer [20 mM-potassium phosphate buffer (pH 7.2) containing 5 mM- MgCl_2 , 1 mM-EDTA and 1 mM-dithiothreitol] and 17% (w/v) sucrose and polyvinylpyrrolidone (1 g of nodules: 0.33 g of polyvinylpyrrolidone: 10 ml of buffer). The homogenate was filtered through four layers of cheesecloth and centrifuged at 400 g for 10 min at 5 °C. The supernatant fraction was centrifuged at 8000 g for 15 min at 5 °C. The 8000 g supernatant contained the cytosol of the plant nodule cells, and the pellet consisted of bacteroids, plant mitochondria and plant membranes. The pellet was resuspended in MEP buffer (2 ml/g original wt. of nodules) plus 17% (w/v) sucrose and layered on to a gradient consisting of 30% (10 ml), 40% (5 ml) and 57% (6 ml) (w/w) sucrose in MEP buffer. The tubes were centrifuged in a SW-28 rotor at 72 000 g for 35 min at 5 °C in a Beckman L8-55 ultracentrifuge. This step removed the mitochondria and plant membranes from the bacteroids; the mitochondria sedimented to the 30/40%-sucrose interface and the bacteroids to the 40/57% interface. The bacteroids were collected and placed on to a second 30:40% (w/w) discontinuous sucrose step gradient and centrifuged for 35 min at 72 000 g at 5 °C as described above. The bacteroid pellet was collected, diluted with 2 vol. of MEP buffer and centrifuged at 8000 g for 15 min at 5 °C. Bacteroids were resuspended in MEP buffer, passed through a French pressure cell at 1.1×10^8 Pa and centrifuged at 30 000 g for 30 min. The resulting supernatant is termed the bacteroid crude extract.

Hydroxyapatite chromatography. Bacteroid crude extracts (approx. 250 mg of protein in 50 ml) were applied on to a hydroxyapatite column (2.5 cm diam. $\times$ 28 cm) intermixed with Sephadex G-25 (3 vol. of hydroxyapatite: 1 vol. of Sephadex G-25) and washed with 5 mM-potassium phosphate buffer (pH 7.2) also containing 5 mM- MgCl_2 , 1 mM-EDTA, 1 mM-dithiothreitol and 1 M-glycerol. Acetyl-CoA synthetase was separated from acetate kinase with a linear gradient (400 ml total volume) of 5–200 mM-potassium phosphate also containing 5 mM- MgCl_2 ,

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1 mM-EDTA, 1 mM-dithiothreitol and 20 % (v/v) glycerol. Fractions were collected and assayed as described below.

Chromatography of acetyl-CoA synthetase on Green A cross-linked agarose. Hydroxyapatite-purified acetyl-CoA synthetase was applied in four separate portions (each approx. 20 ml) to a 30 ml bed volume of Green A cross-linked agarose equilibrated in a buffer of 20 mM-potassium phosphate buffer (pH 7.2), 5 mM-MgCl₂, 1 mM-EDTA, 1 mM-dithiothreitol and 20 % glycerol. After each 20 ml application, the column was washed with 80 ml of the above buffer. After the last 20 ml portion was added, the column was washed with 200 ml of the above buffer. Bacteroid acetyl-CoA synthetase was eluted with a linear gradient (125 ml) of 0–10 mM-ATP in the equilibrating buffer.

Red A chromatography. Fractions from the Green A column containing acetyl-CoA synthetase activity were loaded in four equal portions on to a Red A column (8 ml bed volume), equilibrated in 20 mM-potassium phosphate buffer (pH 7.2)/5 mM-MgCl₂/1 mM-EDTA/1 mM-dithiothreitol. After each application ($\approx$ 15 ml), the column was washed with 20 ml of the above buffer. After the last application, the column was washed with 100 ml of the above buffer. Acetyl-CoA synthetase was eluted with 20 mM-Tris/HCl (pH 8.0)/0.5 M-KCl/5 mM-MgCl₂/1 mM-EDTA/5 mM- β -mercaptoethanol/20 % glycerol/10 mM-ATP. The enzyme preparation could be stored at 0–4 °C in the above solution or recycled through the Green A and Red A columns.

Enzyme assays

Acetyl-CoA synthetase was assayed by incorporation of [¹⁴C]acetate into acetyl-CoA (Roughan *et al.*, 1979). The reaction mixture contained, in addition to enzyme, 1 mM-CoA, 2 mM-ATP, 5 mM-MgCl₂, 1 mM-dithiothreitol, 50 mM-Hepes buffer (pH 8.0) and 1 mM-[¹⁴C]acetate (20 μ Ci/ μ mol). The reaction mixtures were incubated at 30 °C for 1–3 min, and 20 μ l samples were spotted on to a 1 cm square of Whatman no. 1 filter paper. The filter paper was washed four times with ethanol/diethyl ether (3:1, v/v) containing 0.25 % (w/v) trichloroacetic acid. The radioactivity bound ([¹⁴C]acetyl-CoA) to paper was counted in a Beckman LS-230 liquid-scintillation counter. Acetate kinase was assayed by the procedure of Rose (1955) as modified by Skarstedt & Silverstein (1976). The forward direction (acetyl phosphate formation) was measured in a 0.5 ml reaction mixture containing 10 mM-ATP, 10 mM-MgCl₂, 0.3 M-sodium acetate, 0.3 M-hydroxylamine, 100 mM-Tris/HCl (pH 7.5) and enzyme. The reaction was stopped after 30 or 60 min by the addition of 0.7 ml of 10 % (w/v) FeCl₃·6H₂O in 0.7 M-HCl. The resulting iron-acetylhydroxamate complex was measured spectrophotometrically at 505 nm.

Determination of *M_r* by gel-permeation chromatography

A 'Superose' (Pharmacia) f.p.l.c. column (30 ml bed volume) was equilibrated with 100 mM-Tris/HCl (pH 8.0) / 5 mM- β -

mercaptoethanol/20 % glycerol. The column was run at a flow rate of 0.5 ml/min, and 0.5 ml fractions were assayed for enzyme activity. The column was calibrated with the following *M_r* markers: catalase (240 000); aldolase (158 000); bovine serum albumin (67 000); haemoglobin (64 450); hen-egg albumin (45 000); chymotrypsin A (25 000); cytochrome *c* (12 500). The correlation coefficient for the calibration curve was 0.90. Yeast acetyl-CoA synthetase (151 000) was used as a comparative control. Blue Dextran was used to determine the column void volume.

SDS/polyacrylamide-gel electrophoresis

Electrophoresis in the presence of SDS was done as described by Laemmli (1970). The reservoirs contained 0.025 M-Tris/HCl (pH 8.3), 0.192 M-glycine and 0.1 % (w/v) SDS. The resolving gel contained 12.5 % (w/v) acrylamide, 0.33 % (w/v) bisacrylamide, 0.38 M-Tris/HCl (pH 8.8) and 0.4 % SDS. The stacking gel contained 12.5 % acrylamide, 0.016 % bisacrylamide, 0.5 M-Tris/HCl (pH 6.8), and 0.4 % SDS. *M_r* standards were: phosphorylase *b* (97 000); bovine serum albumin (66 200); ovalbumin (45 000); carbonic anhydrase (31 000); soya-bean trypsin inhibitor (21 500); lysozyme (14 400). Protein was detected in the slab gel by using Bio-Rad (Richmond, CA, U.S.A.) silver-staining reagent and protocol. Gels were scanned with a Hoefer model GS 300 Gel Scanner.

Protein determination

Protein was determined by the method of Bradford (1976).

Chemicals and supplies

Buffers and most chemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Polychar (polyvinylpyrrolidone) was purchased from GAF Corp., New York, NY, U.S.A.; before use, it was treated with 10 % (v/v) HCl, neutralized, washed with distilled water, and dried. Sephadex G-10 was purchased from Pharmacia, Piscataway, NJ, U.S.A. Hydroxyapatite, Bio-Gel HTP and silver-staining reagents were purchased from Bio-Rad. [¹⁴C]Acetate (45–60 mCi/mmol) was purchased from Amersham, Arlington Heights, IL, U.S.A. Matrix affinity gels used for screening and purification were obtained from Amicon, Lexington, MA, U.S.A. Perlite and plant pots were purchased from local sources.

RESULTS

Acetyl-CoA synthetase from *B. japonicum* bacteroids was purified over 800-fold from crude extracts after three chromatographic steps (Table 1). Hydroxyapatite chromatography separates acetyl-CoA synthetase from acetate kinase (Preston *et al.*, 1989). The hydroxyapatite column also served to remove exopolysaccharides from the extracts, which would have hindered further purification of acetyl-CoA synthetase (Waters *et al.*, 1985). Low-ionic-strength buffers, e.g. not greater than 20 mM-

Table 1. Purification of bacteroid acetyl-CoA synthetase

Procedure	Total activity (μ mol/min)	Yield (%)	Specific activity (μ mol/min per mg of protein)	Purification (fold)
Crude extract	2.73	100	0.019	1.0
Hydroxyapatite chromatography	2.78	103	0.187	9.5
Green A chromatography	1.23	45	2.15	109
Red A chromatography	2.12	78	16.1	817

Fig. 1. SDS/polyacrylamide-gel electrophoresis and gel scan of purified acetyl-CoA synthetase

The M_r standards ($\times 10^{-3}$) are indicated by the arrows.

potassium phosphate (pH 7.2), removed the exopolysaccharide without eluting enzyme activity.

A number of affinity columns were found to bind acetyl-CoA synthetase. However, using the combination of Green A and Red A cross-linked agaroses eliminated the need to desalt, or to change the buffer or pH of the preparation. Acetyl-CoA synthetase was eluted from the Green A column with a gradient of 0–10 mM-MgATP. The fractions from the Green A column containing acetyl-CoA synthetase activity were applied directly to the Red A column. The presence of MgATP did not interfere with the ability of the enzyme to bind to the Red A column. Acetyl-CoA synthetase was eluted from the Red A column with a solution containing 0.5 M-KCl, 10 mM-ATP, 20 mM-Tris/HCl (pH 8.0), 20% glycerol and 5 mM- β -mercaptoethanol. Acetyl-CoA synthetase was obtained in a highly purified form after these three steps; the resulting enzyme preparation was judged to be over 95% homogeneous by silver-staining of SDS/polyacrylamide gels (Fig. 1).

Purified acetyl-CoA synthetase can be stored at 0–4 °C for at least 1 month without loss of activity in 20 mM-Tris/HCl (pH 8.0) containing 10 mM-ATP, 5 mM-MgCl₂, 1 mM-dithiothreitol (or 5 mM- β -mercaptoethanol) and 20% glycerol. Omission of either ATP or glycerol resulted in a 70% loss of activity within 1–2 weeks. Incubation of acetyl-CoA synthetase activity at 0–4 °C or at room temperature for 20 min in solutions of low ionic strength (less than 1.0 mM) resulted in a loss of activity of greater than 90%. Preincubation with dithiothreitol or β -mercaptoethanol at 5 or 10 mM resulted in a 20% increase in activity relative to no addition of thiol reagents. Oxidized dithiothreitol at greater than 1 mM appeared to inhibit enzyme activity.

Fig. 2. Effect of pH on *B. japonicum* bacteroid acetyl-CoA synthetase activity

Acetyl-CoA synthetase was assayed at the various pH values in 50 mM-2-(*N*-cyclohexylamino)ethanesulphonic acid (○), 50 mM-Hepes (●) or 50 mM-Mes (△). The pH indicated in the Figure was recorded at the end of the assay. The initial and final pH values varied by < 0.2 pH unit.

Fig. 3. Effect of temperature on *B. japonicum* bacteroid acetyl-CoA synthetase activity

Acetyl-CoA synthetase was assayed at the indicated temperatures. Inset: determination of the energy of activation from the data of the main figure.

The apparent M_r of the native enzyme was determined by gel-permeation f.p.l.c. to be 150 000. *B. japonicum* acetyl-CoA synthetase was eluted at the same volume as for the enzyme from yeast (M_r 151 000). Under denaturing conditions, only one polypeptide was observed by SDS/polyacrylamide-gel electrophoresis, with an apparent M_r of $72\,000 \pm 250$. These data indicate that acetyl-CoA synthetase exists as a dimer.

The pH/activity profile of acetyl-CoA synthetase shows a broad optimum, with the highest activity found at pH values greater than 8.0; pH 8.5 usually produced the maximal activity (Fig. 2). The enzyme retained its activity at non-physiological alkaline pH values. The profile was not affected by the type of buffer in which the assay was performed, but the absolute value of the activity varied almost 2-fold. The temperature optimum for the purified enzyme was 50 °C (Fig. 3). Above 55 °C the activity decreased markedly, and incubation of the enzyme for 5 min at 60 °C resulted in a 20% loss of activity. The activation energy of the reaction between 25° and 50 °C was estimated at approx. 33.5 kJ/mol (Fig. 3 inset). The enzyme was not inhibited by 0.5 h preincubations with reagents (1 mM each) that modify thiol groups (iodoacetamide), serine (phenylmethanesulphonyl fluoride) or lysine residues (pyridoxal phosphate). Acetyl-CoA synthetase was, however, rapidly inhibited by diethyl pyrocarbonate, which reacts with histidine and cysteine residues at alkaline pH; diethyl pyrocarbonate at 1.0 mM inhibited activity by 65%.

The K_m values for acetate, CoA and ATP were $146 \pm 17 \mu\text{M}$, $202 \pm 8 \mu\text{M}$ and $275 \pm 23 \mu\text{M}$ respectively. These values were determined at 1 min time intervals, since at low substrate concentrations the assay became non-linear at times longer than 1 min. The Lineweaver-Burk plots were linear for all substrates; no substrate inhibition was observed by acetate and CoA at concentrations up to 2 mM or by ATP at 10 mM. MgCl₂ did show

Fig. 4. Nucleotide specificity of *B. japonicum* bacteroid acetyl-CoA synthetase

Acetyl-CoA synthetase was assayed in the presence of ATP (○), dATP (▲), ADP (□), UTP, XTP and AMP (△).

inhibition at high concentrations where the metal was present as the free ion (i.e. 50 % inhibition at 20 mM; Mg/ATP ratio = 10).

Acetate was the preferred substrate for the enzyme, as propionate and oleate were used only poorly (results not shown). Propionate was estimated to have a K_m value approx. 4 times that for acetate (200 compared with 50 μ M) and a V_{max} about 7 times less (2.5 versus 18 μ mol/min per mg of protein).

Acetyl-CoA synthetase used ATP preferentially and other adenine nucleotides less effectively. Deoxy-ATP ($K_m = 2.3$ mM) and ADP ($K_m = 0.4$ mM) could substitute approx. 30 % and 20 % as well, respectively, as ATP at 2.0 mM each. AMP was completely ineffective. Other purine or pyrimidine triphosphates (GTP, UTP, XTP) could not substitute for ATP (Fig. 4).

The enzyme appeared to show a broad specificity for bivalent metal ions (Fig. 5). The enzyme could use $MnCl_2$ ($K_m = 0.5$ mM) equally as well as $MgCl_2$ ($K_m = 0.3$ mM), and could use $CaCl_2$ (1.2 mM) and $CoCl_2$ (0.2 mM) less effectively, and $FeCl_3$, $NiCl_2$ and $ZnCl_2$ poorly (Fig. 5).

The effect of several univalent cations on acetyl-CoA synthetase was determined. KCl has been shown to activate a number of enzymes, and it increased the activity of *B. japonicum* acetyl-CoA synthetase about 60 % at 5 mM and 80 % at 20 mM. Increasing the concentration of KCl above 20 mM decreases the activity slightly, although at 200 mM-KCl the activity remained 10 % greater than that in the absence of added KCl. NaCl

Fig. 5. Metal-ion specificity of *B. japonicum* bacteroid acetyl-CoA synthetase

Acetyl-CoA synthetase was assayed in the presence of $MgCl_2$ (△), $MnCl_2$ (▲), $CaCl_2$ (○), $CoCl_2$ (●), $NiCl_2$ (■), $FeCl_3$ (□) and $ZnCl_2$ (◻) at the indicated concentrations.

Table 2. Effect of various metabolites on acetyl-CoA synthetase

Concentration of metabolites was 1 mM. Assays were initiated by addition of enzyme. Activities are means $\pm$ range of two determinations; 100 % = 5.76 μ mol/min.

Metabolite	Activity (%)
NAD ⁺	102 $\pm$ 4
NADH	110 $\pm$ 12
NADP ⁺	146 $\pm$ 15
NADPH	105 $\pm$ 16
FMN	23 $\pm$ 10
Pyridoxal phosphate	55 $\pm$ 14
AMP	30 $\pm$ 1
Pyrophosphate	15 $\pm$ 1
Acetyl-CoA	99 $\pm$ 23
Acetoacetyl-CoA	93 $\pm$ 6
Propionyl-CoA	58 $\pm$ 6
Crotonyl-CoA	64 $\pm$ 20
Palmitoyl-CoA	> 0.5
Oleoyl-CoA	> 0.5
Pyruvate	15 $\pm$ 15

decreased the activity by 20–25 % at concentrations of 5, 10 and 20 mM. NH_4Cl increased the activity by 30 % at 5 mM and 70 % at 20 mM.

To determine the possible physiological effectors of acetyl-CoA synthetase activity, a number of cellular metabolites were tested as possible positive or negative regulators. These assays were performed at near- K_m concentrations of acetate, ATP and CoA (70, 100 and 200 μ M respectively), and at 1 mM concentrations of added metabolites. There was no substantial activation or inhibition by the following metabolites: glucose 6-phosphate, fructose 6-phosphate, glucose 1-phosphate, 6-phosphogluconate, fructose 1,6-bisphosphate, phosphoenolpyruvate, glyceraldehyde 3-phosphate, phosphatidylcholine, UDP-glucose, ribose 5-phosphate, citrate, fumarate, succinate, glycerate, malate, maleate, hydroxybutyrate, alanine, arginine, aspartate, asparagine, glutamate, glutamine, tryptophan, leucine, isoleucine or valine. NADP⁺ was the only compound which activated acetyl-CoA synthetase, producing an approx. 50 % stimulation at 1.0 mM. Acetyl-CoA, the product of acetyl-CoA synthetase, did not inhibit the activity, but other short-chain CoA esters inhibited the enzyme by 40 %. Long-chain CoA esters strongly inhibited the enzyme. Pyruvate inhibited the enzyme by about 85 % (Table 2), and may serve to regulate

Fig. 6. Effect of various metabolites on *B. japonicum* bacteroid acetyl-CoA synthetase

Acetyl-CoA synthetase was assayed in the presence of 1 mM each of AMP (○), PP_i (●), FMN (△) or pyridoxal phosphate (▲).

acetyl-CoA formation between acetyl-CoA synthetase and pyruvate dehydrogenase. Commercial pyruvate may contain substantial amounts of acetate, which may account for some of the observed inhibition. Acetyl phosphate caused an apparent 68% inhibition of acetyl-CoA synthetase. However, because of the lability of acetyl phosphate, it could not be accurately determined what proportion of the decrease in activity was due to actual inhibition or to dilution of labelled substrate by acetate resulting from acetyl phosphate breakdown. The enzyme was not inhibited by ADP or any nicotinamide nucleotides. It was, however, inhibited strongly by AMP, PP_i and FMN (Fig. 6). The K_i values for AMP, PP_i, FMN and pyridoxal phosphate were $720 \pm 50 \mu\text{M}$, $222 \pm 42 \mu\text{M}$, $397 \pm 35 \mu\text{M}$ and $1050 \pm 210 \mu\text{M}$ respectively.

DISCUSSION

Bradyrhizobium japonicum is a micro-organism that can grow heterotrophically in culture or can exist in a viable but non-growing form symbiotically within soya-bean root nodules (Sutton, 1983). The symbiotic forms of *B. japonicum*, referred to as bacteroids, metabolize photosynthetically derived carbon compounds from the plant to provide the energy required for the nitrogen-fixation process (Bergersen & Turner, 1967; Burris & Wilson, 1939). Acetyl-CoA is likely to be central to bacteroid metabolism, as the symbiotic forms possess a citric acid cycle, a polyhydroxybutyrate cycle and active fatty acid metabolism (Stovall & Cole, 1978; Karr *et al.*, 1984). In addition, LaRue and co-workers have shown that acetate could support nitrogen-fixation activity in isolated *B. japonicum* bacteroids (Peterson & LaRue, 1981, 1982; Tajima & LaRue, 1982). Previously, we demonstrated the presence of acetyl-CoA synthetase and acetate kinase in the symbiotic and heterotrophically cultured forms of *B. japonicum* (Preston *et al.*, 1989).

B. japonicum bacteroid acetyl-CoA synthetase does not appear to be readily responsive to many of the metabolites tested. Since acetyl-CoA is a biosynthetic precursor at the beginning of many different pathways, its synthesis would not be expected to undergo feedback inhibition by the product(s) of only one of these pathways. The synthesis of acetyl-CoA would, however, be expected to be under cellular control *in vivo*, depending on whether or not the cell, in the free-living or bacteroid state, is in an overall anabolic (actively growing) or catabolic (energy-expending, i.e. nitrogen-fixing) mode. Therefore the enzyme from *B. japonicum* may be controlled at the level of synthesis as a function of the carbon source used for bacterial growth, or as a function of nodule development.

Pyruvate appears to be a potent inhibitor of acetyl-CoA synthetase. Pyruvate may regulate the generation of acetyl-CoA via pyruvate dehydrogenase for purposes of energy generation, as opposed to the generation of acetyl-CoA via acetyl-CoA synthetase for biosynthetic metabolism. High concentrations of pyruvate would increase pyruvate dehydrogenase activity and inhibit acetyl-CoA synthetase, whereas under limiting pyruvate the reverse situation could occur.

Alternatively, the fate of acetate may be under branch-point control (LaPorte *et al.*, 1984), whereby acetyl-CoA synthetase activity is controlled solely by substrate availability and acetate

kinase is directly regulated by allosteric effectors or covalent modification. Acetate kinase from both *Escherichia coli* and *Salmonella typhimurium* undergoes reversible covalent phosphorylation (Fox & Roseman, 1986), and subsequently Fox *et al.* (1986) speculated on the role this regulatory control may play in sugar uptake and citric acid-cycle metabolism. Collectively, the data reported here are congruous with a branch-point model similar to that proposed by LaPorte *et al.* (1984).

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