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Modulation of TCA cycle enzymes and aluminum stress in *Pseudomonas fluorescens*

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Abstract

Oxalic acid plays a pivotal role in the adaptation of the soil microbe *Pseudomonas fluorescens* to aluminum (Al) stress. Its production via the oxidation of glyoxylate necessitates a major reconfiguration of the enzymatic reactions involved in the tricarboxylic acid (TCA) cycle. The demand for glyoxylate, the precursor of oxalic acid appears to enhance the activity of isocitrate lyase (ICL). The activity of ICL, an enzyme that participates in the cleavage of isocitrate to glyoxylate and succinate incurred a 4-fold increase in the Al-stressed cells. However, the activity of isocitrate dehydrogenase, a competitor for the substrate isocitrate, appeared to be diminished in cells exposed to Al compared to the control cells. While the demand for oxalate in Al-stressed cells also negatively influenced the activity of the enzyme α-ketoglutarate dehydrogenase complex, no apparent change in the activity of malate synthase was recorded. Thus, it appears that the TCA cycle is tailored in order to generate the necessary precursor for oxalate synthesis as a consequence of Al-stress.

Keywords: Al tolerance; TCA cycle enzymes; Isocitrate lyase; α-Ketoglutarate dehydrogenase complex; Glyoxylate; Oxalate

1. Introduction

The TCA cycle is a pivotal metabolic pathway in most organisms. It results in the generation of reducing factors that propel the production of energy and also acts as a reservoir of essential metabolic precursors that are channeled towards the biogenesis of essential compounds like amino acids, fatty acids and sugars [1]. This cycle can however, be modified and enzymes can be upregulated or downregulated depending on the needs of a cell. The demand for energy necessitates the complete decarboxylation of acetyl CoA and the formation of the reducing moieties like $FADH_2$ and NADH. On the other hand, the requirement for anaplerotic metabolites usually triggers less decarboxylation and leads to different truncated versions of the TCA cycle. For instance, cellular growth on acetate evokes the inactivation of the enzyme isocitrate dehydrogenase (ICDH) via phosphorylation and the stimulation of acetate fixation with the aid of ICL [2–4]. Fluxes in the environment that may be physical and/or chemical in nature will have a major impact on this metabolic cycle. Decreasing oxygen tension may promote a non-cyclic TCA cycle, while a lack of micronutrients like iron modulates key enzymes like aconitase and succinate dehydrogenase [5,6].

Our laboratory has been studying the biochemical impact of environmental stress on cellular metabolism in an effort to delineate various adaptation mechanisms. We have demonstrated the ability of the soil microbe *Pseudomonas fluorescens* to survive millimolar amounts of Al by eliminating the trivalent metal as an insoluble residue consisting mainly of oxalate and phosphatidylethanolamine (PE) [7,8]. Although oxalic acid has been shown to play an instrumental role in the detoxification of Al in numerous organisms, the precise enzymatic reaction mediating the formation of this acid as a consequence of Al-stress has not been elucidated. Furthermore, de novo synthesis of oxalic acid, prompted by Al-stress has not been shown [9]. However, we have recently demonstrated that oxalic acid is produced via the oxidation of glyoxylate. Indeed, in Al-stressed cells, the glyoxylate-oxidizing activity that is

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associated with the cytoplasmic membrane-component increased 8-fold compared to control cells [7].

To further explore the importance of oxalic acid in the adaptation to Al, we have investigated various enzyme systems that are known to contribute to glyoxylate production. Here, we report that the activities of TCA cycle enzymes are reconfigured in order to promote an increase in the production of glyoxylate. A 4-fold increase in the activity of ICL in the soluble extracts from Al-stressed cells compared to control cells has been shown. The reduction in activities of ICDH and α-ketoglutarate dehydrogenase complex (KGDHC) in the Al-stressed cells and no apparent change in activity of MLS are also discussed.

2. Materials and bacterial cultivation

All chemicals were reagent grade. Serum albumin, oxalate assay kit, lactate dehydrogenase, acetyl CoA, Coenzyme A, 2,4-dinitrophenylhydrazine, dithiobenzoic acid, nicotinamide adenine dinucleotide (oxidized form), nicotinamide adenine dinucleotide phosphate (oxidized form) were obtained from Sigma (St. Louis, MO, USA). The Bradford assay was from Bio-Rad and (2,4-^{13}C) citric acid was from Isotec (Miamisburg, OH, USA). The bacterial strain *Pseudomonas fluorescens* ATCC 13525 from the American Type Culture Collection (Rockville, MD, USA) was maintained and grown in a mineral medium consisting of Na_2HPO_4 (6.0 g), KH_2PO_4 (3.0 g), NH_4Cl (0.8 g), $MgSO_4{\cdot}7H_2O$ (0.2 g) and citric acid (4.0 g) in 1 l of deionized distilled water. Trace elements as previously described were also included [10]. Aluminum chloride (15 mM) was complexed to the citrate prior to sterilization. The pH was adjusted to 6.8 with dilute NaOH. The medium was dispensed in 200-ml amounts in 500-ml Erlenmeyer flasks and inoculated with 1 ml of stationary phase cells grown in a medium unamended with the test metal. The cultures were aerated on a gyratory water bath shaker (model G76, New Brunswick Scientific) at 26°C and 140 rpm. Cellular biomass was monitored by measuring solubilized proteins following digestion with 1 M NaOH by the method of Bradford [11].

2.1. Nuclear magnetic resonance studies

NMR analyses were performed using a Varian Gemini 2000 spectrometer operating at 50.38 MHz for ^{13}C (carbon). Experiments were executed with a 5-mm dual probe (35° pulse, l-s relaxation delay, 8 kilobytes of data and 2000 scans). Chemical shifts were referenced to shifts of standard compounds obtained under the same conditions. Whole cells (250 μg equivalent of solubilized protein) obtained after 40 h of growth in an Al-citrate (15 mM) medium were placed in a phosphate buffer (10% ^2H_2O). The reaction was initiated by addition of labeled Al-citrate (1:1) and citrate [2,4-$^{13}C_2$], respectively. Following a 10-min incubation at 26°C, the cells were centrifuged in order to afford a cellular pellet and a supernatant. The cellular pellet was sonicated and centrifuged at 181 000 *g* for 1 h in order to afford a soluble component and a membrane fraction. All these three components were subjected to ^{13}C NMR proton decoupled analyses following treatment with perchloric acid or heating at 50°C for 10 min. The presence of glyoxylate, succinate and oxalate in these fractions was further confirmed by commercial enzymatic assays utilizing lactate dehydrogenase, succinate dehydrogenase and oxalate oxidase, respectively [12–14].

2.2. Isolation of cellular fractions and enzymatic assays

The cells were washed once with 0.85% NaCl and with 50 mM Tris buffer (pH 7.3), 1 mM phenylmethylsulfonylfluoride (PMSF). Following suspension in 2 ml of the same buffer, the cells were disrupted by sonication. After separation of the cell extract from intact cells by centrifugation, the membranes (both inner and outer) were pelleted from the cell extract by ultracentrifugation (181 000 *g* for 1 h at 4°C). The soluble fraction was collected, and the membrane pellet was washed with 50 mM Tris buffer (pH 7.3) containing 1 mM PMSF and pelleted again by ultracentrifugation (181 000 *g* for 15 min at 4°C). The membrane pellet was resuspended in Tris buffer, and the membranes were dispersed by sonication. ICL activity was assayed in 25 mM Tris–HCl buffer (pH 7.3) containing 5 mM $MgCl_2$, 2 mM isocitrate, and approximately 0.1 mg ml^{-1} soluble protein. Enzyme activity was determined spectrophotometrically by monitoring the production of glyoxylate with 2,4-DNPH at 450 nm as described in [15]. Glyoxylate was used as a standard. ICDH activity was assayed in 25 mM Tris–HCl buffer (pH 7.3) containing 2 mM isocitrate and 1 mM NAD^+ and/or $NADP^+$, 5 mM succinate (to inhibit contaminated ICL activity) and approximately 0.2 mg ml^{-1} membrane protein. ICDH activity was determined by measuring the formation of α-ketoglutarate. The ketoacid was determined spectrophotometrically using 5 mM 2, 4-DNPH. α-Ketoglutarate served as a standard. KGDHC activity was assayed in 25 mM Tris–HCl buffer (pH 7.3) containing 2 mM α-ketoglutaric acid, 0.1 mM Coenzyme A and 1 mM NAD^+ and ~0.2 mg ml^{-1} membrane protein. The disappearance of α-ketoglutarate was followed colorimetrically with the aid of DNPH. Malate synthase activity was assayed in 25 mM Tris–HCl buffer (pH 7.3) containing 2 mM glyoxylate, 0.2 mM acetyl coenzyme A and 0.2 mg ml^{-1} membrane protein. Enzyme activity was determined spectrophotometrically by monitoring both the disappearance of glyoxylate in the presence of DNPH and the disappearance of coenzyme A in the presence of dithiobenzoic acid [16]. These activities were also followed by ^{1}H NMR spectroscopy. In an effort to verify that the enzymatic activities in the Al-stressed were not a result of the interaction of the enzymes directly with Al, following the fractionation of the control cells, Al was added as citrate (0.1–1 mM) and $AlCl_3$ (0.01–0.5 mM) to both the soluble and membrane

components and the activities of ICL, ICDH, KGDHC and MLS were recorded. The ICL and MLS activities were associated with the soluble fraction while ICDH and KGDHC were localized in the membrane components.

2.3. Examination of other enzymes involved in the production of glyoxylate

In order to identify other metabolic routes that may contribute to the production of glyoxylate, the cellular factions were incubated either separately or together with glycolate (10 mM) with or without NAD^+ (3 mM) and pyruvate/glycine, respectively. Oxalate and/or glyoxylate were quantitated by colorimetric assays [14,15].

2.4. ICL activity at various growth intervals, in media with varying concentrations of Al and different metals

Pseudomonas fluorescens was grown and at various time intervals the soluble cellular fractions were analyzed for ICL activity. To evaluate the influence of Al on ICL activity, cells were subjected to different Al concentrations ranging from 0.1 to 15 mM and the production of glyoxylate by the soluble cellular component in the presence of isocitrate was monitored. Furthermore, cells were grown in a citrate medium complexed to various metals like Ga^{3+} (1 mM), Y^{3+} (0.1 mM), Ca^{2+} (5 mM), Pb^{2+} (0.1 mM), and ICL activity was investigated. Cells were obtained at the same growth phase (late logarithmic phase) and 100 μg protein equivalent of soluble fraction were incubated with isocitrate (2 mM) for 15 min.

2.5. Aluminum and modulation of ICL activity

P. fluorescens cultured in a medium supplemented with Al and grown for 37 h (time period when ICL activity was optimal) was harvested and washed. These microbial cells corresponding to 2 mg of protein were transferred in control media without Al and allowed to incubate for 4 h (B). The cells were subsequently harvested and resuspended in fresh Al media for 2, 4 and 6 h (values for 6 h are shown as E in Fig. 4). Similar experiments were repeated in the presence of rifampicin (200 μg/ml) [17] and chloramphenicol (200 μg/ml) [18] in the Al media. These are represented as C and D in Fig. 4. ICL activity in the soluble fraction was monitored at these different time intervals. All experiments were done three times and in duplicate.

3. Results

3.1. ^{13}C-NMR studies of Al-citrate metabolism and Al efflux

The presence of the peak at 43.0 ppm indicative of the [2,4-$^{13}C_2$] resonance of the citric acid in the soluble cellular fraction after 10 min of incubation may be indicative of a direct interaction between the tricarboxylic acid and the microbial system. The intracellular localization of Al has previously been demonstrated [8]. The concomitant appearance of a peak at 83.4 ppm attributable to [2-$^{13}C_1$] of glyoxylate would imply that the cleavage of the tricarboxylic acid contributed to the formation of this moiety. The resonance at 27.7 ppm was assigned to the [$CH_2-^{13}C_1$] of succinate [19]. The peak at 158.8 ppm has been shown to be free oxalic acid [7]. The ^{13}CNMR studies with the membrane component did show a peak at 166.9 ppm characteristic of oxalate bound aluminum [7] and a resonance at 28 ppm characteristic of succinate. The peaks at 121.7 ppm in the membrane component and the resonance at 137.8 ppm in the soluble cellular fraction have not been assigned yet. The supernatant had an oxalate peak 158.8 and a chemical shift at 43 ppm attributable to the unreacted substrate. The presence of glyoxylate, oxalate and succinate was further confirmed by enzymatic assays utilizing lactate dehydrogenase, oxalate oxidase and succinate dehydrogenase, respectively. The NMR pattern in the control experiments was entirely disparate. In the control cells, a peak at 99.9 ppm assigned to oxaloacetate [19] was apparent as were two unattributed resonances at 139.2 ppm and 176.3 ppm in the membrane component (Fig. 1). The soluble fraction did not reveal any significant peaks. These spectra are characteristic of three separate experiments. When control cells (i.e. cells obtained from a citrate medium with no added Al) were incubated with Al-citrate (1:1) the NMR spectra did not reveal any peaks, thus confirming the inability of these cells to deal with Al-citrate.

The presence of labelled succinate and glyoxylate prompted us to examine the obvious candidate involved in the generation of this moiety namely, ICL. The cellular fractions (soluble and membrane) were incubated with isocitrate and the production of glyoxylate was monitored. This enzyme that was primarily localized in the soluble-cellular component, was drastically increased in the Al-stressed cells. At least a 4-fold increase was observed at 37 h of growth, an incubation period corresponding to late logarithmic to early stationary phase in the Al-stressed cells compared to the control cells in the same growth phase (Fig. 2). However, other potential precursors of glyoxylate like glycolate, glycine/pyruvate did not reveal any detectable formation of glyoxylate when incubated with the cell free extracts. Oxalate production was also not observed (data not shown). Thus, it became quite evident that ICL was the driving force mediating the production of oxalate via the cleavage of isocitrate into succinate and glyoxylate. The profile of the activity of ICL was monitored at various time intervals. Although no significant increment was observed in Al-stressed culture itself at different time periods, a marked variation was evident when compared to the control cultures. It is important to note that following the maximal activity observed at 37 h, a noticeable decline was observed subsequently. This

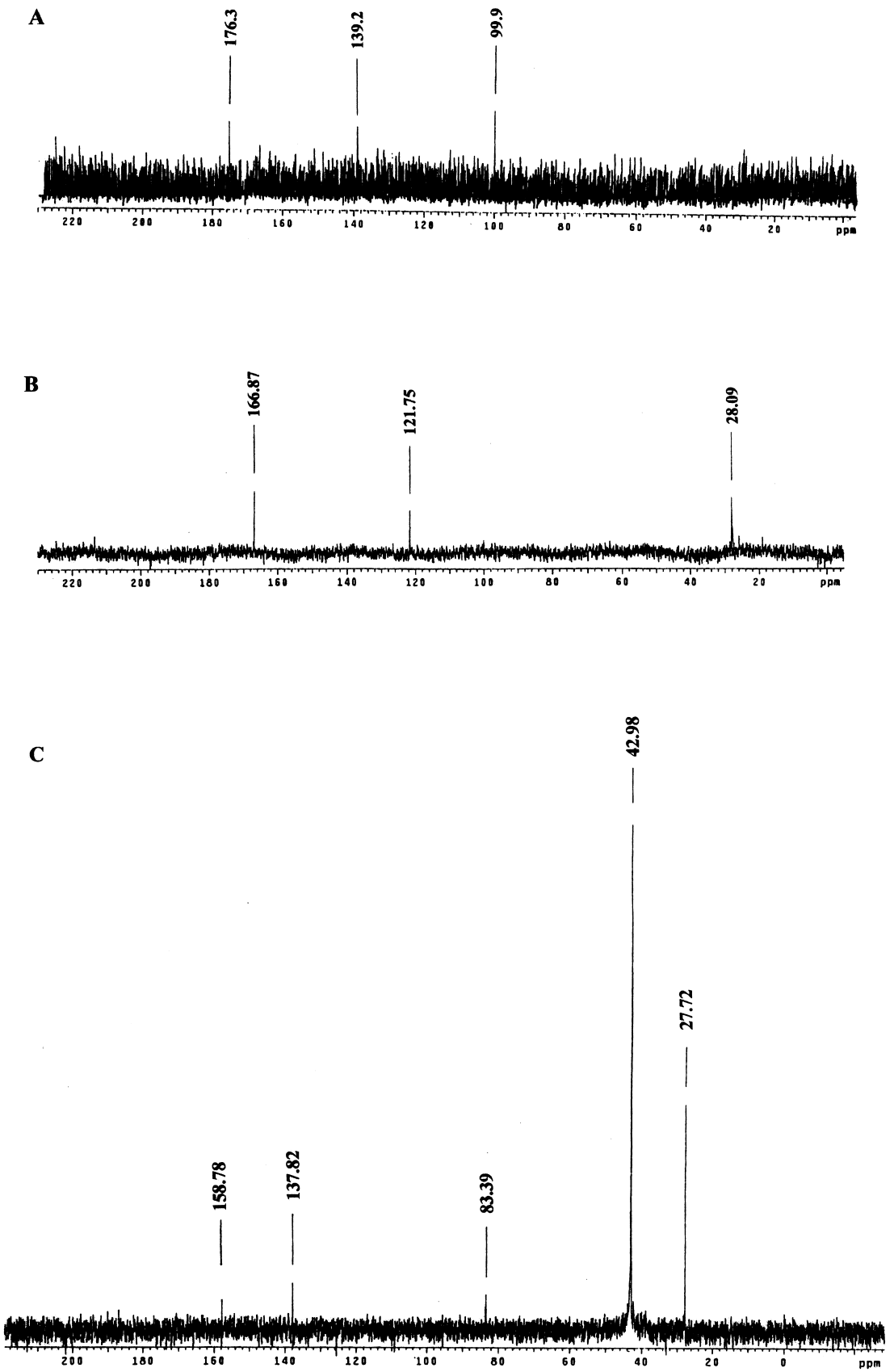


Fig. 1. Proton-decoupled ^{13}C NMR spectra obtained upon incubation of citrate [2,4,- ^{13}C] with *P. fluorescens* from control and Al-supplemented (15 mM) cultures. Cells (late logarithmic phase) were incubated for 10 min and the cellular fractions were analyzed. (A) Membrane fraction from control cells. (B) Membrane components from Al-stressed cells. (C) Soluble fraction from Al-stressed cells.

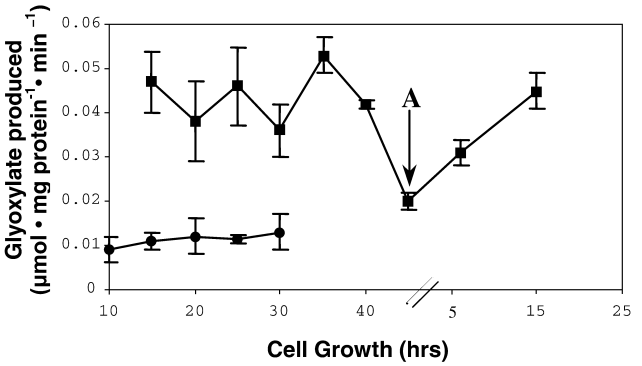


Fig. 2. ICL activity in control and Al-stressed cells obtained at various growth intervals. (A) Cells were transferred in a fresh Al-medium; $n=3$, values are mean±S.E.M.

decrease in ICL activity appeared to coincide with the formation of the gelatinous residue i.e. the time when there was minimal amounts of Al in the cells [8,20]. When these cells were incubated in a fresh Al medium, the ICL activity increased (Fig. 2). In an effort to establish a direct relationship between the activity of ICL and Al-stress, *P. fluorescens* was grown in a medium supplemented with varying amounts of Al at various time intervals. Maximal ICL activity was observed in cells subjected to 10–15 mM Al (Fig. 3). This ICL profile revealed an intricate relationship between the increased glyoxylate forming ability of the soluble fraction from isocitrate and the presence of Al in the culture media. Furthermore, no change of ICL activity was observed when Al as citrate or chloride was added to the soluble-cellular component from the control

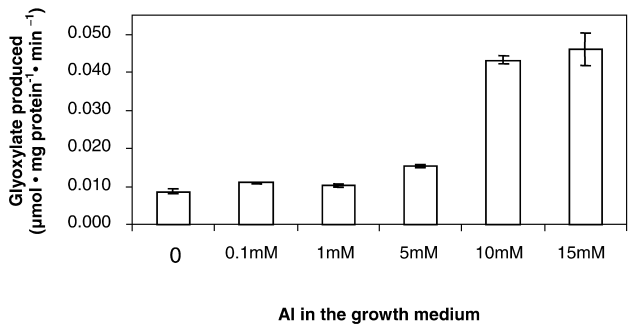


Fig. 3. ICL activity in cultures with varying Al concentrations; $n=3$, values are mean±S.E.M.

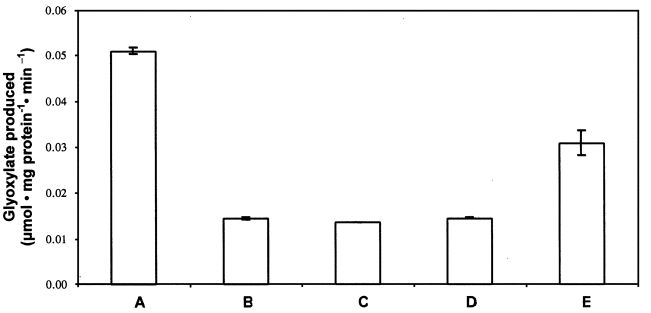


Fig. 4. Modulation of ICL activity: Influence of Al, rifampicin and chloramphenicol. (A) Cells grown at in Al medium; (B) cells from (A) incubated in control medium; (C) Cells from (B) incubated in fresh Al medium in the presence of rifampicin (200 µg/ml); (D) Cells from (B) incubated in fresh Al medium in the presence of chloramphenicol (200 µg/ml); (E) Cells from (B) incubated in fresh Al medium devoid of any inhibitors; $n=3$, values are mean±S.E.M.

cultures (Table 1). To further confirm that Al was indeed the effector and was promoting the enhanced activity of ICL, cells were subjected to Al and transferred to control media for 4 h and resuspended in the Al media. In the control media, Al-stressed cells underwent a sharp decline in glyoxylate forming activity. However, when these cells were transferred to a fresh Al medium, an increase was evident. If Al would have been interacting directly with the enzyme a sudden increase with no decrease would have been observed. These observations prompted us to explore some of the various regulatory channels via which Al may be exerting its influence. Experiments were performed in the presence of rifampicin and chloramphenicol, two chemicals known for their potent inhibitory effects on the transcriptional and translational machineries of prokaryotic systems, respectively. Following the incubation of the Al-stressed cells in the control media (depression of ICL activity), the cells were transferred in the Al media containing either rifampicin or chloramphenicol. A marked decrease in ICL activity was observed in cells subjected to these inhibitors (Fig. 4). Thus, it is quite likely that the regulation of ICL activity may be occurring at the transcriptional level. If the modulation was at the translational stage, rifampicin would have had no impact on ICL activity. Cells obtained in media supplemented with different metals were also analysed for their ICL activities (Table 2). As expected, ICL activity due to its ability to

Table 1
Influence of Al stress on various enzymatic activities

Enzymes	Al-stressed cultures	Control cultures	Control fractions +Al-citrate	Control fractions +$AlCl_3$
Isocitrate lyase	0.051 (±0.001)	0.011 (±0.002)	0.013 (±0.004)	0.011 (±0.001)
Isocitrate dehydrogenase	0.091 (±0.021)	0.134 (±0.033)	0.120 (±0.006)	0.127 (±0.002)
∝-Ketoglutarate dehydrogenase complex	0.011 (±0.011)	0.034 (±0.013)	0.030 (±0.001)	0.045 (±0.002)
Malate synthase	0.131 (±0.047)	0.120 (±0.04)	0.126 (±0.024)	0.132 (±0.04)

Units: µmol of substrate consumed or products formed mg protein^{-1} min^{-1}) S.D. are given in parentheses, $n=3$.

Table 2
ICL activity in cultures grown in various metal stresses

Metal stress	Glyoxylate produced[a] (%)
None	21±3
Al^{3+}	100±1
Ga^{3+}	23±5
Ca^{2+}	19±2
Pb^{2+}	20±6
Y^{3+}	28±5

[a] 0.051 μmol glyoxylate produced mg protein^{-1} min^{-1} was taken as 100%. Mean±S.D. are given, $n=3$.

divert citrate, the sole carbon source, to anaplerotic reactions, was present in all the cellular soluble fractions monitored. However, a marked increase was observed only in Al-stressed cells. These findings led us to probe some of the key enzymes that may have a direct impact on the concentration of glyoxylate. ICDH, a competitor for isocitrate, was an obvious choice. This enzyme showed a decrease in activity in Al-stressed cells. KGDHC also showed a sharp diminution (3-fold) in activity in the membrane fraction from stressed cells. However, MLS, an enzyme that may channel the excess of glyoxylate produced in the Al-stressed cells towards the anaplerotic reactions, did not show any change in activity and was predominantly localized in the soluble cellular component (Table 1). It is important to note that ICL, ICDH and MLS were not affected by the presence of Al-citrate or $AlCl_3$ in the cellular preparations from control cells. However, the activity of KGDHC from control cells did increase.

4. Discussion

The foregoing results clearly point to a major restructuring of the TCA cycle as a consequence of an environmental pollutant like Al. This crucial metabolic pathway appears to be an important supplier of the precursor that plays an important role in the adaptation to the trivalent metal. Glyoxylate that is utilised in the biogenesis of oxalate is obtained predominantly by the decomposition of isocitrate, a cleavage mediated by ICL. ICL is a key enzyme that contributes to various anaplerotic reactions and allows numerous organisms to thrive in environments containing nutrients like acetate or fatty acids. Its intervention allows the generation of glucogenic metabolites [21]. For instance, the prolific growth and the adaptability of the pathogenic microbe, *Mycobacterium* has been attributed to its ability to invoke the participation of this enzyme [22]. Seeds are known to have lipids as the major source of energy reserve and develop primarily due to their capacity to modulate the activity of ICL during different stages of morphogenesis [23]. Thus, the presence of this enzyme that cleaves isocitrate into glyoxylate and succinate provides numerous evolutionary advantages to a wide variety of organisms. The glyoxylate is usually converted to malate with the aid of the MLS, while succinate may become a source of reducing power and consequently contributes to the production of energy. Malate is subsequently diverted towards numerous biosynthetic reactions leading to such products as glucose, amino acids and fatty acids [2].

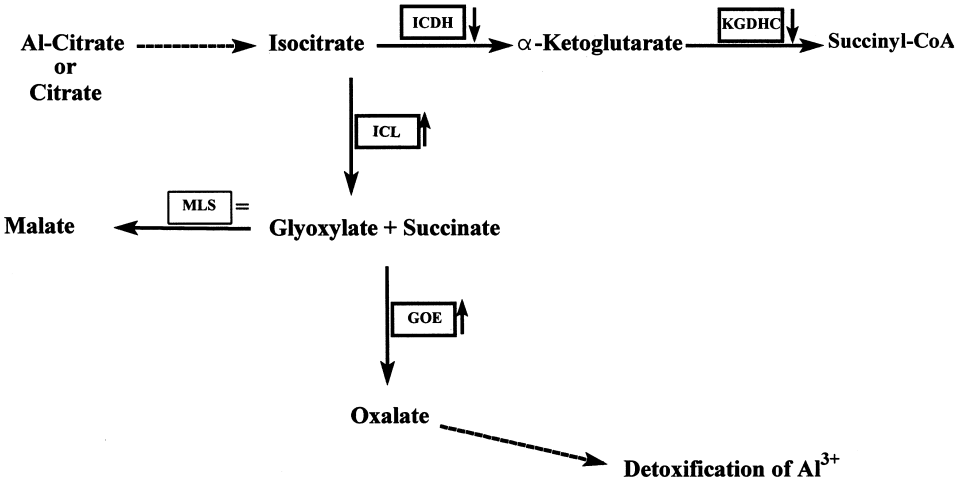
In the present study, a 4-fold increase in ICL activity is observed in Al-stressed cells. However, it is important to note that the glyoxylate is not directed primarily to the generation of malate but is oxidized to oxalate, an important ingredient in the Al-detoxification stratagem in this microbe. Although the production of glyoxylate is accelerated during Al-stress via the activity of ICL, the cells assure its conversion to oxalate by upregulating the activity of the glyoxylate oxidizing enzyme. There is an 8-fold increase in the activity of this enzyme in Al-stressed cells compared to the control [7]. MLS, the other competitor for this substrate did not appear to be affected in Al-stressed cells and its activity is unchanged compared to the control cells. Thus, it is not unconceivable that this strategy promotes the production of oxalate in order to circumvent Al toxicity without completely inhibiting the formation of anaplerotic products via MLS. Thus, the enhanced ICL activity is funneled towards the formation of oxalate. In this instance, it appears that the activity of ICL is not directly coupled to the activity of MLS, an occurrence that is not common in biological systems [24].

The activity of ICL remained relatively unchanged throughout the growth of *Pseudomonas fluorescens* in the Al-stressed media. However, a sharp decline was observed, when most of the trivalent metal was sequestered as an insoluble residue composed primarily of PE, oxalate and phosphate. Hence, it is likely that following the detoxification of Al, as the demand for glyoxylate subsides, the enzyme contributing to the formation of this metabolite is downregulated. Al does not appear to be interacting directly with ICL in a manner that would affect its activity. The soluble cellular fraction (ICL containing component) from the control cells, i.e. cells not exposed to Al, did not show either a decrease or increase in glyoxylate forming activity from isocitrate in the presence of Al-citrate (up to 1 mM) or $AlCl_3$ (up to 0.5 mM). Similar experiments performed with whole cell lysate (soluble and membrane fractions) did confirm the inability of Al to stimulate ICL through its direct interaction with intracellular constituents of the cells. Furthermore, commercial ICL from *E. coli* was not affected by the presence of Al-citrate or $AlCl_3$. These data clearly indicate that the elevation of ICL activity observed was most probably due to cells responding to Al rather than the metal interacting directly with this enzyme. However, the possibility that this toxicant influenced other enzymes and/or metabolites during growth and consequently contributed to the modulation of ICL may also be plausible. The inability of other metals to trigger a similar change confirms the pivotal role of Al in this process and also indicates that the microbe has

different adaptive responses to different metals [25]. Although further experiments have to be performed in order to confirm the veracity of this postulation, the inhibition of ICL activity in cells treated with rifampicin lends support to a possible transcriptionally regulated mechanism. However, it is interesting to note that MLS, a competitor for the substrate glyoxylate was not affected by the presence of Al and rifampicin in the cultures (data not shown).

The activities of two key enzymes of the TCA cycle that were markedly decreased were ICDH and KGDHC. ICDH decarboxylates isocitrate with the concomitant formation of α-ketoglutarate and the reducing factors, NADPH or NADH. This enzyme would compete for the substrate generating the precursor for the production of oxalate, an essential component of the Al detoxification machinery in this system. Hence, a reduction in the activity of ICDH would, in essence, target isocitrate to ICL and lead to the production of glyoxylate. If indeed, Al is eliciting this response, following the detoxification of the trivalent metal ICL should return to the original state, i.e. when the cells were growing in a control medium. Indeed, at 45 h when most of the toxicant is sequestered exocellularly, the activity of this enzyme reverts back to near control conditions. Environmental stresses such as nutrient stress and temperature change have been shown to modulate ICDH activities. Organisms on an acetate diet, have been shown to decrease their ICDH activity via a kinase-mediated phosphorylation [26,27]. The influence of temperature triggers the expression of structurally different ICDH isozymes [28]. The reduction of KGDHC in Al-stressed cells would also indirectly lead to the diversion of isocitrate towards the conversion of oxalate via the oxidation of glyoxylate. This enzyme has been shown to undergo an increase in activity in vitro by addition of Al-lactate [29]. An increase was also observed when $AlCl_3$ was added to the cellular fraction from the control cells. A marked diminution of KGDHC activity is observed in neurological diseases and is postulated to be due to free radical formation and abnormalities in metal metabolism [30]. However, in the present study, it appears to be intricately linked to a flux of isocitrate. Its decrease in activity favours the generation of glyoxylate via ICL. Scheme 1 depicts a possible modulation of the TCA cycle evoked by Al-stress.

In conclusion, it appears that the TCA cycle has been reconfigured as a consequence of Al stress. As the adaptation to this environmental toxicant necessitates the production of oxalate, this cellular system utilizes isocitrate as the generator of glyoxylate. Consequently, this process is promoted via the enhanced activity of ICL and a



ICL : isocitrate lyase

ICDH : isocitrate dehydrogenase

KGDHC : αketoglutarate dehydrogenase Complex

MLS : malate synthase

GOE: Glyoxylate oxidizing enzyme

(↑ increase, ↓ decrease, = unchanged compared to control)

Scheme 1. Modulation of TCA cycle enzymes and the production of oxalate triggered by Al-stress.

decrease in the activity of ICDH and KGDHC. Although more studies have to be performed before the molecular mechanisms participating in this adaptation strategy are delineated, it is quite evident that this metal stress evokes a major readjustment of the energy-producing machinery of the cell and commandeers various enzymes towards the production of glyoxylate.

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