SHORT COMMUNICATION

The Regulation of Phosphoenolpyruvate Carboxykinase and the NADP-linked Malic Enzyme in *Aspergillus nidulans*

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It has previously been suggested that the synthesis of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) in *Aspergillus nidulans* is regulated by a repression–derepression mechanism involving a glycolytic intermediate, and not by induction. Results obtained using compounds that enter the tricarboxylic acid cycle via 2-oxoglutarate, and that can supply both a carbon and a nitrogen source for *A. nidulans*, suggest it is more likely that the synthesis of phosphoenolpyruvate carboxykinase is inducible, and only weakly regulated by carbon catabolite repression. A similar study of the regulation of the NADP-linked malic enzyme (EC 1.1.1.40) indicates that it too may be inducible.

INTRODUCTION

The gluconeogenic enzyme phosphoenolpyruvate carboxykinase (EC 4.1.1.32; PEPCK) catalyses the conversion of oxaloacetate to phosphoenolpyruvate. In both *Neurospora crassa* and *Aspergillus nidulans*, the synthesis of PEPCK is strongly regulated, and activity is low in sucrose-grown mycelia but high in acetate-grown mycelia (Flavell & Fincham, 1968; Beever, 1975; Armitt et al., 1976). It has been proposed that the synthesis of PEPCK in both these organisms is regulated by a glycolytic intermediate, high activity in acetate-grown mycelia resulting from derepression rather than induction (Beever, 1975; Armitt et al., 1976). McCullough & Roberts (1974) showed that the NADP-linked malic enzyme (EC 1.1.1.40; NADP-ME) of *A. nidulans* converts malate to pyruvate and is required for growth on carbon sources metabolized via the tricarboxylic acid cycle. They suggested that this enzyme might be inducible by a C₄ dicarboxylic acid.

We have been interested in the regulation of the synthesis of enzymes involved in the utilization of acetate in *A. nidulans* as an extension of the intensive analysis of the regulation of the acetamidase (Hynes, 1978, 1979). These studies have indicated that PEPCK and NADP-ME syntheses are regulated separately from the syntheses of acetyl-CoA synthetase (EC 6.2.1.1) and the glyoxylate cycle enzymes malate synthase (EC 4.1.3.2) and isocitrate lyase (EC 4.1.3.1) (unpublished results). The syntheses of PEPCK and NADP-ME appear to be inducible, but not by the same induction mechanism or metabolite, and to be relatively weakly regulated by carbon catabolite repression.

METHODS

*Media.* The media used were those of Cove (1966). Basic medium, designated –CN, lacked both a carbon and a nitrogen source.

*Strains.* All strains used were derived from the Glasgow strain of Pontecorvo et al. (1953). The wild-type strain used was of genotype *biA1; niiA4* (Pateman et al., 1967). The *facA303* strain was of genotype *biA1; acrA1;
amd9; facA303; sb3; nicB8; riboB2, and was derived from crosses in the laboratory. The gdhB101 strain was of genotype biA1; gdhB101; nilA4 and was derived as a mutant isolated from the wild-type strain by virtue of inability to grow on L-glutamine as the sole carbon source. Complementation analysis showed allelism with the gdhB mutant of Arst et al. (1975) (unpublished results). Full meanings of the gene symbols are given by Clutterbuck & Cove (1974).

**Growth of strains.** Strains were grown in 1% (w/v) sucrose + 20 mM-ammonium tartrate medium for 16 h, and aseptically transferred to the treatment medium for a further 6 h before harvesting and enzyme determinations.

**Enzyme determinations.** A 0.2 g sample of mycelium was ground with glass beads in 2.7 ml extraction buffer and centrifuged to produce a cell-free extract. PEPCK activity was determined by the method of Armitt et al. (1976) and NADP-ME activity by the method of McCullough & Roberts (1974). Protein was determined by the Lowry method.

**RESULTS**

PEPCK activity was determined in wild-type mycelia after transfer to a range of media (Table 1). It was increased in mycelia transferred to medium containing acetate, compared with medium either lacking a carbon source or containing glycerol as the carbon source. The presence of sucrose prevented this increase. Acetamide, L-threonine, ethanol and D-quinate, which are all potential sources of acetyl-CoA, also led to increased activities of PEPCK; the increase being related to the relative abilities of these compounds to serve as sole carbon sources for *A. nidulans*. PEPCK activity was also high in mycelia transferred to medium containing carbon sources such as L-proline, L-glutamate or γ-aminobutyric acid that enter the tricarboxylic acid cycle via 2-oxoglutarate, and that require gluconeogenic enzymes for their utilization as carbon sources. The PEPCK activity in mycelia transferred to medium containing malate, citrate or succinate as sole carbon sources was only twofold higher than in mycelia transferred to medium lacking a carbon source (data not shown). However, these compounds are not good carbon sources for *A. nidulans* and may not rapidly enter the cells.

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Addition(s) to -CN medium</th>
<th>Specific activity [nmol product min⁻¹ (mg protein)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>10 mM-NH₄Cl</td>
<td>PEPCK: 14 7 15 8</td>
</tr>
<tr>
<td>Wild-type</td>
<td>25 mM-acetate + 10 mM-NH₄Cl</td>
<td>220 8 31 11</td>
</tr>
<tr>
<td>Wild-type</td>
<td>25 mM-acetamide</td>
<td>65 17 ND ND</td>
</tr>
<tr>
<td>Wild-type</td>
<td>25 mM-L-threonine</td>
<td>33 ND 18 ND</td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.5% (v/v) ethanol + 10 mM-NH₄Cl</td>
<td>120 ND 98 ND</td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.5% (v/v) D-quinate + 10 mM-NH₄Cl</td>
<td>210 ND 20 ND</td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.5% (v/v) glycerol + 10 mM-NH₄Cl</td>
<td>8 ND 12 ND</td>
</tr>
<tr>
<td>Wild-type</td>
<td>25 mM-L-proline</td>
<td>99 47 330 63</td>
</tr>
<tr>
<td>Wild-type</td>
<td>25 mM-L-proline + 10 mM-NH₄Cl</td>
<td>91 10 ND ND</td>
</tr>
<tr>
<td>Wild-type</td>
<td>25 mM-L-glutamate</td>
<td>86 ND 24 ND</td>
</tr>
<tr>
<td>Wild-type</td>
<td>25 mM-γ-aminobutyric acid</td>
<td>90 ND ND ND</td>
</tr>
<tr>
<td>facA303</td>
<td>25 mM-acetate + 10 mM-NH₄Cl</td>
<td>25 ND 8 ND</td>
</tr>
<tr>
<td>facA303</td>
<td>25 mM-L-proline</td>
<td>91 ND 430 ND</td>
</tr>
<tr>
<td>gdhB101</td>
<td>25 mM-L-proline</td>
<td>4 4 34 ND</td>
</tr>
</tbody>
</table>
A critical test to determine whether the synthesis of PEPCK was directly subject to strong carbon catabolite repression or whether the effects of carbon catabolite repression were indirect and due to effects on inducer formation was possible using compounds that can serve as both a carbon and a nitrogen source and enter the tricarboxylic acid cycle via 2-oxoglutarate. L-Proline is metabolized at a high rate, and enzymes for its utilization are subject to both carbon catabolite and nitrogen metabolite repression (Kinghorn & Pateman, 1973, 1974; Arst & MacDonald, 1975). The absence of either a good carbon source or a good nitrogen source allows synthesis of the enzymes of L-proline metabolism. In sucrose medium with L-proline as the sole nitrogen source, the enzymes necessary to convert L-proline to 2-oxoglutarate and ammonium are synthesized as they are derepressed for nitrogen metabolite repression. However, their synthesis remains repressed in sucrose + L-proline + ammonium medium as both carbon catabolite repression and nitrogen metabolite repression operate. In medium in which L-proline provides both the carbon and the nitrogen source, both carbon catabolite repression and nitrogen metabolite repression are relieved.

PEPCK activity was determined in wild-type mycelia grown in sucrose + L-proline medium in both the presence and absence of ammonium. PEPCK activity is not required for growth in either medium, and carbon catabolite repression operates, but in the presence of ammonium, L-proline would not be metabolized via 2-oxoglutarate at a significant rate, due to nitrogen metabolite repression operating as well. As the activity of PEPCK was nearly fivefold higher in mycelia grown in sucrose + L-proline medium compared with sucrose + L-proline + ammonium medium (Table 1), the synthesis of the PEPCK enzyme cannot be directly subject to strong carbon catabolite repression. The very low PEPCK activity in mycelia grown in sucrose + L-proline + ammonium medium must thus be largely the result of carbon catabolite repression and nitrogen metabolite repression of enzymes involved in inducer formation from L-proline, rather than direct carbon catabolite repression of PEPCK synthesis. The sum total of carbon catabolite repression of all enzymes involved in converting L-proline to the true inducer, plus the direct effect on PEPCK synthesis, is represented by the difference in PEPCK activity in mycelia transferred to L-proline medium in the presence and absence of sucrose, and this was only twofold. In addition, PEPCK activity was higher in mycelia grown in sucrose + L-proline medium than in mycelia grown in sucrose + ammonium medium, further indicating induction.

In a gdhB101 mutant, which lacks NAD-linked glutamate dehydrogenase activity and so cannot metabolize L-proline further than L-glutamate, there was no detectable increase in PEPCK activity after transfer to medium containing L-proline as the sole carbon source (Table 1). In addition, in a facA303 mutant, which lacks acetyl-CoA synthetase activity, PEPCK activity remained low in mycelia transferred to acetate medium, but increased to the same level as in the wild type in mycelia transferred to L-proline medium. Thus acetate and L-proline need to be metabolized to allow increased synthesis of PEPCK, and the simple absence of hexose metabolites per se is insufficient to result in high PEPCK activity.

A similar approach was used to study the regulation of NADP-ME (Table 1). Activity was increased up to twofold in mycelia transferred to acetate, acetamide, L-threonine or D-quinate medium compared with transfer to medium either lacking a carbon source or containing glycerol. The presence of sucrose prevented this increase in acetate-grown mycelia, and no increase was observed in the facA303 strain lacking acetyl-CoA synthetase. The presence of ethanol resulted in approximately sixfold higher enzyme activities than those in glycerol medium or in medium lacking a carbon source. Considering compounds metabolized via 2-oxoglutarate, the presence of L-glutamate resulted in about twofold higher NADP-ME activity compared with glycerol-grown mycelia, while growth in the presence of L-proline resulted in more than 20-fold higher enzyme activities. This effect of L-proline was dependent on metabolism via the NAD-linked glutamate dehydrogenase since it was virtually abolished in the gdhB101 strain.

NADP-ME activities were about twofold higher in mycelia grown in medium lacking a carbon source compared with medium containing sucrose. This indicated that some carbon
catabolite repression might be operating. This was further supported by the observation that the presence of sucrose greatly reduced the effect of L-proline on enzyme activities.

**DISCUSSION**

Armit et al. (1976) found that the PEPCK activity in *acuG* mutant strains (which lack fructose-1,6-bisphosphatase activity) was fivefold higher than in the wild-type strain after growth in acetate medium. They interpreted this as supporting their view that the synthesis of PEPCK is regulated by a repression–derepression mechanism in that, in *acuG* mutant strains, no metabolites above fructose 6-phosphate could be formed to effect repression. However, this observation is also compatible with the view that PEPCK synthesis is inducible, the higher levels of PEPCK in acetate-grown *acuG* mycelia being attributable to inducer accumulation. NADP-ME activity was also found to be eightfold higher in *acuG* mutants and in *acuF* mutants (which lack PEPCK activity) than in the wild-type strain (Armit et al., 1976). This too can be interpreted as evidence for the proposal that NADP-ME is subject to induction, these lesions resulting in inducer accumulation.

The data indicate that PEPCK is probably inducible and is only subject to weak regulation by carbon catabolite repression. The use of L-proline as a source of induction in the presence and absence of sucrose has provided a means of distinguishing between the direct effects of carbon catabolite repression on PEPCK and on inducer formation. Compounds that result in PEPCK induction are those metabolized via the tricarboxylic acid cycle and the level of induction is roughly correlated with the ability of these compounds to serve as sole carbon sources.

The situation for NADP-ME is much less clear since enzyme activities in the presence of different carbon sources are not simply correlated with their ability to support growth. For example, it is not obvious why growth in ethanol should result in higher enzyme activities than growth in acetate. However, again using L-proline as a source of induction in the presence and absence of sucrose, it is clear that induction rather than carbon catabolite repression by hexoses is the major source of regulation. The much greater induction by L-proline than L-glutamate of NADP-ME is paralleled by the greater ability of L-proline to induce NAD-linked glutamate dehydrogenase (Hynes & Kelly, 1977). The data indicate that the inducing metabolite or metabolites are unlikely to be the same for PEPCK and NADP-ME, but further work is required to determine their nature.

Regulation of these two enzymes by carbon catabolite repression thus does not appear to be as important as regulation by induction. In *A. nidulans*, *cre* mutants have been isolated which are altered in the response of various enzymes to the presence of sources of carbon catabolite repression such as glucose and sucrose. Enzymes affected include acetyl-CoA synthetase, isocitrate lyase and malate synthase (Kelly & Hynes, 1977). We have found that these *cre* mutants are not altered in PEPCK or NADP-ME regulation (unpublished results).

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**REFERENCES**


Short communication

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