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Production of Glutamine Synthetase by *Proteus mirabilis* in Short Time Periods

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It has been demonstrated for *Proteus mirabilis* that 40 mM urea, in the presence of glucose, restrain the production of glutamine synthetase without modifying the glutamate dehydrogenase induction. Glutamine synthetase is derepressed when urea is removed of the medium.

The enzyme which catalyzes the urea hydrolysis to ammonium and carbon dioxide, the urease, is induced in *Proteus mirabilis* by urea (10). Adding the produced ammonium to an α-ketoglutarate molecule, the ammonium can be used by the bacteria to synthetize glutamate. This reaction is catalyzed by a NADP(H)linked glutamate dehydrogenase (7), the glutamate causing a feed back inhibition in the urease, the first enzyme of the process. The possibility of a second incorporation of the ammonium derived from the urea, this time on glutamate to synthetize glutamine is the subject of this communication.

Materials and Methods

Proteus mirabilis NCIB 5887 is cultivated at 37° with constant aeration in a Fildes medium modified by Sandys (5), to which carbon and nitrogen are added under the conditions specified in each case. The culture growth is measured as an increase in the optical density at 910 nm.

The loss of urea in the media is measured by the p-dimethylamine benzaldehyde method (1), the removal of ammonium by Nessler's method (9) and the loss of glucose by the glucose oxydase method (8).

Cell-free extracts are prepared as shown previously (7), in the presence of 0.14 M 2-mercaptoetanol.

The glutamate dehydrogenase activity has been measured by oxidation of the NADPH, as a decrease of the optical density at 340 nm (4). The glutamine synthetase activity is estimated as a γ-glutamyl hydroxamate production, using hydroxilamine as substratum (2).

Protein estimation is carried out by the LOWRY *et al.* method (6).

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Results and Discussion

The *Proteus mirabilis* growth is maximum in media containing 0.1 M glucose and 40 mM urea; the cultures double their dry weight in the first eight hours of incubation. The presence of glucose induces a NADP-linked glutamate dehydrogenase (fig. 1); this result is in accordance with those of HALPERN and UMBARGER for *Escherichia coli* (3). The substitution of glucose by different organic acids, glutamate included, stabilizes or decreases the intracellular glutamate dehydrogenase level.

The use of 80 mM urea in the media which contain 0.1 M glucose stop both the growth and the glutamate dehydrogenase synthesis.

Nevertheless, under the conditions specified in Figure 1, the glutamine synthetase activity of the cultures decreases to level zero after a five hour incubation. This decrease of activity is much quicker when 40 mM is used instead of urea. When 0.5 mM ammonium is used, the production of glutamine synthetase increases although the bacterial population does not show any growth during the first five hours (fig. 2).

In order to know the role of the urea in the production of glutamine synthetase,

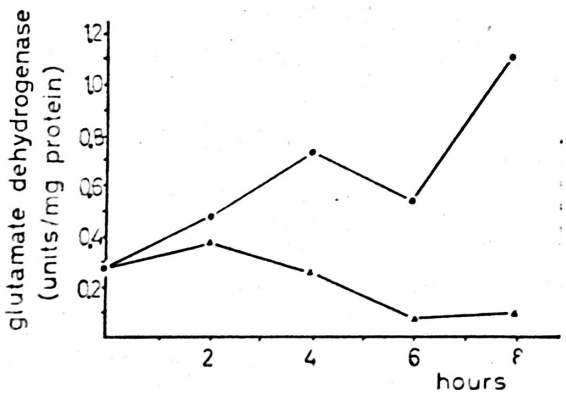

Fig. 1. *Time course of the induction of glutamate dehydrogenase by glucose in* Proteus mirabilis.

At time zero, glucose was added up a final concentration of 0.1 M. Urea was utilized as nitrogen source. The specific activity of glutamate dehydrogenase was determined in the extracts of the corresponding cultures at the indicated times. ●—● cells growing on 40 mM urea; ■—■ cells growing on 80 mM urea.

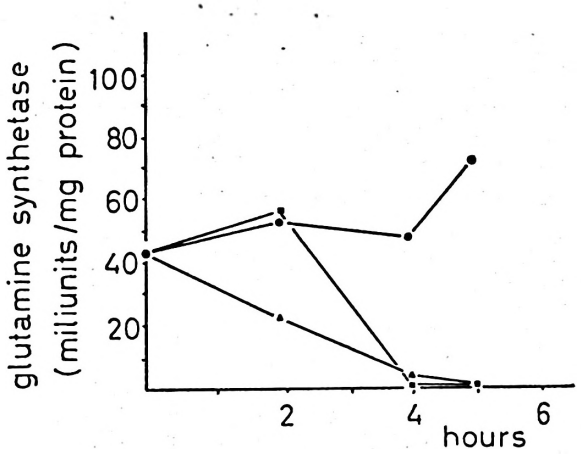

Fig. 2. *Time course of the synthesis of glutamine synthetase by* Proteus mirabilis *in function of nitrogen source.*

0.1 M glucose was utilized as carbon source. ●—● cells growing on 0.5 mM NH_4^+; ■—■ cells growing on 40 mM NH_4^+; ▲—▲ cells growing on 40 mM urea.

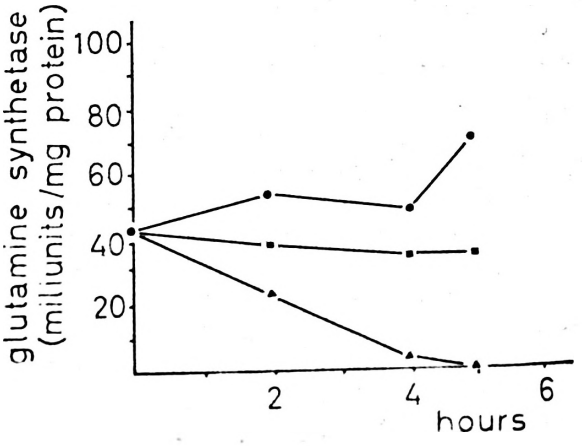

Fig. 3. *Time course of the repression of glutamine synthetase by urea in* Proteus mirabilis.

0.1 M glucose was utilized as carbon source. ●—● cells growing on 0.5 mM NH_4^+; ■—■ cells growing on 0.5 mM NH_4^+ + 40 mM urea; ▲—▲ cells growing on 40 mM urea.

experiments shown in Figure 3 were planned. The enzyme synthesis increases when 0.1 M glucose and 0.5 mM ammonium are present in the culture media and it is stabilized when besides both those nutrients, 40 mM urea is added to the culture medium.

In both cases there is no growth during the five hour's experiment. The glutamine synthetase activity decreases to level zero when cells grow on 0.1 M glucose and 40 mM urea. This culture is in logaritmic phase of growth after five hours of incubation. After this period of time there is no exhaustion of any of the nutrients of the medium.

We have wanted to analize the possibility of this occurring through an enzyme inactivation by urea. This fact has been described by WOOLFOLK *et al.* (11) for the glutamine synthetase of the *Escherichia coli*. A 1 M urea concentration causes the *in vitro* inactivation of the enzyme. The fact that the bacteria use a much smaller concentration (25 time less) of urea and that the cells only consume after five hours of incubation half the urea present in the medium (20 μmoles/ml) seems to exclude this hypothesis.

The possibility of an enzyme inactivation seems also to be excluded through preincubation of the extracts with ATP.

We may affirm that in our experiments there has been a repression of the glutamine synthetase due to the presence of urea. The enzyme is derepressed when the urea is removed to the medium. This would nullify the claim of the biosynthetical way formulated as: urea $\longrightarrow$ NH_4^+ $\longrightarrow$ glutamate $\longrightarrow$ glutamine. The regulation mechanism seems to be at a finer level than the action of urea on the glutamate dehydrogenase.

Resumen

Proteus mirabilis puede producir ureasa inducida por urea. Las concentraciones de urea que permiten esta síntesis no alteran la inducción de glutamato dehidrogenasa por glucosa, lo que permite pensar que la hidrólisis de la urea aboca, en alguna extensión, en síntesis de glutamato. Sin embargo, la misma concentración (40 mM) reprime la producción de glutamina sintetasa, siendo el enzima derreprimido cuando se suprime la urea del medio de cultivo. Esto hace concluir que la vía de aminación directa termina en la síntesis de glutamato, no pudiendo llegar a glutamina.

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