Vol. 92, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1980 Pages 134-140

RELATION BETWEEN STRUCTURE AND FUNCTION OF *Neurospora crassa* GLUTAMINE SYNTHETASE

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Received November 16,1979

Summary

Two distinct monomers, α and β participate in the structures of different oligomers of *Neurospora crassa* glutamine synthetase (EC 6.3.1.2). In ammonium-limited cultures a tetrameric form composed mainly of α monomers was found. In excess of nitrogen an octameric form composed mainly from β monomers is the predominant oligomeric state. The presence of both monomers was observed in intermediate oligomeric forms.

Introduction

The nitrogen source regulates the concentration (1) and the *de novo* synthesis of glutamine synthetase (GS) in *Neurospora crassa* (2). On glutamate as nitrogen source the rate of synthesis of GS is 10-fold higher than in glutamine and corresponds to a similar difference in the specific mRNA levels of the enzyme (3). *N. crassa* GS purified from mycelia grown on glutamate is structured in an octameric form composed of monomers with a molecular weight of 48,000 (4). In Fed-batch ammonium-limited cultures the activity of GS increased and instead of an octamer, mainly a tetrameric form of GS was found (5). This lower oligomeric state of GS was also found in glutamine auxotrophs of *Neurospora* (6). Since these mutants grow in limited ammonium, it is possible that the two oligomeric states of GS correspond to different gene products (6), and have a different function in ammonia fixation.

Recently it has been found that two different monomers contribute to the activity of GS (Sánchez, *et al*; submitted for publication, 1979). These

0006-291X/80/010134-07$01.00/0

monomers can be separated in acrylamide gel electrophoresis in SDS-urea where one of them (α) runs slightly slower than the other (β).

In this paper we report the relation that exists between these monomers with the oligomeric state and the function of GS in Neurospora crassa.

Material and Methods

Strains and Chemicals. - Neurospora crassa wild-type strain 74-A, the glutamic acid dehydrogenase deficient mutant am-1 and the glutamine auxotroph gln-1a, were obtained from the Fungal Genetics Stock Center at the Humboldt State University Foundation, Arcata, Calif. U.S.A. The glutamine auxotroph gln-1c was obtained in our laboratory and is an allele of the auxotrophs previously reported (6). All chemicals used were analytical grade.

Growth Conditions. - Batch cultures of N. crassa were grown after inoculating conidia in Vogel's minimal medium (7) with 1.5% sucrose. The nitrogen source was glutamate at 25°C, or 37°C and glutamine at 37°C. Fed-batch ammonium-limited cultures at 25°C with a constant growth rate, were achieved as reported (5), except that the conidia were not previously incubated in the absence of nitrogen. Growth was determined as described (1).

Determination of Glutamine Synthetase Activity. - Glutamine synthetase measured as transferase activity was assayed as described by Ferguson and Sims (8) in cell-free extracts of Neurospora prepared as in a previous work (1).

Immunoprecipitation of in vivo-labeled Glutamine Synthetase. - The cultures were pulsed with [^{3}H] leucine for one hour before harvesting. The cell-free extracts were sedimented in sucrose gradients (4) and fractions of the principal peaks of activity were precipitated with $(NH_4)_2SO_4$ at 70% saturation, resuspended and dialyzed overnight. Aliquots were immunoprecipitated with antibody against GS as described (2).

Electrophoresis and Fluorography. - The immunoprecipitates were subjected to acrylamide slab gel electrophoresis in the presence of SDS and 7 M urea (Sánchez, et al; submitted for publication, 1979), stained with Coomassie blue (9) and treated for fluorography (10).

Purification of Glutamine Synthetase. - Conidia obtained from slants with glutamine (10 mM) as nitrogen source, were used to inoculate cultures with glutamate at 25°C. After 3 hs the germinated conidia were filtered, washed and dried with acetone (1). From this powder the octameric GS was purified as previously reported (4). The tetrameric GS from the glutamine auxotroph gln-1c was purified as reported (4) from cultures grown on glutamate at 37°C, except for the following modifications. The cell-free extract was prepared in buffer A diluted 10-fold, this buffer was used to equilibrate the DEAE-cellulose column, which was eluted with Buffer A (Buffer A contains 50 mM imidazole, 50 mM glutamic acid, 80

mM $MgSO_4$, 50 mM K_2SO_4, 0.5 mM EDTA, 5 mM 2-mercaptoethanol, 25 mM $NaHSO_3$, pH 7.2). The fractions with activity were pooled and made 2.25 mM $MnCl_2$ and after added to the sepharose-anthranilic acid column previously equilibrated with Buffer A 2.25 mM $MnCl_2$, the enzyme was eluted with 40 mM AMP in this buffer. The protein was precipitated with $(NH_4)_2SO_4$ at 70% saturation and resuspended and dialyzed against Buffer A.

Results and Discussion

To study the distribution of the two different monomers of GS in the different oligomeric forms of this enzyme, the wild-type 74-A strain was grown on batch cultures at 25°C with glutamate as nitrogen source and in Fed-batch cultures ammonium-limited. In these conditions the GS is mainly found as an octamer (1) and tetramer (5), respectively. In addition the 74-A strain and the gln-1c mutant strain were grown in glutamine

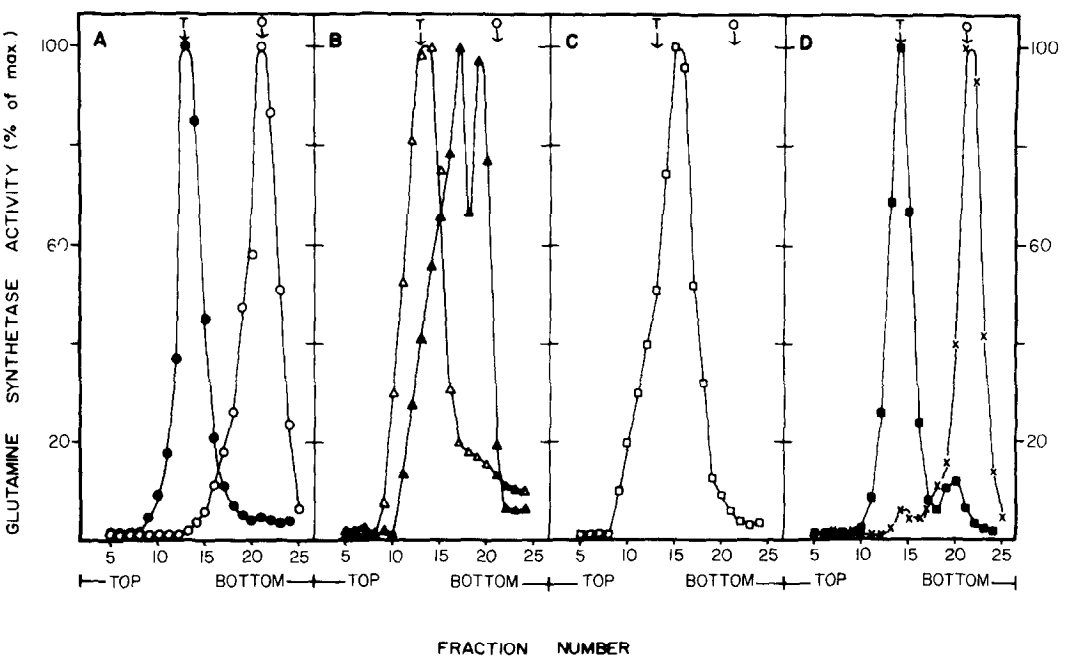

Fig. 1. Sucrose gradient sedimentation of glutamine synthetase from crude extracts: A, wild-type grown at 25°C on 5 mM glutamate (o) or ammonium-limited (●) B, at 37°C on 5 mM glutamine the wild-type (▲) and the mutant strain gln-1c (△) and C, the am-1 strain at 25°C ammonium-limited (□). D, purified GS from cultures grown on glutamate at 25°C from the wild-type (x) and from the gln-1c at 37°C (■). T tetramer, O octamer.

at 37°C and the am-1 mutant strain in limited-ammonium. The glutamine auxotrophs were grown on glutamine at 37°C since in this condition they had a sizable activity of GS structured in a tetramer (6). All the cultures were pulsed with [^{3}H]leucine as described in methods.

In Fig. 1 is presented the transferase activity in sucrose gradients from extracts of these cultures. As expected the tetrameric GS was present in extracts of the wild-type ammonium-limited and in the gln-1c (Fig. 1 A,B), the octamer was found in the 74-A strain grown on glutamate (Fig. 1 A). On the other hand in the wild-type grown on glutamine at 37°C, in addition to the octamer, a well defined peak of activity was found in the sucrose gradient left and next to this oligomer (Fig. 1 B). A different oligomer of GS, that banded next and right to the tetramer, was also found in the am-1 mutant strain ammonium-limited (Fig. 1C). The bands of activity immunoprecipitated, stained with Coomassie blue after acrylamide gel electrophoresis in SDS-urea, are shown in the top of Fig. 2. It is clear that the octamer is composed mainly by β monomers (Fig. 2c) and the tetramer by α monomers (Fig. 2d). Both monomers were found in the intermediate oligomeric forms that banded in sucrose gradient between the tetramer and the octamer (Fig. 2a,b). As these oligomers (am-1 strain and wild-type grown on glutamine at 37°C) band closer to the octameric form, an increase in the β monomers and a decrease in the α monomers was apparent. Only in the case of the wild-type strain grown in glutamine at 37°C the monomers corresponded to the pool of two peaks of activity of the sucrose gradient.

The fluorography of the gel (Fig. 2 bottom) shows that the distribution of α and β monomers resembles very closely what is seen after staining the gel (Fig. 2 top), and emphasizes that the newly synthesized

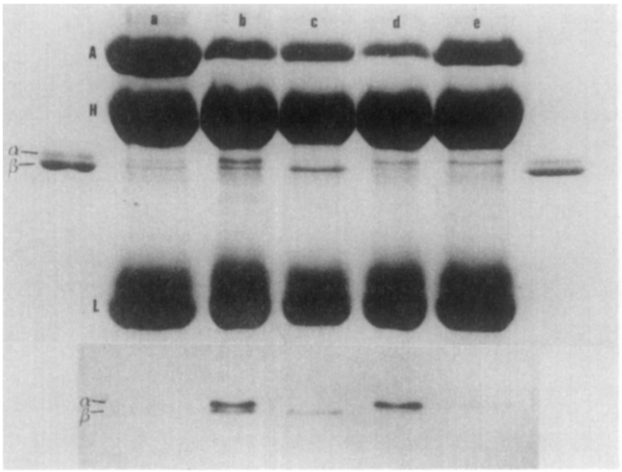

Fig. 2. Top panel Gel after coomassie-blue staining: a) wild-type on glutamine, b) am-1 strain ammonium-limited, c) wild-type on glutamate, d) wild-type ammonium-limited and e) gln-1c strain on glutamine. In the first and the last tracks of the gel purified non-labeled GS from glutamate grown cultures was run as a marker. For other conditions see Fig. 1 and methods. Bottom panel: gel after fluorography. A albumin, H γ-globulin heavy chain, L γ-globulin light chain, α and β monomers of GS.

α's are incorporated almost exclusively in tetramers and the β's in octamers. In contrast, hybrid monomers were found in the oligomers intermediate between the tetramer and the octamer.

It has been possible to obtain growth conditions in which the octamer can be purified with only traces of α monomers (compare Fig. 2 and 3). These data indicate that the α monomers are not required for the structure and activity of the octamer. The tetramer purified from gln-1c mutant strain is composed only of α monomers (Fig. 3).

The presence of some α monomers in the octamer and of some β monomers in the tetramer, of the wild-type strain may be the result of monomer hybridization, or that the equilibrium of α's favors more the arrangement in tetramers than in octamers, and viceversa for the β's. The fact that the α monomers of the mutants strain gln-1a and gln-1c in

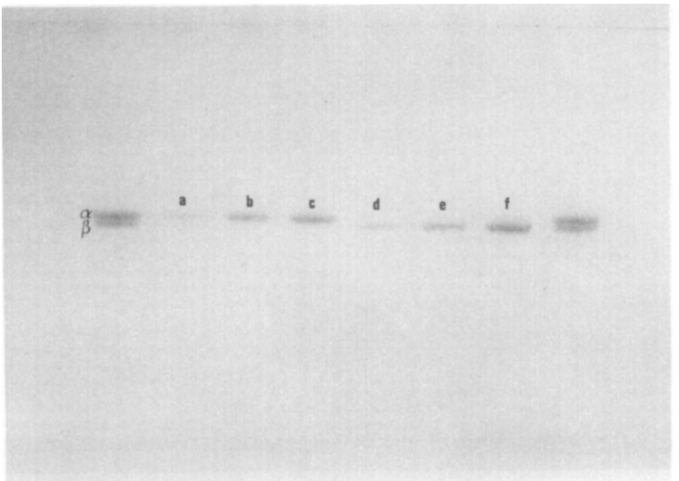

Fig. 3. Gel after coomassie-blue staining of the purified preparations of GS obtained from gln-1c strain (a, b and c) and from wild-type (d, e and f). The cultures were grown as described in methods. In the first and the last traks a mixture of α and β monomers were run as markers.

addition to the tetramer, are able to form octamers in a low proportion, and that the monomers of the wild-type strain behave oppositively (Fig. 1d), is in favour of the equilibrium hypothesis. This equilibrium would also depend of the intracellular conditions. It is in intermediate oligomeric forms of GS where both monomers are found in an important proportion, then it is possible that these intermediate states are composed by hybrids of α and β monomers, which appear when the nitrogen content is neither limiting nor in excess. Experiments are in progress to purify the wild-type tetramer and test in vitro if the presence of this form in low ammonium and the octamer in nitrogen excess, are related with a different affinity for ammonium of these oligomers. Recent evidence has demonstrated that glutamate synthase (GOGAT) is present in ammonium-limited cultures (Hummelt, et al; submitted for publication, 1979). This enzyme together with the tetrameric GS participates in the assimilation of low ammonium concentrations.

Acknowledgments: This work was supported in part by CONACyT, México.

We are indebted to Dr. Rafael Palacios for advice and criticism throughout this work.

REFERENCES

1. Vichido, I., Mora, Y., Quinto, C., Palacios, R., and Mora, J. (1978). J. Gen. Microbiol. 106: 251-259.
2. Quinto, C., Mora, J., and Palacios, R. (1977). J. Biol. Chem. 252: 8724-8727.
3. Sánchez, F., Campomanes, M., Quinto, C., Hansberg, W., Mora, J., and Palacios, R. (1978). J. Bacteriol. 136: 880-885.
4. Palacios, R. (1976). J. Biol. Chem. 251: 4787-4791.
5. Limón-Lason, J., Lara, M., Resendiz, B., and Mora, J. (1977). Biochem. Biophys. Res. Commun. 78: 1234-1240.
6. Dávila, G., Sánchez, F., Palacios, R., and Mora, J. (1978). J. Bacteriol. 134: 693-698.
7. Vogel, H.J. (1964). Am. Nat. 98: 534-546.
8. Ferguson, A.R., and Sims, A.P. (1974). J. Gen. Microbiol. 80: 159-171.
9. Martin, A.F., Prior, G., and Zak, R. (1976). Anal. Biochem. 72: 577-585.
10. Bonner, W.M., and Laskey, R.A. (1974). Eur. J. Biochem. 46: 83-88.