ACTIVITY OF THE HEXOSE MONOPHOSPHATE SHUNT IN A MUTANT OF *SACCHAROMYCES CARLSBERGENSIS* LACKING NADP DEPENDENT GLUTAMATE DEHYDROGENASE ACTIVITY

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1. Introduction

In yeast, as in many other fungi, two glutamate dehydrogenase activities occur [1]. One enzyme is specific for NAD (EC 1.4.1.4). This enzyme is preferentially synthesized under conditions favouring the breakdown of glutamate [2, 3]. The other enzyme is specific for NADP (EC 1.4.1.2), and is involved in the biosynthesis of glutamate [4–6]. Since other pathways for the assimilation of NH_4^+ are less active [6], it is generally assumed that in yeast the synthesis of glutamate by the NADP dependent glutamate dehydrogenase constitutes the major route of NH_4^+ assimilation.

The recent isolation and characterization of mutants lacking the NADP dependent glutamate dehydrogenase activity proves that these assumptions are correct ([7], see also Materials and methods).

It was observed several times that under certain conditions the presence of NH_4^+ in the medium can be correlated with an increased activity of the hexose monophosphate (HMP) shunt [8, 9]. It is assumed that this higher activity is due to an increased production of $NADP^+$ by the action of the NADP dependent glutamate dehydrogenase. This theory is verified in a mutant lacking this enzyme.

2. Materials and methods

In these experiments the following strains derived from *Saccharomyces carlsbergensis N.C.Y.C. 74* were used [10]; the haploid parent strain with mating type α, carrying the marker lys_2, and the diploid parent strain with the genotype a/α, lys_2, *LYS*, ade_1, *ADE*. Mutagenesis by 1-nitrosoimidazolidone-2 of the haploid parent strain was performed as described before [10]. Cells were plated on minimal medium with both glutamate (1%) and $(NH_4)_2SO_4$ (2%) as nitrogen source. Colonies were replicated on the same medium without glutamate. 80 Mutants were isolated which did not grow on the latter medium. After testing for the presence of NADP dependent glutamate dehydrogenase only 3 mutants were found with a very low level of this enzyme (about 3% of the wild type). One of these mutants, with the genotype α, lys_2, *glu*, was crossed with a strain of the genotype *a*, ade_1, *GLU*. Random spore analysis of this hybrid indicated that the *glu* phenotype resulted from one mutational event. From this cross a strain was selected with the genotype *a*, ade_1, *glu*. This strain was crossed with the original *glu* mutant, resulting in a diploid strain homozygous for *glu* (a/α, lys_2, *LYS*, ade_1, *ADE*, *glu*, *glu*). The diploid parent strain has a generation time of about 2 hr on liquid minimal medium with or without 0.05 M glutamate, whereas for the diploid *glu* mutant a generation time was observed of 8 hr on minimal medium, and 3.5 hr on the same medium with 0.05 M glutamate. These results clearly indicate that the production of glutamate by the action of the NADP dependent glutamate dehydrogenase under these conditions is the major route of NH_4^+ assimilation. Unless indicated otherwise, cells were grown on minimal medium containing 2% glucose and salts and vitamins as described by Roman [11]. Nutrilites for the auxotrophic markers were added. Sporulation was induced

Table 1
The effect of $(NH_4)_2SO_4$ on the activity of the HMP shunt in protoplasts of the diploid parent strain and of the diploid *glu* mutant.

	Parent strain	Parent strain $+(NH_4)_2SO_4$ (2 mM)	*glu* mutant	*glu* mutant $+(NH_4)_2SO_4$ (2 mM)
CO_2 produced by the HMP shunt after 120 min (pmoles)	84	224	96	108

Protoplasts were used in a concentration corresponding to an absorbance of 15 at 500 nm, which is equivalent to a protein concentration of 5 mg per ml, and incubated at 30° in a medium containing 0.015% casamino acids, 1.2% glucose, 12% mannitol and 1.5 μCi (0.52 μM) [1-^{14}C]glucose or 1.5 μCi (0.44 μM) [6-^{14}C]glucose.

on 0.4% sodium acetate. For solid media 1% Oxoid agar No. 1 was added.

Cell extracts were prepared ultrasonically. Protein was determined according to Lowry et al. [12]. The NADP dependent glutamate dehydrogenase activity was determined as described by Holzer and Schneider [1]. The preparation of the protoplasts and the determination of the activity of the hexose monophosphate (HMP) shunt (the production of $^{14}CO_2$ from [1-^{14}C]-glucose minus that of [6-^{14}C]glucose) were performed as described before [8, 13].

3. Results and discussion

In short term experiments the stimulation of the HMP shunt is probably only found if the cells are depleted of their nitrogen pool by protoplast formation [8] or by washing, and incubating in a medium without a nitrogen source [9] since in intact cells immediately after harvesting, no such stimulation of the HMP shunt was observed.

From the results presented in table 1 it is clear that the stimulation of the HMP shunt, observed previously in protoplasts of wild type cells of *S. carlsbergensis* [8], is not present in protoplasts of the diploid *glu* mutant. These results prove that the availability of $NADP^+$ under these conditions is the limiting factor for the activity of the HMP shunt. In yeast NADP is present predominantly in its reduced form [9], the increase in the production of $NADP^+$ by the action of the NADP dependent glutamate dehydrogenase leading to an increase in the activity of this pathway of glucose metabolism.

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References

[1] H. Holzer and S. Schneider, Biochem. Zeit. 329 (1957) 361.
[2] G. Hierholzer and H. Holzer, Biochem. Zeit. 339 (1963) 175.
[3] H. Westphal and H. Holzer, Biochim. Biophys. Acta 89 (1964) 42.
[4] I. Nuñez de Castro, M. Ugarte, A. Cano and F. Mayor, European J. Biochem. 16 (1970) 567.
[5] I. Witt and H. Holzer, Biochem. Zeit. 339 (1964) 255.
[6] K.W. Thomulka and A.G. Moat, J. Bacteriol. 109 (1972) 25.
[7] M. Grenson and C. Hou, Biochem. Biophys. Res. Commun. 48 (1972) 749.
[8] K.W. van de Poll, Proc. Kon. Ned. Akad. Wet. B73 (1970) 342.
[9] H. Holzer and I. Witt, Biochim. Biophys. Acta 38 (1960) 163.
[10] A.M.A. ten Berge, Mol. Gen. Genetics 115 (1972) 80.
[11] H. Roman, C.R. Lab. Carlsberg 26 (1956) 299.
[12] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[13] K.W. van de Poll, A.A.G. Verwey and V.V. Koningsberger, Proc. Kon. Ned. Akad. Wet. B71 (1968) 344.