BBA 75228

DEREPRESSION OF A PROLINE TRANSPORT SYSTEM IN *SACCHAROMYCES CHEVALIERI* BY NITROGEN STARVATION

JAIME SCHWENCKE AND NIEVE MAGAÑA-SCHWENCKE

Instituto de Química, Departamento de Ciencias, Universidad de Chile, Casilla 130-V, Valparaíso (Chile)

(Received October 20th, 1968)

SUMMARY

1. *Saccharomyces chevalieri* cells grown in complex medium or in synthetic medium containing $(NH_4)_2SO_4$ as the sole nitrogen source show a reduced capacity to accumulate [^{14}C]proline.

2. The uptake of proline is greatly enhanced by incubating the cells without further growth in a nitrogen-free medium, with galactose as the sole energy source. The enhancement in uptake capacity by nitrogen starvation is not affected by anaerobic or fully aerobic conditions. Also, the increase in proline pool is without effect.

3. Cycloheximide, blasticidine-S and sparsomycin inhibit the derepression. Also, the amino acid analogs *p*-fluorophenylalanine and 5-methyltryptophan prevent the increase of proline uptake. Benzimidazole, proflavine and ethidium bromide are effective inhibitors of the derepression.

4. Other amino acids do not show such an increase in accumulation capacity after nitrogen starvation. For some amino acids which show a moderate transport enhancement, such an increase is not abolished by cycloheximide.

5. Results are discussed in terms of the idea that the biosynthesis of a protein necessary for the transport of proline is repressed during growth of *Saccharomyces chevalieri* and that derepression occurs when the cells are subjected to nitrogen starvation.

INTRODUCTION

While studying the characteristics of a proline transport system in a strain of *Saccharomyces chevalieri* (ref. 1), we observed that the capacity of yeast cells to accumulate [^{14}C]proline was almost null when grown in complete medium or in mineral medium containing $(NH_4)_2SO_4$ as the sole nitrogen source. However, if cells unable to accumulate proline were incubated in a nitrogen-free medium (N-free medium) with galactose as the sole energy and carbon source, proline-uptake capacity gradually increased and reached a maximum after 3 h of nitrogen starvation (N starvation).

At least two mechanisms could adequately explain the N-starvation results. First, the depletion of the yeast amino acid pool in the presence of a metabolizable carbon source, will allow an increased uptake of a number of amino acids[2]. This effect

has been first reported by HALVORSON, FRY AND SCHWEMMIN[3] and has been confirmed by other workers (for reviews on yeast amino acid pools see SPIEGELMAN, HALVORSON AND BEN-ISHAI[4] and HOLDEN[5]).

Alternatively, one may assume that the main mechanism responsible for the enhanced proline accumulation after N starvation is the synthesis of a proline transport system which is normally repressed under usual culture conditions. It is interesting to note in this connection that evidence for repressibility of amino acid uptake has been reported in bacteria[6].

The results of the study undertaken to establish the exact nature of this phenomenon are presented in this paper.

MATERIALS AND METHODS

Yeast strains, growth conditions

Strain C_1, a representative of *S. chevalieri*, was used. It originated from culture NRRL-Y of the Northern Regional Laboratories and was obtained through the courtesy of DR. BORIS ROTMAN. In this work the galactose-adapted cells[7] have been used. The yeast cells were routinely grown in standing test tubes at 30° in a complete medium prepared by adding to 1 l of water: 20 g D-galactose; 5 g bacto peptone; 2.5 g yeast extract; 6 g $(NH_4)_2SO_4$; 0.25 g $CaCl_2 \cdot 2H_2O$; 0.25 g $MgSO_4 \cdot 7H_2O$; and 2 g KH_2PO_4. For some experiments a modified BURKHOLDER[8] mineral medium, prepared by omitting the asparagine and replacing glucose with 20 g/l of D-galactose, was used.

Compounds

DL-[*carboxy*-^{14}C]Proline; DL-[*carboxy*-^{14}C]phenylalanine; DL-[*carboxy*-^{14}C]-aspartic acid; DL-[*carboxy*-^{14}C]lysine; DL-[*carboxy*-^{14}C]serine; DL-[*carboxy*-^{14}C]methionine; DL-[*carboxy*-^{14}C]leucine and L-[*carboxy*-^{14}C]isoleucine were purchased from Calbiochem. DL-[$^{14}C_2$]Hydroxyproline was obtained from the Radiochemical Centre, Amersham, England. The specific activity of all amino acids was adjusted to 4 mC/mmole.

Ingredients for media were purchased from Calbiochem. D-Galactose was a product of Pfanstiehl. Benzimidazole was obtained from Nutritional Biochemicals Corp. DL-5-Methyltryptophan was a product of Koch–Light, Colnbrook, England. DL-*p*-Fluorophenylalanine was obtained from Mann Research Laboratories. Proflavine hemisulfate was purchased from British Drug Houses Laboratory Chemicals, Poole, England, and purified as previously indicated[9].

Cycloheximide was a generous gift of DR. C. A. FENWICK of the Upjohn Co. Blasticidin-S was kindly provided by DR. HIROSHI YONEHARA, Institute of Applied Microbiology, University of Tokyo. Sparsomycin was a gift of DR. C. G. SMITH of the Upjohn Co., and ethidium bromide was made available to us by DR. DICKINSON of Boots Pure Drug Co.

Nitrogen starvation

Exponentially growing cells were harvested by centrifugation, washed three times and adjusted to $2 \cdot 10^7$ cells/ml (hemocytometer) in 66 mM KH_2PO_4 (pH 5.5) containing 10 mM D-galactose (N-free medium). The prepared cells were incubated at 30° in a rotatory shaker (Rollordrum, New Brunswick) at 60 rev./min for 3 h

unless otherwise indicated. 2-ml samples were removed at intervals to measure uptake of proline. During the whole N-starvation period the cells showed no growth, although they remained viable even after 4 h of incubation.

Uptake experiments

2 ml of suspension ($2 \cdot 10^7$ cells/ml) were preincubated at 30° for 5 min with 2.1 μg of cycloheximide *plus* the additions, if any, and the amount of N-free medium necessary to give a vol. of 2.9 ml. At zero time, 0.1 ml of DL-[*carboxy*-^{14}C]proline was added (final [^{14}C]proline concentration $9.1 \cdot 10^{-7}$ M) and the cells incubated in a rotatory shaker (60 rev./min) at 30°. Incubation was stopped after 15 min by filtering through a Millipore filter (pore size, 0.45 μ) the filter was washed three times with 5-ml portions of room-temperature, distilled water, and dried and counted in a thin-window gas-flow counter (Nuclear Chicago Corp.). The efficiency of the counting method is 30%. 30 to 40% of the radioactivity added as [^{14}C]proline was recovered in the cells after washing. Of this amount, 5% of the counts were associated with cell debris, and 10% was incorporated into high molecular weight substances.

Measurement of amino acid pool and L-proline pool

2-ml aliquots of cells subjected to N starvation were withdrawn at different times, centrifuged rapidly, resuspended in distilled water and boiled 10 min. The extracts obtained were used to measure total free amino acid content by the ninhydrin method of MOORE AND STEIN[10]. Extracts obtained from the pellets of 50-ml aliquots of cell suspension ($2 \cdot 10^7$ cells/ml) were used to measure the free L-proline pool by bioassay[1].

RESULTS

Kinetics of derepression in N-free medium

Cells grown in complete medium were resuspended in N-free medium and incubated aerobically in a shaker (Rollordrum, New Brunswick Corporation); the uptake of [^{14}C]proline measured at appropriate intervals. As shown in Fig. 1a, the capacity of yeast cells to accumulate radioactive proline increased up to 180 min of N starvation, after an initial lag period of 30 min. No significant difference in the shape of the curve was observed if the N starvation was carried out in a completely organic buffer *plus* 10 mM D-galactose (Tris–maleic buffer). Furthermore, when cells grown in mineral medium were N starved, the same results were obtained but a longer (60 min) lag period was observed (Fig. 1b). Finally if cells grown in complete medium under vigorous aerobic conditions were N starved, they gave essentially identical results.

Effect of a metabolizable carbon source

The increase in proline accumulation capacity due to N starvation requires the presence of a metabolizable carbon source. In a concentration of 10 mM, galactose provides the necessary energy. When 10 mM glucose is added instead of galactose, one observes that galactose is a better carbon source than glucose although the results are quite similar (Fig. 2). Omission of the carbon source prevents the full development of the phenomenon.

Effect of some amino acids

When N starvation was carried out in the presence of a number of amino acids at a final concentration of 0.1 mM, no significant variation was found in the development of proline-uptake capacity. Further, addition of casein hydrolysate (50 μg/ml) was also without effect (Table I).

Variation of amino acid pool during N starvation

Samples were taken at appropriate intervals and both the amino acid pool and the accumulation of proline were measured.

The results obtained demonstrate that the total free-amino acid pool declines to about half its initial value in the first 30 min but afterwards remains fairly constant.

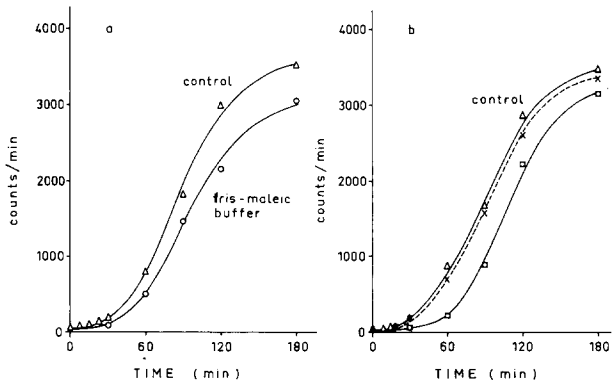

Fig. 1. Kinetics of derepression by N starvation. a. Samples of cell suspension were taken at intervals and their capacity to accumulate [^{14}C]proline was assayed as described under MATERIALS AND METHODS. △—△, 66 mM KH_2PO_4 (pH 5.5) *plus* 10 mM D-galactose; ○—○, 66 mM Tris–maleic buffer (pH 5.5) *plus* 10 mM D-galactose. b. All cells were resuspended in 66 mM KH_2PO_4 (pH 5.5) *plus* 10 mM D-galactose and then N starved. △—△, cells anaerobically grown in complete medium; ×----×, cells aerobically grown in complete medium; □—□, cells anaerobically grown in mineral medium.

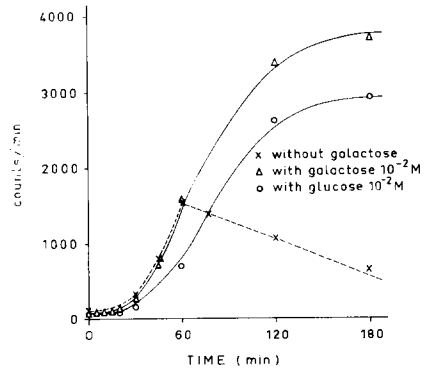

Fig. 2. Effect of a carbon source on the kinetics of derepression in N-free medium. Samples of cell suspension in 66 mM KH_2PO_4 (pH 5.5) with or without additions were taken at intervals and their capacity to accumulate [^{14}C]proline was assayed as described under MATERIALS AND METHODS. △—△, *plus* 10 mM D-galactose; ○—○, *plus* 10 mM D-glucose; ×----×, without any carbon source.

TABLE I

EFFECT OF SOME AMINO ACIDS ON DEREPRESSION

N starvation was carried out as described under MATERIALS AND METHODS with or without the amino acid. Uptake of [^{14}C]proline was measured after 180 min of starvation (for details see MATERIALS AND METHODS). Values represent the means of three separate experiments.

Amino acid added (0.1 mM)	*Accumulated radioactivity (counts/min)*
None	3112
DL-α-Alanine	3204
DL-Asparagine	3350
DL-Aspartic acid	2908
DL-Cysteine	2837
Glycine	3184
DL-Isoleucine	3315
DL-Lysine	3258
DL-Phenylalanine	3068
DL-Serine	3441
Casein hydrolysate* (enzymatic)	3049

* 50 μg/ml.

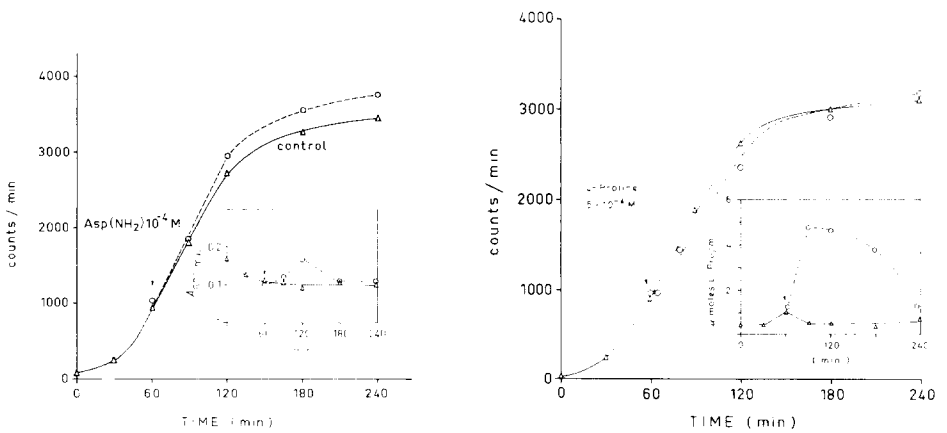

Fig. 3. Effect of replenishment of amino acid pool on derepression kinetics. N starvation was carried out as described in MATERIALS AND METHODS. At the time indicated by the arrow, asparagine was added to a portion of the cell suspension to obtain a final concentration of 0.1 mM, then incubation was continued. Samples were taken to measure [^{14}C]proline uptake. △—△, control, no additions; ○···○, *plus* asparagine. In the inset: variation of amino acid pool. Total amino acid pool was measured in duplicate samples of cell suspension (see MATERIALS AND METHODS for details). △—△, control, no additions; ○···○, *plus* asparagine.

Fig. 4. Effect of the variation of L-proline pool on derepression kinetics. N starvation was carried out as described in MATERIALS AND METHODS. At the time indicated by the arrow, L-proline was added to a portion of the cell suspension to obtain a final concn. of 0.5 mM, then incubation was continued. Samples were taken to measure [^{14}C]proline uptake. △—△, control, no additions; ○···○, *plus* L-proline. In the inset: variation of free L-proline pool. L-Proline pool was measured by bioassay with *E. coli* 55/1 as described under MATERIALS AND METHODS. Each point represents the mean of three determinations. △—△, control, no additions; ○···○, *plus* L-proline. L-Proline was calculated as μmoles/ml of intracellular water.

The effect of amino acid pool replenishment was tested by adding asparagine, which is used as a nitrogen source by *S. chevalieri*[8]. It can be seen from Fig. 3 that even when the amino acid pool was replenished, the increase in proline-uptake capacity was not found to be inhibited. Similar results were obtained when 0.1 mM glutamine or 50 μg/ml casein hydrolysate was added instead of asparagine. Furthermore, the free L-proline pool was also analysed, and as shown in Fig. 4, after an initial increase, it remains fairly constant during the whole N-starvation period. Moreover, when L-proline was added to the N-starvation medium, a great increase in the intracellular free proline pool is observed, although the enhancement in proline uptake capacity is practically unaltered.

Effect of anaerobiosis and oxygenation

The possibility that oxygenation may have some specific relation to the increased uptake of proline was tested by inducing N starvation either in anaerobiosis or after bubbling with pure oxygen for 15 min. Proline-uptake capacity was measured at the end of N starvation. Only small differences in the amount of proline were found (Table II).

TABLE II

EFFECT OF OXYGENATION AND ANAEROBIOSIS ON DEREPRESSION

The cell suspension in N-free medium was divided into four portions which were subjected to the following treatment before the onset of N starvation. With portion 1, N starvation was carried out as usual. Portion 2 was bubbled with pure oxygen for 15 min. Portion 3 was covered with 10 cm of vaseline. Portion 4 was bubbled 5 min with pure *n*-butane, then subjected to a vacuum for 5 min, and the whole procedure was repeated 8 times; finally the cell suspension was covered with vaseline. With the exception of the tube containing portion 1, all others were carefully closed before the onset of the N-starvation period. [^{14}C]Proline accumulation was measured after N starvation. Values represent the means of three independent experiments.

Treatment before N starvation	*Accumulated radioactivity (counts/min)*
None (control)	3217
plus O_2	3179
Vaseline	3248
n-Butane/vacuum/vaseline	3018

Kinetics of derepression in the presence of inhibitors of protein biosynthesis

The effect of some inhibitors of protein biosynthesis on the increase of proline-uptake capacity due to N starvation was assayed. The concentrations used were those inhibiting the growth of our yeast strain after 24 h, by between 50% and 80%.

As shown in Fig. 5, $2.5 \cdot 10^{-6}$ M cycloheximide added at the onset of N starvation completely and permanently inhibited the increase in proline-uptake capacity. When added later, total inhibition was obtained 15 min after its addition.

Fig. 6 shows that in the presence of 10^{-5} M blasticidin-S added 60 min after the beginning of N starvation, the increase in proline-uptake capacity was slowed down and completely inhibited about 30 min after the addition of the antibiotic. Further, the addition of 10^{-4} M sparsomycin gave essentially the same results, although the increase in proline accumulation capacity is completely inhibited only 60 min after addition of the antibiotic.

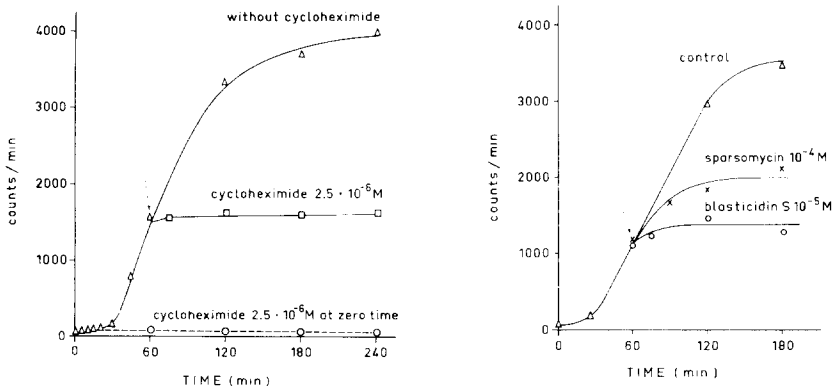

Fig. 5. Effect of cycloheximide on derepression kinetics. The antibiotic was added to a portion of the cell suspension at the concentration indicated; at zero time (○ ···○); at the time indicated by the arrow (□—□); control without inhibitor (△—△). [^{14}C]Proline uptake was determined in all cases in aliquots taken at the indicated intervals as described under MATERIALS AND METHODS.

Fig. 6. Derepression kinetics in the presence of blasticidin-S and sparsomycin. The drugs were added at the times indicated by the arrow to portions of the cell suspension in N-free medium. The antibiotic concentration is given in the figure. The uptake of [^{14}C]proline was assayed as described under MATERIALS AND METHODS. ○—○, blasticidin-S; ×—×, sparsomycin; △—△, control, no additions.

Time course of derepression in the presence of amino acid analogs

If *p*-fluorophenylalanine is added 60 min after the onset of N starvation, there is no further increase in accumulation capacity. 5-Methyltryptophan also prevents this increase but only after a lag period of 60 min (Fig. 7).

Time course of derepression in the presence of inhibitors of nucleic acid biosynthesis

In view of the significant inhibition observed for proline accumulation increase in the presence of amino acid analogs and protein biosynthesis inhibitors, it was of

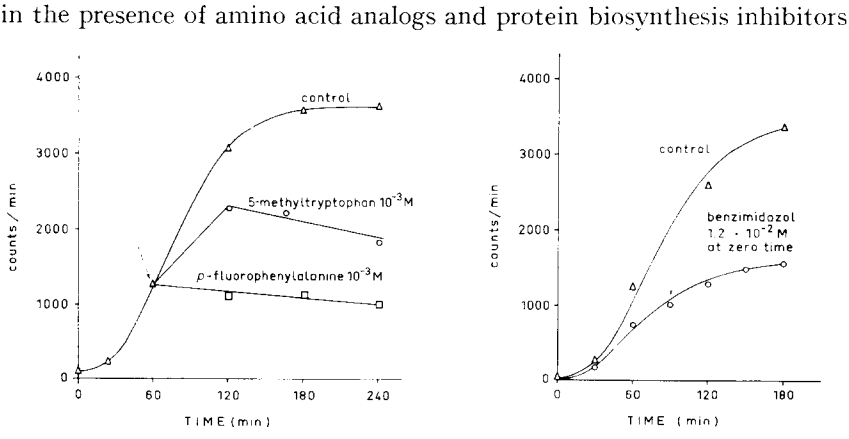

Fig. 7. Time course of derepression in the presence of amino acid analogs. Conditions as those described in Fig. 5. ○—○, 5-methyltryptophan; □—□, *p*-fluorophenylalanine; △—△, control no additions.

Fig. 8. Time course of derepression in the presence of benzimidazole. Benzimidazole at the concentration indicated was added to a portion of cell suspension in N-free medium. The uptake of [^{14}C]proline was assayed as described under MATERIALS AND METHODS with samples withdrawn at intervals. ○—○, analog added at zero time; △—△, control, no additions.

interest to determine whether similar results could be obtained by interfering with nucleic acid biosynthesis.

Results given in Fig. 8 indicate that if N starvation is run in the presence of 1.5 mg/ml ($1.27 \cdot 10^{-2}$ M) benzimidazole, the observed enhancement of proline uptake

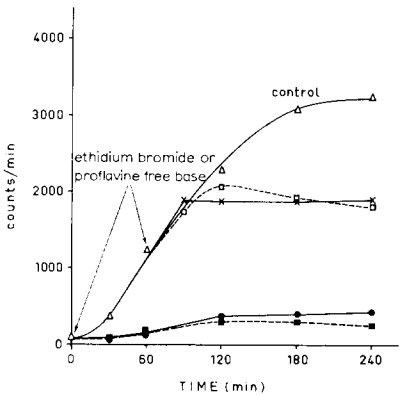

Fig. 9. Time course of derepression in the presence of inhibitors of DNA-primed RNA polymerase. The final concentrations of the inhibitors added were 70 μM for ethidium bromide and 100 μM for proflavine-free base. Other conditions are as those described for Fig. 5. Inhibitors added at zero time: ■----■, ethidium bromide; ●—●, proflavine. Inhibitors added 60 min after zero time: □ ---□, ethidium bromide; ×—×, proflavine. No inhibitors added: △—△, control.

TABLE III

EFFECT ON N STARVATION ON THE UPTAKE BY *S. chevalieri* OF SOME AMINO ACIDS

N starvation was carried out as described in MATERIALS AND METHODS. To a portion of the cell suspension cycloheximide was added to a final concn. of 2.6 μM. Samples of cell suspension starved with (SC) or without (S) cycloheximide were taken after 3 h of N starvation, and [^{14}C]-amino acid accumulation was assayed as described for [^{14}C]proline. The uptake of [^{14}C]amino acids in nonstarved cells (NS) was also measured. The ratio S/NS gives the times that accumulation was enhanced by N starvation, without added cycloheximide. The ratio SC/NS gives the times that accumulation was increased by N starvation, in the presence of cycloheximide. The results are the means of three separate experiments.

[^{14}C]*Amino acid*	*Accumulated radioactivity (counts/min)*			*Ratio S/NS*	*Ratio SC/NS*
	Nonstarved cells	*Starved cells*			
		Without cycloheximide	*With cycloheximide*		
	(NS)	*(S)*	*(SC)*		
DL-Asparagine	160	736	185	4.6	1.2
L-Alanine	300	290	310	1.0	1.0
L-Isoleucine	517	805	810	1.6	1.6
DL-Leucine	698	1163	874	1.7	1.3
DL-Lysine	1986	2820	2666	1.4	1.4
DL-Methionine	596	1387	1422	2.3	2.4
DL-Phenylalanine	426	1972	1874	4.6	4.4
DL-Hydroxyproline	107	127	120	1.2	1.1
DL-Proline	37	2960	190	80.0	5.1
DL-Serine	446	1617	842	3.6	2.9

is slowed down. At the end of the N-starvation period, benzimidazole reduced the usual increase in proline uptake capacity to 50%.

Proflavine (free base), added at a concentration of 10^{-4} M at zero time, showed a more profound effect (Fig. 9). Derepression was inhibited up to 87%. If ethidium bromide ($7 \cdot 10^{-5}$ M) was added at zero time a 90% inhibition of the increase in proline uptake was obtained. However, if proflavine or ethidium bromide was added 60 min after the derepression began, its inhibitory effect became detectable only 30 min after addition.

Effect of N starvation on the uptake capacity for other amino acids

Uptake capacity for various amino acids was assayed at zero time and after 180 min of N starvation. As a control, yeast cells were N starved in the presence of $2.5 \cdot 10^{-6}$ M cycloheximide to measure the increase in uptake capacity which may be due to depletion of amino acid pool and uptake assayed after 180 min of N starvation.

Among the amino acids tested (Table III) only proline showed a substantial increase in uptake capacity after N starvation (80 times more). This increase could be effectively blocked by cycloheximide. Other amino acids showed moderate increases in uptake capacity, but these increases were not inhibited by cycloheximide.

DISCUSSION

The transport systems for amino acids are generally considered to be constitutive in the organisms investigated so far. However, there are reports which indicate that some amino acid transport systems may be either inducible[11,12] or repressible[6]. Nevertheless, to the best of our knowledge, no report has been published concerning derepression of an amino acid accumulating system in yeasts.

The results reported here support the idea that the system for proline uptake in *S. chevalieri* is normally repressed in yeast cells. Our results cannot be explained by depletion of preexisting pools[2,4,5]. Furthermore, if one increases the L-proline pool or replenishes the total amino acid pool during N starvation, the increase in proline-uptake capacity is either unaltered or slightly enhanced rather than inhibited.

The fact that glucose, which is known to inhibit the biosynthesis of inducible enzymes[13], does not prevent the increase in proline accumulation while galactose is a necessary carbon source for the development of the phenomenon, argues against the involvement of catabolite repression. Further, glucose catabolites (pyruvate, lactate) and amino acids proved to have no effect when added to the N-starvation medium (Table I).

Oxygen has been reported to stimulate induced enzyme synthesis (see refs. 14–17). Nevertheless, the results presented in Table II and the fact that aerobically grown cells were also repressed rule out such oxygen effect.

The enhanced capacity for proline uptake may also be related to the biosynthesis of a protein. This possibility was tested with inhibitors of protein biosynthesis in yeasts such as cycloheximide[18,21], sparsomycin[22,23] and blasticidin-S[24,25]. All of them severely restrict the increase in accumulating capacity. Further evidence is also provided by our results with *p*-fluorophenylalanine and 5-methyltryptophan, which are known inhibitors of induced enzyme synthesis[26,27].

Since both protein and RNA synthesis are required for derepression[28], inhibitors

of RNA biosynthesis were also tested. Benzimidazole, which impairs yeast growth[29] by interfering with nucleic acid synthesis[30], effectively prevents the increase in proline uptake. Also, proflavine[9] and ethidium bromide[31,32] which are specific inhibitors of DNA-primed RNA biosynthesis, were tested. If mRNA is biosynthesized as a result of release of repressive control, these inhibitors should produce a different effect when added at the onset of N starvation or later. As shown in Fig. 9, both inhibitors proved to give the expected results.

Taken together our results strongly suggest that for the uptake capacity to develop during N starvation, the biosynthesis of a protein is an indispensable step. However, degradation of a specific inhibitory protein may also be postulated, since it is well known that proteolysis occurs during N starvation[33]. However, although most inhibitors of protein biosynthesis terminate protein degradation during N starvation, *p*-fluorophenylalanine does not[34]. The clear inhibition of the phenomenon in its presence rules out the idea of the degradation of an inhibitory protein.

The probability that derepression by N starvation is a general phenomenon has been ruled out, at least for the amino acids assayed, by the fact that none of the amino acids tested showed substantial increase in uptake comparable to proline.

Although relatively little information exists concerning the control of transport-system biosynthesis in microorganism, their regulation by induction and repression in bacteria has been reported and reviewed[35]. Furthermore, BENKO, WOOD AND SEGEL[36] have recently suggested that the regulation of methionine transport in fungi may involve derepression.

Our findings support the idea that control of transport-system biosynthesis may prove to be a more generalized phenomenon than it is usually considered.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of Mr. GUSTAVO GONZÁLEZ in some of the experiments.

The authors thank Miss CARMEN L. VALENZUELA for valuable technical assistance.

REFERENCES

1 N. MAGAÑA-SCHWENCKE AND J. SCHWENCKE, *Biochim. Biophys. Acta*, 173 (1969) 313.
2 H. O. HALVORSON AND B. D. COWIE, in A. KLEINZELLER AND A. KOTYK, *Membrane transport and Metabolism*, Academic Press, London, 1960, p. 479.
3 H. HALVORSON, W. FRY AND D. SCHWEMMIN, *J. Gen. Physiol.*, 38 (1955) 512.
4 S. SPIEGELMAN, H. O. HALVORSON AND R. BEN-ISHAI, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, The Johns Hopkins Press, Baltimore, Md., 1955, p. 124.
5 J. T. HOLDEN, *Amino Acid Pools*, Elsevier Publishing Company, Amsterdam, 1962, p. 82.
6 Y. INUI AND H. AKEDO, *Biochim. Biophys. Acta*, 94 (1965) 143.
7 O. WINGE AND C. ROBERTS, *Comp. Rend. Trav. Lab. Carlsberg*, 24 (1948) 263.
8 P. R. BURKHOLDER, *Am. J. Botany*, 30 (1943) 206.
9 B. H. NICHOLSON AND A. R. PEACOCKE, *Biochem. J.*, 100 (1966) 50.
10 S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 176 (1948) 367.
11 J. A. BOEZZI AND R. D. DE MOSS, *Biochim. Biophys. Acta*, 49 (1961) 471.
12 W. R. BIBB AND W. R. STRAUGHN, *J. Bacteriol.*, 87 (1963) 815.
13 B. MAGASANIK, *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961) 249.
14 A. SCHULTZ, L. ATKIN AND C. FREY, *J. Am. Chem. Soc.*, (1940) 2271.
15 J. LEIBOWITZ AND S. HESTRIN, *Biochem. J.*, 36 (1942) 772.
16 S. SPIEGELMAN, *J. Cellular Comp. Physiol.*, 25 (1945) 121.

17 V. E. CHESTER, *Biochem. J.*, 92 (1964) 318.
18 M. R. SIEGEL AND H. D. SISLER, *Biochim. Biophys. Acta*, 87 (1964) 70.
19 H. FUKUHARA, *Biochem. Biophys. Res. Commun.*, 18 (1965) 297.
20 S. R. DE KLOET, *Biochem. Biophys. Res. Commun.*, 19 (1965) 582.
21 L. FELICETTI, B. COLOMBO AND C. BAGLIONI, *Biochim. Biophys. Acta*, 119 (1966) 120.
22 S. P. OWEN, A. DIETZ AND G. W. CAMIENER, *Antimicrobial Agents Chemotherapy*, (1962) 772.
23 I. H. GOLDBERG AND K. MITSUGI, *Biochemistry*, 6 (1967) 383.
24 S. TAKEUCHI, K. HIRAYAMA, K. UEDA, H. SAKAI AND H. YONEHARA, *J. Antibiotics, Tokyo, Ser. A*, 11 (1958) 1.
25 H. YAMAGUCHI AND N. TANAKA, *J. Biochem.*, 60 (1966) 632.
26 H. O. HALVORSON AND S. SPIEGELMAN, *J. Bacteriol.*, 64 (1952) 207.
27 H. O. HALVORSON, S. SPIEGELMAN AND R. L. HINMAN, *Arch. Biochem. Biophys.*, 55 (1955) 512.
28 F. JACOB AND J. MONOD, *Proc. Intern. Congr. Biochem., 5th*, Pergamon Press, Oxford, 1963, p. 132.
29 D. W. WOOLLEY, *J. Biol. Chem.*, 152 (1944) 225.
30 G. A. LE PAGE AND J. L. GREENLESS, *Cancer Res. Suppl.*, 3 (1955) 102.
31 M. J. WARING, *Biochim. Biophys. Acta*, 87 (1964) 358.
32 M. J. WARING, *Biochim. Biophys. Acta*, 114 (1966) 234.
33 J. MANDELSTAM, *Biochem. J.*, 69 (1958) 110.
34 D. SCHLESSINGER AND F. BEN-HAMIDA, *Biochim. Biophys. Acta*, 119 (1966) 171.
35 A. B. PARDEE, in M. P. STULBERG, *Enzymatic Aspects of Metabolic Regulation*, U. S. Department of Health, Education and Welfare, Washington, 1967, p. 249.
36 P. V. BENKO, T. C. WOOD AND I. H. SEGEL, *Arch. Biochem. Biophys.*, 122 (1967) 783.