Metabolism of lysine-chromium complex in *Saccharomyces cerevisiae*

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Abstract *Saccharomyces cerevisiae* which cannot utilize lysine as a sole nitrogen source is shown to metabolize a Lysine – Cr³⁺ (1:1) complex synthesized, as a combined nitrogen and carbon source. It induces rapid uptake of lysine and prevents loss of viability, in contrast with free lysine. That complexation with trivalent chromium has the effect of profoundly influencing intracellular distribution and metabolism of the liganded amino acid is demonstrated.

Keywords *Saccharomyces cerevisiae* · Lysine · Lysine-chromium complex · Metabolism

Introduction

Chromium has been recognized as an essential element ever since Schwartz and Mertz [1] elucidated its significance in glucose metabolism. Toepfer et al [2] extracted a low molecular weight, organic chromium complex from yeast, known as the Glucose Tolerance Factor (GTF), containing liganded glutamic acid, glycine, cysteine and nicotinic acid [2]. GTF is a known requirement for normal carbohydrate metabolism in animals and humans [3]. Importantly, GTF also enhances the rate of glucose metabolism in yeast [4]

When yeast is grown in a medium containing trivalent chromium salts, it incorporates 80–87% of the chromium assimilated into an organically bound form [5]. This fraction includes GTF and, most likely, other chromium complexes with intracellular ligands such as amino acids, nucleotides and metabolites, whose nature and biological roles are unknown. For example, a NADP-Cr (III) complex was also found to occur in yeast [6]. A recently synthesized (phenylalanine)₃Cr (III) has also been reported to have GTF-like activity [7].

S. cerevisiae exhibits several unusual metabolic features. Asparagine, glutamine, glutamic acid and arginine are “preferred” amino acids which can support rapid yeast growth as sole nitrogen sources [8]. On the other hand, lysine cannot do so [9] and even inhibits yeast growth by mechanisms not well defined [10]. We show herein that complexation of lysine with chromium strongly and positively influences lysine metabolism in yeast.

Materials and methods

A Lysine-chromium (1:1) complex, hereinafter designated as LyCr was synthesized by reacting equimolar amounts

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(500 μ moles) in a volume of 6 mL at pH 3.5 in a water bath at 45 $\pm$ 1°C for 60min. Complex formation was monitored by the specific increase in absorbance at 570nm [$\Delta A^{570} = A^{570}(\text{Lys} + \text{Cr}^{3+}) - A^{570}(\text{Cr}^{3+})$] which plateaued out by 60min. ΔA^{570} is a characteristic, quantifiable parameter of amino acid– Cr^{3+} complexation [11]. A modified Job's plot [12] with varying concentrations of lysine reacting with a fixed Cr^{3+} level confirmed that ΔA^{570} maximized at (1:1) molar ratio establishing the stoichiometry. Infrared spectra of the complex synthesized, revealed absorption bonds at 609 cm^{-1} and 1129 cm^{-1} (of Cr–O of carboxyl and of (S–O) stretching vibrations of sulfate respectively) and weakened (N–H) vibration shifts from 2100 cm^{-1} in the free ligand to 2043 cm^{-1} in the complex indicating the nature of bonding of chromium to carboxyl as well as amino groupings of lysine.

A haploid *Saccharomyces cerevisiae* NCIM 3558 strain isolated in this laboratory [13] was grown on a minimal salts medium [9] supplemented, as required, with lysine or LyCr (10mM) in place of ammonium sulfate in the complete basal medium (CBM: Glucose 1%+2mM NH_4^+).

The metabolism of lysine and LyCr was then examined by transferring pregrown log phase yeast cells (starved for 24 h), on to a medium containing either lysine or LyCr (at 10mM as sole N & C source in each case). Subsequent growth and lysine uptake from the medium were followed respectively by absorbance changes and a specific lysine assay [14].

Analysis for chromium uptake in *S. cerevisiae* cells was performed by separation, washing and analysis of cells for Chromium taken up during growth by a diphenylcarbazide

procedure based on Feigl [15]. Cytosolic and vacuolar content of lysine was estimated by a copper permeabilisation procedure [16].

Results and discussion

Lysine is not one of the preferred amino acids for yeast. In the case of the laboratory isolate, *S. cerevisiae* NCIM 3558 as well preliminary experiments showed that lysine is a very poor growth substrate when required to provide both nitrogen and carbon: in contrast, LyCr (at 10mM) was able to support yeast growth and proliferation.

The remarkable feature of the growth pattern is that it shows that, unlike free lysine, LyCr is well utilized for growth as a nitrogen as well as a carbon source. It has hitherto been believed that the biological role of chromium in yeast cells is mainly connected with carbohydrate metabolism [17]. Also, although relatively fewer studies have examined the efficacy of amino acid nitrogen as well as carbon as growth substrates, during yeast growth on single amino acids, some nitrogen sources could also be considered as preferred carbon sources as well for the cells [18]. Thus, eventual growth attained also reflects quantitatively the metabolism of the amino-nitrogen as well as the carbon skeleton of the amino acid. Although several aspects of lysine metabolism in *S. cerevisiae* remain enigmatic, it may be mentioned, that enzymes that metabolize lysine are strongly induced when lysine functions as a sole carbon source [19]. Consequently, the critical step would be the

Fig. 1 Growth of *Saccharomyces cerevisiae* on lysine and lysine chromium complex

Log phase cells ($A^{660}=0.85$; 24 hrs. old) grown on CBM, washed and resuspended in (CBM-N-C) medium containing Lysine or LyCr (1μ mole/ml). Other growth condition as in Fig. 1. Lysine remaining in the medium for cells in Lysine (○—○) and in LyCr (●—●). Growth of cells during lysine Lys (△—△) and LyCr (▲—▲) utilization

Fig. 2 Utilisation of Lysine and Lysine-Cr complex by *S. cerevisiae* NCIM 3558

ability of yeast to utilize lysine nitrogen. This, evidently, does occur with LyCr though not with free lysine.

The same pattern of results as shown in figure 1 was obtained in the case of fully aerobic cultures grown in an orbitek shaker (at ~150 rpm) as well, indicating that this is an intrinsic feature of the metabolism of lysine and LyCr in this strain.

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The results displayed in figure 2 show that pregrown cells take up free lysine to only a small extent, unlike LyCr which is well assimilated, metabolized and supported further growth. This is the first instance wherein such an uptake enhancement results consequent on amino acid complexation with chromium (III). It may be mentioned, in this context, that the only other comparable example is enhanced uric acid uptake and better growth from chromium-uric acid complexes [20].

Analysis of chromium uptake by cells during growth on LyCr showed that the complex was taken up, cleaved in the cells, lysine posited intracellularly and the chromium loosely bound to the cell walls. The chromium was thereafter, apparently, returned to the medium since the residual chromium in the washed cell fraction accounted for 40% of the expected based on amino acid taken up.

Of the lysine uptake (Fig. 2), 8μ moles /mg dry weight of cells was taken up from LyCr in 96 h, of which only

1.9μ moles (24%) was vacuolar. In contrast, over 90% of lysine is known to be stored in vacuoles when it cannot support growth. This shows that LyCr mediates retention of lysine in the cytosol rather than deposition in vacuoles. It has been suggested [21] that cytosolic amino acids, but not vacuolar pools are metabolizable in *S. cerevisiae*. This LyCr dependent shift in intracellular lysine would appear to be the mechanism by which LyCr, unlike free lysine, enables yeast to metabolize the amino acid.

Finally, the decrease in cell density (A^{660}) revealed (Δ - Δ , Fig. 2) is indicative of loss of viability due to free lysine which is also arrested by metabolism promoted by LyCr. It is known that yeast cells limited for nutrients other than carbon, are growth arrested in the G_0 state and are unable to remain viable [22]. This explains why LyCr supported growth counteracts loss of viability due to lysine. The results of the study thus demonstrate, for the first time, the remarkable influence of chromium complexation on lysine metabolism in yeast.

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