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Uptake of chromium(III) and chromium(VI) compounds in the yeast cell structure

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Abstract

The study presented in this article investigated the influence of different Cr(III) and Cr(VI) compounds in the cultivation medium on the uptake and localization of chromium in the cell structure of the yeast *Candida intermedia*. The morphology of the yeast cell surface was observed by the scanning electron microscopy. Results demonstrated that the growth inhibitory concentration of Cr(III) in the cultivation medium induced changes in the yeast cell shape and affected the budding pattern, while inhibitory concentration of Cr(VI) did not cause any visible effects on morphological properties of the yeast cells. The amount of total accumulated chromium in yeast cells and the distribution of chromium between the yeast cell walls and spheroplasts were determined by atomic absorption spectroscopy. No significant differences were found neither in total chromium accumulation nor in the distribution of chromium in yeast cell walls and spheroplasts between the two of Cr(VI) compounds. Conversely, substantial differences between Cr(III) compounds were demonstrated in the total uptake as well as the localization of chromium in yeast cells.

Introduction

Chromium represents one of those elements that have been extensively studied in the field of bioaccumulation with microorganisms for the last few years, although it attracts attention in other organisms as well, in particularly humans due to it's toxicity. Two oxidation states have attracted the highest attention, +3 and +6. Metabolism for chromium(VI) is relatively well known, since in the case of chromium(III) the question of it's transport across biological membranes and mechanisms of complexation still remain to be elucidated.

Hexavalent chromium compounds, which have many industrial applications, are very strong oxidizing agents which can easily penetrate living cells causing cell or tissue damage and which are capable of carcinogenic and mutagenic activities. Several studies have been undertaken in order to elucidate complex mechanisms of chromium(VI) toxicity. (Lu & Yang 1995; Rapoport & Muter 1995; Krauter *et al.* 1996; Beveridge *et al.* 1997; Gharieb & Gadd 1998; Belagyi *et al.* 1999; Raspor *et al.* 1999; Shi *et al.* 1999)

On the other hand, chromium has been recognized as an essential dietary supplement in animal and human nutrition, providing beneficial effects in the carbohydrate and lipid metabolism. Moreover, 'organically bound' forms of chromium with higher biological activity proved to be more suitable as dietary supplements than inorganic chromium compounds, the existence and the exact structure of such organic compounds, however, have not been identified yet. In this respect, yeasts are suitable eukaryotic microorganisms for research, since they are capable of accumulating

chromium from aqueous solution and storing it in the cells. Characterization of such uptake process is important not only for understanding chromium transport and localization in yeast cells, but also for possible use of chromium enriched yeast biomass as a food or feed supplement. (Sumrall & Vincent 1997; Anderson 1998; Mertz 1998; Stearns 2000; Vincent 2000) Since chromium(III) compounds are known to be much less toxic than chromium(VI) compounds, Cr(III) has been widely used in these experiments, which included many different organisms, media, chromium compounds in different concentrations, various cultivating methods and parameters (Rapoport & Muter 1995; Wang & Shen 1995; Hegóczki *et al.* 1997; Schmieman *et al.* 1997; Demirci & Pometto 2000; Ding *et al.* 2000).

The purpose of our research was to study the effect of different chromium(III) ($CrCl_3 \cdot 6H_2O$, $KCr(C_2O_4)_2 \cdot 3H_2O$) as well as chromium(VI) compounds ($K_2Cr_2O_7$, $Na_2Cr_2O_7 \cdot 2H_2O$) on morphological properties and chromium uptake and distribution between the cell walls and spheroplasts of the yeast *Candida intermedia*. The budding yeast *C. intermedia* was selected on the basis of good growth characteristics and well studied metabolism with high potential of chromium accumulation (Batič *et al.* 1996; Raspor *et al*. 1999).

Materials and methods

Microorganism

In our experiments we used yeast *Candida intermedia* ZIM 156, obtained from the Collection of Industrial Microorganisms of Biotechnical Faculty, University of Ljubljana.

Media

In order to avoid possible interactions of chromium compounds with complex components of the medium (e.g., yeast extract, malt extract, bactopepton, amino acids), chemically defined medium (CDM) reported by Davies *et al*. (1985) was used, the amino acids, however, were omitted. The CDM consisted of: 10 g glucose, 4.5 $(NH_4)_2SO_4$, 0.1 KCl, 0.1 NaCl, 0.5 $MgSO_4 \cdot 7H_2O$, 0.1 $CaCl_2 \cdot 2H_2O$, 1.0 KH_2PO_4, 0.005 H_3BO_3, 0.002 $CuSO_4 \cdot 5H_2O$, 0.002 $FeCl_3 \cdot 6H_2O$, 0.01 $ZnSO_4 \cdot 7H_2O$, 0.002 Na_2 $MoO_4 \cdot 2H_2O$, 0.001 KI, 0.004 Ca-D-pantothenate, 0.02 myo-inositol, 0.004 thiamine $\cdot$ HCl, 0.004 nicotinic acid, 0.004 pyridoxine $\cdot$ HCl, 0.002 riboflavin, $0.05 \cdot 10^{-3}$ folic acid, $0.05 \cdot 10^{-3}$ (+)-biotin per liter of distilled water. The vitamins were added to the medium aseptically from stock solution that was filter sterilised (Sartorius, 0.45 μm), after sterilizing the rest of the medium in autoclave. The pH of the CDM was adjusted to 4.0 with 1 M HCl. The CDM was used for inoculum preparation and for control cultures.

Agar plates for storing and maintaining the viability of yeast culture at 28 °C were prepared by addition of 20 g l^{-1} agar to the CDM.

For the experiments of chromium uptake and distribution in yeast cells the CDM was supplemented separatelly by the following chromium(III) compounds: $CrCl_3 \cdot 6H_2O$, $KCr(C_2O_4)_2 \cdot 3H_2O$ or chromium(VI) compounds: $Na_2Cr_2O_7 \cdot 2H_2O$, $K_2Cr_2O_7$. Chromium compounds were added from stock solutions and appropriate volumes were used to give the initial concentration of 1 mM Cr(III) or 20 μM Cr(VI) in the medium. To avoid precipitation of chromium in the medium, pH was adjusted to 4.0 with 1 M HCl.

For purposes of the scanning electron microscopy (SEM) examinations the initial chromium concentrations in the CDM were 1 mM and 10 mM in the case of $CrCl_3 \cdot 6H_2O$ addition and 1 mM as $K_2Cr_2O_7$ was added to the medium.

Culture conditions

Inoculum for all cultivations was prepared by transferring 3-day old culture from agar plate to 100 ml CDM followed by cultivation in shaking flasks (28 °C, 200 rpm) to the late exponential phase of growth (corresponding to OD_{650} 1.8). 500 ml CDM containing single chromium compounds was inoculated by 30 ml inoculum and the flasks were agitated on a rotary shaker (200 rpm) at 28 °C for 12 h. At least four separate cultivations were performed for each chromium compound ($CrCl_3$, $KCr(C_2O_4)_2$, $K_2Cr_2O_7$, $Na_2Cr_2O_7$) and for control culture, where chromium compounds were omitted.

The same procedure was applied for cultivating yeasts in order to observe possible morphology changes as a result of the presence of chromium(III) or chromium(VI) in the medium by scanning electron microscopy.

Washing of the cells

After 12-h cultivation, the broth was centrifuged (5 min, 4000 rpm) and the supernatant discarded. The

pellet was washed three times with 0.015 M phosphate buffer (pH = 4.0) in order to remove loosely associated fraction of chromium from yeast cell surface. An aliquot of washed yeast cells was dried (at 105 °C to constant mass) and used for determination of total accumulated chromium in whole yeast cells. Spheroplasts were prepared from another aliquot, which was followed by the detection of chromium in cell walls and spheroplasts.

The washing step for SEM visualization of yeast cells is described in the 'scanning electron microscopy' section in more detail.

Determination of total accumulated chromium in whole yeast cells

Dry yeast biomass was digested with addition of 1 ml of 65% (v/v) HNO_3 to 20 mg dry biomass and heating for 30 min at 140 °C. After cooling down, samples were diluted with bidistilled water up to 10 ml and the content of chromium was analysed by electrothermal atomic absorption spectrometry (ETAAS), using HITACHI Z-8270 Polarized Zeeman Atomic Absorption Spectrophotometer. The measurements were carried out under the optimised measurement conditions (Ščančar & Milačič 2002). Chromium standard solutions were prepared by using appropriate dilutions of a stock Cr(III) standard solution for AAS analysis (1000 ± 2 mg Cr l^{-1} in 5% HNO_3, Merck). Limit of detection calculated on a 3*s* basis (a value three times the standard deviation of the blank) was 0.15 μg Cr g^{-1} dry wt.

The amount of total chromium was calculated per gram of dry weight (μg Cr g^{-1} dry wt.)

Determination of chromium in yeast cell walls and spheroplasts

Spheroplasts were prepared by the adapted protocol of Curran & Bugeja (1996), which includes enzymatic degradation of yeast cell walls. Washed cells (~0.5 mg wet weight) were suspended in 5 ml of freshly prepared and filtered spheroplasting solution (Sartorius, 0.45 μm) comprising of 15 mg of lysing enzymes from *Trichoderma harzianum* and 6 μl 14.3 M of β-mercaptoethanol in MP buffer. MP buffer was made of 1 M D-sorbitol, 0.1 M NaCl, 0.01 M acetic acid in distilled water; pH was adjusted with 1 M NaOH to 5.5. The suspension was incubated at 28 °C for 45 min with constant mixing. Spheroplast formation was visually monitored under light microscope. Efficiency of spheroplast formation was checked by spheroplast bursting in hypo osmotic solution (0.1 ml of spheroplast suspension was diluted in 0.9 ml of distilled water).

The suspension, after spheroplast formation, was centrifuged (5 min at 4000 rpm) to obtain digested cell walls in the supernatant while spheroplasts remained in the pellet. The pellet was washed three times with 5 ml of the MP buffer and the supernatants were collected.

The concentration of chromium in cell walls and subsequent washings were determined directly by ETAAS (described above), which was followed by calculation of the amount of chromium per gram of dry weight (μg Cr g^{-1} dry wt.).

The pellet representing washed spheroplasts was dried (at 105 °C to a constant mass) and digested with 65% (v/v) HNO_3 applying the same procedure as described for digestion of whole yeast cells. Samples were diluted with bidistilled water up to 10 ml and their chromium content was analysed with ETAAS (described above).

The amount of chromium in spheroplasts was calculated per gram of dry weight (μg Cr g^{-1} dry wt.).

Scanning electron microscopy (SEM)

Surface properties of yeast cells were surveyed by scanning electron microscope JSM 840A after 12-h cultivations in the presence of 1 mM or 10 mM Cr(III) and 1 mM Cr(VI). Protocol for specimens' preparation was based on the method introduced by Hanschke & Schauer (1996), but modified for our purposes.

The samples of cultivation broth were concentrated by centrifugation (3 min, 5000 rpm).

In order to remove rests of cultivation broth and so to ensure a clean surface yeast cells were washed ten times in 0.1 M sodium phosphate buffer (pH = 7.2), followed by one wash in 0.1 M sodium cacodylate buffer (pH = 7.2).

After that, cells were resuspended in fixans A containing 1% glutaraldehyde and 0.4% formaldehyde (depolimerised from paraformaldehyde) in 0.1 M cacodylate buffer (fixans was adjusted to osmolarity of cca 400 mosmol). After 4 h, cells were transfered for a next 12 to 24 h in a subsequent B solution containing fixans A with 1% of tannic acid added. After fixation was finished cells were washed out twice in 0.1 M cacodylate buffer.

A drop of a sample was driped to a raund cover slip and left in a wet chamber for 30 min. To get a stronger

cell adhesion the surface of cover slip was treated by Alcian blue.

Samples were then dehydrated in a series of increasingly concentrated ethanol solutions (30%, 50%, 70%, 80%, 90% and 100%), followed by acetone solution, which was used for dehydration and as transitional fluid. To obtain a stronger signal in SEM cells were impregnated during a procedure of dehydration by uranylacetate (50% ethanol containing 2% uranylacetate).

Cover slips were transferred in Balzers CPD 030 into a CO_2 atmosphere and dried at a critical point.

Finally, dry samples were sputtered by gold in a BalTec SCD 050 sputtercoater and examined by scanning electron microscope JSM 840A at an accelerating voltage of 12 kV.

Results

Effect of chromium(III) and chromium(VI) on yeast cell morphology

Through observation of yeast cell surface, using scanning electron microscopy, after 12-h cultivation in chemically defined medium containing chromium compounds, we tried to establish whether the presence of chromium(III) and chromium(VI) in the medium specifically affects the morphological properties of the yeast cells' surface.

C. intermedia cells grown without chromium (Figure 1a) appear in pairs or short chains, as a result of bipolar budding, since individual cells or small flocks are rarely found in culture (micrographs are not shown). The usual cell shape ranges from round to ellipsoidal/oval and its length from 3 to 5 μm. Ring shaped bud scars could be clearly seen on the surface of mother cells. The occurrence of impurities on the cell surface (Figure 1a) could result from secretion of macromolecules such as extracellular polysaccharides.

The presence of 1 mM Cr(III) in the medium (this concentration was also used for experiments of chromium localization in yeast cells), which was not inhibitory for yeast growth (results are not shown), did not induce changes of yeast cell shape or of their size (Figure 1b). No significant changes could be perceived on the yeast cell surface.

On the other hand, the same concentration of chromium(VI) (1 mM Cr(VI) in the form of $K_2Cr_2O_7$) strongly inhibited yeast growth, and yet it was still not reflected in any significant changes of the morphological properties of yeast cells observed by SEM (Figure 1c).

Finally, we used 10-times higher concentration of Cr(III) in the medium (10 mM $CrCl_3$), which strongly inhibited yeast growth (results are not shown) and consequently, as could be seen on Figure 1d, specifically affected morphological properties of the yeasts, primarily the shapes of the cells as well as their budding pattern. When compared to the control samples (Figure 1a), longer and more tubular shaped daughter cells and elongated neck connections could be noticed.

Effect of different chromium sources on total accumulation of chromium in yeast cells

Total accumulation of chromium in yeast cells was determined by ETAAS after carrying out a series of 12-h cultivations in the medium containing different chromium compounds in amounts which did not inhibit yeast growth (1 mM Cr(III) and 20 μM Cr(VI)), which was followed by the washing step with phosphate buffer in order to remove loosely bound chromium from yeast cell surface (Blackwell *et al.* 1999; Demirci & Pometto 2000) and by HNO_3 treatment.

The average results of total chromium uptake by yeasts are presented in Table 1 (total (det)). Yeast cells accumulated 491.8 μg Cr g^{-1} dry wt. in the case of $CrCl_3$ as the source of Cr(III) in the medium, which was approximately 100-times higher if compared to the uptake when $KCr(C_2O_4)_2$ was used and the initial concentration of Cr(III) in the medium was the same.

Conversely, no significant differences were found in total chromium accumulation between the two of Cr(VI) compounds, 18.4 μg Cr g^{-1} dry wt. was taken up by the cells in the case of $Na_2Cr_2O_7$ and 21.0 μg Cr g^{-1} dry wt. in the case of $K_2Cr_2O_7$.

Distribution of accumulated chromium in yeast cells

The results of chromium localization in yeast cells (Table 1) varied when different chromium(III) compounds were used as a source of chromium(III) in the cultivation medium.

The major portion of the accumulated chromium in yeast cells, when grown in the presence of $CrCl_3$, was accumulated in the spheroplasts, while in the case of $KCr(C_2O_4)_2$ the chromium was almost evenly distributed between the cell walls and spheroplasts.

There were no essential differences in the distribution of chromium between yeasts cell walls and spheroplasts in case of two of chromium(VI) compounds.

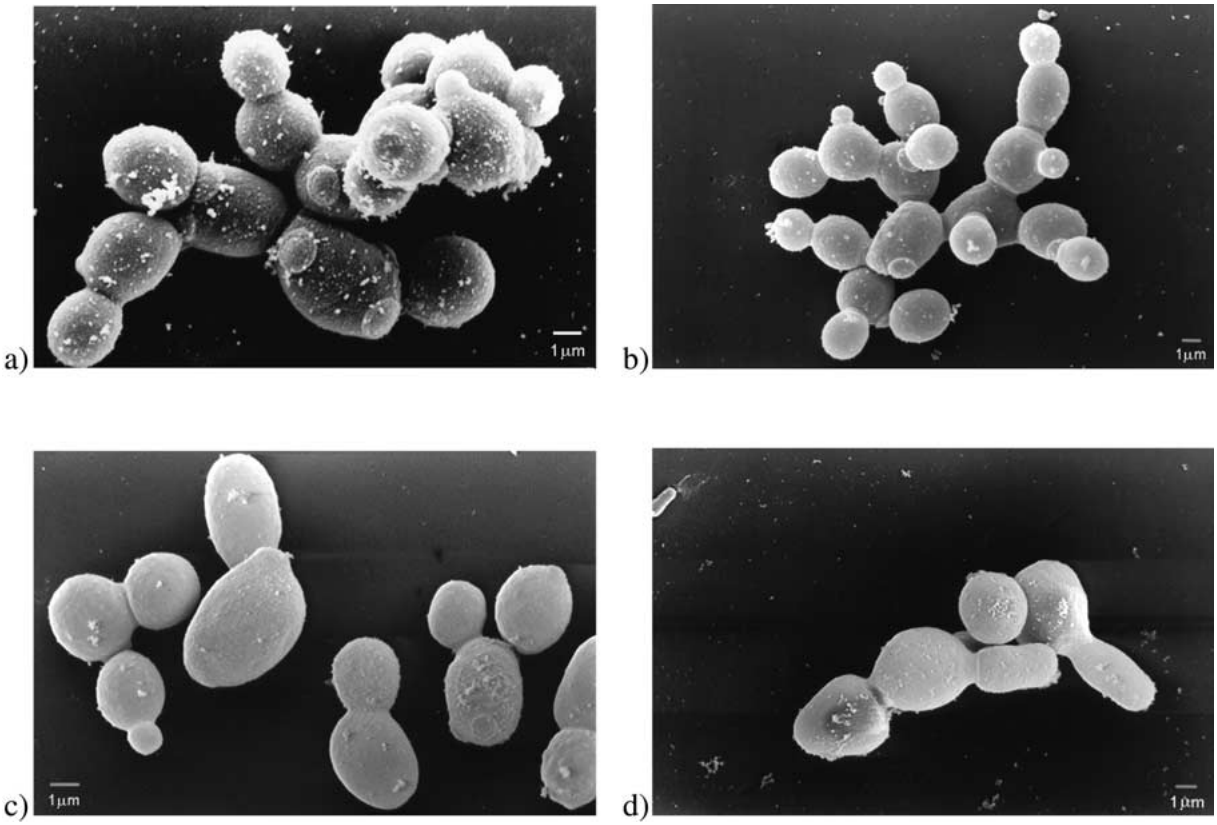

Figure 1. Scanning electron micrographs of *Candida intermedia* cells after 12-h cultivation in (a) CDM – control culture, (b) CDM containing 1 mM Cr(III), (c) CDM containing 1 mM Cr(VI), (d) CDM containing 10 mM Cr(III).

The minor part of the total chromium accumulated in the yeast cells was in both cases incorporated in the cell walls, while the major part was bound in the spheroplasts.

In all four chromium compounds the portions of chromium in the washings were rather small when compared to those in the cell walls and protoplasts.

Since the results obtained for chromium distribution in yeast cells were quite surprising, additional checking was undertaken. The amounts of chromium determined separately in the cell walls, spheroplasts and washings after using the protocol of spheroplast preparation were summed up (Table 1 – (sum) total) and these data were compared to the results of total accumulated chromium in yeast cells obtained after digestion of cells with HNO_3 (Table 1 – total (det)). A fairly good match between the total amount of chromium in yeast cells and sum total of chromium in particular compartments was obtained, ranging from $\pm$ 0.1% for $K_2Cr_2O_7$ to $\pm$ 16.0% for $KCr(C_2O_4)_2$.

Finally, the amounts of chromium determined in single fractions of the cells were represented as percentages of total chromium and shown on Figure 2.

Discussion

Mineral nutrition and bioaccumulation could be studied on different levels, but there are not many studies, which would deal with morphological changes of yeast cells. For that reason, we addressed single cell morphology, what presents a novelty in elucidation of chromium penetration into the cell.

Investigations of the cells *C. intermedia* grown for 12 h in shaking flasks in the medium containing 1 mM Cr(III), using scanning electron microscopy, did not reveal any differences when compared to the control samples without chromium. On the other hand, inhibitory concentration of Cr(III) (10 mM) showed some specific changes in the yeast cell shape and the budding pattern. Chromium in the oxidation state of +3 was used in many studies that explored the metal

Table 1. Total accumulation and distribution of chromium in yeast cells after 12-hour cultivation in the presence of different chromium compounds.

Initial Cr conc. in the medium	Chromium source	Cr concentration in specific cell fractions (μg g^{-1} dry wt.)				
		Spheroplasts	Cell walls	Washings	Total (sum*)	Total (det**)
1 mM Cr(III)	$CrCl_3$	368.1 ± 28.7	69.0 ± 5.5	29.9 ± 10.8	467.8 ± 31.2	491.8 ± 13.6
	$KCr(C_2O_4)_2$	2.4 ± 0.3	2.4 ± 0.7	0.4 ± 0.0	5.2 ± 0.7	4.4 ± 0.4
20 μM Cr(VI)	$Na_2Cr_2O_7$	12.3 ± 2.0	3.8 ± 0.5	1.7 ± 0.2	17.8 ± 2.1	18.4 ± 1.3
	$K_2Cr_2O_7$	14.9 ± 3.0	4.3 ± 0.6	1.8 ± 0.7	21.0 ± 3.2	21.0 ± 4.3

*the amount of chromium which was summed up (sum) from the amounts in spheroplasts, cell walls and washings
**the amount of chromium which was experimentally determined (det) in yeast cells as total accumulated chromium

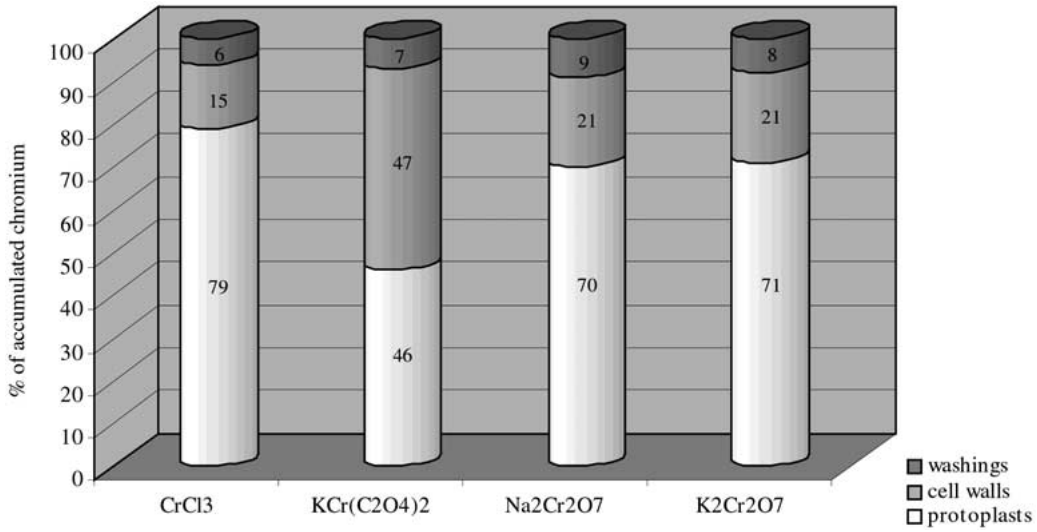

Figure 2. Distribution of accumulated chromium in yeast cells, expressed as % of Cr in cell walls, protoplasts and washings, after 12-h cultivation in the presence of 1 mM Cr(III) (as $CrCl_3$ or $KCr(C_2O_4)_2$) and 20 μm Cr(VI) (as $K_2Cr_2O_7$ or$Na_2Cr_2O_7$).

uptake by microbial cells (Sakurai *et al.* 1978; Bianchi *et al.* 1983; Shen & Wang 1993; Hegóczki *et al.* 1997; Paš *et al.* 1999; Demirci & Pometto 2000; Ding *et al.* 2000) mostly, however, in concentrations, which were not inhibitory to growth, since the principal aim was to produce the highest possible amounts of microbial (yeast) biomass enriched with chromium. Furthermore, no literature data on morphological investigations of yeast cells cultured in the presence of high (inhibitory) amount of chromium(III) could be found.

On the other hand, Muter *et al.* (2001) reported some similar changes in the surface structures and cell shape of the yeast *Candida utilis*, which was, however, grown in the presence of Cr(VI) (2 to 6 mM). They discussed the possibility of ascribing those changes to the damage of the cell wall structure (for example glucan component which is responsible for cell shape) as a result of Cr(VI) sorption to the cell walls. The results of our experiments with *C. intermedia* using toxic concentration of Cr(VI) in the medium (1 mM) are not consistent with their observations, since they do not demonstrate any similar effects of Cr(VI) on cell morphology. It appears that higher amounts of Cr(VI) in growth medium exert different effects on morphological properties of yeast cells when observed by scanning electron microscopy, although all concentrations have the same inhibitory effect on yeast growth. Accordingly, the concentration of Cr(VI) determines whether the toxicity of Cr(VI) will be reflected in morphological changes of the yeast cells and in which manner.

The main purpose of yeast cultivations in the media containing different chromium compounds was to obtain equal amounts of yeast biomass after 12 h, regardless of the compound used or the chromium content in the medium. Based on the previous experiments (results are not shown) and the well known

fact, that chromium(VI) compounds are much more toxic to living organisms than chromium(III) compounds (Lu & Yang 1995; Rapoport & Muter 1995; Krauter *et al.* 1996; Beveridge *et al.* 1997; Gharieb & Gadd 1998; Belagyi *et al.* 1999; Raspor *et al.* 1999; Shi *et al*. 1999), the used concentrations of Cr(III) and Cr(VI), which still did not inhibit yeast growth, proved to be different. Thus, a direct comparison between total chromium amounts accumulated in yeast cells after cultivation in the presence of different chromium source would not seem reasonable, however a simple calculation could be introduced in order to assess whether there were any differences in this respect. Since the initial Cr(III) concentration in the medium was 1 mM, which is 50-times higher than the initial concentration of Cr(VI) (20 μM), we could theoretically presume, that the total amount of accumulated chromium in the case of Cr(III) as a source of chromium would be 50-times higher than in the case of Cr(VI). As indicated previously, no significant differences were found in total chromium uptake between the two of Cr(VI) compounds. In both cases yeast cells accumulated about 20 μg Cr g^{-1} dry wt. (Table 1) and that is only 25-times less when compared to the total chromium accumulation in the case of $CrCl_3$ and even 4-times more than in the case of $KCr(C_2O_4)_2$. For that reason, 50-times higher initial chromium(III) concentration in yeast growth medium, when compared to Cr(VI), did not result in 50-times higher total accumulation of chromium in yeast cells after 12-h cultivation. This results could be explained by lower bioavailability of Cr(III) ions in the medium (Hughes & Poole 1991) and by hindered transport across the yeast cell membranes (Arslan *et al.* 1987; Hughes & Poole 1991; Ramana & Sastry 1994; Lu & Yang 1995; Rapoport & Muter 1995; Beveridge *et al.* 1997; Schmieman *et al.* 1997; Belagyi *et al.* 1999; Nies 1999). Additionally, a notable difference in the total chromium uptake by yeast cells, when cultivated in the presence of two different Cr(III) compounds ($CrCl_3$ and $KCr(C_2O_4)_2$) yet at the same concentration of Cr(III), could most probably be linked to their chemical properties and behaviour in the liquid medium. As the initial pH of the medium was acidic (pH = 4.0), the positively charged hexaaqua ion $[Cr(H_2O)_6]^{3+}$, which is regular octahedral, occurs in the medium, when commercially available chromium chloride in the form of dark green trans-$[CrCl_2(H_2O)_4]Cl \cdot 2H_2O$ was used as a source of chromium. On the other hand, $KCr(C_2O_4)_2$ in aqueous solution behaves completely different, since a negatively charged anionic complex $[Cr(ox)_3]^{3-}$ appears (Cotton & Wilkinson 1988).

Based on the results obtained for the total chromium accumulation in the yeast cells using different chromium(III) and chromium(VI) compounds, further experiments were performed to determine the location of accumulated chromium in yeast cells.

Even though the mechanisms of Cr(III) and Cr(VI) uptake in yeast cells have not been completely clarified, it is well known, that cell membranes are impermeable to chromium(III), while Cr(VI) enters most microbial cells in the form of chromate (CrO_4^{2-}) and bichromate ($Cr_2O_7^{2-}$) ions via the transport mechanisms of structurally similar physiologically important anions, such as SO_4^{2-} or PO_4^{3-} (Arslan *et al.* 1987; Ramana & Sastry 1994; Lu & Yang 1995; Rapoport & Muter 1995; Schmieman *et al.* 1997; Belagyi *et al.* 1999; Nies 1999).

By determining chromium amounts in cell walls and spheroplasts of the yeast *C. intermedia* we consequently expected that the greater part of accumulated chromium(III) would remain in the cell walls in contrast to bichromate anions, which should accumulate mainly in the spheroplasts. Furthermore, different chromium(III) and chromium(VI) compounds were also studied in this respect.

The results of chromium distribution in the yeast cells obtained for Cr(VI) compounds are consistent with our predictions, since the main part of total accumulated chromium (70 and 71% in the case of $Na_2Cr_2O_7$ and $K_2Cr_2O_7$, respectively) was found in the spheroplasts (Figure 2). Additionally, the cationic part of Cr(VI) compounds does not influence the uptake of bichromate anions across the yeast cell membrane into the cell interior. Some previous reports on the localization of chromium in yeast cells showed that chromate accumulated mainly in the cytosolic fraction of the cells (Gharieb & Gadd 1998).

On the contrary, no general conclusions could be made for Cr(III) compounds. In the case of $CrCl_3$ the greater part of total chromium in the yeast cells (79%) accumulated in the spheroplasts (Figure 2), which is comparable to the results obtained for Cr(VI) compounds. These results do not correspond with the possible explanation suggested earlier in this article, which claimed that the relatively smaller total chromium uptake, if compared to the initial concentration of chromium in the medium, when using $CrCl_3$, may be the result of hindered Cr(III) transport across the yeast cell membranes. At this point, one could speculate, that yeast cell membranes are still not

completely impermeable to chromium(III) ions, this assumption, however, should be subjected to further scrutiny. Additionally, some data from corresponding literature demonstrate that Cr(III) cations could be located in the negatively charged regions of the yeast plasma membrane (Belagyi *et al.* 1999).

The results of chromium localization in the yeast cells in the case of $KCr(C_2O_4)_2$ showed that chromium was almost evenly distributed between the cell walls and spheroplasts (Figure 2). Compared to other chromium compounds that were investigated, the cultivations of the yeasts in the presence of $KCr(C_2O_4)_2$ resulted in the smallest total chromium uptake and in the greatest portion of accumulated chromium in the cell walls (47% of total chromium). It is evident that the uptake and cellular distribution of chromium differed when different Cr(III) compounds were used as a source of chromium in the yeast growth medium. As indicated before, these findings could be the result of different chemical properties of chromium(III) compounds, which may consequently lead to specific behaviour in the growth medium as well as specific interactions with microbial cells, which may at the same time open some new questions about the transport of Cr(III) into cells.

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