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Hexavalent chromium stimulation of riboflavin synthesis in flavinogenic yeast

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Abstract

Flavinogenic yeast overproduce riboflavin (RF) in iron-deprived media. In optimal growth media supplemented with Fe, hexavalent chromium 'Cr (VI)' treatment led to elevated RF synthesis in all cases of 37 flavinogenic strains studied. The level of RF production exceeded the rate observed at iron-deficient conditions. At sublethal Cr concentrations the RF oversynthesis over time correlated well with the growth-inhibitory adaptational period as manifested by the prolonged lag phase. The consecutive logarithmic biomass growth was accompanied by a drop in RF biosynthesis. Cr (VI)-induced RF overproduction was not a result of cellular iron level decrease. The treatment of yeast with Cr (VI) led to the stimulation of GTP-cyclohydrolase and RF-synthase activities, the key enzymes of the RF biosynthesis pathway.

Introduction

A great majority of microorganisms can synthesize riboflavin (RF) from simple medium components. Some bacteria, yeast, and yeast-like fungi are able to overproduce RF under specified cultivation conditions in quantities which exceed their physiological requirements. This characteristic makes it possible to obtain the vitamin on the industrial scale. The yield of flavinogenesis depends on both the cultivation medium content and the physico-chemical environmental conditions such as temperature, pH or redox potential. Dramatic changes of flavinogenesis activity can be caused by the use of various inhibitors, antimetabolites and other toxic agents (Demain 1972).

RF biosynthesis rate in many microorganism species can be modulated by metals. For example, Co at 10^{-4} M stimulates RF production by *Pichia guilliermondii* and many other yeasts, whereas a deficiency of Mg (II) ions can induce flavinogenesis in several *Aspergillus niger* strains (Enari 1955; Shukla & Prabku 1961). In turn, Zn (II) presence leads to the enhanced RF synthesis in various yeast (*Candida guilliermondii*, *C. parapsilosis*, *C. flareri*) as well as in *Asp. niger* (Knusel 1957; Naik 1964). However, the detailed mechanisms of the observed effects have not as yet been explained.

So far, iron is the only metal whose influence on RF metabolism in yeast such as *Candida, Pichia, Debaryomyces* and *Schwanniomyces,* seems to be well understood. Fe-deficient media (Shavlovsky & Logvinenko 1988a) cause the derepression of enzymes of the RF biosynthesis pathway, which in turn leads to RF overproduction. Some of the strains exhibit a particularly strong effect of the enhanced flavinogenesis resulting from iron deprivation. Such strains are called flavinogenic yeasts.

The vitamin B_2 synthesis rate is strictly correlated with the level of enzyme derepression. It has been

postulated (Shavlovsky & Logvinenko 1988b) that RF synthesis is controlled by an iron-regulated repressor protein. The genetic control of RF biosynthesis has been studied extensively in yeast *P. guilliermondii* (Shavlovsky & Logvinenko 1988a). Genes *RIB*1-*RIB*7, coding for the enzymes involved in consecutive flavinogenesis stages, have been identified. The regulation of synthesis of these enzymes is complex and involves both negative (genes *RIB*80, *RIB*81, and *HIT*) and positive (genes *RIB*83 and *RIB*84) control mechanisms. In this study we present experimental evidence that in the case of flavinogenic yeasts yet another metal – chromium (VI) – is involved in modulation of riboflavin biosynthesis.

The interaction of chromium compounds with living cells is a subject of extensive studies due to environmental pollution by Cr and extreme toxicity of this metal (Cieslak-Golonka 1995; Costa 1997; Dilon *et al.* 1998; Lay & Levina 1998). Bioremediation of Cr contamination is of a special interest since in a hexavalent form Cr compounds can easily penetrate cell membranes through anion transport channels (Cohen *et al.* 1993; Costa 1997). Then, immediately after entering the cell cytoplasm, various intermediates are formed as a result of reduction by cellular redox systems. These intermediates are responsible for genotoxic and severe metabolism-perturbing effects. The reported yeast flavinogenesis stimulation by Cr (VI) might be an important stress reaction of cells against sublethal concentrations of this heavy metal.

Materials and methods

Thirty-seven flavinogenic and 17 non-flavinogenic yeast strains were used in experiments. The yeast strains studied belonged to various systematic groups such as *Pichia*, *Candida*, *Debaryomyces*, *Schwanniomyces*, *Saccharomyces*, *Zygosaccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Hansenula* and *Torulopsis*. Also, a mutant strain of *Pichia guilliermondii* was used, characterized by genotype *rib*81, which reveals an impaired RF biosynthesis regulation by iron (Babyak *et al.* 1993).

Yeast cultures were typically grown in 100 ml Erlenmeyer flasks on a gyro-shaker (200 rpm) at 30 °C for 3–5 days, either in synthetic (Sibirny *et al.* 1977) or rich (optimal growth) media. The latter were obtained by supplementation of a synthetic salt medium with 0.2% yeast extract and 0.2% peptone (Gibco). Iron (0.2 mg/l) was provided with Mohr salts. Fe-deficient media contained less than 0.01 mg Fe/ml. Iron was removed from the media with 8-hydroxyquinoline. Sterile chromium (VI) $K_2Cr_2O_7$ and (III) $Cr_2(SO_4)_3 \times 16H_2O$, salts were added directly to the cultivation media at concentrations given in Results.

Biomass check was done turbidimetrically, as optical density at 540 nm (OD_{540}). Yeast dry mass, expressed in mg per ml of the cell suspension, was calculated based on appropriate calibration curves as $OD_{540} \times$ Dilution /1.9. Quantitative estimation of the total chromium and iron content in cells was carried out by means of atomic absorption spectroscopy (AAS) after sample mineralization using a Varian spectrometer model Spectr AA-20B. Riboflavin was assayed fluorimetrically with an EF-3M fluorimeter.

The activity of GTP-cyclohydrolase and RF-synthase was determined according to the method of Shavlovsky (Shavlovsky *et al.* 1980; Trach et al. 1982). For cell permeabilization, DMSO (Sigma) was used (Logvinenko *et al.* 1977). Protein determination in cellular extracts was made according to the method of Lowry (Lowry *et al.* 1951).

All of the chemicals were of analytical grade. Whenever required, fully sterile conditions were applied.

Results

Our previous studies on Cr accumulation (Fedorovich *et al.* 1999a,b) showed that some of the strains which belong to the group of flavinogenic yeast could overproduce riboflavin (RF) when the cultures were grown in the presence of hexavalent (and not trivalent) chromium. In this work we have investigated the influence of Cr (VI) on the RF synthesis in 37 flavinogenic and 17 non-flavinogenic yeast. The yeast were grown in optimal media fully supplemented with Fe ions so as not to induce any flavinogenesis by iron deprivation in the medium.

In all of the cases of flavinogenic strains, chromium (VI) applied at sublethal concentrations of 0.2–0.6 mM, which caused a significant growth inhibition, led to the dramatic increase in the vitamin B_2 production. At the same time, nonflavinogenic yeast did not reveal such an effect. The results obtained for the most typical strains are listed in Table 1. Apart from the Cr effect – the RF synthesis levels at iron-deficient conditions are also included. Table 1 also shows that Cr (VI)-stimulated RF biosynthesis was in most of the cases higher than that induced by the

Table 1. The influence of iron and chromium (VI) on riboflavin biosynthesis by flavinogenic and nonflavinogenic yeasts

Yeast strain	Level of riboflavin produced, mg/g d.w.			
	synthetic medium		rich medium	
	+ Fe	− Fe	+ Cr (VI)	− Cr (VI)
Flavinogenic yeasts				
Pichia guilliermondii ATCC 9058	0.14	11.2	9.1	<0.20
Pichia guilliermondii rib 81 131-6	3.3	8.9	12.0	1.5
Pichia guilliermondii VKM 1257	0.39	19.6	16.4	<0.20
Candida rhagii VKM U-1520	1.25	8.5	11.3	<0.20
Candida species UBFM U-454	0.75	4.3	9.8	<0.20
Candida famata	0.10	10.7	32.2	<0.20
Debaryomyces klöckeri U-102	0.10	11.2	27.6	<0.20
Debaryomyces hansenii	0.15	21.3	70.0	<0.20
Schwanniomyces occidentalis	0.10	11.6	72.4	<0.20
Nonflavinogenic yeasts				
Candida boidinii T-2A	0.10	0.07	0.12	<0.1
Candida pulcherrima	0.17	0.34	0.3	<0.1
Hansenula polymorpha	0.04	0.30	0.14	<0.1
Saccharomyces cerevisiae 3A-171	0.12	0.36	0.21	<0.1

In rich media supplemented with Cr (VI), the stimulation of RF production by chromium was observed at Cr (VI) concentrations within the concentration range of 0.3-0.5 mM, which were sublethal for particular strains.

lack of Fe ions. It should be noted, however, that in synthetic media the effect of chromium-enhanced RF production was less manifested and was significantly weaker than in the case of iron deficiency. The above results imply different metabolic background of the observed phenomena.

In a more detailed approach, in which flavinogenic yeast was exemplified by *Pichia guilliermondii* strain ATCC 9058, flavinogenesis was investigated under various cultivation conditions, i.e. in optimal and synthetic media, and at several Cr (VI) concentrations. The results are presented in Figures 1 and 2. As seen in Figure 1, RF was oversynthesized only in optimal growth medium, containing yeast extract and peptone, at Cr concentrations high enough to inhibit the growth of the yeast culture significantly. The results shown in Figure 2 prove that the yeast's culture resistance to chromium tended to increase over time of the prolonged incubation. Both flavinogenesis and culture growth kinetics reveal a biphasic characteristics, which was particularly visible at higher Cr concentrations. The initial lag-phase was prolonged and it depended strongly on the Cr (VI) content in the medium. This stage was accompanied by the substantial activation of vitamin B_2 production. Following this the yeast entered a phase of intense growth, finally reaching biomass densities close to the control level. During this phase the rate of flavinogenesis dropped significantly relative to the previous adaptational lag period.

At higher chromium concentrations, as shown in Figure 3, the yeast was still able to adapt to the presence of this metal but the initial lag phase was prolonged up to 9, and 11 days for Cr (VI) at 0.35 mM, and 0.55 mM, respectively. RF production correlated well with the time range of growth inhibition caused by Cr: the lower the level of biomass formed, the higher the amount of riboflavin synthesized (cf. the 9-day growth at Cr (VI) above 0.4 mM given as open circles in Figure 3, and the respective RF level represented as white bars).

The strong correlation between the phase of growth inhibition and the Cr concentration in the medium is further documented by the experiment presented in Figure 4. Circles represent the dependence of the length of the lag phase and bars reveal the RF amount released to the medium at given Cr (VI) content.

It should be stressed that, for all the studied yeast strains, Cr (III) never induced any flavinogenesis. Furthermore, for such yeast as *P. guilliermondii rib*80-R2,

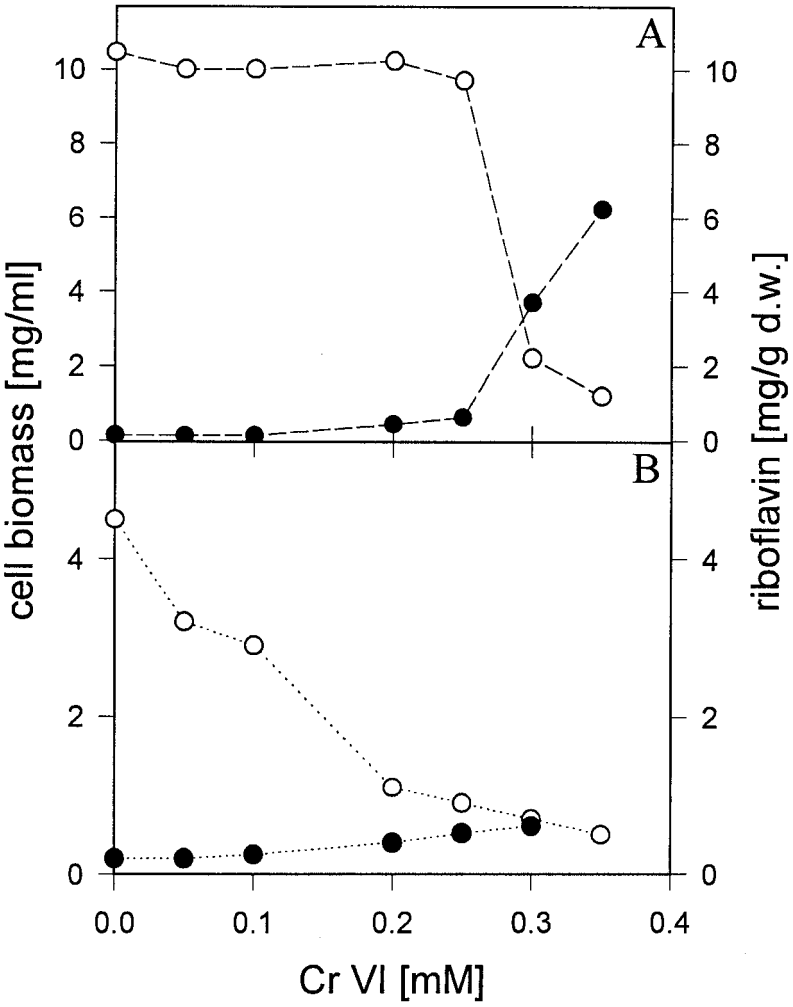

Fig. 1. Cr (VI) influence on culture growth and RF synthesis by *Pichia guilliermondii* ATCC 9058 in iron supplemented optimal (A) and synthetic (B) media. Open circles represent biomass produced after 3 d incubation with Cr (VI); full circles represent the amount of RF synthesized by the yeast culture.

P. guilliermondii hit, and *P. ohmeri* 1253, which can synthesize excess RF constitutively, Cr (III) even had an inhibitory effect (data not shown).

The question of whether or not the enhanced RF synthesis induced by Cr (VI) is caused by a drop of intracellular Fe concentration was highly interesting. Such iron deficit might be a result of Fe ion transport mechanism perturbation which in turn could be the effect of chromium presence and be directly responsible for the derepression of RF synthesis genes. However, as exemplified by typical strains in Figure 5, it is clear that in media supplemented with 0.3 mM Cr (VI) the cellular Fe levels were not decreased at all and in some cases (e.g. *P. guilliermondii* L2) were even higher than under optimal control conditions.

In search of the detailed mechanisms of chromium(VI)-stimulated RF production we also verified that the biosynthesis of riboflavin was triggered by the derepression of a gene coding either for GTP-cyclohydrolase, a key enzyme of the flavinogenesis pathway, or for RF-synthase, the enzyme which catalyses the final step of riboflavin synthesis. Such a mechanism has been reported for iron-deficient conditions, where the drop in intracellular Fe level led to the induction of the mentioned enzymes (Shavlovsky & Logvinenko 1988b). In our study, preliminary *in situ* determinations discovered that – even though no elevated enzyme activities could be detected in cellular extracts obtained from Cr(VI)-treated cultures – *P. guilliermondii* strain 1453 revealed up to 5-fold in-

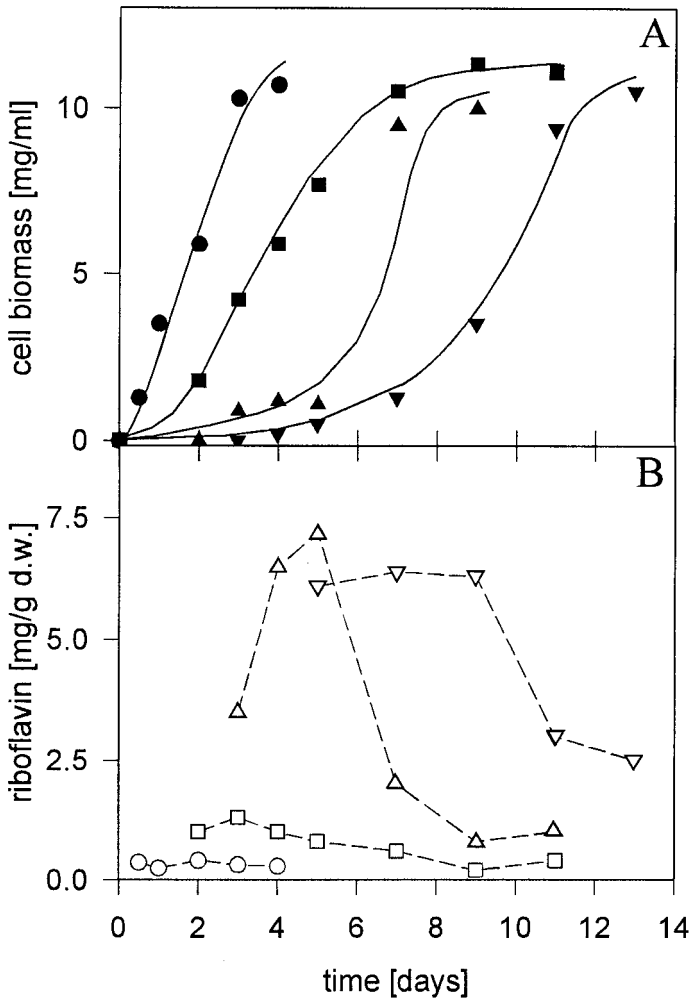

Fig. 2. Growth kinetics (A, full symbols) and RF production (B, open symbols) during treatment of *Pichia guilliermondii* ATCC 9058 cell culture with chromium VI at concentrations 0.2 mM (squares), 0.3 mM (up triangles), and 0.4 mM (down triangles). Circles represent the control experiment.

crease in both GTP-cyclohydrolase and RF-synthase activity (data not shown). Such results imply that in fully viable, compartmentalized cells, chromium may indeed lead to the derepression of genes involved in the regulation of RF production. However, the above observations need to be further confirmed by a more rigorous genetic approach and by the extensive enzymatic screening of a variety of other strains.

Discussion

Biosynthesis of riboflavin by yeast can serve as a suitable model for studies of the mechanisms of gene expression regulation by metals. The control of expression of flavinogenesis genes in the yeast *Pichia guilliermondii* includes the regulatory elements of both positive and negative mode of action as well as Fe (II) ions (Shavlovsky & Logvinenko 1988a,b). Among other factors involved in the above mechanism are cAMP and FAD. The latter is a final RF biosynthesis product which acts as an allosteric regulator of the key flavinogenesis enzyme, GTP-cyclohydrolase. The rate of RF production by yeast is also strictly dependent on a pool of accessible intracellular riboflavin precursor, GTP.

The results presented in this study prove that, apart from Fe(II), the RF biosynthesis by yeast can be strongly affected by hexavalent chromium. It should be noted, however, that this effect was observed only

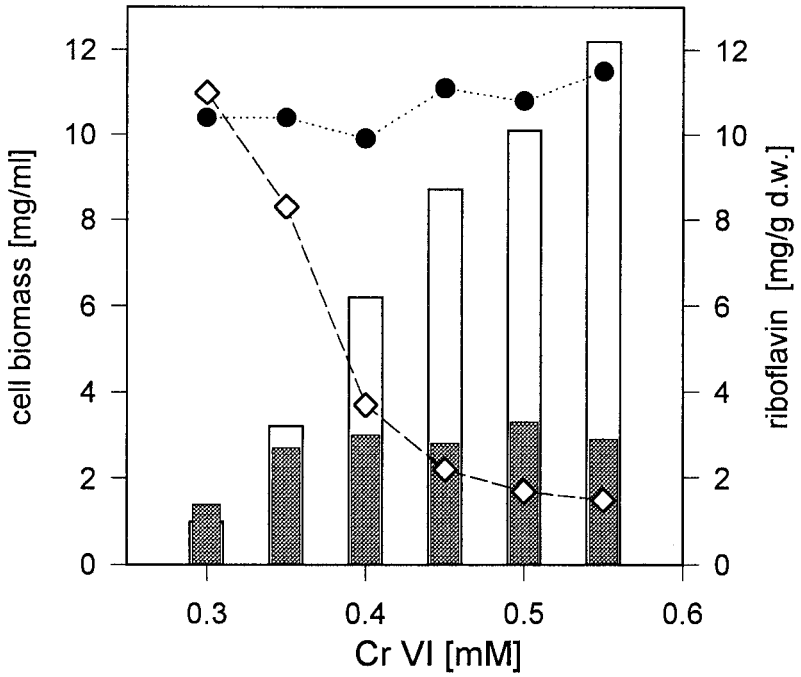

Fig. 3. Long-term cultivation of *P. guilliermondii* ATCC 9058 at high Cr (VI) concentrations. Light and dark symbols represent 9 and 11-day incubation, respectively, lines – the amount of biomass formed (left scale), and bars – the RF synthesis (right scale).

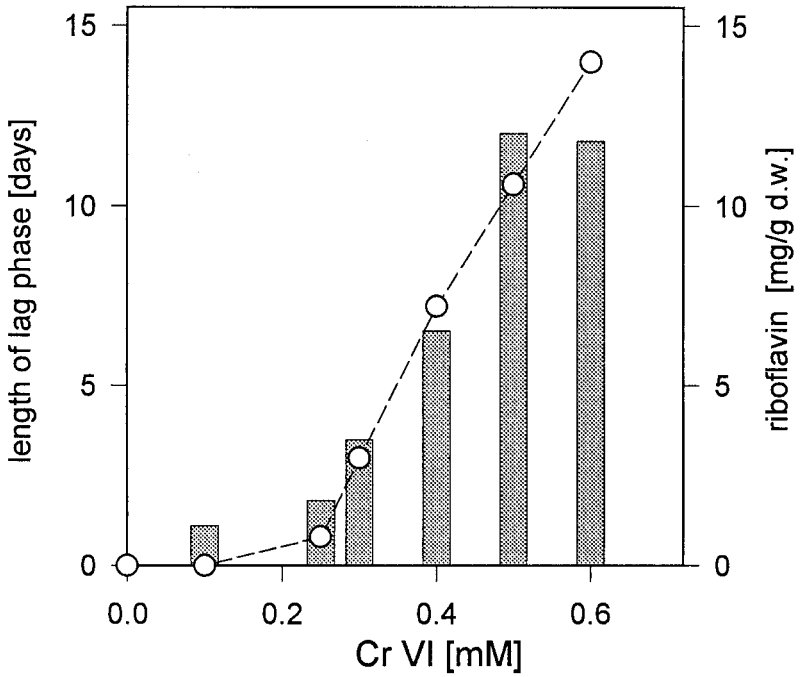

Fig. 4. Correlation between the length of the lag-phase (circles, left scale) of *P. guilliermondii* ATCC 9058 grown at various Cr (VI) concentrations and the level of RF produced (bars, right scale).

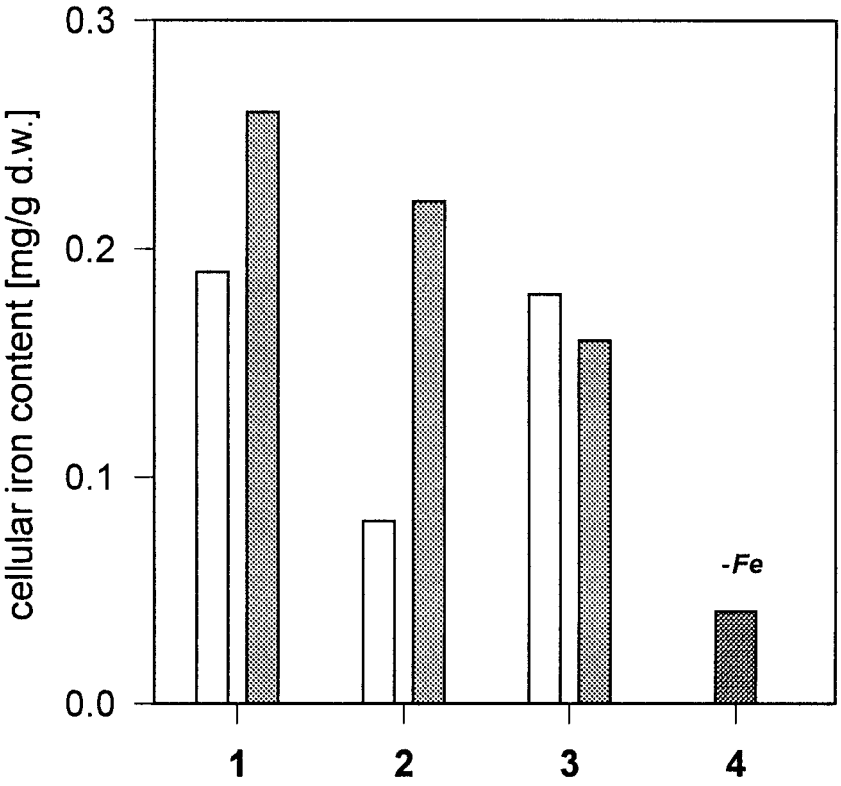

Fig. 5. Iron content in the cells of flavinogenic yeast: (1) *Pichia guilliermondii* ATCC 9058, (2) *Candida flareri*, and (3) *Schwanniomyces occidentalis*. Yeast were grown in optimal medium supplemented with Fe (light bars) and after 1-day treatment with 0.3 mM Cr (VI) (dark bars). Bar (4) represents the average value obtained for all the strains at iron-deficient conditions.

in the case of the flavinogenic yeasts, i.e. the strains which respond by extensive flavinogenesis to iron deprivation. The level of RF produced by flavinogenic strains was in most cases much higher in the presence of Cr (VI) than under Fe-deficient conditions. The amount of the released RF depended both on the Cr (VI) concentration and on the content of the medium. In general, the higher the concentration of chromium, the longer the lag phase of hampered growth revealed. In addition, during that phase of culture physiological adaptation, yeast cells tended to synthesize the excess riboflavin.

It may be suggested that cell-accumulated Cr (VI) leads to the cellular total Fe ion deficit, as was described for *Neurospora crassa* (Ramana & Sastry 1994). However, this was not the case for the chromium treatment of the studied yeast strains where the cellular iron content kept constant or even elevated. As reported earlier (Fedorovich *et al.* 1997, 1999a), the iron-deficient media which stimulated flavinogenesis in *P. guilliermondii* led to approx. fivefold decrease of intracellular Fe content. The results presented in this paper show that Cr (VI) supplemetation does not perturb the mechanisms of iron uptake by yeast. Still, the Cr(VI)-induced RF synthesis might involve the derepression of flavinogenesis enzymes by some interaction of Cr with the intracellular pool of iron, e.g. the complexation of ferrous and/or ferric ions, or due to a simple redox mechanism involving the oxidation of ferrous ions by Cr (VI), all finally leading to the relative local Fe (II) deficit. The above suggestions were supported by *in situ* observations of the increased activity of the enzymes involved in RF biosynthesis such as GTP-cyclohydrolase and RF synthase, the effect similar to that caused by iron starvation. However, we suspect that a detailed mechanism of Cr(VI)–Fe(II) interaction in a cytoplasm is much more complex and may involve certain proteins participating in redox reactions. Such an opinion is based on our observations that Cr presence in the yeast cell leads to the induction of new proteins (not shown).

It is well documented that the bioremediation of Cr (VI) in a variety of living organisms involves an immediate and rapid reduction of this metal to lower

valencies right after entering cellular matrix (Arslan 1987; Liu *et al.* 1995; Wang & Chen 1995; Corbett *et al.* 1998; Appenroth *et al.* 1999). The reduced Cr intermediates may act as an oxidative stress and may be responsible for chromium toxicity (Shi & Dalal 1994; Luo *et al.* 1996; Lay & Levina 1998). It was also shown that *E. coli* responded to oxidative stress with elevated GTP-cyclohydrolase activity (Koh *et al.* 1996). However, as yet not much is known about chromium involvement in oxidative stress reactions in yeast and this topic is now under extensive study. Thus, it cannot be excluded that the extensive flavinogenesis observed in some yeast strains as a reaction to Cr (VI) treatment is a resistance mechanism leading to higher cell survival. This postulate is supported by the results revealing that vitamin B_2 supplementation in animal tissues reduced the toxicity of hexavalent chromium (Appenroth *et al.* 1996). Furthermore, our recent observations prove that the addition of exogenous riboflavin leads to the increase in yeast cell viability in the presence of sublethal Cr (III) and Cr (VI) compounds.

In order to shed more light on the suggested mechanisms of Cr (VI) effects on riboflavin production, elaborate studies are required concerning the kinetics of Cr transport, cellular accumulation, binding, localization and possible exclusion, generation of oxidative stress as well as regarding the links between Cr and Fe metabolism. Both the detailed enzymatic analyses of yeast RF synthesis induced by chromium and the aspects of cellular Fe-Cr interaction are now being thoroughly investigated and will be published in consecutive papers.

Conclusions

(1) Flavinogenic yeasts overproduce RF at sublethal Cr (VI) concentrations in media supplemented with iron. Cr (III) does not stimulate flavinogenesis.

(2) Cr (VI)-stimulated RF synthesis level exceeds that induced by Fe ion deficit.

(3) Chromium causes a temporary growth inhibition characterized by a prolonged lag phase during which RF is oversynthesized. The total amount of the synthesized RF correlates with the duration time of this phase which in turn depends on Cr concentration. The adaptational lag phase is followed by cell culture proliferation together with a dramatic drop of RF biosynthesis rate.

(4) Cr (VI) treatment does not affect negatively the cellular total iron level. The putative mechanism of Cr-induced RF synthesis *in vivo* may involve the derepression of the enzymes of flavinogenesis pathway reactions, namely GTP-cyclohydrolase and RF-synthase.

(5) Riboflavin oversynthesis may be a stress-like reaction leading to the enhanced yeast resistance to hexavalent chromium.

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References

Arslan P, Beltrame M, Tomasi A. 1987 Intracellular chromium reduction *Biochim Biophys Acta* **931**, 10–15.

Appenroth D, Shulz O, Winnefeld K. 1996 Riboflavin can decrease the nephrotoxic effect of chromate in young and adult rats. *Toxicol Lett* **87**, 47–52.

Appenroth KJ, Bischoff M, Gabrys H, Stockel J, Swartz HM, Walczak T, Winnefield K. 1999 Kinetics of chromium (V) formation and reduction in fronds of the duckweed *Spirodela polyrhiza* – a low-frequency EPR study. *J Inorg Biochem*, in press.

Babyak LY, Sibirny AA, Shavlovsky GM. 1993 The selection and some characteristics of mutants *Pichia guilliermondii rib*81 with defects of riboflavin biosynthesis regulation. *Cytologia Genetika* **27**, 28–31.

Cieslak-Golonka M. 1995 Toxic and mutagenic effects of chromium(VI) A review. *Polyhedron* **15**, 3667–3689.

Corbett GE, Dodge DG, O'Flaherty E, Liang J, Throop L, Finley BL, Kerger BD. 1998 *In vitro* reduction kinetics of hexavalent chromium in human blood. *Environ Res* **78**, 7–11.

Costa M. 1997 Toxicity and carcinogenicity of Cr(VI) in animal models and humans. *Crit RevToxicol* **27**, 431–440.

Cohen MD, Kargacin B, Klein CB, Costa M. 1993 Mechanisms of chromium cancerogenicity. *Crit Rev Toxicol* **23**, 255–268.

Demain AL. 1972 Riboflavin oversynthesis. *Annu Rev Microbiol* **26**, 369–388.

Dillon CT, Lay PA, Bonin AM, Cholewa M, Legge GJF, Collins TJ, Kostka KL. 1998 Permeability, cytotoxity and genotoxicity of chromium (V) and chromium (VI) complexes in V79 chinese hamster lung cells. *Chem Res Toxicol* **11**, 119–129.

Enari TM. 1955 Effect of cobalt and iron on riboflavin by *Candida guilliermondii. Acta Chem Scand* **9**, 1726–1729.

Fedorovich DV, Kityk IV, Dzala VI, Protchenko OV, Shavlovsky GM. 1997 Accumulation and redox transformations of iron in the yeast *Pichia guilliermondii* and its flavinogenic mutants. *Microbiologia* **66**, 60–64.

Fedorowicz D, Krzeminska H, Babjak L, Kaszycki P, Koloczek H. 1999a Chromium toxicity in yeast *Pichia guiliermondii. Current Genetics* **35**, 448.

Fedorowicz D, Babiak L, Krzeminska H, Kaszycki P, Kołoczek H. 1999b Stimulation of flavinogenesis by chromium (VI) in chromium-resistant mutants of the yeast *Pichia guilliermondii. Folia Histochem Cytobiol* **37 S 1**, 60.

Fedorovich DV, Protchenko OV, Lesuisse E. 1999 Iron uptake by the yeast *Pichia guilliermondii*. Flavinogenesis and reductive iron assimilation are co-regulated processes. *BioMetals* **12**, 295–300.

Knusel F. 1957 Die Biosynthese des Riboflavins bei *Candida guilliermondii* (A. Cast) Langeron und guerra und einigen verwandten Arten. Einfluss von Saurenelementen, ins besonders von Eisen und Zink. *Arch Microbiol* **27**, 219–226.

Koh JS, Choin J, Lee JH, Roe JH. 1996 Regulation of the ribA gene encoding GTP cyclohydrolase II by the soxRS locus in *Escherichia coli. Mol Gen Genet* **251**, 591–598.

Lay PA, Levina A. 1998 Activation of molecular oxygen during the reactions of chromium (VI/V/IV) with biological reductants: Implications for chromium-induced genotoxicities. *J Am Chem Soc* **27**, 6704–6714.

Liu KJ, Jiang J, Shi X, Gabryś H, Walczak T, Swartz HM. 1995 Low-frequency EPR study of chromium (VI) in living plants. *Biochem Biophys Res Comm* **206**, 829–834.

Logvinenko EM, Trach VM, Kashchenko VE, Zakalskij AE, Koltun LV, Shavlovsky GM. 1977 Study of activities of some yeast flavinogenic enzymes *in situ. Biochimia* **42**, 1649–1654.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951 Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**, 265–275.

Luo H, Lu Y, Mao Y, Shi X, Dalal NS. 1996 Role of chromium(IV) in the chromium(VI)-related free radical formation, dG hydroxylation, and DNA damage. *J Inorg Biochem* **64**, 25–35.

Naik MS, Das NB. 1964 Effect of cooper and zink deficiency on the synthesis of protein and riboflavin by *Aspergillus niger. Indian J Exptl Biol* **2**, 59–64.

Ramana VV, Sastry KS. 1994 Chromium toxicity in *Neurospora crassa. J Inorg Biochem* **56**, 87–95.

Shavlovsky GM, Logvinenko EM. 1988a Flavin overproduction and its molecular mechanisms in microorganisms. *Prikl Biochim Microbiol* **24**, 435–447.

Shavlovsky GM, Logvinenko EM. 1988b The participation of iron in the regulation of protein synthesis in microorganisms. *Uspiechi Mikrobiologii* (*Adv Microbiol*) **22**, 108–133.

Shavlovsky GM, Logvinenko EM, Bendorf R, Koltun LV, Shlee D, Reinbothe H. 1980 First reaction of riboflavin biosynthesis – catalysis by a guanosine triphosphate cyclohydrolase from yeast. *Arch Microbiol* **124**, 255–259.

Shukla JP, Prabku KA. 1961 Studies in the production of riboflavin by *Aspergillus niger*. *J Sci Industr Res* **20**, 40–43.

Sibirny AA, Sharova VP, Kshanovskaja BV, Shavlovsky GM. 1977 Selection of genetic line of the yeast *Pichia guilliermondii* capable of producing large amounts of spores. *Cytologia Genetika* **11**, 330–333.

Shi X, Dalal NS. 1994 Generation of hydroxyl radical by chromate in biologically relevant systems: role of Cr(V) complexes versus tetraperoxochromate(V) *Environ Health Perspect* **102**, 231–236.

Trach VM, Logvinenko EM, Shlee D, Reinbothe H. 1982 Einflus von Transkriptionsinhibitoren auf die Derepression der Riboflavinsynthase in der Hefen *Torulopsis candida*. *Biochem Physiol Pflanzen* **177**, 585–592.

Wang Y-T, Shen H. 1995 Bacterial reduction of hexavalent chromium *Industrial Microbiol* **14**, 159–163.