ELSEVIER

Biochimica et Biophysica Acta 1421 (1999) 175–182

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Effect of hexavalent chromium on eukaryotic plasma membrane studied by EPR spectroscopy

Joseph Belagyi [a], Maya Pas [b], Peter Raspor [b], Miklós Pesti [c], Tibor Páli [d,*]

[a] *Central Research Laboratory, University Medical School, Pécs, Hungary*
[b] *Food Science and Technology Department, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[c] *Department of Genetics and Microbiology, Janus Pannonius University, Pécs, Hungary*
[d] *Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary*

Received 11 May 1999; received in revised form 15 July 1999; accepted 28 July 1999

Abstract

The effect of Cr(VI) anion on an ergosterol-producing strain of eukaryotic yeast *Candida albicans* and its mutant with ergosterol-less membrane was studied with EPR spectroscopy. 5- and 14-doxyl stearic acid spin probes were used to label the protoplast membrane after removal of the cell wall. In control experiments, the mutant strain exhibited larger rigidity in the membrane than its parental strain. Addition of Cr(VI), at a minimum inhibitory concentration of 0.6 mM, increased the rotational mobility of the spin labels significantly and decreased the temperature of the structural changes in both strains, in the temperature range between 0 and 30°C. The ergosterol-less mutant, having a membrane composition with increased polyunsaturated fatty acid content, exhibited higher Cr(VI) sensitivity. Treatment of the membrane with Cr(VI) for 10 min already resulted in an increase in membrane fluidity. An EPR signal of Cr(V) was detected which reached maximum amplitude after 120 min of treatment with Cr(VI). Further chemical reduction of Cr(V) in the absence of extracellular Cr(VI) led to a lack of detectable paramagnetic chromium intermediates within 200 min.

Keywords: Chromium VI; Spin label EPR; Membrane fluidity; *Candida albicans*

1. Introduction

Chromium VI compounds are known to be potent mutagenic, toxic and carcinogenic agents, as shown by epidemiological studies [10]. Numerous aspects of the complex effect of Cr(VI) on eukaryotic cells and tissues have already been studied extensively [3,8,12,19]. Chromium VI penetrates into the cell as a tetrahedral chromate (CrO_4^{2-}) anion using a non-specific anion carrier, the so-called permease system, which transports a number of anions with a tetrahedral configuration, such as SO_4^{2-} and PO_4^{2-} [24]. In contrast to Cr(III), the anion Cr(VI) has a very weak association with the negatively charged polynucleotide chains, as revealed by in vitro studies with purified DNA [23]. The carcinogenic effect of Cr(VI) within the cell may therefore be explained by redox processes. Cr(VI) is reduced to Cr(III) thereby generating unstable intermediates, i.e., Cr(V) and Cr(IV) ions [2]. Recent studies have also shown that free

Abbreviations: EPR, electron paramagnetic resonance; 5-SASL, 5-(4′,4-dimethyl′-oxazolidine-*N*-oxyl) stearic acid; 14-SASL, 14-(4′,4-dimethyl′-oxazolidine-*N*-oxyl) stearic acid

* Corresponding author. Fax: +36-62-433133;
E-mail: tpali@nucleus.szbk.u-szeged.hu

0005-2736/99/$ – see front matter
PII: S0005-2736(99)00129-7

radicals, such as hydroxyl free radicals, are generated during the reduction process. Singlet oxygen, superoxide anion, thiyl and organic free radicals are all responsible for the DNA damage observed earlier [1,9,18]. The end product of Cr(VI) reduction is Cr(III) that binds to purified DNA in vitro, forming adducts, DNA-DNA cross links, and DNA-protein cross links reducing the fidelity of DNA polymerase [21,22].

This multiplicity of interactions between chromate anions and cellular processes has been demonstrated by in vivo and in vitro experiments. However, interactions of Cr(VI) with plasma membranes have not been investigated until now. In order to get more information about the interaction of chromium ions with the components of the biological membranes after addition of Cr(VI), cell growth and EPR experiments were performed in the present study on an ergosterol non-producing yeast membrane mutant and its parental strain to elucidate the importance of membrane components in this interaction. The lipid components of the mutant strain have already been characterized both qualitatively and quantitatively [16]. The enzymatic and transport properties of membrane proteins have been shown to be sensitive both to the membrane lipid composition and to the mobility of the lipid chains [15]. Because the lipid bilayer is the major permeability barrier of the membranes, potential perturbation of this structure by chromium in the presence of membrane proteins may also influence the membrane properties. This process should then be dependent on the lipid composition, and therefore the physical state of the membrane, which is known to be different in the two strains [20]. Spin probes, providing fluidity data, incorporated directly into the plasma membrane of the two strains and paramagnetic forms of reduced Cr(VI) were measured by EPR in the present work to address these questions.

2. Materials and methods

2.1. Chemicals

$K_2Cr_2O_7$ and other chemicals were of analytical grade and purchased from Reanal Co. (Hungary). $K_2Cr_2O_7$ solutions were always freshly prepared. 5-SASL was purchased from Sigma Co. (Germany). 14-SASL was kindly donated by Derek Marsh (Max-Planck-Institut für biophysikalische Chemie Göttingen, Germany).

2.2. Strains and culture conditions

The ergosterol-deficient membrane mutant of *Candida albicans*, *erg-2* (ATCC 44831), originated from the adenine auxotroph ergosterol-producing strain *33 erg*$^+$ (ATCC 44829), as described earlier [15]. The following media were used throughout the experiments: complete medium (YG): 0.5% yeast extract, 1% glucose, 25 μg/ml adenine; minimal medium (MM): 1% glucose, 0.5% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 25 μg/ml adenine, 2 μg/ml biotin, 400 μg/ml thiamine (pH 6.0). Media were solidified with 2% agar when required. Four day old stock cultures were used to prepare overnight mid-log phase cultures. Minimal inhibitory concentrations of $K_2Cr_2O_7$ were determined by a twofold serial dilution test on YG and MM agar, as described previously [15]. The inhibitory effect of $K_2Cr_2O_7$ on growth at different concentrations was measured in liquid MM cultures for 60 h at 30°C on a shaker at 200 rpm. The starting cell number was 1×10^6 cells/ml, the cell numbers were determined using a hemocytometer.

2.3. Preparation of samples for spin labelling experiments

Mid-log phase cells were collected by centrifugation and washed two times with 0.6 M KCl solution. For protoplast formation, the suspension was incubated with 2% lyophilized snail enzyme, prepared from *Helix pomatia* in 0.6 M KCl as osmotic stabilizer. In order to perform spin labelling of the plasma membrane of vegetative cells, cell wall-free protoplasts were used. After 2 h incubation at 37°C, the protoplasts were washed three times in stabilizer solution and diluted fivefold in 0.6 M KCl prior to addition of spin probes. For control experiments, a 13 μl aliquot of a solution of 5-SASL or 14-SASL (5 mg/ml in ethanol) was added to 300 μl of cell suspension, and the mixture was gently shaken for 10 min at room temperature to facilitate spin probe incorporation. The protoplast suspension was then

sedimented (at $3000 \times g$ for 3 min) and resuspended in 100 μl 0.6 M KCl. The suspension was filled into a 100 μl capillary tube, then centrifuged again at 4°C and the supernatant was removed. Capillary tubes contained 5×10^8 protoplasts in each experiment. Under these conditions, no isotropic triplet arising from unincorporated spin probes was detected.

In order to study the effect of Cr(VI) on plasma membranes, 0.6 mM $K_2Cr_2O_7$ dissolved in 0.6 M KCl was used. Addition of $K_2Cr_2O_7$ did not change the pH (8.2) of the solution. $K_2Cr_2O_7$ solution was added to the protoplast suspension following the incorporation of spin labelled fatty acids into the membrane. After 120 min of Cr(VI) treatment, the cell suspension was washed again with 0.6 M KCl.

2.4. Electron paramagnetic resonance measurements

Electron paramagnetic resonance spectra were recorded with an ESP 300E spectrometer (Bruker, Germany), equipped with an ER 412VT temperature regulator. The EPR spectra of fatty acid-free radical spin labels 5-SASL and 14-SASL incorporated into the membranes were taken in the temperature range of 0–30°C on both control and chromium-treated samples. The conventional EPR spectra were obtained using 5 mW microwave power, and 100 kHz field modulation with an amplitude of 0.1–0.2 mT, and a 10 mT scan width. The parallel and perpendicular hyperfine splitting parameters partially averaged by limited rotational motion ($A_{\parallel}$ and $A_{\perp}$, respectively) of the spin label molecules, known to be related to the orientational order parameter in membranes [4,13], were used to study the effects of Cr(VI) on the fluidity of protoplast membranes [4,13]. In the case of slow motion at lower temperatures, where the inner splitting ($2A_{\perp}$) could not be derived clearly from the spectra, the splitting of the outermost extrema ($2A_{\parallel}$) was used. However, the varying large EPR signal arising from Cr(V) of samples treated with chromium, and for which subtraction was not a rational approach, overlapped with the high-field extrema of the 5-SASL spectrum. A simple parameter was therefore introduced instead of $2A_{\parallel}$ to characterize the changes in the mobility of probe molecules as a function of temperature. This parameter, designated by $\Delta H(+1)$, was defined as the distance between the low-field maximum and the cross-over point of the central hyperfine peak. Unfortunately, the accuracy of this spectral parameter is smaller than that of $2A_{\parallel}$ normally measured without a disturbing spectral component, but the systemic error can be estimated to be not larger than the g-value anisotropy [6] and to not influence temperature-dependent differences.

In order to characterize the difference between the strains and the effect of Cr(VI), the break in the plot of the spectral parameter $\Delta H(+1)$ against temperature was approximated by two straight lines. The straight lines were obtained using a computer program that calculated the breakpoint by searching for the minimum of the residual sum of squares of the two regression lines. According to Jones and Molitoris, an approximate F value was calculated to verify that a broken line gave a significantly better fit than a single straight line [7]. Data analysis showed that the two-line model resulted in a significantly better fit in all cases.

3. Results and discussion

3.1. Characterization of the membrane

The plasma membrane-Cr(VI) interaction was investigated using an ergosterol-producing wild-type *33 erg*$^+$ strain of *C. albicans* and its ergosterol-free membrane mutant, *erg-2* [16]. In comparison to *33 erg*$^+$, the *erg-2* has the following features: (i) *33 erg*$^+$ accumulated ergosterol (71.5%) and ergosta-7-en-3β-ol (14.3%), while *erg-2* membrane contained fecosterol (55.6%) and zymosterol (42.7%) as the main sterols, indicating a block of the Δ^8-Δ^7 isomerase; (ii) alterations in sterol composition induced an adaptation in phospholipid composition resulting in decreased amounts of phosphatidylcholine and phosphatidylserine (described as fluidizing lipids) and in an increased amount of phosphatidylinositol, phosphatidylethanolamine and phosphatidic acid (considered to be rigidifying lipids) in the mutant [20]; (iii) as regards the fatty acid composition, a significant increase of polyunsaturated fatty acids of 16:2, 16:3 and 16:8 was detected in *erg-2* [20] which might be attributable to an elevated level of lipid peroxidation and, thus, metal sensitivity [5]. These two well-characterized yeast strains seemed to be useful to

investigate the dynamic properties of the membranes and their significance in the interaction with Cr(VI).

Multiplication of both strains was fully blocked by 0.6 mM Cr(VI) for growth in minimal liquid medium (data not shown). In complete medium, 5 mM Cr(VI) was required to block cell multiplication (Figs. 1 and 2) for both strains. Cell viability was not decreased during the first 2 h required for the EPR measurements. The *erg-2* mutant was more sensitive than the parental *33 erg*$^+$ strain both in minimal and complete medium (cf. Figs. 1 and 2 for the complete medium) at lower Cr(VI) concentrations. These experiments suggest that the differences in the composition of the membrane of the two strains play a role in the interactions between chromate anions and cellular processes. On the basis of these observations, application of 0.6 mM $K_2Cr_2O_7$, which

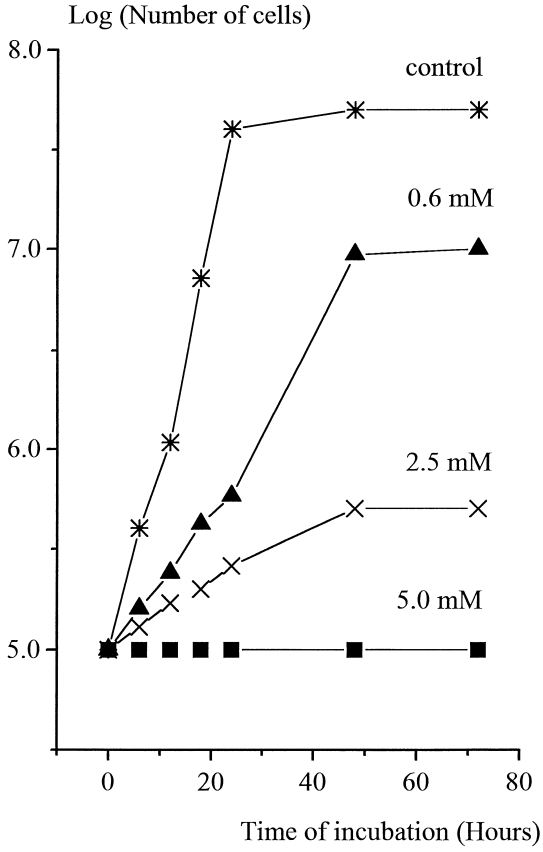

Fig. 1. Effect of $K_2Cr_2O_7$ on growth of an ergosterol-producing strain *33 erg*$^+$ incubated in complete medium for 72 h at 30°C. Symbols: (∗) no $K_2Cr_2O_7$; (▲) 0.6 mM $K_2Cr_2O_7$; (×) 2.5 mM $K_2Cr_2O_7$; (■) 5.0 mM $K_2Cr_2O_7$.

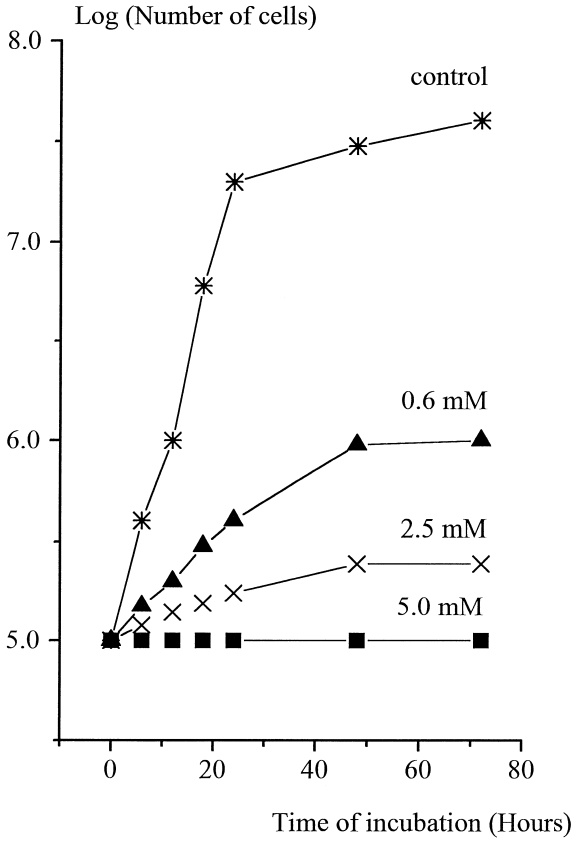

Fig. 2. Effect of $K_2Cr_2O_7$ on growth of mutant *erg-2* in complete medium at 30°C. Symbols: (∗) no $K_2Cr_2O_7$; (▲) 0.6 mM $K_2Cr_2O_7$; (×) 2.5 mM $K_2Cr_2O_7$; (■) 5.0 mM $K_2Cr_2O_7$.

was already effective in complete medium, seemed to be justified to carry out measurements on the interaction of the plasma membrane with Cr(VI) ions by EPR. The EPR measurements were done on yeast extract-free solutions. Integrity of the plasma membrane did not change during treatment with 0.6 mM $K_2Cr_2O_7$, neither lysis nor shrinking of protoplasts was observed.

3.2. Interaction of Cr(VI) with membrane components

Samples were prepared by pre-treatment of the cells in 0.6 mM Cr(VI) for 120 min in order to receive maximum concentration of Cr(VI) in the cells [24]. An EPR signal arising from Cr(V), with $g = 1.9554$, was detected that evidenced rapid reduction of Cr(VI) to Cr(V) [2] (Cr(VI) has no paramagnetism). The concentration of the Cr(V) species gen-

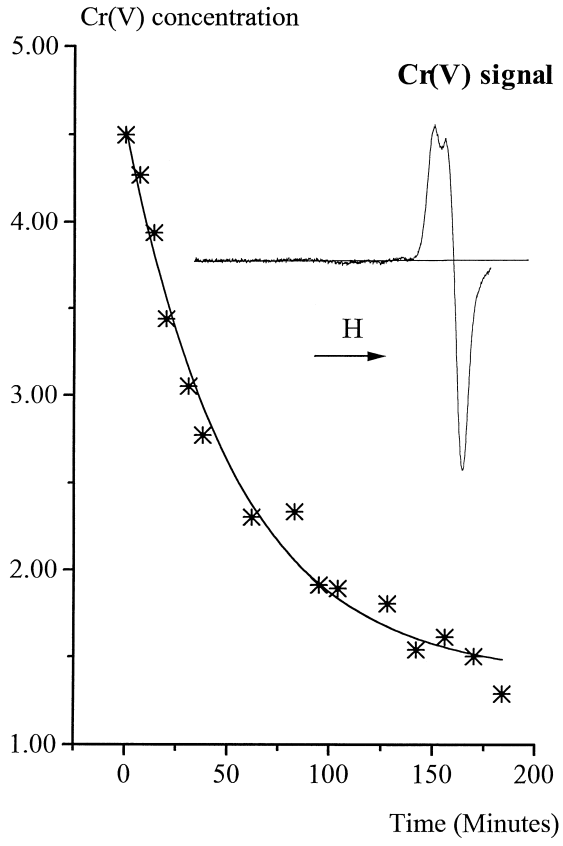

Fig. 3. Time dependence of the concentration of Cr(V) in protoplast preparations at 14°C after 120 min treatment with and then removal of extracellular Cr(VI). The double integral of the Cr(V) EPR signal is plotted against time. Inset shows the Cr(V) signal ($g = 1.9554$) over a scan range of 10 mT.

erated did not remain constant, the double integral of the newly formed Cr(V) species decreased in time due to further chemical reactions (Fig. 3). The semilogarithmic plot of the signal intensity can be approximated by two straight lines (not shown) with different slopes evidencing that the rapid reduction process of Cr(VI) to Cr(V) was followed by slower chemical reactions resulting in the reduction of Cr(V) to Cr(IV) and then to its ultimate form Cr(III) [3,19]. Nearly 200 min was needed to reduce Cr(VI) to Cr(III) in the absence of extracellular Cr(VI). Cr(IV) has again no paramagnetism but we could assign a very broad peak to Cr(III) (data not shown) after incubating samples directly in Cr(III) at high concentrations (since its spectrum is hardly detectable at room temperature below millimolar concentrations). These chemical reactions clearly show the fast incorporation of Cr(VI) ions into the membrane, and give further evidence that the treatment of cells with 0.6 mM $K_2Cr_2O_7$ did not significantly impair the membrane function, at least during the time of incubation and spectroscopic measurements. A slow, temperature-dependent decrease in the intensity of the 5-SASL EPR signal with time was also observed but was found not to affect the spectral shape during the 30 min temperature scans. Since the stearic acid spin label is a free radical, this was very likely a consequence of the metabolic activity, or is due to the effects of various biological reductants in the membranes.

3.3. Effect of Cr(VI) treatment on membrane fluidity

Both spin labels incorporated into the membrane of the protoplasts reflected different mobilities de-

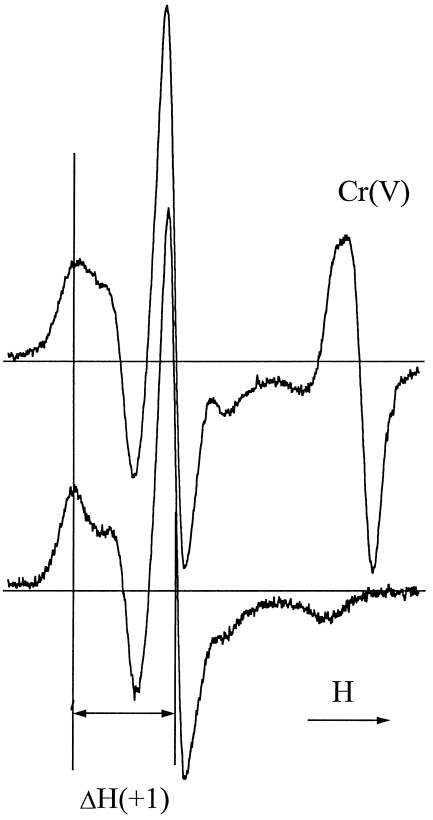

Fig. 4. Representative EPR spectra of 5-SASL incorporated into protoplast membranes of the yeast strain *erg-2* recorded at 14°C. Upper trace: EPR spectrum recorded after 120 min treatment with Cr(VI); bottom: EPR spectrum of a control sample of the same strain. $\Delta H(+1)$ was defined as the distance between the low-field maximum and the crossover point of the central line. The field scan was 10.0 mT.

pending on the strain and the mobilities were affected to different extents by addition of Cr(VI) (cf. Fig. 4 for strain *erg-2*). Ergosterol-producing strain *33 erg*$^+$ exhibited larger fluidity compared to the mutant *erg-2*; the spectral parameter $\Delta H(+1)$ and the distance between the outermost extrema (hyperfine splitting $2A_{\|}$) that characterize the mobility of the 5-SASL and 14-SASL spin labels were smaller for samples of strain *33 erg*$^+$ (note: larger values mean lower fluidity), over the whole temperature range studied in these experiments. At about 16°C and 18.5°C, characteristic changes in the spectral parameter $\Delta H(+1)$ were measured in control samples (no Cr(VI) addition) of the strains (Fig. 5). The straight lines through the data points were obtained by least squares fitting. The temperature of the structural transition for strain *33 erg*$^+$ was about 3°C lower than that of *erg-2*. The break in the temperature profile was independent of whether the spectral parameter $\Delta H(+1)$ versus temperature plot was measured at increasing temperature up to 40°C or at decreasing temperatures from 40°C to 0°C; no hysteresis was observed. The logarithm of the spin label concentration (spectral double integral) as a function of time could not be approximated by straight lines, showing that the reduction process could not be described by a simple first order chemical reaction. The decrease in spin label signal was independent of the addition of chromate to the protoplast membranes. This means that different chemical processes are responsible for the reduction of Cr(V) to lower oxidation states and the reduction of 5-SASL to the diamagnetic species.

The breaks in the temperature plots, measured at

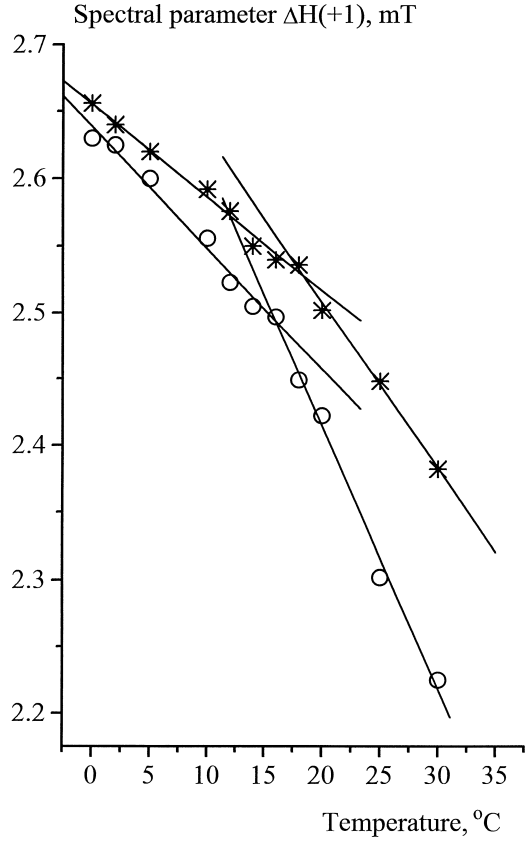

Fig. 5. Plot of the spectral parameter $\Delta H(+1)$ as a function of temperature. Protoplast samples prepared from strains *33 erg*$^+$ and *erg-2* were spin labelled with 5-SASL. Symbols: (○) strain *33 erg*$^+$; (∗) strain *erg-2*. The characteristic change at 18°C in the plot for *erg-2* is about 3°C greater than the change for *33 erg*$^+$.

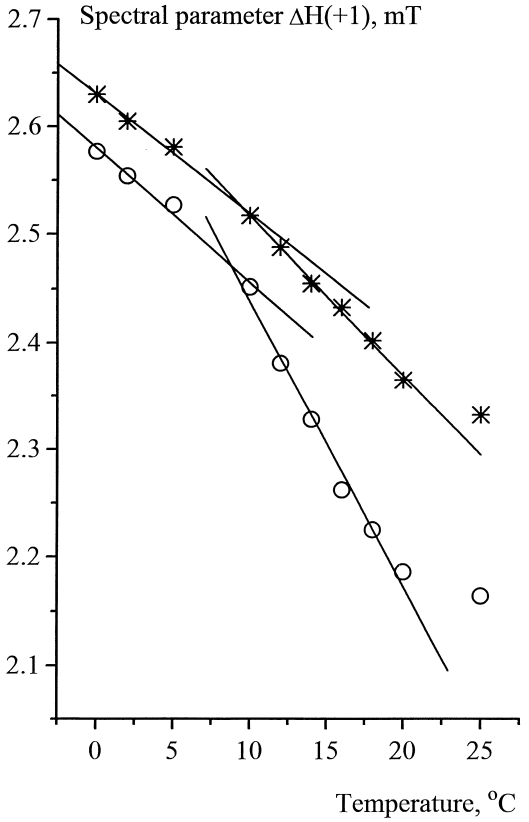

Fig. 6. Effect of Cr(VI) treatment (0.6 mM, 120 min) on spin label mobility in yeast protoplast membranes. The spectral parameter $\Delta H(+1)$ decreases after addition of Cr(VI) ions. The protoplast membranes were labelled with 5-SASL. Symbols: (○) strain *33 erg*$^+$; (∗) strain *erg-2*.

about 18.5°C and 16°C in samples of the two strains, decreased very significantly after treatment with Cr(VI); the change was about $\Delta T = 8$°C (Fig. 6). The differences between the characteristic EPR parameters of the strains were reduced after treatment with Cr(VI). It has been shown earlier that the uptake kinetics of Cr(VI) ions in cell fractions of yeast was linear during an incubation period of 30 s to 12 min [14]. In our case, a shorter incubation (10 min instead of 120 min) of the membranes with 0.6 mM $K_2Cr_2O_7$ resulted in a smaller change in fluidity. The apparent rotational correlation time of the 5-SASL label in samples of *erg-2* decreased from 22.2 ns to 17.2 ns after 10 min treatment with Cr(VI), whereas addition of Cr(VI) to the *erg-2* sample induced a decrease of τ_2 from 22.2 ns to 10.6 ns after 120 min treatment at 10°C. The rotational correlation time was calculated according to the Goldman equation assuming a rigid limit of $2A_{\parallel} = 6.648 \pm 0.038$ ($n = 4$) mT and a rigid limit value of the low-field linewidth of 0.277 mT ($n = 3$) [4]. These values were obtained on control samples of *erg-2* measured at −20°C. At lower temperatures the hyperfine splitting did not increase, but a significant spectral broadening was observed. The experiments with 10 min incubation of the membrane suspensions with Cr(VI) might reflect purely the Cr(VI) ion-membrane interaction. Addition of Cr(VI) for 120 min further increased the mobility of 5-SASL in the membrane, which could be partly the result of metal-induced peroxidation [5] or toxic intermediates of Cr(VI) reduction [19].

The 14-SASL spin label, for which the nitroxide moiety is located in the more hydrophobic environment of the membrane, also showed significant differences between strain *33 erg*$^+$ and *erg-2*; addition of Cr(VI) increased rotational mobility. The order parameter (S) calculated from the spectra also showed a characteristic change in the plot against temperature at 15°C (data not shown), but the difference between the two strains seemed to be smaller in comparison to the outer region of the membranes, which might be due to a non-uniform distribution of the different chromium species along the membrane normal.

In the case of the 5-SASL spin probe, where the nitroxide moiety is situated near the surface of the membranes, the spectra reflect the changes close to the lipid-water interface. The Cr(III) cations that cannot penetrate the protoplast membrane have a preferred localization at the negatively charged regions of the membrane [2,11], and might tend to create bridges between phospholipid molecules and the side chains of negatively charged amino acid residues. These interactions reduce the stability of the head group regions of the protoplast membrane, and can affect the boundary layer of phospholipids around the membrane proteins. In such a way, the receptor and transport activities that are usually associated with the protein components of the membrane can be modulated by the altered properties of the lipid matrix [11]. In contrast to Cr(III) ions, Cr(VI) ions are able to pass across the membrane [2,3,24]. They readily interact with components of the membrane, and might perturb the hydrophobic region of the membrane also, as reported by the 14-SASL probes. The decrease in the Cr(V) EPR signal makes it difficult to verify that the change in spectral parameters observed after treatment with Cr(VI) ions at the hydrophilic region is produced entirely by Cr(VI) ions. Cr(III) ions are very effective perturbants of the outer region of the membrane [2,17], and Cr(VI) ions are reduced to Cr(III). However, due to its spectral properties, a strong EPR signal from Cr(III) ions is hardly expected. The spectral changes detected by 14-SASL probes support the view that specific or aspecific interactions of Cr(VI) ions with membrane components could independently contribute to the fluidity change of the membrane induced by the treatment.

In summary, we have shown that Cr(VI) uptake and reduction was fast but further reduction of Cr(V) was relatively slow. It was demonstrated that the effect of Cr(VI) on cell viability depends on the lipid composition of the membrane and the effect on membrane fluidity was found to be time dependent having been observable already on the time scale of Cr(VI) uptake. In addition, the relative effect of Cr(VI) on membrane fluidity was found to be dependent on the depth in the membrane. The changes in the local structure produced by Cr(VI), its unstable intermediates, the presence of Cr(III) ions at the outer and inner headgroup regions of the protoplast membrane, as well as the changes in the electric charge of the cell surface could affect the physiological function of the cells. The altered permeability for different substances, the toxicity of substances and

the ion binding potential will influence the signalling processes, the cell-cell interactions, and hence the viability of cells.

Acknowledgements

This work was supported by Grants PKFP 508/1997 and 463/1997 from the Ministry of Culture and Education Hungary. EPR measurements were supported by Grants OTKA CO123, T020593 and T029458 from the National Research Foundation, Grant AMFK 110/96 from the Ministry of Culture and Education and Grant T-06 737/96 from the Ministry of Health, Hungary. The authors thank E. Palotai and E. Greiner for skillful technical assistance.

References

[1] J. Aiyar, H.J. Berkovits, R.A. Floyd, K.E. Wetterhahn, Chem. Res. Toxicol. 3 (1990) 737–741.
[2] P. Arslan, M. Beltrame, A. Tomasi, Biochim. Biophys. Acta 931 (1987) 10–15.
[3] M.D. Cohen, B. Kargacin, C.B. Klein, M. Costa, CRC Rev. Toxicol. 23 (1993) 225–281.
[4] S.A. Goldman, G.V. Bruno, J.H. Freed, J. Phys. Chem. 76 (1972) 1858–1860.
[5] N.G. Howlett, S.V. Avery, Appl. Environ. Microbiol. 63 (1997) 2971–2976.
[6] J.S. Hyde, J.R. Pilbrow, J. Magn. Reson. 41 (1980) 447–457.
[7] R.H. Jones, B.A. Molitoris, Anal. Biochem. 141 (1984) 287–290.
[8] S.A. Katz, H. Salem, J. Appl. Toxicol. 13 (1993) 217–224.
[9] C.B. Klein, K. Frenkel, M. Costa, Chem. Res. Toxicol. 4 (1991) 592–604.
[10] S. Langard, Am. J. Ind. Med. 17 (1990) 189–215.
[11] A. Leonard, R.P. Lauwerys, Mutat. Res. 77 (1980) 227–231.
[12] M.E. Losi, C. Amrheim, W.T. Frankenberger, Rev. Environ. Cont. Toxicol. 136 (1994) 91–121.
[13] D. Marsh, in: E. Grell (Ed.), Electron Spin Resonance: Spin Labels in Membrane Spectroscopy, Springer, Berlin, 1981, pp. 52–198.
[14] B.-I. Ono, Curr. Genet. 6 (1982) 71–77.
[15] M. Pesti, J.McA. Campbell, J.F. Peberdy, Curr. Microbiol. 5 (1981) 187–190.
[16] M. Pesti, L. Horváth, L. Vigh, T. Farkas, Acta Microbiol. Hung. 32 (1985) 305–313.
[17] M. Pesti, M. Pas, P. Raspor, J. Belagyi, 8th International Conference of Bacteriology and Applied Microbiology, Jerusalem, 1996, Abstracts, p. 21.
[18] X. Shi, Z. Dong, N.S. Dalal, P.M. Ganett, Biochim. Biophys. Acta 1226 (1994) 65–72.
[19] X. Shi, A. Chiu, C.T. Chen, B. Halliwell, V. Castranova, V. Vallyathan, J. Toxicol. Environ. Health 2 (1999) 87–104.
[20] M. Shinitzky, P. Henkart, Int. Rev. Cytol. 60 (1980) 187–242.
[21] E.T. Snow, Environ. Health Perspect. 92 (1991) 75–81.
[22] E.T. Show, L.-S. Xu, Biochemistry 30 (1991) 11238–11245.
[23] M.J. Tsapakos, K.I. Wetterhahn, Chem.-Biol. Interact. 46 (1983) 265–272.
[24] H.J. Wiegand, H. Ottenwalder, H.M. Bolt, Arch. Toxicol. 57 (1985) 31–34.