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In vitro effects of sodium fluoride and sodium dichromate on dynamic properties of human erythrocyte membrane

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Abstract

Sodium fluoride (NaF) and sodium dichromate ($Na_2Cr_2O_7$) are two different toxic compounds which are used as a dental caries prophylactic and as an oxidising agent in various industrial areas, respectively. However, accidental fluoride and chromate poisoning is not a rare occurrence, even death may result from cardiac or respiratory failure. In the present work, alterations produced by NaF, $Na_2Cr_2O_7$ and temperature changes in the molecular dynamics of the human erythrocyte membrane were studied, in vitro, by the spin-labelling ESR technique. Human intact erythrocyte cells spin labelled with 5- and 16-doxyl stearic acids (5-DSA and 16-DSA) and treated with 40 μM NaF and 5 μM $Na_2Cr_2O_7$ at 37°C were used to quantify membrane fluidity. This was performed by measuring the changes in the order parameter (S), correlation time (τ) and phase transition temperature using recorded electron spin resonance (ESR) spectra. Experimental results show that 5 μM $Na_2Cr_2O_7$ and 40 μM NaF do not produce any significant effects on the order parameter of 5-DSA spin label while they cause appreciable changes in the correlation time of the same label. As for 16-DSA, while $Na_2Cr_2O_7$ does not produce any measurable effect on the order parameter of this label, NaF does in a certain extent. Although weak, the effects of both compounds on the correlation time of 16-DSA are found to be well above the experimental error limits. Change in temperature was observed to alter significantly S and τ parameters which show biphasic character in the temperature range of 5–50°C. Activation energies of the hydrocarbon chains above and below transition temperatures were also determined for untreated and NaF or $Na_2Cr_2O_7$ treated erythrocyte cells and the effect of NaF and $Na_2Cr_2O_7$ on these energies and transition temperatures were discussed.

Keywords: Sodium fluoride; Sodium dichromate; Human; Erythrocyte; ESR

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1. Introduction

Fluoride is a normal component of body fluids and soft tissues. The normal concentrations of total fluorides in plasma and concentration of ionic fluoride in the serum are well documented [1]. However, most of the fluoride in the body is deposited in the bones and teeth. NaF (hereafter SF) is very frequently used as an additive to the drinking water and to the diet, or as a solution painted directly on the teeth, because its fluoride anion prevents dental caries. Accidental fluoride poisoning is not a rare occurrence. Large doses cause muscular weakness and clonic convulsions, followed by respiratory and cardiac failure and death within a few hours. Chronic fluoride poisoning may arise from continued ingestion of fluoride and has occurred in workers handling fluorides and in communities using drinking water containing particularly high concentrations of natural fluorides [2,3].

Development of anaemia has been demonstrated in experimental animals chronically exposed to toxic amounts of fluoride [4,5]. Fluoride in vitro and in vivo has been reported to cause enhanced generation of superoxide radicals ($O_2^{\bullet -}$) and lipid peroxidation in polymorphonuclear leukocytes and in tissues of fluorosed animals [5–7] leading to alteration in cell membrane function and structure. The exposure of red cells to the high level of fluoride in the erythropoietic tissue and in blood of fluoretic patients during the circulation have been shown to induce structural and functional changes in erythrocyte cell membrane. On the other hand, in a concentration of 1%, SF was considered the most efficient and suitable preservative for postmortem blood samples, preventing the generation of alcohol in blood samples [8].

As for $Na_2Cr_2O_7$ (hereafter SDC), it was found to produce extreme sensitisation of the skin [9]. Ingestion of large amounts causes symptoms which include vomiting and diarrhoea, gastrointestinal haemorrhage and signs of hepatic and renal impairment. It appears that dichromate is retained in the body and excreted in urine over a period of months [10]. Hexavalent chromium compounds are more dangerous than di- or trivalent compounds.

The chromate anion has been reported to be rapidly taken up by human erythrocyte cells and reduced intracellularly to trivalent chromium which is accumulated in a large extent in the cells. Hexavalent chromium anion reacts with intracellular electron donors such as the thiol groups of glutathione or haemoglobin and it produces structural and functional changes by damaging membrane constituents. It modifies even the kinetics of its own transport through the red cell membrane [11,12]. In the literature, it has been shown that at chromate concentrations higher than 1 mM spectrin crosslinks but not the anion transporter protein in the erythrocyte membrane [13].

In the present work, in vitro effects of SF, SDC and temperature on the order and molecular dynamics of the human erythrocyte membrane were studied by electron spin resonance (ESR) spectroscopy using 5- and 16-doxyl stearic acid as spin labels in order to get more insight into the molecular mechanisms giving rise to the structural and functional changes in the human erythrocyte membrane by these compounds.

2. Materials and methods

2.1. Membrane model

Human erythrocytes were used as a membrane model system, since the membranes of erythrocytes are the best understood of all cellular membranes in terms of molecular composition and function. Fresh human blood, drawn from healthy volunteers was collected in citrate–phosphate dextrose (CPD) in polyvinyl chloride containers and stored at 4°C for 1–3 h. The blood was then washed with 4°C phosphate-buffered saline (PBS) solution of mM composition NaCl 145, KCl 5, $NaPO_4$ 5 with a pH of 7.4, and then centrifuged at 3500 rev./min for 10 min. The buffy coat was carefully collected by aspiration and discarded. This step was repeated at least three successive

times. The erythrocytes were then suspended in PBS to an haematocrit of 20%.

2.2. Spin-labelling and treating with sodium fluoride and sodium dichromate

Fatty acid spin-labels of 5-doxylstearic acid and 16-doxylstearic acid, which have a stable nitroxide radical ring at the C-5 and C-16 positions (counted from the carboxyl group of the acyl chain), respectively, were used. Stearic acid spin-labels were dissolved in ethanol. To 3 μg of probe, evaporated under nitrogen stream an amount of membranes corresponding to 0.3 mg lipids were added to keep spin-label concentrations low enough and therefore to avoid any spin–spin interaction. At room temperature, the time needed for the spin-label incorporation into membranes was very short and, after 5–10 min, the spin-labels were completely associated with the membranes. At the end of the spin labelling step, labelled erythrocytes were washed twice with phosphate-buffered saline solution to remove unincorporated spin-labels.

Spin-labeled erythrocytes of final haematocrit 20% were treated with SF and SDC of concentrations 40 and 5 μM, respectively, for 10, 25, 50, 100, 150 and 200 min at 37°C in a shaking thermostat bath. The 40- and 5-μM concentration values were chosen to stay roughly in the twofold limits of normal concentrations of the F^- and Cr^{6+} ions in plasma. After the treatment, the erythrocytes were suspended in 10 volumes of PBS solution at 4°C and centrifuged at $3000 \times g$ for 3 min, and the pellet was sucked into a 50-μl micropet capillary and sealed at both ends with Critoseal. Care was taken to avoid direct contact of the cell suspension with Critoseal so as to avoid leakage of Mn^{2+} ions from the seal into the cell suspension.

2.3. ESR spectroscopy

Spectra were recorded on a Varian E-line 9″ spectrometer operated at 9.5 GHz, with TE_{102} cavity resonator, 3300 field set, 100-kHz field modulation, 1.25 G peak-to-peak modulation amplitude and 10 mW microwave power. The difficulties in determining the intensities of the low- and high-field lines were overcome either by increasing the gain of the spectrometer or by setting the modulation amplitude at 5 G without apparent changes in the $2T'_{\parallel}$ values. Sample temperature inside the microwave cavity was monitored with a digital temperature control system (Bruker ER 4111-VT). The latter gives the opportunity of measuring the temperature with an accuracy of ± 0.5°C at the site of sample. The temperature of the samples were changed between 5 and 50°C with a 5°C increment. To establish a complete thermal equilibrium, samples were kept for 5 min at each measuring temperature before recording the spectra.

2.4. Spectral analysis

ESR spectra obtained from membranes of intact human erythrocyte cells with the spin labels 5-DSA and 16-DSA show contributions mostly from spin-labels of restricted motion with negligible contributions from free-moving spin labels. The ESR spectra were evaluated by calculating the motionally averaged nitrogen hyperfine tensor components $2T'_{\parallel}$ and $2T'_{\perp}$ from which the order parameter, S, were calculated according to the following equation [14]

$$S = \frac{T'_{\parallel} - T'_{\perp} - C}{T'_{\parallel} + 2T'_{\perp} + 2C} \times \frac{T_{zz} + T_{xx} + T_{yy}}{T_{zz} - 1/\ 2(T_{xx} + T_{yy})} \quad (1)$$

where $T'_{\parallel}$ and $T'_{\perp}$ are the hyperfine parameters (in gauss) measured directly from experimental spectra and T_{xx}, T_{yy}, T_{zz} are the single crystal hyperfine tensor principal elements of the relevant spin-label. The constant C is a correction factor and is given as

$$C = 1.45\left[1 - \frac{T'_{\parallel} - T'_{\perp}}{T_{zz} - 1/2(T_{xx} + T_{yy})}\right]$$

Due to the anisotropy of the erythrocyte membrane the calculated order parameters are not true order parameters, but the apparent order

parameter as well as $2T'_{\parallel}$ measurements may be used to obtain information on the dynamic behaviour of the membrane. No measurable clustering effect of spin-labels was observed for spin-label/membrane lipid ratio (1/100) adopted in the present work. That is, the values of the parameters were not affected by spin–spin interaction and clustering.

The rotational correlation time, τ, was calculated using the following equation [15]

$$\tau = 3.418 \times 10^{-10} \times \Delta H(0) \left[\sqrt{\frac{h(0)}{h(-1)}} - \sqrt{\frac{h(0)}{h(1)}} \right] \quad (2)$$

where $h(0)$, $h(1)$ and $h(-1)$ are the peak height of the centre, low-field and high-field lines, respectively, and $\Delta H(0)$ is the width of the central line. Eq. (2) is suitable for a rod-like molecule and especially for perpendicular resonance spectra. Because of assumptions made in the derivation of Eq. (2), its validity is questionable for rotational correlation times longer than approximately 2 ns.

For a rod-like molecule of radius, r, and length, l, the rotational correlation time is related to the rotational viscosity as

$$\tau = \frac{2\pi l \eta r^2}{kT} \quad (3)$$

where η is the viscosity, T is the absolute temperature and k is the Boltzmann constant. On the other hand, the activation energy E_{ac} of rotational viscosity is given by Andrade's equation [16] for viscosity, that is,

$$\eta = B \exp[E_{ac}/RT] \quad (4)$$

where R and T are the gas constant and absolute temperature, respectively. From the free volume theory, B may be given by [17]

$$B = B_0 T^{3/2} \quad (5)$$

Using above equations, we have constructed Andrade plots for the activation energies of microviscosity of the labelled erythrocyte membrane untreated and treated with SF and SDC.

2.5. Chemicals

5-DSA and 16-DSA stearic acid spin-labels were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). SF, SDC and all other chemicals used for sample preparation and treatment were of reagent grade from Fluka (Bushs, Switzerland).

2.6. Statistics

Experiments were performed four to seven times and standard deviations were calculated. All calculated order parameters and correlation times are reported as mean ± S.D. of the number of experiments performed. The S.D. values were determined using paired or non-paired Student's t-test depending on appropriateness. P values of less then 0.05 were considered significant changes. The phase transition temperatures and activation energies were determined using the linear regression analysis 'break-points' computer program.

3. Results

At the end of each treatment period four to seven samples prepared from untreated (control) and SF- or SDC-treated erythrocyte cells incubated at 37°C were used to determine an appropriate treatment time. Analysis of the spectra recorded at 37°C for control and treated samples have shown that a treatment time of 60 min was enough for both SF and SDC to produce their ultimate effect on the order parameter (S) and correlation time (τ). Therefore, a treatment time of 60 min was adopted for the rest of the present work for both compounds.

Fig. 1 shows ESR spectra recorded at 37°C for SDC-treated erythrocyte cells labelled with 5-DSA and 16-DSA stearic acids together with their controls. From recorded spectra it was clear, as expected [14,18], that ESR spectra of 5-DSA labelled erythrocyte cells were very different from those labelled with 16-DSA. However, the same was not

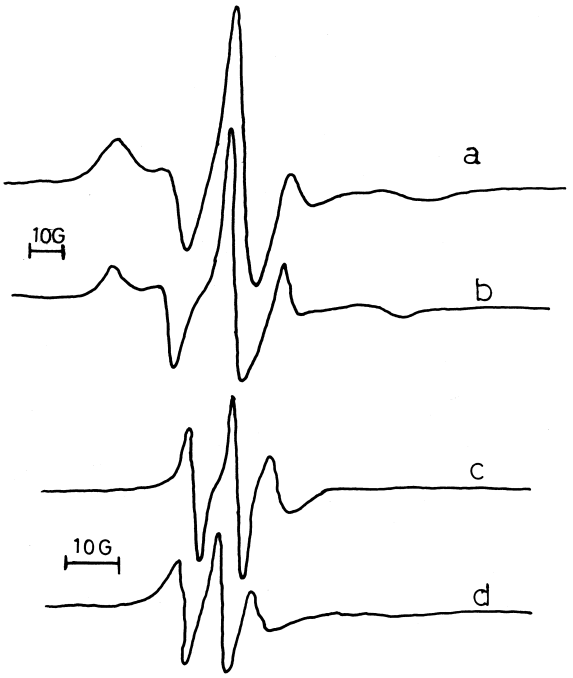

Fig. 1. ESR spectra recorded at 37°C for erythrocyte cells treated with 5 μM SDC over a period of 60 min. (a) Control (erythrocyte + 5-DSA); (b) treated (erythrocyte + 5-DSA + 5 μM SDC); (c) control (erythrocyte + 16-DSA); (d) treated (erytrocyte + 16-DSA + 5 μM SDC).

true as for the effects of SF and SDC on the shape of spectra. The results of the detail analysis of these spectra recorded for control and treated samples labelled with 5-DSA and 16-DSA stearic acids are given in Table 1 and their illustration as histograms are shown in Fig. 2 to make the comparison easy. From those data it is clear that 5 μM SDC and 40 μM SF do not produce any measurable effects on the order parameter of 5-DSA while they cause appreciable changes in the correlation time of the same label. On the other hand, while SDC does not produce any significant effect on the order parameter of 16-DSA label, SF does in a certain extent. Although weak, the effects of both compounds on the correlation time of 16-DSA spin label are well above the experimental error limits.

It is well known that temperature greatly affects the behaviour of model and biological membranes. In previous studies [19–21], it has been observed that in the 0–50°C temperature range the erythrocyte membrane undergoes discontinuous changes in the nitroxide spin-labels freedom of motion which is an indicative of protein-dependent structural transitions. Thus, the effect of temperature on the structural features of the untreated and treated erythrocyte membrane was also investigated by changing the sample temperature from 5°C up to 50°C and the spectra were recorded with a temperature increment of 5°C.

From recorded spectra S and τ parameters were determined for the control and SF- or SDC-treated erythrocyte membrane using appropriate equations given in Section 2. The data obtained are presented as plots in Figs. 3 and 4. The magnitude of the changes in the measured S and τ values for untreated and treated cells were of the same order as those found by many investigators who have compared membranes of biologically altered cells [22,23]. Both order parameter and correlation time decrease rapidly with increasing measuring temperature for both control and treated samples in the temperature range of

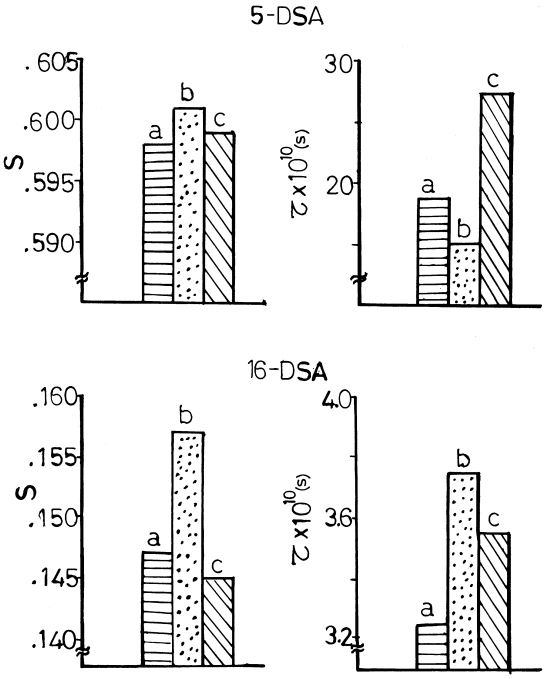

Fig. 2. Representation of the data given in Table 1 as histograms. (a) Control; (b) SF; and (c) SDC treated.

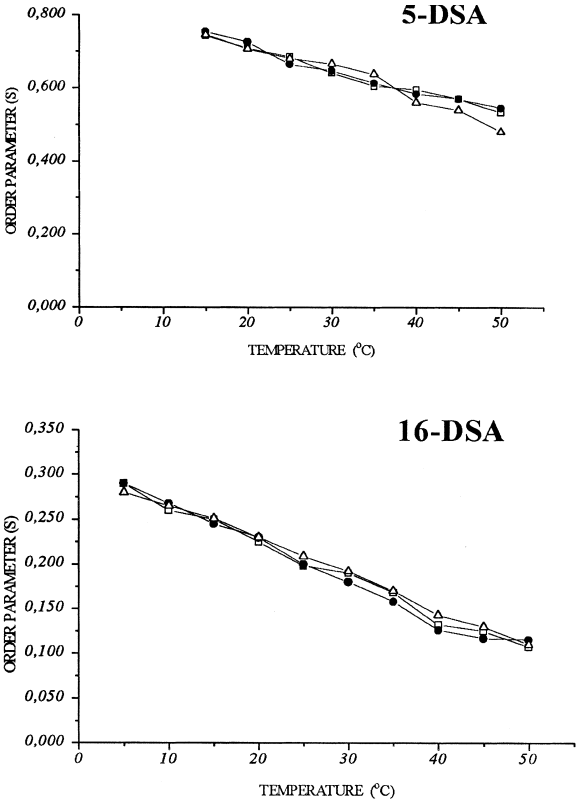

Fig. 3. Variations of order parameters with temperature for untreated (control) and treated erythrocyte cells labelled with 5-DSA and 16-DSA. □, Control; ●, 40 μM SF-treated; △, 5 μM SDC-treated.

5–50°C. This means that increase in temperature brings about an increase in the motional freedom of spin labels. Using rotational correlation time data, ln $(\tau \times \sqrt{T})$ vs. $1/T$ curves for the control and treated erythrocyte membranes were also plotted and they have been observed to be composed of two approximately straight lines with a break point. The straight lines at lower temperature were steep (gel phase) and those at higher temperature had a gentle slop (liquid crystalline phase).

The activation energies and thermotropic transition temperatures of control and treated samples were calculated using a linear regression analysis 'break points' computer program assuming that $l = 22.63$ Å and $r = 1.08$ Å for 5-DSA and 16-DSA spin label molecules. As is known, the slopes of these straight lines are related to the activation energies of micro-viscosity of the studied membranes. The equation of the straight lines obtained from regression analysis, activation energy and thermotropic phase transition temperature values are given in Table 2.

4. Discussion

The analysis of motion of spin-labels in the model and biological membranes is a sensitive and reliable indicator of the physical state of these membranes. ESR studies give principally the rotational correlation time (τ) and order parameter (S) which represent the degree of hydrocarbon chains long-range alignments along the

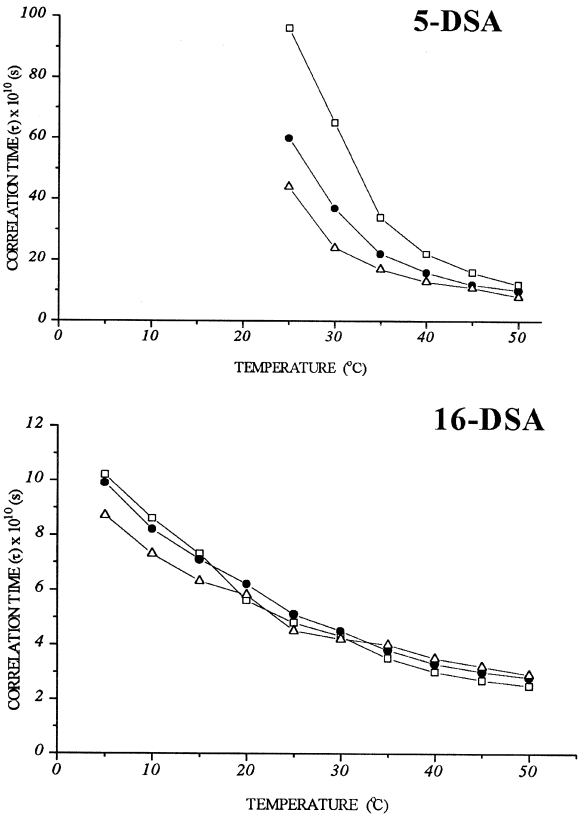

Fig. 4. Variations of correlation time with temperature for untreated (control) and treated erythrocyte cells labelled with 5-DSA and 16-DSA. □, Control; ●, 40 μM SF-treated; △, 5 μM SDC-treated.

membrane normal and the motional state of these chains [14,24]. However, the rotational correlation time should also be related to the short-range alignments of hydrocarbon chains because when the short-range alignments are increased the distance among hydrocarbon chains are reduced. This reduction in distance causes an increase in chain–chain interactions which give rise, in turn, to an increase in correlation time.

Spin labelling of the erythrocyte membrane with 5-DSA or 16-DSA at 37°C, was shown to be completed in great extent in a time interval of 10 min after addition of spin-label molecules to erythrocyte cell samples. On the other hand, treatment time longer than 60 min with SF and SDC was observed not producing any significant effect on the shape of ESR spectra and on the spectral parameters of 5-DSA and 16-DSA spin-labels incorporated into erythrocyte cell membrane. This means that the distribution of the ions through the membrane; inside and outside surfaces of the cells and their ultimate effects on the membrane physical properties are largely completed over a treatment time of 60 min.

In biological and model membranes the rotational correlation time and order parameter, generally decrease gradually as the depth of spin-label from the polar surface of the membrane increases. In accordance with this expectation, relatively big differences in the values of correlation times and order parameters of 5-DSA and 16-DSA spin labels (see Table 1) inserted in untreated and treated erythrocyte cell membranes were observed at 37°C. These differences are due to the fact that deeper in the hydrocarbon region, where the nitroxide group of 16-DSA penetrates, exists a more fluid environment than that closer to the membrane surface where the nitroxide groups of 5-DSA probes [14,23]. Similar increases in the motional freedom of spin-probes were reported in the literature [18,20,25,26] for spin-labels probing the same membrane depths as in our study on erythrocyte membranes.

Biomembranes are major sites of lipid peroxidative damage which may cause alterations in erythrocyte cell membrane function and structure. Fluoride in vitro and in vivo causes enhanced generation of superoxide radicals ($O_2^{\bullet-}$) and lipid peroxidation [6,7]. Lipid peroxidation in erythrocyte cells from humans exposed to toxic levels of fluoride has been reported to be nearly twofold greater compared to controls [2]. This increase in lipid peroxidation suggests that fluoride causes oxidative damage to erythrocyte cell membrane. This damage could be expected to alter the erythrocyte cell membrane lipid fractions, protein profiles and enzymes. Total cholesterol and phospholipid fractions have been shown to increase and three new protein bands have been observed to appear in the regions of 20, 66 and 93 kDa in erythrocyte ghost of fluoretic patients [2].

Table 1
Results of the analysis of the ESR spectra recorded at 37°C for control and 40 μM SF- or 5 μM SDC-treated erythrocyte cells[a]

Sample	5-DSA		16-DSA	
	S	$\tau \times 10^{10}$ (s)	S	$\tau \times 10^{10}$ (s)
Control	0.598 (0.004)	18.9 (0.9)	0.147 (0.004)	3.25 (0.08)
40 μM SF-treated	0.601 (0.002)	15.1 (0.8)	0.157 (0.003)	3.75 (0.07)
5 μM SDC-treated	0.599 (0.004)	27.5 (0.7)	0.145 (0.003)	3.55 (0.09)

[a]All values are reported as means of the number of experiments performed. Numbers in brackets are standard deviations

The peroxidation of erythrocyte membrane phospholipids and accumulation of lipid peroxides are expected to modulate the membrane fluidity and consequently the membrane function. Changes in erythrocyte membrane cholesterol level affect cell rigidity, membrane viscosity and transport function because cholesterol which is the major lipid constituent of the erythrocyte membrane, interacts with different classes of membrane phospholipids. The observed changes in the order parameters and correlation times of 40-μM SF-treated erythrocyte cell membranes (see Table 1) indicate that S is not a good parameter to measure the effect of lipid peroxidation at the membrane surface. However, τ parameter can be used to detect the changes produced in the membrane, fluidity, upon SF treatment. A decrease of nearly 20% (see Table

1) in rotational correlation time shows that at the surface of the membrane, fluidity is increased after 40 μM SF treatment. As for the core of the membrane, both S and τ parameters are sensitive to the changes due to lipid peroxidation and they are increased in this region. In other words, the fluidity in the core is decreased after SF treatment. This result is in agreement with the fact that total cholesterol and phospholipid fractions increase in erythrocyte cells from humans exposed to fluoride. Nevertheless, basing on the observed changes of erythrocyte membrane lipid profile in chronic fluorosis, a compensative mechanism operating in erythrocyte of fluorosed humans resisting the fluidising effect of fluoride has been suggested in the literature [2]. An increase in the high molecular weight proteins (93 kDa) and in the polymerised products of lipid peroxidation in the erythrocyte of fluoretic patients might be another possible source of membrane fluidity increase.

The chromate anion which is a strong oxidant is rapidly taken up by human erythrocyte cells and reduced intracellularely to compounds of trivalent chromium. It reacts with cellular electron donors and may induce changes in membrane constituents especially in erythrocyte cell membrane proteins [12,27]. At concentration of 1–10 mM, chromate has been observed to modify its own transport kinetics through erythrocyte cell membranes, to cause crosslinking of membrane-associated proteins and to increase erythrocyte cell interior pH [13]. The modified chromate uptake kinetics is attributed to the crosslinking of spectrin which is a cytoskeletal protein attached to the anion transporter band 3 protein [28].

From the results given in Table 1 it is seen that, as in the case of SF treatment, S is not a good parameter to measure the alterations created neither at the surface nor in the core of erythrocyte membrane after treatment of these cells with 5 μM SDC. However, τ parameters experience relatively big increases. These increases are more pronounced at the surface than at the core of the membrane (45.5% and 9.2%, respectively). We believe that the observed decreases in the freedom of 5-DSA and 16-DSA spin labels incorporated in SDC-treated erythrocyte membrane originate both from crosslinking of spectrin to anion transport band 3 protein and from polymerised products of lipid peroxidation distributed through the membrane.

Calculated spectral parameters S and τ were observed to vary with temperature, as expected. These variations are shown in Figs. 3 and 4 for untreated (control) and SF- and SDC-treated erythrocyte cells. The magnitude of changes in the measured S and τ values for untreated and treated cells were of the same order as those found in the literature for erythrocyte membranes [18–20,26,29]. Since spin-label molecules used in the present work (5-DSA and 16-DSA) are composed of very flexible hydrocarbon chains, it seems unreasonable to assume that these spin labels can be treated as rigid rod-like molecules. For this reason, the activation energy relevant to rotational viscosity was obtained from Andrade plots without calculating the rotational viscosity itself. These plots (not given here) showed the existence of phase transition of hydrocarbon chain regions. It is clear from the data given in Figs. 3 and 4 that changes in temperature have a greater effect on rotational correlation time than on the order parameter in both hydrophobic core and on the surface of the membrane. However, this effect is more pronounced for 5-DSA spin label which probes the surface of the membrane.

Erythrocyte membranes have been reported to undergo more than one thermotropic phase transition using ESR and fatty acid spin-labelling techniques together [18–20,26]. Low temperature phase transition was not observed in the present work due to the fact that in the 0–20°C temperature range proper determinations of experimentally measured quantities [$T'_{\parallel}$, $T'_{\perp}$, $h(1)$, $h(0)$, $h(-1)$, $\Delta H(0)$] from recorded spectra were not possible. Furthermore, in this temperature range, the validity of the assumptions made in deriving Eqs. (1) and (2) which were used to calculate S and τ parameters, is questionable.

Inspection of the data given in Table 2 shows that low and high temperature rotational activation energies at the surface of the erythrocyte membrane are much bigger than those relevant to the hydrophobic core of the membrane. This result is in agreement with the fact that the core

of the erythrocyte membrane is more fluid. Namely, in this region, the hydrocarbon chains undergo, much easily, from one phase to another while at the surface these transitions are more difficult. Although, treatment with 40 μM SF or 5 μM SDC does not affects significantly low and high temperature activation energies of 5-DSA spin label, it causes a decrease (2°C) and an increase (4°C) in the transition temperature of SF- and SDC-treated erythrocyte membranes, respectively. As for the activation energies and transition temperatures relevant to 16-DSA spin label, they are affected drastically after treatment. While 5 μM SDC induces an increase of nearly two folds in the high temperature activation energy of 16-DSA spin label, 40 μM SF causes more than twofold and fourfold increases in the low and high temperature activation energies, respectively. Treatment of erythrocyte cells with SF and SDC brings about an increase of 4°C in the transition temperature of the membrane hydrophobic core.

From a structural standpoint, below the transition temperature, the hydrocarbon chains in the membrane are in relatively rigid all-*trans* conformation. When the temperature is increased up to the transition point, the hydrocarbon chains disorder by undergoing very rapid *trans*-gauche rotational isomerisation along the chains. The results of the present and similar works reported in the literature suggest that the spectrin–actin network and proteins that link the skeletal network to the membrane are important in mediating this *trans*-gauche rotational isomerisation along the hydrocarbon chains and that protein unfolding could be at the origin of this rotational isomerisation [20]. Experimental results presented in this investigation for polar and hydrophobic core membrane regions, which are monitored by 5-DSA and 16-DSA spin labels, respectively, show that the rotational isomerisations occur at approximately 36°C and 40°C in these regions, respectively (see Table 2). Treatment of erythrocyte cells with 40 μM SF or 5 μM SDC causes increases in these temperatures.

In conclusion, the observed effects of fluoride and chromate on erythrocyte cell membranes seem to be of secondary order with respect to the toxicologies of SF and SDC, since the effects observed in the present work occur at concentrations of 40 μM and 5 μM, respectively, which are fairly below the mutagenic and clastogenic fluoride and chromate concentrations. However, fluoride and chromate at lower concentration may

Table 2
Straight lines; activation energies and thermotropic transition temperatures calculated from correlation time data for control and treated erythrocyte cells labelled with 5-DSA and 16-DSA

Spin label	Sample	Straight lines[a] and activation energies[b]	
		Low temperature	High temperature
5-DSA	Control	$y = 9054.95x - 49.3349$ 18.0 (±1.0)	$y = 5245.9x - 36.9929$ 10.4 (±1.0)
	SF-treated	$y = 9489.566x - 50.262$ 18.9 (±0.9)	$y = 6122.024x - 39.499$ 12.2 (±1.0)
	SDC-treated	$y = 8744.530x - 48.627$ 17.4 (±0.9)	$y = 4893.688x - 36.071$ 9.7 (±0.8)
16-DSA	Control	$y = 3069.47x - 31.7334$ 6.1 (±0.9)	$y = 1849.35x - 27.8398$ 3.7 (±0.8)
	SF-treated	$y = 2714.939x - 30.497$ 5.4 (±0.8)	$y = 3636.172x - 33.4$ 7.2 (±0.9)
	SDC-treated	$y = 2201.655x - 28.818$ 14.4 (±1.0)	$y = 7632.653x - 45868$ 15.2 (±1.0)

[a] Derived from correlation time data.
[b] Figure given below each straight line is the corresponding activation energy value in kcal/mol, figures in brackets are estimated errors of the related quantities and $x = 1/T$.

exert effects on biomembranes of other tissues more sensitive to modifications than erythrocytes which are rather specialised cells [13,28].

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