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# Vanadyl ions increase the order parameter of plasma membranes without changing the rotational relaxation time

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**Differential polarized phase fluorometry of 1,6-diphenyl-1,3,5-hexatriene showed that vanadyl ions ($VO^{2+}$) increased its limiting anisotropy (order parameter) in crude plasma membranes from brown adipose tissue of the golden hamster (*Mesocricetus auratus*). This was about $10^3$ times larger than the effect of $Ca^{2+}$ and was several times greater than the action of $Co^{2+}$. Vanadate anions were without any effect. During the membrane treatment with $VO^{2+}$, the rotational relaxational time of diphenylhexatriene did not change. This results suggest a possible positive influence of tetravalent vanadium on the stability of cell membranes.**

## Introduction

The interaction of divalent ions with plasma membrane phospholipids might have important consequences for the functional properties of the cells [1–4].

In the present communication we followed the effect of cobalt and vanadium ions on motional freedom of plasma membrane lipids. Both elements are of biological importance, $Co^{2+}$ affects the function of membrane receptors and channels [5,6]. The biological effect of vanadium depends on its oxidation state, pentavalent vanadate ($VO_3^-$) strongly inhibits many phosphohydrolases, e.g. $(Na^+ + K^+)$- and $Ca^{2+}$-ATPase and phosphatases [8,11] whereas divalent vanadyl ($VO^{2+}$) hyperpolarizes the intact cells [12] and mimics the action of insulin [12–15]. It is not clear, however, whether among the physiological effects of $Co^{2+}$ and $VO^{2+}$ the direct influence on membrane lipid phase may exist.

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The fluidity or microviscosity of membranes is often estimated from the steady-state fluorescence anisotropy of diphenylhexatriene incorporated into the hydrophobic bilayer; but the term fluidity seems to be inadequate to describe the motion of diphenylhexatriene inside the membrane because of non-zero limiting anisotropy [16]. The motion of diphenylhexatriene is, however, well described by limiting anisotropy ($r_\infty$) and by its rotational relaxation time $\tau_c$ [17]. Both parameters are assessed from the steady-state anisotropy values, fluorescence lifetime and differential tangent [18,19].

These parameters of the diphenylhexatriene incorporated into crude membrane fragments were measured before and after application of $Co^{2+}$ and $VO^{2+}$ and were compared with the actions of $Ca^{2+}$ and $VO_3^-$.

## Materials and Methods

Golden hamsters were used for isolation and purification of crude membrane fractions from intercapsular brown adipose tissue [20]. Labelling of samples by diphenylhexatriene (DPH) and 1-(4-

(trimethylamino)phenyl)-6-phenylhexa-1,3,5-triene (TMA-DPH) was made according to Shinitzky and Barenholz [21] and Kuhry et al. [22], respectively. All measurements were performed at 22 ± 0.5 °C. The exciting wavelength was 360 nm in all measurements and the fluorescence emission was isolated from Rayleigh and Raman scattering by use of a Scott KV 420 filter.

The fluorescence lifetimes, differential tangents and steady-state fluorescence anisotropy were measured on an SLM subnanosecond spectrofluorometer [24,25], the last with the light modulation and radio frequency electronics turned off. The limiting anisotropy and rotational relaxation time were calculated according to Lakowicz et al. [19] (Equation 18).

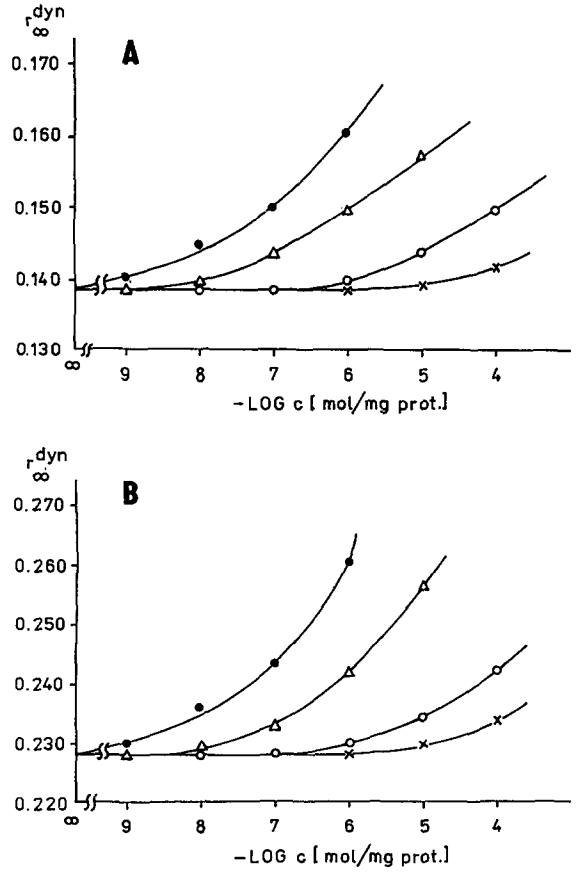


Fig. 1. Influence of $Ca^{2+}$ (○), $Co^{2+}$ (△), $VO^{2+}$ (●) and $VO_4^{3-}$ (×) when added to crude plasma membranes of brown adipose tissue in 0.25 mol/l sucrose plus $10^{-2}$ mol/l Tris-HCl (pH 7.4) on the limiting anisotropy ($r_\infty^{dyn}$). The limiting anisotropy was obtained from the measurements of dynamic depolarization, fluorescence lifetime and steady-state anisotropy of DPH (A) and TMA-DPH (B), respectively. The concentration of protein [23] in all experiments was 100 μg/ml. Abscissa: concentrations of ions ($c$) are expressed in terms of mol per mg of membrane proteins corresponding to 100-times higher concentrations in mol/l). Temperature 22 °C.

## Results

Fig. 1A shows anisotropy changes of DPH-treated membranes in the presence of $Co^{2+}$, $VO^{2+}$, $Ca^{2+}$ and $VO_4^{3-}$. All cations increased the limiting anisotropy $r_\infty^{dyn}$; most potent was $VO^{2+}$, which exhibited the same effect as $Ca^{2+}$ but at a concentration three orders lower and was about 10-times more efficient than $Co^{2+}$. EDTA $10^{-3}$ mol · $l^{-1}$ cancelled the effect of these ions almost completely (data not shown). Measurements with TMA-DPH (Fig. 1B) suggest the same or greater changes of anisotropy, but starting from a higher level. The higher values obtained with TMA-DPH are in good agreement with the theory of membrane fluidity gradient [26–28] because of the

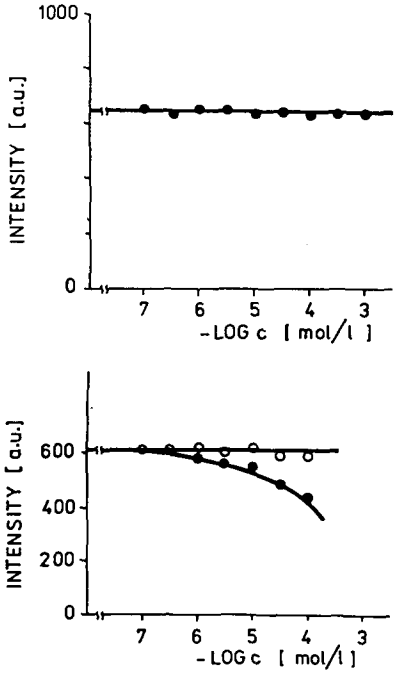


Fig. 2. The influence of $VO^{2+}$ (●) and $Ca^{2+}$ (○) on the fluorescence intensity and anisotropy of DPH in ethanol (upper part) and in the membranes (lower part). Concentration of DPH was $2 \cdot 10^{-5}$ mol/l in ethanol and $1 \cdot 10^{-6}$ mol/l in membranes, respectively.

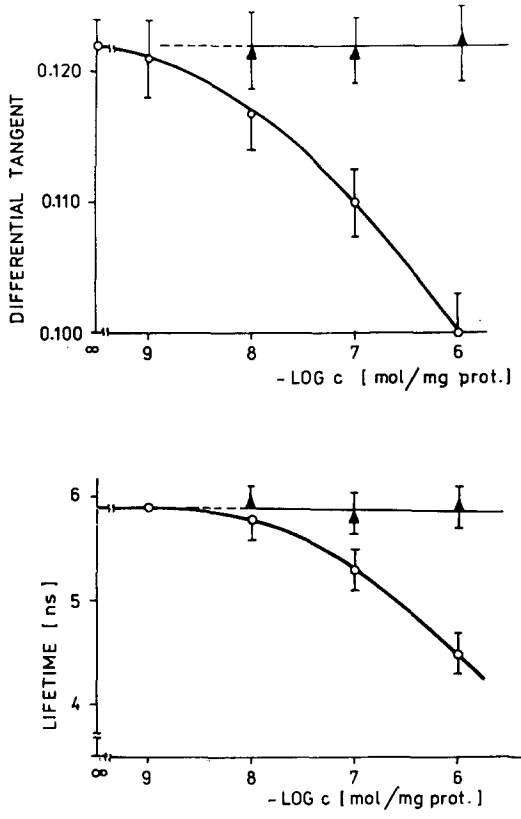


Fig. 3. The change of differential tangent and lifetime of DPH in plasma membranes in the presence of $VO^{2+}$ (○) and $Ca^{2+}$ (▲), respectively. For all experiments, the values are means (± S.E.) of three different experiments made in triplicates. Abscissa: concentration of ions ($c$).

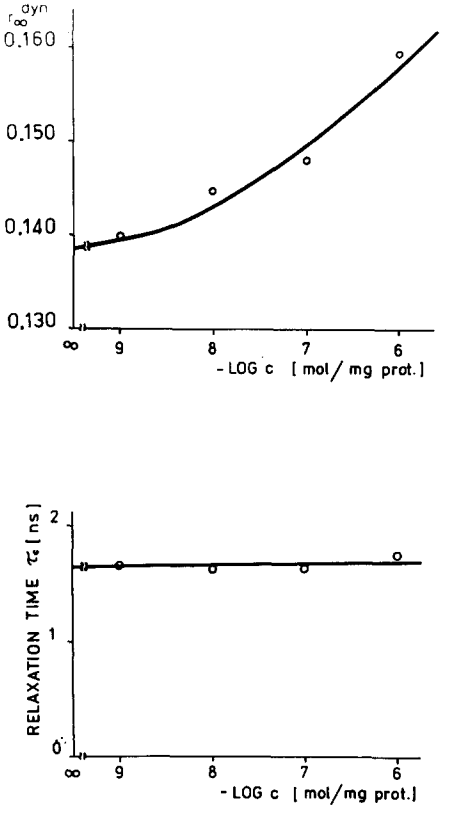


Fig. 4. The limiting anisotropy ($r_\infty^{dyn}$) and the rotational relaxational time ($\tau_c$) of DPH obtained from dynamic measurements for different concentrations of $VO^{2+}$.

distribution of TMA-DPH only in the surface area of the membrane. $VO_4^{3-}$ was without effect.

Together with the increase of anisotropy, we observed also a decrease of the total DPH fluorescence intensity in the presence of $VO^{2+}$ (Fig. 2). In principle, there are three possible explanations for the latter effect: (1) Fluorescence quenching of DPH by $VO^{2+}$. The quenching due to the Förster energy transfer can, however, be neglected because there is no overlap of DPH emission spectrum (peak about at 430 nm) and the absorption spectrum of $VOSO_4$ (> 720 nm). (2) $VO^{2+}$ can penetrate the membrane and then collisional quenching with DPH may occur. This is also excluded, because no intensity change was observed when only DPH and $VO^{2+}$ were present in the medium (Fig. 2). (3) Change in the probe environment. To check this possibility, dynamic fluorescence measurements were made. Fig. 3 shows that the average lifetime of the excited state of DPH decreased with higher concentration of $VO^{2+}$ but it did not change during treatment with the same amount of $Ca^{2+}$. Two component mathematical analysis distinguished the change in ratio between shorter ($\tau_1 = 2.3 \pm 0.3$ ns) and longer ($\tau_2 = 7.5 \pm 0.4$ ns) lifetimes of DPH in membranes after adding $VO^{2+}$; this resulted in the shorter average lifetime of DPH and also in the lower fluorescence intensity observed with $VO^{2+}$. Similarly we observed the decrease of the differential tangent with the increase of $VO^{2+}$ concentration (Fig. 3). From the values of steady-state anisotropy, lifetimes and differential tangents we calculated $r_\infty^{dyn}$ and $\tau_c$. Fig. 4 displays these two quantities as a function of $VO^{2+}$ concentration. It is clear that $r_\infty^{dyn}$ determined from the dynamic

measurement also increased with $VO^{2+}$. This is similar to $r_\infty$ calculated from the steady-state values only. It can be also seen from Fig. 4 that $VO^{2+}$ (up to $10^{-4}$ mol · $l^{-1}$, i.e. up to $10^{-6}$ mol · $mg^{-1}$ of protein) does not affect the rotational relaxation time of DPH.

## Discussion

DPH molecules are distributed centrally between the fatty acid chains with their long axes normal to the plane of the membrane. On the other hand, the cationic derivative TMA-DPH remains localized exclusively near the surface of the membrane [22]. It therefore reflects the changes on the membrane-water interface. Because of the observation that $VO^{2+}$ has the same or greater effect on the limiting anisotropy $r_\infty^{dyn}$ of TMA-DPH than on that of DPH, it seems that $VO^{2+}$ acts mainly on the surface of membrane. It is possible that $VO^{2+}$ may be localized preferentially in the more negatively charged (i.e. inner [29,30]) monolayer of the plasma membrane. The higher $r_\infty^{dyn}$ for TMA-DPH is consistent with a more restricted order in the inner monolayer of the adipose cell plasma membrane and is also in agreement with earlier work [27,31]. The cation, $VO^{2+}$, would tend to bind more to the negatively charged cytoplasmic monolayer – precisely where the TMA-DPH would also tend to be localized. In this way $VO^{2+}$ may create bridges in a manner similar to that proposed for $Ca^{2+}$ [1]. An interesting fact is that this phenomenon occurs in the case of $VO^{2+}$ at about a concentration three orders lower; this shows that the structure of the membrane is easily stabilized by $VO^{2+}$. This is also indicated from the dependence of $r_\infty^{dyn}$ on concentration. On the other hand, the kinetic parameters, represented by the rotational relaxational time $\tau_c$ are not affected by $VO^{2+}$. Other investigators have also shown the insensitivity of DPH rotational relaxation time to a number membrane changes that dramatically alter $r_\infty$ [19,31]. This means that the microviscosity that is derived from $\tau_c$ does not change within the experimental errors. The changes in the structural arrangement of the lipid compartment at the membrane can consequently also influence the functional properties of membrane bound proteins (see, for example, Ref. 3). In the case of $VO^{2+}$ and to a lesser extent $Co^{2+}$ (but not $VO_4^{3-}$), one should take this possibility into consideration when the biological effects of vanadium salts are discussed (see, for example, Refs. 32, 33).

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