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Absorption of Tungsten Carbonyl Anions into the Lipid Bilayer Membrane of Mouse Myeloma Cells

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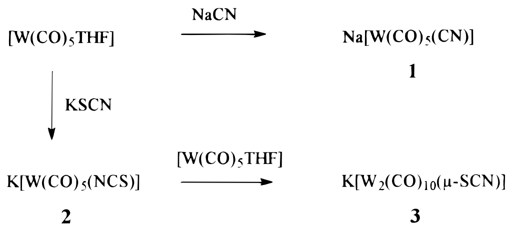
Living cells are surrounded by a lipid bilayer membrane which maintains the physical and chemical integrity of the cell and at the same time protects the cell from adverse influences of the environment. The membrane must, however, allow for the molecular communication of the cell with its surroundings, e.g. for diffusion of nutrients and metabolites. This can occur either by transport through specific channels and carriers or by diffusion through the bilayer itself.[2] From a study of such tranport phenomena, a detailed understanding of the structure and properties of the lipid bilayer can be gained.[3]

Advances in the field of electromanipulation of cells offer the opportunity to study ion transport phenomena in living cells by noninvasive electrical methods.[4] One of them is the so-called electrorotation: Cells, embedded in a culture medium of known conductivity (σ_e), are subjected to a rotating electric field of variable frequency from a few hertz to several megahertz. At low frequencies (<1 MHz) the cells begin to rotate in the opposite direction (antifield rotation), whereas at high frequencies they rotate in the same direction as the field does (cofield rotation; see below for a typical rotation spectrum). The electrical capacity of the membrane, C_m, can be determined by studying the variation, with σ_e, of the frequency of maximum antifield rotation (f_c), because this rotation is due to the charging of the plasma membrane. For low σ_e, the theory gives the following relationship

$$f_c a = \sigma_e/(\pi C_m) + aG_m/(2\pi C_m) \qquad (1)$$

where a and G_m are the cell radius and the conductance of the membrane, respectively.[5] Moreover, from an analysis of electrorotation spectra, important information such as partition coefficients of membrane-soluble ions and their translocation rates across the plasma membrane can also be extracted.[6] Experiments on artificial lipid bilayers showed that the translocation of lipophilic ions absorbed by the membrane (mobile charges) can greatly increase C_m and G_m.[7]

Here, we report the influence of three different (lipophilic) tungsten carbonyl salts, $Na[W(CO)_5(CN)]\cdot 3H_2O$ (**1**), $K[W(CO)_5(NCS)]$ (**2**), and $K[W_2(CO)_{10}(\mu\text{-SCN})]$ (**3**), on electrorotation of mouse myeloma Sp2 cells. Tungsten compounds of this type were chosen for their ease of changing charge, size, and shape of the anions. Compounds **1** and **2** were prepared by irradiating $[W(CO)_6]$ in THF to produce a solution of $[W(CO)_5THF]$[8] and adding a stoichiometric amount of NaCN or KSCN, respectively. **3** was obtained similarly from **2** and $[W(CO)_5THF]$ (eq 2).



(2)

The compounds were purified by crystallization from THF/ether (with a trace of water added for **1**) and characterized by elemental analyses, IR, and ^{13}C-NMR.[9] The ^{13}C resonances (recorded in acetone-d_6) are virtually identical to those of the Et_4N^+ salts.[10] The infrared spectra (recorded in THF) in the ν(CO) region, however, show additional bands due to the simultaneous presence of both contact-ion-paired and solvent-separated forms of these salts.[11]

1−**3** are reasonably soluble in water, unlike the usually prepared Et_4N^+ and Ph_4P^+ salts.[10] The stability of the aqueous solutions was checked by UV/vis and IR spectroscopy. **2** and **3** slowly decomposed in the course of several days to $[W(CO)_6]$ and KSCN, while **1** seemed to be indefinitely stable in water, even without rigorous exclusion of atmospheric oxygen. Their toxicity was found to be fairly low: At concentrations up to 10 μM these salts did not inhibit growth of the myeloma cells, and even at 100 μM the count of live cells was not significantly diminished over a period of four days.

In a typical rotation experiment the cells were suspended in a hypotonic medium (150 mM inositol) whose conductivity was adjusted to a value of 10−40 μS cm^{-1} by addition of an appropriate amount of KOH−HEPES (pH 7.4). After incubation with the carbonyl salt (20 min at 0.5−90 μM), the rotation spectrum was recorded. A typical example, together with control data, is shown in Figure 1. The shift of f_c from 26 kHz (control) to 6.3 kHz is brought about by mobile charges introduced by **1** into the membrane. f_c Depends on the concentration of the added carbonyl salt (Figure 2). The membrane capacity C_m was calculated by linear regression of $f_c a$ values against σ_e (eq 1; Figure 3). Figure 4 shows that the tungsten carbonyl anions of **1**−**3** are able to increase C_m while

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(9) **1**: off-white solid; IR (cm^{-1}; THF) 2123 (sh) and 2115 (vw) (CN), 2055 (w), 1933 (sh), 1923 (vs), 1896 (m), and 1882 (sh) (CO). **2**: yellow solid; IR (cm^{-1}; THF) 2105 (vw) (CN), 2061 (w), 1935 (sh), 1929 (vs), and 1882 (m) (CO). **3**: yellow solid; IR (cm^{-1}; THF) 2139 (vw) (CN), 2061 (w), 1939 (s), 1926 (vs), and 1885 (m) (CO).

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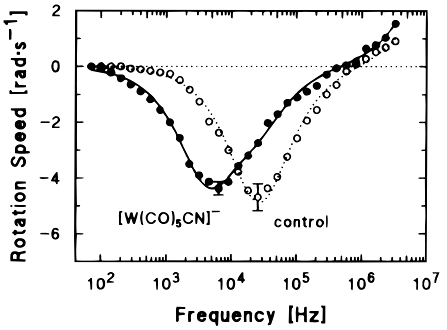

Figure 1. Rotation spectra of Sp2 cells in the presence of 12 μM **1** (●, $n = 5$) and of untreated cells (○, $n = 3$). In both cases, medium conductivity and osmolality were 42 μS cm^{-1} and 150 mOsm, respectively.

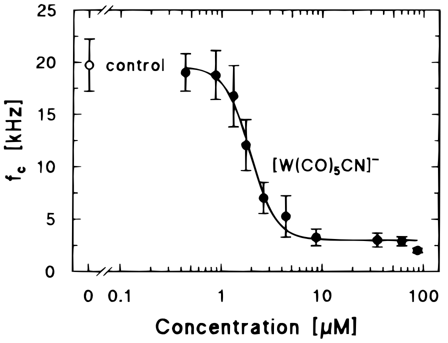

Figure 2. Dependence of f_c on the concentration of **1** added to 10^5 cells/ml for 20−30 min at $\sigma_e = 41 \pm 3$ μS cm^{-1}. Each point represents a mean ±SD from 20 cells.

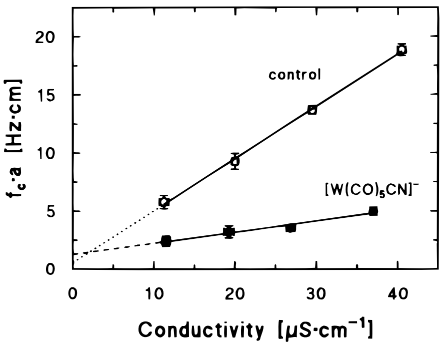

Figure 3. Typical dependence of the normalized quantity $f_c a$ on the medium conductivity. In this particular experiment, control cells and cells treated with **1** (10 μM) showed $C_m = 0.7$ and 3.3 μF cm^{-2}, respectively (eq 1).

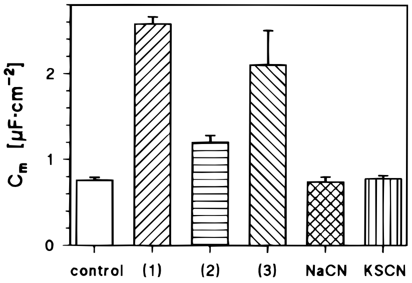

Figure 4. Variation of C_m upon addition of 5.0 μM metal carbonyl salts (**1**−**3**), NaCN, and KSCN. The area-specific membrane concentrations of **1**−**3** estimated using eq 3 were 95, 23, and 70 pmol cm^{-2}, respectively.

the highly hydrophilic salts NaCN and KSCN, as expected, do not affect C_m at all. The area-specific concentrations N were calculated using the equation

$$N = 2(\Delta C_m)RT/F^2 \qquad (3)$$

where ΔC_m is the C_m increment due to the mobile charges. The values obtained for **1**−**3** were 0.95, 0.23, and 0.70 pmol cm^{-2}, respectively.

This study shows that electrorotation yields useful data on the solubility of charged molecules in the plasma membrane of living cells. Metal carbonyl anions such as **1**−**3** appear to give particularly large effects. As a possible explanation, a significant hydrophilicity of the carbonylate anions might be assumed which parallels their readiness to undergo ion pairing with alkali metal cations.[12] This would lead to a pronounced affinity of these anions for the interface between the aqueous solution and the lipid bilayer membrane. This working hypothesis will have to be checked by employing other metal carbonyl salts which have been modified accordingly. Furthermore, the observation of such large effects as seen here is an ideal instrument to check and further develop existing models of mass transport through lipid membranes. Work along these lines is in progress in our laboratories.

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