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# C0310 NO3- SELECTIVE MINI-ELECTRODES AS A TOOL TO INVESTIGATE THE NO3- TRAFFIC IN CHLAMYDOMONAS REINHARDTII D. ()

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## 1 Resumen

Ion selective $NO_3^-$ mini-electrodes were used to measure the external $NO_3^-$ concentration in *C. reinhardtii* liquid cultures. Electrodes were prepared using glass capillaries (1.5 mm external diameter). Capillaries were cut in 10 cm long pieces, dehydrated for 45 minutes in an oven and silanized by addition of dimethyldichlorosilane in bencene 0.1% (V/V). Once silanized, the capillaries were baked again for 30 minutes. Once cold the capillaries were backfilled with the $NO_3^-$ ionophore (Fluka: 72549), which contains PVC (5.75% w/w) dissolved in tetrahydrofurane. Then, the $NO_3^-$ mini-electrodes were stored in dark in a desiccator until tetrahydrofurane gets evaporated. Before use, $NO_3^-$ selective mini-electrodes were backfilled with 0.1 M $NaNO_3$ and 0.1 M KCl and connected to a high-impedance differential amplifier (WPI FD223). Mini-electrodes were calibrated in N-free Beijerinck medium, which contains 0.1 mM $Cl^-$. In those conditions, electrodes calibration slope was 54 mV/p$NO_3^-$ in the range 1 - 1000 µM $NO_3^-$. The mini-electrodes were used to continuous monitoring of the external $NO_3^-$ concentration in liquid culture of different *C. reinhardtii* strains, incubated in N-free Beijerinck medium supplemented with 100 µM $NO_3Na$. Previous to the assays, strains were N starved for 6 days. In the light, wild type strain uptakes $NO_3^-$ at a rate of 15 nmol $NO_3^-\cdot 10^6$ $cells^{-1}\cdot h^{-1}$, in the dark this rate was one third of this figure. After 5 h, the external $NO_3^-$ levelled off at 10 µM in the light and around 30

µM in the dark. *C. reinhardtii* cells cultured in the presence of 2 mM $NO_3NH_4$ do not show significant $NO_3^-$ uptake nor a mutant strain, defective in nitrate transport and having an active nitrate reductase. However, a mutant strain lacking the nitrate reductase shows an enhanced $NO_3^-$ uptake rate, compared with the value obtained for the wild type in the light.

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