

Determination of transmembrane pH difference in chloroplasts using the effect of concentrational broadening of tempoamine EPR spectrum

Sergei G.Magnitsky, Maryanna Masarova, Alexander N.Tikhonov

The method for measuring the transmembrane pH difference (ΔpH) in chloroplasts is relied on the effect of exchange broadening the EPR spectra of spin label Tempoamine (TA). This effect results from the TA accumulation in the bulk phase of the intrathylakoid volume in response to the light-induced generation of ΔpH . The broadening of the spectra, caused by TA spin-exchange interactions, reveals itself only if the TA concentration exceeds the "threshold" level $[\text{TA}]^0$. Having experimentally determined value $[\text{TA}]^0_{\text{in}}=2.5\text{-}4\text{ mM}$ for TA molecules located inside the thylakoids, we were able to find such an external concentration of TA ($[\text{TA}]^0_0$) which corresponds $[\text{TA}]_{\text{in}}=[\text{TA}]^0_{\text{in}}$, and then to calculate $\Delta\text{pH}=\lg[\text{TA}]^0_{\text{in}}/[\text{TA}]^0_0$. The ΔpH values determined by this method ($\Delta\text{pH}\approx 2.6\text{-}2.9$) are consistent with the previous measurements of ΔpH by conventional methods. The advantage of our method is the possibility to measure the bulk-to-bulk ΔpH values without the knowledge of a vesicle internal volume.