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EPR study of surface potential and buffer capacity of thylakoid membranes

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In this work we describe a new method for the determination of the partial buffer capacity of the thylakoid membranes (on the outer surface). This method is based on the selective modification of the membrane surface electric potential and, consequently, the value of the local pH at the membrane interface. Membrane surface potential was measured with charge amphyphylic spin labels (CAT_9 and 16-doxylstearate). To modify a surface potential, small aliquots of charged compounds (negatively charged – SDS, positively charged – CTAB) and Mg^{2+} ions were added to chloroplast suspension. In the dark, the buffer capacity of the outer surface of the thylakoid membrane was estimated as 80 mol/mol P700×pH. T this value is about half of the total buffer capacity of thylakoid membrane ($\Delta\Psi_s \approx$-15mV), we were able to evaluate the number of protons transferred inside the thylakoids (about 50 H^+ per one P700 at pH 8). The data obtained point to the heterogeneity of membrane-bound proton accepting groups. The large portion (about 50%) of protons consumed by thylakoids during illumination bound to sequestered proton-accepting groups which are out equilibrium with protons located in the bulk phase of the thylakoid lumen.