

INTERACTION BETWEEN CYTOCHROME *b₆f* COMPLEX AND PLASTOCYANIN – SPECTROSCOPIC STUDIES

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Two independent spectroscopic techniques: steady-state absorption spectroscopy and stopped-flow kinetics have been applied to study the interaction between the active cytochrome *b₆f* complex and plastocyanin both isolated from the same source - *Spinacia oleracea* plants. As in kinetic experiments the equilibrium of the electron transfer reaction can not be monitored directly, the ratio of apparent rate constants of (forward) cytochrome *f* oxidation and (backward) cyt *f* reduction by oxidised and reduced Pc have been used, respectively, which should be a close approximation to the equilibrium constant *K*. The second order rate constants *k*₂ for forward and backward electron transfer between cytochrome *f* and plastocyanin have been found between 1.4-2×10⁷ M⁻¹s⁻¹ and 8-10×10⁶ M⁻¹s⁻¹, respectively, giving the value of an equilibrium constant of about 2±0.4. This value translates into a difference in redox potential between plastocyanin and cytochrome *f* of cytochrome *b₆f* complex of ca. 17 mV. We propose a method of evaluation of the final plastocyanin concentration after the electron transfer reaction between cytochrome *bf* complex and plastocyanin that overcomes the interference by the strong chlorophyll absorption in the spectral region where oxidised plastocyanin has its low extinction absorption band. The value of *K*=1.7±0.3 has been estimated from steady-state experiments in which the initial and final concentrations of participating components after mixing have been estimated via differential spectra analysis or spectra deconvolution. The data from both experiments, in the system devoid of quinol being the electron donor to cytochrome *b₆*, suggest that in case of electron transfer from cytochrome *f* to plastocyanin electron transfer can either bypass cytochrome *f* or the Rieske Iron-Sulfur protein can be reduced prior to its movement to the quinol binding site of cytochrome *b₆*. Effects of the ionic strength on the equilibrium constant are analysed. The role of the Rieske protein in forward and backward electron transfer reactions will be discussed.