O15

The Electron Self-Exchange Rate Constant for Stellacyanin

STEFAN DAHLIN, BENGT REINHAMMAR

Department of Biochemistry and Biophysics, University of Göteborg and Chalmers Institute of Technology, S-412 96 Göteborg, Sweden

and MICHAEL T. WILSON

Department of Chemistry, University of Essex, Colchester CO4 3SQ, U.K.

Stellacyanin is a blue copper-containing protein, which like other low-molecular-weight blue proteins, contains a single copper ion in a unique coordination site. These proteins take part in various redox reactions and the metal is rapidly changing valence. The 'blue' copper ion is also present in the more complex copper oxidases, laccase, ceruloplasmin and ascorbate oxidase, where it serves as the primary electron-accepting site. It has therefore attracted much interest and the reduction of this metal has been studied extensively, in particular in azurin, plastocyanin [1] and stellacyanin [2–4].

In 1963, R. A. Marcus published a theory concerning the rates of electron transfer in oxidation–reduction reactions [5]. Since then, numerous investigations have shown that this theory is applicable to redox-reactions involving proteins, provided they react via an outer-sphere mechanism. An important conclusion drawn by Marcus is that one can predict the rate constant for a second order electron transfer reaction from the equilibrium constant of the reaction and the one-electron self-exchange rate constants of the individual components. Attempts to determine this self-exchange constant for stellacyanin have previously been made, using Marcus' theory and data from reactions with low-molecular weight compounds [2, 3] or two forms of cytochrome *c* [4]. The present work presents a way to determine the true self-exchange constant for stellacyanin without the use of redox-compounds or different redox proteins.

A new method was developed for the exchange of the naturally occurring isotopic mixture of 70% ^{63}Cu and 30% ^{65}Cu for the pure isotopes ^{63}Cu or ^{65}Cu. Thus, two isotopically pure forms of stellacyanin, one containing only ^{63}Cu and the other containing only ^{65}Cu, were obtained in high yields. ^{63}Cu-stellacyanin and ^{65}Cu-stellacyanin show differences in their electron paramagnetic resonance (EPR) spectra (Fig. 1). The largest difference occurs at about 0.32 T and was used to follow the electron transfer between the two isotopic forms of the protein. Reduced ^{65}Cu-stellacyanin and oxidized ^{63}Cu-stellacyanin were mixed, allowed to react for various times and then rapidly frozen in order to quench the reaction. This was achieved with a rapid-freeze apparatus. The reaction mixtures were analyzed with EPR and the differences between spectra of various reaction times were measured. These differences allowed us to calculate the pseudo first-order reaction rate constants and to determine the self-exchange rate constant for stellacyanin. A preliminary value of this constant was found to be 1×10^5 M^{-1} s^{-1}. This value should be compared to the values previously reported for other reaction-partners: 6×10^3 M^{-1} s^{-1} from electron transfer with *Pseudomonas* cytochrome c_{551} [4], 2.9×10^4 M^{-1} s^{-1} with horse heart cytochrome *c* [4], 1.7×10^5 M^{-1} s^{-1} with [Ru-pentaaminepyridine]$^{3+}$ [3], 2.3×10^5 M^{-1} s^{-1} with [Fe(EDTA)]$^{2-}$ [3] and 3.0×10^5 M^{-1} s^{-1} with [Co(phenanthroline)$_3$]$^{3+}$ [3].

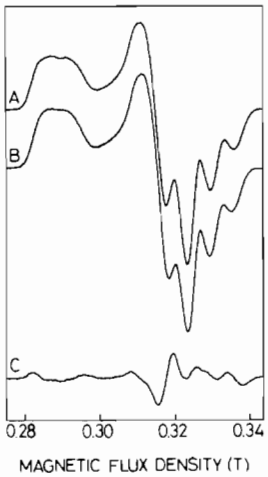

Fig. 1. EPR spectra of the two isotopically pure forms of stellacyanin. A: ^{63}Cu-stellacyanin, B: ^{65}Cu-stellacyanin and C: The difference between these two spectra, A minus B. The spectra were recorded under the following conditions: Microwave frequency 9.25 GHz, microwave power 30 dB and amplitude modulation 0.001 T. The temperature was 20 K.

1 S. Wherland and I. Pecht, *Biochemistry, 17*, 2585 (1978).
2 J. V. McArdle, C. L. Coyle, H. B. Gray, G. S. Yoneda and R. A. Holwerda, *J. Am. Chem. Soc., 99*, 2483 (1977).
3 D. Cummins and H. B. Gray, *J. Am. Chem. Soc., 99*, 5158 (1977).
4 M. T. Wilson, M. C. Silvestrini, L. Morpurgo and M. Brunori, *J. Inorg. Biochem., 11*, 95 (1979).
5 R. A. Marcus, *J. Phys. Chem., 67*, 853 (1963).