STRUCTURES AND FUNCTIONS OF CAROTENOIDS BOUND TO REACTION CENTRES FROM PURPLE PHOTOSYNTHETIC BACTERIA

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15,15'-Cis carotenoids bound to the photosynthetic reaction centre (RC) of purple bacteria serve a photoprotective function [1]. This has been studied using carotenoids reconstituted into carotenoidless RCs from *Rb. sphaeroides* strain R26.1 [2]. This approach has revealed the effect of the position of the energy level of the carotenoid triplet state on quenching the potentially harmful primary donor bacteriochlorophyll triplet state [3]. These studies have all assumed that the reconstituted carotenoids occupy the same binding site in the RC. We have investigated this assumption by determining X-ray crystal structures of carotenoidless, wild-type carotenoid-containing, and 3,4-dihydrospheroidene-reconstituted RCs [4]. The structural studies have confirmed that the carotenoid occupies the same binding site in the reconstituted RCs as it does in the wild-type protein. Having proven that reconstitution places the carotenoid in the same position as in the wild-type RCs, we have investigated the effect of the reconstituted carotenoid on the electrostatic environment of the RC "special pair," primary donor, dimer bacteriochlorophylls using electroabsorption (Stark) spectroscopy [5]. The electrostatic field around the special pair is changed by 10% in the presence of the carotenoid.

The structural study emphasized the importance of the methoxy-group of the carotenoids for binding to the RCs [4]. Thus, We have investigated the functional consequences of reconstituting a set of methoxy-containing spheroidene analogues with different numbers of conjugated double bonds (n). Nanosecond flash photolysis has been used to monitor the formation of carotenoid triplets as a function of the number of conjugated double bonds and revealed the photoprotective effect of the bound carotenoids. The carotenoid triplet signals were analyzed by singular value decomposition of the full time and wavelength data set. Carotenoid triplet formation was detected for n = 8 to 11. Reconstitution was not successful with carotenoids having n less than 8, suggesting that the absence of the conjugated double bond at position C7'=C8' is required for binding. Future work will examine whether carotenoids with n = 7 can bind to the RC and quench the primary donor triplet state, or whether the energetic cut-off for this reaction is at n = 8.

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[1] H. A. Frank and R. J. Cogdell, In: *Carotenoids in Photosynthesis*, A. J. Young, G. Britton (eds.), Chapman & Hall, London, 253–326 (1993).

[2] H. A. Frank, In: *The Photochemistry of Carotenoids: Applications in Biology*, H. A. Frank, R. J. Cogdell, A. Young, G. Britton (eds.), Kluwer Academic Publishers, London, 235–244 (1999).

[3] R. Farhoosh, V. Chynwat, R. Gebhard, J. Lugtenburg and H. A. Frank, *Photochem. Photobiol.*, **66**, 97–104 (1997).

[4] A. W. Roszak, K. McKendrick, A. T. Gardiner, I. A. Mitchell, N. W. Isaacs, R. J. Cogdell, H. Hashimoto, H. A. Frank, *Structure*, **12**, 765−773 (2004).

[5] K. Yanagi, M. Shimizu, H. Hashimoto, A. T. Gardiner, A. W. Roszak, and R. J. Cogdell, *J. Phys. Chem. B* **109**, 992−998 (2005).