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Coupling of electron transfer and proton uptake in the reaction center mutant L210DN of *Rhodobacter sphaeroides*

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By coupling a light-induced electron transfer to proton uptake, photosynthesis transforms light energy into chemical energy. The system used in this study is the photosynthetic reaction center from the purple nonsulfur bacterium Rhodobacter sphaeroides. This reaction center is a transmembrane pigment protein complex of ~100 kDa molecular mass. Upon light excitation, an electron is transferred from the primary donor P (bacteriochlorophyll *a* dimer) via intermediate acceptors to the primary quinone Q_A and finally to the secondary quinone Q_B. Asp210 in the L-subunit has been shown to be an important component of the proton transfer pathway to Q_B. Mutation of Asp210 to Asn leads to a deceleration of the re-oxidation of Q_A^- in the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ transition. We crystallized the mutant protein to obtain static structural information. The structure of this mutant reaction center has been solved at 2.5 Å resolution by molecular replacement using the atomic coordinates of the wild type reaction center (pdb code 1AIG). The structure has been refined, leading to a crystallographic R factor of 21.2% using reflections between 38 and 2.5 Å. The current model contains the chains L, M, and H and 10 bound cofactors with good stereochemistry. There are no major structural differences to the wild type protein. We found Q_B well-defined in the distal position and a water molecule distribution comparable to wild type structures. In addition, we characterised the kinetic features of this mutant using time-resolved fourier-transform infrared (FTIR) spectroscopy. Previous FTIR measurements led to the suggestion of a mechanism of electron transfer involving a putative intermediary electron donor X. As for the wild type reaction center we found that Q_B^- formation and Q_A^- oxidation do not proceed synchronously. We characterised the IR spectral features of the intermediary electron donor X.

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Asymmetric distribution of cations binding sites in high resolution crystal structures of double-stranded gramicidin D complex with Na^+ and K^+

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Gramicidin D is a mixture of six linear pentadecapeptides isolated from *Bacillus brevis*, exhibiting antibiotic activity against gram-positive bacteria. The peptides consist of alternating D- and L-amino acids in the seqence HCO-L-X_1-Gly_2-L-Ala_3-D-Leu_4-L-Ala_5-D-Val_6-L-Val_7-D-Val_8-L-Trp_9-D-Leu_{10}-L-Y_{11}-D-Leu_{12}-L-Trp_{13}-D-Leu_{14}-L-Trp_{15}-$NHCH_2CH_2OH$, where X=Val or Ile and Y=Trp, Tyr or Phe. The main composite is gramicidin A, having X=Val and Y=Trp. Due to alternating D, L seqence, all side chains are positioned on one side of the gramicidin backbone forcing the peptide to coil. Hydrophilic side chains of the helical gramicidin enable the gramicidin to build easily into a cell membrane. Gramicidin's antibiotic activity is due to intramembrane channel formation allowing monovalent cations and alkali metals to escape from cells disrupting ion balance [1].

Earlier we have determined the crystral structures of Rb^+ and Cs^+ complex with gramicidin D at 1.14 and 0.86 Å resolutions respectiely [2].

Now, we present three complexes with NaI, KI and KSCN, studied with synchrotron radiation at 100K. The resolutions achieved were 1.25 (NaI), 0.80 (KI) and 0.95 Å (KSCN). The present R factors are 15.8, 11.1 and 14.7% respectively. The most surprising observation in case of Na^+ and K^+ complexes is significant asymmetry of cations occupations of two halves of the dimeric fragment of the gramicidin channels. Usually there is one site of lower occupation of one side and two or three sites of higher occupations on the other side. According to our hypothesis the asymmetry originated during formation of a gramicidin - cation complex. The binding of a cation at one end of the dimer (called gramicidin channel) results in higher preference of one orientation of the dimer while joining the growing crystal.

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