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THE ROLE OF CARBOXYLIC ACID RESIDUES IN THE PROTON PUMP MECHANISM OF BACTERIORHODOPSIN (bR):  
INVESTIGATION BY TIME RESOLVED AND STATIC IR DIFFERENCE SPECTROSCOPY WITH (3-13C) LABELLED  
ASPARTIC ACID

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In order to analyse reactive amino acids participating in the pathway of proton transfer we labelled bR with (3-13C) aspartic acid. For the three carbonyl stretching vibrations, identified by Siebert et al. (1), time resolved and static FTIR studies showed the expected isotope shift of about 40 cm<sup>-1</sup>. One of them - Asp 1 - disappears during the life time of the L Intermediate, whereas the two others, Asp 2 and Asp 3, appear in the life time of the M intermediate. In addition, we identified carbonyl stretching vibrations of glutamic acids in the K and M intermediate, which showed the expected shift in D<sub>2</sub>O but no shift for labelled bR.

From the symmetric and asymmetric vibrations of the ionised form we have evidence that at least one ionised Aspartic acid residue is influenced in the BR-K and BR-L transition and protonated during the L to M transition. We interpret this as a charge separation (2) of the protonated Schiff Base from the counterion Asp 212 in K and a protonation of the counterion in the L to M transition.

1) Siebert, F., Mäntele, W., und Kreutz, W. 1982, FEBS LETT 141, 83-87 M.

2) Honig, B., Ebrey, T.E., Callender, R.H., Dinur, U., and Ottolenghi, M., 1979, Proc. Natl. Acad. Sci. USA, 76, 2503-2507