LETTER

Stoichiometry of proton pumping by the *cbb*$_3$-type oxygen reductase in whole cells of *Rhodobacter capsulatus* at pH 7 is about 0.5 H^+ per electron

Recently, we reported (1) that the stoichiometry of proton pumping is about 0.4–0.5 proton per electron for members of the C family of respiratory oxygen reductases, also called the cbb_3-type cytochrome *c* oxidases. This amount (0.5) is about half of the value obtained with the canonical A family of respiratory oxygen reductases, which includes the mitochondrial cytochrome *c* oxidases. We pointed out that the low stoichiometry correlates with the absence of the D channel for proton input and speculated that these properties may be an evolutionary adaptation to aerobic respiration under conditions of low oxygen. The measurements were obtained with intact cells at pH values near pH 6, using the electron donor, *N*,*N*,*N*',*N*'-tetramethyl-*p*-phenylenediamine (TMPD). However, TMPD has a pK_a of 6.5 (2) and, hence, oxidation of TMPD at pH 6 will result in proton release. This proton release could be misinterpreted as proton pumping, leading to a high apparent proton/electron stoichiometry. It is also possible that protonated TMPD could act as an uncoupler and result in an apparent low value of proton-pumping stoichiometry. The validity of our reported proton-pumping stoichiometries has been properly questioned by Rauhamäki et al. (3). We have, therefore, repeated the proton-pumping measurements at pH values greater than 7. For the caa_3 A-family oxygen reductase from *Thermus thermophilus* YC1001, the measured proton/electron stoichiometry is decreased from about 1.1 (pH 5.8) to 0.7 ± 0.6 (n = 2) (pH 7.5). This value (0.7) is somewhat lower than the expected value of 1 for this enzyme. Measurements with the cbb_3 C-family oxygen reductase from *Rhodobacter capsulatus* (KZ1) show the same proton pump stoichiometry for pH > 7 as was previously reported at pH 6: H^+/e^- = 0.4 ± 0.15 (n = 9). Hence, we confirm that proton pump stoichiometry of the cbb_3-type oxygen reductase, at least from *R capsulatus*, is about 0.5 proton per electron as previously reported (1) and is about half of the value obtained with the A-family oxygen reductases. Possibly, the artifacts at pH 6 fortuitously cancel out.

Rauhamäki et al. (3) have convincingly demonstrated that the cbb_3-type oxygen reductase, at least that from *Rhodobacter sphaeroides*, can pump a full one proton per electron, so we must agree that the absence of the D channel for proton input does not correlate with a mechanistic limitation of the proton pump stoichiometry. The absence of the D channel, which carries all of the pumped protons of the A-family oxygen reductases, does not change the capacity for proton pumping in the cbb_3 C-family enzymes. Our data indicate, however, that under some circumstances in vivo the enzyme does not pump protons to its full capacity. This latter point is of particular importance for how microbes have evolved to optimize their respiratory systems for particular ecological niches.

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The authors declare no conflict of interest.

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