Supporting Information

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SI Materials and Methods: *N,N′*-dicyclohexylcarbodiimid (DCCD) Inhibition of ATPase activity and $^{22}Na^+$ Binding to the Purified V_1V_o-ATPase

V_1V_o-ATPase and V_1-ATPase were purified as described previously (1, 2). For the $^{22}Na^+$-binding assay, reaction mixture containing 1 μM purified V_1V_o-ATPase and 15 μM $^{22}NaCl$ (3 cpm/nL) in Buffer A (20 mM MES-Tris, 20% glycerol, and 0.05% n-dodecyl β-D-maltoside; pH 6.0) was incubated for 2 h at room temperature, which was sufficient to saturate Na^+ binding to the enzyme. The time course of the DCCD inhibition experiment was initiated by the addition of 0.2 mM DCCD to the incubation mixture at room temperature. Free $^{22}Na^+$ was rapidly separated using a Dowex-50 method at various time intervals (1). For the ATPase assay, a reaction mixture containing 1 μM purified V_1V_o-ATPase (or V_1-ATPase) and 15 μM NaCl for the ATPase-activity assay in Buffer A was incubated for 2 h at room temperature, which was sufficient to saturate Na^+ binding to the enzyme. The time course of the DCCD inhibition experiment was initiated by the addition of 0.2 mM DCCD, 5 mM ATP, and 5 mM $MgSO_4$ to the incubation mixture at room temperature. The concentration of hydrolyzed ATP was measured (2). These inhibition curves followed pseudo-first-order kinetics. The rate constants (k_{DCCD}) of the inhibition by DCCD were calculated by fitting the linear part of the data plotted as residual activities versus incubation time. The slope of the line represents the negative of the rate constant for inhibition. The measurement was repeated three times, data were averaged, and the standard deviation was calculated.

SI Results: DCCD inhibition of V_1V_o-ATPase

We previously reported that DCCD inhibited the ATPase-activity, transport activity, and $^{22}Na^+$-binding ability of purified *Enterococcus hirae* V_1V_o-ATPase (3, 4). Inhibition by DCCD was specifically prevented by the presence of high-concentration (25 mM) of Na^+, suggesting that Na^+ bound to the binding site protects against DCCD reaction to E139 (Fig. 2*B*), and that DCCD does not react with other regions to inhibit V_1V_o-ATPase, such as the nucleotide-binding site of the V_1 moiety (1, 3). Here, we examined the inhibition rates of ATPase-activity and $^{22}Na^+$ binding by 0.2 mM DCCD in the presence of 15 μM Na^+ (Fig. S1). Under these conditions, V_1-ATPase was not significantly inhibited by DCCD: 80% of the ATPase activity remained after 60 min. On the other hand, the ATPase activity of purified V_1V_o-ATPase was rapidly inhibited by DCCD, and 90% of the activity was lost in 10 min in the presence of a low concentration of Na^+. In contrast, $^{22}Na^+$ binding ability to the purified whole enzyme decreased very slowly, with 60% of the binding remaining even after 10 min incubation. The inhibition time courses of these residual activities followed pseudo-first-order kinetics, and the slopes of the linear part of the data plotted as logarithm of the residual activity versus incubation time showed the pseudo-first-order rate constants (ATPase activity, $k_{DCCD} = 5.2 \times 10^{-3} \pm 5 \times 10^{-4}$ [S^{-1}]; $^{22}Na^+$ binding, $k_{DCCD} = 0.8 \times 10^{-3} \pm 1 \times 10^{-4}$ [S^{-1}]) (see Fig. S1, *Inset*).

1. Murata T, Takase K, Yamato I, Igarashi K, Kakinuma Y (1999) Properties of the V_1V_o Na^+-ATPase from *Enterococcus hirae* and its V_0 moiety. *J Biochem* 125:414–421.
2. Arai S, et al. (2009) Reconstitution in vitro of the catalytic portion ($NtpA_3$-B_3-D-G complex) of *Enterococcus hirae* V-type Na^+-ATPase. *Biochem Biophys Res Commun* 390:698–702.
3. Murata T, Igarashi K, Kakinuma Y, Yamato I (2000) Na^+ binding of V-type Na^+-ATPase in *Enterococcus hirae*. *J Biol Chem* 275:13415–13419.
4. Murata T, Takase K, Yamato I, Igarashi K, Kakinuma Y (1997) Purification and reconstitution of Na^+-translocating vacuolar ATPase from *Enterococcus hirae*. *J Biol Chem* 272: 24885–24890.

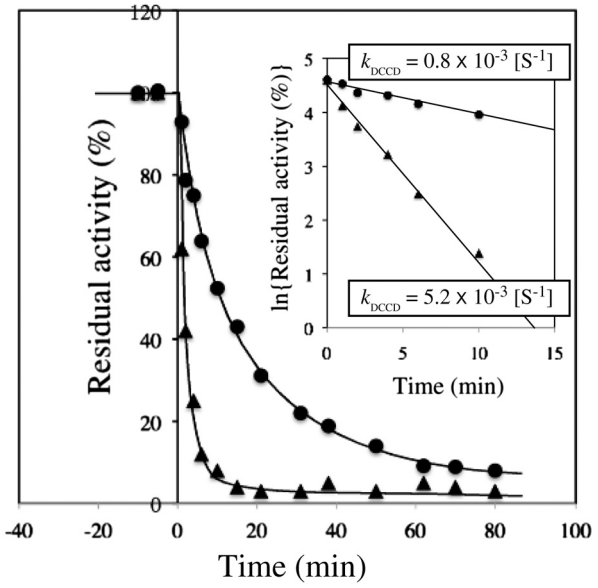


Fig. S1. DCCD inhibition of $^{22}Na^+$ binding (●) and ATPase activity (▲) of purified V_1V_o-ATPase. The time course was initiated by the addition of 0.2 mM DCCD. The inhibition rate constant (k_{DCCD}) was estimated from a semilogarithmic plot (*Inset*).

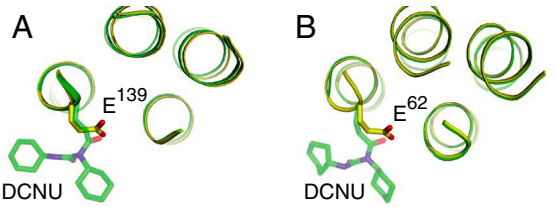
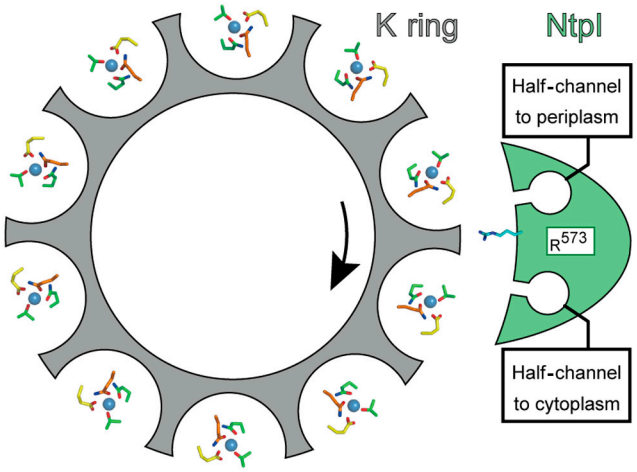


Fig. S2. Comparison of the K ring with the c ring from the F-ATPase of *Spirulina platensis*. Close-up top views of the ion-binding sites of the wild-type rings (yellow) and DCCD-modified rings (green) are superposed. (*A*) K ring of *E. hirae* V-type Na^+-ATPase. (*B*) C ring of *S. platensis* F-type H^+-ATPase. The conserved glutamate residues that are modified by DCCD are shown in stick representation. DCNU, dicyclohexyl-N-acylurea.



Movie S1. Animation of a model for the ion-transport mechanism of Na^+-transporting V-ATPase.
Movie S1 (MOV)

Table S1. Data collection and crystallographic analysis

	Na^+-bound K ring modified with DCCD	Na^+-unbound K ring modified with DCCD
Wavelength, Å	1.0000	1.0000
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions, Å	$a = 120.2$, $b = 125.2$, $c = 211.6$	$a = 120.4$, $b = 124.9$, $c = 208.2$
Resolution, Å	48.41–2.40 (2.46–2.40)	48.79–3.14 (3.22–3.14)
No. of reflections	118,450	51,928
R_{merge}*†	0.091 (0.605)	0.106 (0.723)
Completeness,* %	99.7 (99.1)	94.8 (95.9)
Redundancy*	4.7 (3.7)	3.7 (3.7)
$\langle I\rangle/\sigma\langle I\rangle$*	11.2 (1.8)	10.2 (1.8)
Overall *B* factor, $Å^2$	57.3	35.3
R factor, %	22.5 (28.1)	21.1 (34.2)
Free *R* factor, %	23.1 (29.4)	24.8 (40.8)
rmsd bonds, Å	0.009	0.011
rmsd angles, °	1.199	1.391

*Statistics for the highest resolution bin are shown in parentheses.
†$R_{merge} = \sum_{hkl}\sum_{I}|I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_{i} I_i(hkl)$.