Na\(^+\) Binding of V-type Na\(^+\)-ATPase in Enterococcus hirae*

Received for publication, November 22, 1999, and in revised form, January 24, 2000

Takeshi Murata§§, Kazuei Igarashi¶, Yoshimi Kakinuma¶, and Ichiro Yamato‡

From the §Department of Biological Science and Technology, Science University of Tokyo, 2641 Yamazaki, Noda-shi, Chiba 278-8510, Japan and the ¶Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Ion motive ATPases that do not form phosphorylated intermediates are divided into two types: F\(_{0}\)F\(_{1}\)-ATPase (F-ATPase) and V\(_{0}\)V\(_{1}\)-type ATPase (V-ATPase). F-ATPase functions as an ATP synthase in mitochondria, chloroplasts, and oxidative bacteria (1). V-ATPase functions as a proton pump in acidic organelles, in plasma membranes of eukaryotic cells (2), and in bacteria (3). Because V-type and F-type ATPases resemble each other both structurally and functionally, it is accepted that the reaction mechanism and energy coupling mechanism of the two ATPases are similar (2, 4, 5).

The "rotation catalysis" mechanism, proposed by P. D. Boyer (6), has now been verified as the mechanism of F-ATPase; the energy of ATP hydrolysis is converted into the physical force in the form of rotation of the \(\gamma\) subunit, with three ATP hydrolyses/rotation (7–10). Therefore, an important question is how the physical rotation and \(\Delta H\) transport are connected. Two different models for the mechanism of ion translocation through the F\(_{0}\) portion have been proposed for the \(\Delta H\) -ATPase of Escherichia coli (11–14) and the Na\(^+\)-ATPase of Propionigenium modestum (5, 15). To fully understand the transport mechanism, characterization of the ion binding reaction of the F\(_{0}\) portion is essential. However, in both cases, it was difficult to examine the ion binding to the F\(_{0}\) portion directly, because for E. coli the ion was a proton and for P. modestum the affinity was too low (\(K_m = 0.8 \text{ mM}\)) (16).

A V-type ATPase transports Na\(^+\) or Li\(^+\) in the eubacterium Enterococcus hirae (17). The enzyme consists of nine subunits encoded by a Na\(^+\)-responsive operon (designated ntp) (18, 19). The ATPase activity and Na\(^+\) uptake activity of purified E. hirae V-ATPase were absolutely dependent on the presence of Na\(^+\) (20, 21), with \(K_m\) values for Na\(^+\) of 20 \(\mu\text{M}\) and 4 \(\mu\text{M}\) and a \(K_v\) value for Na\(^+\) of 40 \(\mu\text{M}\). These low \(K_m\) and \(K_v\) values enabled measurement of ion binding to this ATPase. In this study, we report the Na\(^+\) binding properties of E. hirae Na\(^+\)-ATPase. This is the first direct demonstration of cation binding in the studies of V- and F-ATPases.

MATERIALS AND METHODS

Strains and Culture—The E. hirae strains ATCC 9790 (wild type strain) and 25D/pCemtp18, a mutant defective in the production of F\(_{0}\)F\(_{1}\)-ATPase harboring a plasmid containing the whole Na\(^+\)-ATPase (ntp) operon (20) were used in this study. Cells of ATCC 9790 were cultured at 37 °C in KTY medium (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 1% glucose, and 1% \(K_2\text{HPO}_4\)) (19). Cells of 25D/pCemtp18 were cultured at 37 °C in KTY medium containing 0.5 \(\mu\text{M}\) NaCl supplemented with 10 \(\mu\text{g}\)/ml erythromycin.

Preparation of Membrane Vesicles and Purification of Na\(^+\)-ATPase—Membrane vesicles were prepared as described previously (20). Purification of Na\(^+\)-ATPase was performed using anion exchange and gel filtration chromatographies as described previously (21). Concentration of the purified sample was carried out using ultrafiltration (Amicon, YM 100 filter).

Measurement of Na\(^+\) Binding to Membrane Vesicles—The incubation mixture contained 0.1 mg of protein of membrane vesicles in 50 \(\mu\text{L}\) of A buffer (50 mM Tris-HCl, 5 mM MgCl\(_2\), 20% glycerol, 1 mM dithiothreitol, pH 7.5) and 22NaCl (final concentration, 20 \(\mu\text{M}\); 35,000 cpm/\(\mu\text{L}\)). The concentration of contaminating Na\(^+\) in the mixture before addition of 20 \(\mu\text{M}\) 22NaCl was measured using flame photometry. The mixtures including the membrane vesicles from ATCC 9790 and 25D/pCemtp18 had Na\(^+\) concentrations of 4 and 8 \(\mu\text{M}\), respectively. We took into account the contaminating Na\(^+\) to estimate the total concentration of Na\(^+\) in the mixture. The mixture was incubated for 60 min at room temperature; this period was sufficient to saturate the Na\(^+\) binding sites of the membrane (data not shown). The mixture was applied to a spin column of 400 \(\mu\text{L}\) Dowex-50 WX-200 (H\(^+\) form, Sigma) preloaded with K\(^+\) and the free 22Na\(^+\) was quickly separated from bound Na\(^+\) on membrane vesicles by centrifuging the spin column at 10,000 \(\times\) g for 5 s at room temperature and washing the column once with 200 \(\mu\text{L}\) of A buffer (Dowex-50 method). The eluted fractions from the column were combined. The radioactivities in the eluted and trapped fractions were determined using a \(\gamma\)-scintillation counter (Aloka, Tokyo). The amount of specific binding of Na\(^+\) to the membrane vesicles was calculated by subtracting the amount of nonspecific binding that was measured by diluting the radioactivity in the reaction mixture with 10 \(\mu\text{L}\) nonradioactive NaCl. In addition to the Dowex-50 method, an ultra centrifugation method was also used (22). The mixture described above was centrifuged at 100,000 \(\times\) g for 30 min at 4 °C, and the supernatant was regarded as having only free 22Na\(^+\). The measurement was repeated five times and averaged, and the standard deviation (S.D.) was calculated.

Measurement of Na\(^+\) Binding to Purified Samples—The molecular mass of the Na\(^+\)-ATPase is estimated as 664 kDa, when calculated from the molecular mass of each subunit and the subunit ratio (20). Thus, 1

* This work was supported by a grant-in-aid (to T. M., I. Y., and Y. K.) for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence and reprint requests should be addressed:
Tel.: 81-471-24-1501 (ext. 4425); Fax: 81-471-25-1841; E-mail: murata@yl05hp.tb.noda.sut.ac.jp.
mg of the purified enzyme corresponds to 1.5 nmol. The incubation mixture contained 0.02 mg of protein (final concentration, 0.6 mM) of the purified enzyme and $^{22}$NaCl (final concentration, 20 mM; 35,000 cpm/nmol) in 50 mM of A buffer plus 0.02% dodecyl maltoside (B buffer). The same mixture, but with various concentrations of $^{22}$NaCl, was used for determination of the Na$^+$ concentration dependence of Na$^+$ binding. The concentration of contaminating Na$^+$ in the mixture before the addition of $^{22}$NaCl was found to be 2 μM by flame photometry. The contaminating Na$^+$ was taken into account to estimate the total concentration of Na$^+$ in the mixture. The mixture was incubated for 60 min at room temperature, which was long enough to saturate the Na$^+$ binding sites of the purified enzyme. The free $^{22}$Na$^+$ was quickly separated by the Dowex-50 method using B buffer as the washing solution, and the amount of Na$^+$ bound to the enzyme was calculated as described above. The equilibrium-dialysis method was also applied using a microequilibrium-dialysis apparatus as described previously (23). The purified enzyme (final concentration, 3 μM ATPase) in 50 mM of B buffer was placed in a chamber, and $^{22}$NaCl (final concentration, 20 mM; 35,000 cpm/nmol) in 50 mM of B buffer was placed in another chamber. The concentration of contaminating Na$^+$ in the mixture including 3 μM purified enzyme was found to be 5 μM and was taken into account to estimate the total concentration of Na$^+$. The difference in radioactivities in the two chambers after dialysis for 3 h at 4°C was used to calculate the specific binding to the purified sample. S.D. was calculated for five repeated experiments.

Chasing experiments were started by adding 10 mM nonradioactive NaCl with or without 2 mM ATP (or ATP-S$^3$) to the incubation mixture equilibrated with 20 μM $^{22}$Na$^+$, and free $^{22}$Na$^+$ was quickly separated by the Dowex-50 method at various time intervals. The release of $^{22}$Na$^+$ bound to the purified enzyme was because of the exchange reaction with nonradioactive Na$^+$; the release of "Na$^+$ from the enzyme was dependent on the concentration of added nonradioactive Na$^+$ (data not shown). If the Na$^+$ binding to Na$^+$-ATPase is a simple binding reaction, the exchange rate constant ($k_{\text{exchange}}$) should correspond to the dissociation rate constant ($k_d$) of Na$^+$ in the binding reaction. In this paper, we used the empirical $k_{\text{exchange}}$ values, because we do not know the detailed mechanism of the Na$^+$ binding reaction.

**Specific Binding of Na$^+$ to Na$^+$-ATPase**—The Na$^+$-ATPase activity in the membrane vesicles prepared from strain 25D/pCemtp18 was 5 μmol of P/min/mg of protein. Specific binding of Na$^+$ to the same membrane vesicles was 0.8 ± 0.1 nmol/mg of protein when assayed in the presence of 20 μM $^{22}$Na$^+$. A similar value of Na$^+$ binding was obtained using the centrifugation method (0.8 ± 0.1 nmol/mg of protein). Because E. hirae V-ATPase is induced in response to an increase in intracellular Na$^+$ concentration (19), the amount of this enzyme is limited when cultured in Na$^+$-starved KTY medium (19). Na$^+$-ATPase activity in the membrane vesicles of 9790 (wild type strain) grown in KTY medium was not detectable; the amount of Na$^+$ binding to the membrane vesicles was 0.04 ± 0.01 nmol/mg of protein. These findings suggest that more than 93% of the amount of Na$^+$ binding to membrane vesicles from 25D/pCemtp18 was because of the Na$^+$-ATPase.

The Na$^+$-ATPase activity of the purified enzyme was 26 μmol of P/min/mg of protein, which represents an ~5-fold purification from the membrane vesicles, because specific activities of the ATPase in the solubilized and membrane-embedded states were similar (data not shown). Specific binding of Na$^+$ to the purified sample in the presence of 20 μM $^{22}$Na$^+$ was 4.0 ± 0.5 nmol/mg protein. A similar value (4.2 ± 0.4 nmol/mg protein) was obtained using the equilibrium dialysis method. The specific binding of Na$^+$ to the purified sample was about five times higher than that to the membrane vesicles, which was consistent with the purification efficiency. These findings indicate that the purified Na$^+$-ATPase retained a Na$^+$ binding capacity similar to that in the native state.

**Number of Na$^+$ Ions Bound/Enzyme**—Fig. 1 shows the NaCl concentration dependence of the Na$^+$ binding to purified Na$^+$-ATPase. The Scatchard plot (Fig. 1, inset) of this data has an intercept on the abscissa at 6.2, indicating that about 6 mol (S.D. ± 1) of Na$^+$ bind/mole of enzyme. The slope of the Scatchard plot indicates that the dissociation constant ($K_{\text{D},\text{Na}}$) is 15 μM (S.D. 15 ± 5). This value is similar to the higher (20 μM) of the two $K_m$ values for Na$^+$ of the ATPase activity (20, 21).

**Effect of Inhibitors on Na$^+$ Binding**—Nitrate is known to inhibit V-type ATPase probably by dissociating the V$_1$ complex of the ATPase (25). This reagent inhibited the Na$^+$-ATPase activity of both the purified sample and the membrane vesicles (Table I, row 2), but it did not affect the Na$^+$ binding of the purified sample or membrane vesicles. This result is consistent with a model in which the ion binding sites reside in the membrane-embedded portions of F-ATPase (5, 14) and V-ATPase (21, 26). Another potent inhibitor of V-type ATPase, destruxin B (27), also had no effect on the Na$^+$ binding, although it inhibited the ATPase activity (Table I, row 3).

DCCD strongly inhibits F- and V-ATPases by covalently reacting with an acidic amino acid residue of the proteolipid of these ATPases (26, 28). Because the coupling ion, Na$^+$, prevented the inhibition by DCCD (21), the reactive amino acid was considered to reside at the ion binding site of the F$_0$ or V$_0$ portion. Preincubation with the purified sample (0.6 μM) and 0.1 mM DCCD in the absence of Na$^+$ lowered the Na$^+$ binding capacity to about half of its normal level, whereas about 85% of the Na$^+$-ATPase activity was inhibited (Table I, row 4). By preincubation with a large excess of DCCD (1 mM), the Na$^+$ binding of the purified enzyme was reduced to 14 ± 8%, and the Na$^+$-ATPase activity was reduced to 2% (Table I, row 5). In the presence of 0.1 or 1 mM DCCD, the Na$^+$ binding of the membrane vesicles was reduced less than that of the purified enzyme (Table I, rows 4 and 5). As expected, neither the Na$^+$ binding nor the Na$^+$-ATPase activity of the purified sample or membrane vesicles was inhibited by preincubation with 1 mM DCCD in the presence of 25 mM Na$^+$ (Table I, row 6). This suggests that the Na$^+$ binding sites are near the DCCD reactive sites in the V$_0$ portion, in agreement with previous data (21).

The ATPase activity of this enzyme was strongly inhibited by AMP-PNP ($K_i$ = 400 μM) (Table I, row 7) and ATP-S$^3$ ($K_i$ = 80 μM) (Table I, row 8). These two ATP analogs are considered to bind to the ATP binding site of F$_2$-ATPase (29) and probably bind to the ATP binding site of V$_1$-ATPase. Therefore, these reagents may affect the Na$^+$ binding properties of the enzyme. However, they did not have much effect on the Na$^+$ binding capacity (Table I, rows 7 and 8). Neither 2 mM ADP nor 2 mM ADP plus P$_i$ affected the Na$^+$ binding capacity (Table I, rows 9 and 10). This is consistent with a previous report that the V-ATPase reaction was not inhibited by the reaction products ADP and P$_i$ (30).

**Release of Bound Na$^+$ from the ATPase**—Fig. 2A shows the time course of release of $^{22}$Na$^+$ bound to the purified enzyme following the addition of a large excess of nonradioactive NaCl. Semilogarithmic plots of the data appear to show three release phases and exchange rate constants ($k_{\text{exchange}}$): a fast component (about 60% of the total amount of bound Na$^+$); $k_{\text{exchange}}$ >
**$^{22}\text{Na}^+$ Binding of E. hirae V-type Na$^+$-ATPase**

1.7 min$^{-1}$, a slow component (about 30% of the total; $k_{\text{exchange}} = 0.16 \text{ min}^{-1}$), and the slowest component (about 10% of the total; $k_{\text{exchange}} = 0.05 \text{ min}^{-1}$) (Fig. 2B). The observed fast exchange rate suggests that about 60% of the bound Na$^+$ is freely exchangeable with Na$^+$ in the solution. When 10 mM LiCl instead of NaCl solution was used in a chasing experiment, a similar time course of $^{22}\text{Na}^+$ release and similar $k_{\text{exchange}}$ values were obtained. However, when K$^+$ or Rb$^+$ was used instead of Na$^+$, essentially no release was observed (data not shown). The cation specificity of chasing was the same as that of stimulation of the transport reaction (20, 21). This suggests that the binding of Na$^+$ is related to the transport reaction.

When the exchange reaction was assayed with 10 mM nonradioactive NaCl solution containing 2 mM ATP, the slow component ($k_{\text{exchange}} = 0.16 \text{ min}^{-1}$) disappeared and most of the bound Na$^+$ quickly exchanged ($k_{\text{exchange}} > 4.8 \text{ min}^{-1}$). Because ions were being translocated in the presence of ATP, the bound Na$^+$ involved in the enzyme reaction of Na$^+$-ATPase should be easily exchanged with nonradioactive Na$^+$ in the solution. Even under these conditions, the slowest component ($k_{\text{exchange}} = 0.05 \text{ min}^{-1}$) was still present (Fig. 2, C and D), indicating that this slowest component was not related to the enzyme reaction of Na$^+$-ATPase.

ATPγS inhibited the ATPase activity but not the Na$^+$ binding capacity of the enzyme (Table I, row 8). Interestingly, when 2 mM ATPγS was used in place of ATP in the chasing experiment, most of the bound Na$^+$ quickly exchanged ($k_{\text{exchange}} > 4.7 \text{ min}^{-1}$), although the slowest component ($k_{\text{exchange}} = 0.05 \text{ min}^{-1}$) remained, as it did in the case when ATP was added (data not shown). This suggests that the conformational change brought about by binding of ATPγS at the ATP binding site in the V$_1$ portion affects the ion binding properties of the V$_0$ portion, resulting in the disappearance of the slow component ($k_{\text{exchange}} = 0.16 \text{ min}^{-1}$) of the exchange rates.

The exchange rate constant ($k_{\text{exchange}}$) of Na$^+$ bound to DCCD-treated Na$^+$-ATPase was 0.05 min$^{-1}$ (Fig. 2, E and F), corresponding to that of the slowest component, which was not related to the enzyme reaction of the Na$^+$-ATPase. This suggests that all the Na$^+$ binding sites of the purified enzyme that are involved in Na$^+$ transport reaction react with DCCD.

**DISCUSSION**

In this study, the $^{22}\text{Na}^+$ binding of a V-type Na$^+$-ATPase (that of E. hirae) was measured. This is the first direct demonstration of cation binding in the studies of V- and F-ATPases. Several of the properties of the Na$^+$ binding of this enzyme are similar to those that previously reported for the enzyme’s ATPase activity and transport activity (20, 21). These include the cation specificity, the affinity for Na$^+$, and the reactivity to DCCD. Therefore we concluded that the Na$^+$ binding we measured in this study is involved in the transport reaction of the Na$^+$-ATPase. The number of bound Na$^+$/ATPase was estimated as 6±1. With the addition of 10 mM nonradioactive NaCl, about two-thirds of the bound Na$^+$ was released quickly, and the remainder was released more slowly (Figs. 1 and 2).

In the rotating model of F-type ATPase, the F$_0$ portion is thought to contain 12 proteolipid 8-kDa subunits, called the c subunits, that form a rotor ring (31) and each is postulated to have one H$^+$ binding site (32). The proteolipid (NtpK) of E. hirae V-type ATPase is about 16 kDa and is considered to have one cation binding site (Glu-139). The Na$^+$ transport activity disappeared in the strain with the E139N mutation, which was induced by site-directed mutagenesis (33). In this study, the number of Na$^+$ binding sites was found to be 6±1. This suggests that the V$_0$ portion of the Na$^+$-ATPase contains six proteolipid (NtpK) subunits. Consistent with this estimate, the number of H$^+$-conducting proteolipid subunits is thought to be six/V-ATPase (26, 34).

The reason that 0.1 mM DCCD in the absence of Na$^+$ strongly inhibited the ATPase activity of the purified enzyme but had only a moderate effect on Na$^+$ binding capacity (Table I, row 4) is unclear. This discrepancy may be because the ATPase activity is inhibited by only a single DCCD molecule. This has been shown to be the case with an F-ATPase (28), and so it seems likely that it also occurs with the

**FIG. 1.** Na$^+$ concentration dependence of Na$^+$ binding to purified Na$^+$-ATPase. The inset shows the Scatchard plot of the specific binding of Na$^+$ to the purified enzyme. $\theta$ is defined as the number of moles of bound Na$^+$/mol of the enzyme.

**TABLE I**

| Reagent | Relative Na$^+$-ATPase activity | Relative binding capacity | Relative Na$^+$-ATPase activity | Relative binding capacity |
|---------|---------------------------------|--------------------------|---------------------------------|--------------------------|
| 1       | No addition                     | 100                      | 100                             | 100                      |
| 2       | 50 mM KNO$_3$                   | 22                       | 95 ± 3                          | 95 ± 9                   |
| 3       | 0.1 mM Destruxin B              | 18                       | 96 ± 5                          | 97 ± 6                   |
| 4       | 0.1 mM DCCD (−25 mM NaCl)       | 15                       | 48 ± 8                          | 40 ± 5                   |
| 5       | 1 mM DCCD (−25 mM NaCl)         | 2                        | 14 ± 8                          | 3 ± 4                    |
| 6       | 1 mM DCCD (+25 mM NaCl)         | 89                       | 90 ± 5                          | 92 ± 5                   |
| 7       | 2 mM AMP-PNP                    | 16                       | 92 ± 7                          | 96 ± 7                   |
| 8       | 2 mM ATPγS                      | 3                        | 96 ± 7                          | 8 ± 1                    |
| 9       | 2 mM ADP                        | 72                       | 93 ± 6                          | 8 ± 1                    |
| 10      | 2 mM ADP, 2 mM Pi              | 70                       | 98 ± 4                          | 72 ± 8                   |
V-ATPase. Because the other subunits in the Na\(^{+}\)-ATPase that are not modified by DCCD should retain a Na\(^{+}\) binding capability, the reduction of binding would be expected to be intermediate.

Our finding that about two-thirds of the bound Na\(^{+}\) was released quickly (fast component) and the remainder was released more slowly (slow component) (Fig. 2) suggested that about two-thirds of the Na\(^{+}\) binding sites of the Na\(^{+}\)-ATPase are readily accessible from the aqueous phase. The slow component disappeared in the presence of ATP or ATP\(_{S}\), suggesting that all or most Na\(^{+}\) binding sites of the enzyme are freely accessible from the aqueous phase under these conditions. In addition, the binding of an ATP analog (ATP\(_{S}\)) affected the existence of the slow component of release of the bound Na\(^{+}\).

We believe that this indicates that the slow component must be involved in the transport reaction. The present findings concerning the Na\(^{+}\) binding reaction of the V-type Na\(^{+}\)-ATPase in E. hirae should be useful in understanding the mechanisms of cation transport through membrane-embedded portions of V- and F-ATPases.

REFERENCES

1. Senior, A. E. (1990) Annu. Rev. Biophys. Chem. 19, 7–41
2. Nelson, N., and Taiz, L. (1989) Trends. Biochem. Sci. 14, 113–116
3. Gogarten, J. P., Kibak, H., Dittrich, P., Taiz, L., Bowman, K. J., Bowman, B. J.,

FIG. 2. Release of \(^{22}\text{Na}\)\(^{+}\) bound to the purified Na\(^{+}\)-ATPase following the addition of excess nonradioactive Na\(^{+}\). Chasing experiments were started at “time 0” by adding 10 mM NaCl into the mixture containing purified Na\(^{+}\)-ATPase that was incubated with 20 \(\mu\text{M}^{22}\text{Na}\)\(^{+}\). The exchange rate constant (\(k_{\text{exchange}}\)) was estimated from semilogarithmic plots (B, D, and F). A, C, and E show the time courses of \(^{22}\text{Na}\)\(^{+}\) release under various conditions. A, in the absence of ATP. C, in the presence of 2 mM ATP. ATP was added simultaneously with 10 mM NaCl at time 0. E, DCCD-treated purified Na\(^{+}\)-ATPase. The ATPase was treated with 1 mM DCCD beforehand for 10 min in the absence of NaCl (see the legend to Table I).
22Na\(^+\) Binding of E. hirae V-type Na\(^+\)-ATPase

Manolson, M. F., Poole, R. J., Data, T., Oshima, T., Konishi, J., Denda, K., and Yoshida, M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 6661–6665

4. Nelson, N. (1992) *Biochim. Biophys. Acta** **1100**, 109–124

5. Dimroth, P., Wang, H., Grabe, M., and Oster, G. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 4924–4929

6. Boyer, P. D. (1993) *Biochim. Biophys. Acta** **1140**, 215–250

7. Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K., Jr. (1997) *Nature* **386**, 299–302

8. Dimroth, P., Wang, H., Grabe, M., and Oster, G. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 4924–4929

9. Nelson, N. (1992) *Biochim. Biophys. Acta** **1100**, 109–124

10. Yasuda, R., Noji, H., Kinosita, K., and Yoshida, M. (1998) *Cell* **93**, 1117–1124

11. Junge, W., Lill, H., and Engelbrecht, S. (1997) *Trends. Biochem. Sci.* **22**, 420–423

12. Dmitriev, O. Y., Altendorf, K., and Fillingame, R. H. (1995) *Eur. J. Biochem.* **233**, 478–483

13. Vik, S. B., and Antonio, B. J. (1994) *J. Biol. Chem.* **269**, 30364–30369

14. Abrahams, J., Leslie, A., Lutter, R., and Walker, J. (1994) *Nature* **370**, 621–628

15. Singh, S., Turina, P., Bustamante, C., Keller, D. J., and Capaldi, R. (1996) *FEBS Lett.* **397**, 30–34

16. Miller, M. J., Oldenburg, M., and Fillingame, R. H. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4900–4904

17. Takase, K., Yamato, I., Igarashi, K., and Kakinuma, Y. (1999) *Biochim. Biophys. Acta** **13419**, 23661–23666
Na⁺ Binding of V-type Na⁺-ATPase in *Enterococcus hirae*
Takeshi Murata, Kazuei Igarashi, Yoshimi Kakinuma and Ichiro Yamato

*J. Biol. Chem.* 2000, 275:13415-13419.
doi: 10.1074/jbc.275.18.13415

Access the most updated version of this article at [http://www.jbc.org/content/275/18/13415](http://www.jbc.org/content/275/18/13415)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 14 of which can be accessed free at [http://www.jbc.org/content/275/18/13415.full.html#ref-list-1](http://www.jbc.org/content/275/18/13415.full.html#ref-list-1)