The resolution-reconstitution approach has been employed in order to gain information as to the subunit composition of the Neurospora plasma membrane H⁺-ATPase. Proteoliposomes prepared from sonicated asolectin and a highly purified, radiolabeled preparation of the 105,000-dalton hydrolytic moiety of the H⁺-ATPase by a freeze-thaw procedure catalyze ATP hydrolysis-dependent proton translocation as indicated by the extensive 9-amino-6-chloro-2-methoxyacridine fluorescence quenching that occurs upon the addition of MgATP to the proteoliposomes, and the reversal of this quenching induced by the H⁺-ATPase inhibitor, vanadate, and the proton conductors, carbonyl cyanide m-chlorophenylhydrazone and nigericin plus K⁺. ATP hydrolysis is tightly coupled to proton translocation into the liposomes as indicated by the stimulated hydrolysis by carbonyl cyanide m-chlorophenylhydrazone and nigericin plus K⁺. The maximum stimulation of ATPase activity by proton conductors is about 3-fold, which indicates that at least two-thirds of the hydrolytically active ATPase molecules present in the reconstituted preparation are capable of translocating protons into the liposomes. Furthermore, as estimated by the extent of protection of the reconstituted 105,000-dalton hydrolytic moiety against tryptic degradation by vanadate in the presence of Mg⁺⁺ and ATP, the fraction of the total population of ATPase molecules that are hydrolytically active is at least 91%. Taken together, these data indicate that at least 61% of the ATPase molecules present in the reconstituted preparation are able to catalyze proton translocation. This information allows an estimation of the amount of any polypeptide in the preparation that must be present in order for that polypeptide to qualify as a subunit that is required for proton translocation in addition to the 105,000-dalton hydrolytic moiety, and an analysis of the radiolabeled ATPase preparation by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and urea rules out the involvement of any such polypeptides larger than 2,500 daltons. This indicates that the Neurospora plasma membrane H⁺-ATPase has no subunits even vaguely resembling any that have been found to be associated with other transport ATPases and that if this enzyme has any subunits at all other than the 105,000-dalton hydrolytic moiety, they must be very small.

It is now fairly clear that most of the membranous structures in cells contain ion-translocating ATPases that function either to synthesize ATP in response to a transmembrane electrochemical proton gradient or, conversely, to hydrolyze ATP and generate transmembrane ion gradients which are in turn used to energize solute transport and a variety of cellular signalling mechanisms as well. The ion-translocating ATPases that have been characterized thoroughly enough to permit classification can be subdivided into two major categories. The first of these comprises the proton-translocating F₃F₅H⁺-ATPase/ATP synthases of mitochondria, bacteria, and chloroplasts, which are characterized by a relatively complex subunit structure, the absence of a covalent phosphoryl-enzyme intermediate in the catalytic cycle, and ready reversibility for ATP synthesis in the presence of a transmembrane electrochemical proton gradient of sufficient magnitude (1). The second category of ion-translocating ATPases is the aspartylphosphoryl-enzyme intermediate family, which is characterized by a more simple subunit structure, the existence of a catalytically competent covalent phosphoryl-enzyme intermediate, apparently less ready reversibility under physiological conditions, and a broader range of transported ions. Well characterized members of this class include the Na⁺/K⁺-translocating ATPase of most animal cell plasma membranes (2), the Ca⁺⁺-translocating ATPase of sarcoplasmic reticulum (3), the H⁺/K⁺-translocating ATPase of gastric mucosa (4), and the electrogenic proton-translocating ATPase of the fungal plasma membrane (5, 6, 10, 11). The fungal enzyme has been the primary subject of experimental attention in this laboratory for several years (7–14).

An understanding of the molecular mechanisms by which the ion-translocating ATPases transduce the chemical energy of ATP hydrolysis into transmembrane electrical and/or chemical potential differences is a major goal of contemporary biological science. An important prerequisite to the attainment of such a goal for any transport ATPase is knowledge of the subunit composition of that enzyme, but unfortunately, such knowledge has not been easy to obtain. Although it is possible to state that the subunit composition of an ATPase is relatively complex or relatively simple, a precise description of the number of individual subunits that constitute a functional transport unit is available for virtually none of these enzymes. A possible exception is the bacterial F₃F₅H⁺-ATPase/ATP synthase (1). The major impedance to progress along these lines in the case of F₃F₅H⁺-ATPases has been the great complexity of these enzymes; in the case of the aspartylphosphoryl-enzyme intermediate ATPase family, confusion has arisen primarily from the recognition that small (Mₖ)
6,000-13,600) proteolipid molecules are present in most of the standard ATPase preparations and attendant suggestions that such proteolipids may play a functional role in the transport process (15-17).

Theoretically, the resolution-reconstitution approach, pioneered for membrane proteins by Racker and his associates (18), is an ideal approach to determining the subunit composition of the transport ATPases. In principle, it should be possible to simply resolve a functional ATPase preparation into its individual components and then assay for necessary components on the basis of reconstitutivity of function. In practice, however, this approach has not been lucrative, probably because no conclusions can be drawn without an estimate of the reconstitution efficiency, and such information is quite difficult to acquire. In this article, we report reconstitution of the Neurospora plasma membrane H\(^+\)-ATPase, describe an experimental approach for estimating the efficiency of the reconstitution process, and provide quantitative information as to the composition of the ATPase preparation used for the reconstitution studies. In light of the results obtained, the probable subunit composition of this enzyme is then discussed.

**Experimental Procedures**

**Growth of Radiolabeled Cells**—Cells of the cell wall-less \( \text{fz, sg, oe-1} \) strain of Neurospora crassa were maintained by daily transfer of 1 ml of an overnight culture into 50 ml of fresh medium followed by rotary shaking (150 rpm) at 30 °C. The maintenance growth medium was Vogel’s N medium (19) supplemented with 2% (w/v) d-mannitol, 0.75% (w/v) yeast extract, and 0.75% (w/v) nutrient broth. The day before the ATPase isolation procedure was carried out, the cells contained in 20 ml of an overnight culture were pelleted by centrifugation (1100 \( \times \) g, 20 min, room temperature) under sterile conditions and resuspended in 10 ml of radioactive growth medium (see below), and the resulting cell suspension was added to an additional 490 ml of radioactive growth medium. The culture was then grown overnight and the optical density of the culture increased 6-fold. The radioactive transport molecules and the number of proteolipid molecules is the proteolipid can be drawn because the number of functional enzyme’s transport function with an efficiency of 100% virtually rules out the participation of the proteolipid in the transport process for the reconstitution studies. In light of the results obtained, the probable subunit composition of this enzyme is then discussed.

**Reconstitution of the H\(^+\)-ATPase**—The ATPase was reconstituted in asolectin liposomes by a procedure closely modeled after the procedure of Dufour et al. (22). Two hundred and forty \( \mu l \) of the liposome suspension was mixed with 340 \( \mu l \) of Buffer I and 20 \( \mu l \) of the purified ATPase solution (2.5 \( \mu g \) of protein) in a polypropylene tube on ice. The tube was then capped and placed in liquid \( \text{N}_2 \). Just prior to use in the various assays, the mixture was thawed in a 30 °C water bath for 2.5 min.

**Measurement of ATP Hydrolysis Catalyzed by the Reconstituted H\(^+\)-ATPase**—Assay mixtures contained 100 \( \mu l \) of the reconstituted ATPase preparation, 850 \( \mu l \) of Buffer I, 50 \( \mu l \) of 0.2 M disodium ATP/MgSO\(_4\) (pH 6.8 with \( \text{Tris} \)), and the indicated additions in a total volume of 1 ml. The reactions were started by the addition of the reconstituted ATPase preparation. After incubation for 10 min at 30 °C, the reactions were terminated by the addition of 5 ml of room temperature 7% (w/v) sodium dodecyl sulfate solution followed by the addition of 400 \( \mu l \) of 2.5% (w/v) (NH\(_4\))\(_2\)MoO\(_4\), 4\( \text{H}_2\)O in 5 N \( \text{H}_2\)SO\(_4\) and then the addition of 40 \( \mu l \) of a solution containing 1-aminoo-3-naphthol-4-sulfonic acid (1.92 mg/ml), Na\(_2\)S\(_2\)O\(_3\) (11.54 mg/ml), Na\(_2\)SO\(_4\) (11.54 mg/ml), and \( \text{NaCl} \) (100 mg/ml). The samples were mixed by vortexing after each addition. After 20 min at room temperature, the \( A_{400} \) was determined, and after subtraction of the values obtained in appropriate zero time controls, the amount of \( \text{P}i \) liberated during the incubations was estimated by comparison with a KH\(_2\)PO\(_4\) standard treated in identical fashion.

**ACMA Fluorescence Assay**—Conditions of the ACMA fluorescence assay were virtually identical to those of the ATPase assay except that the assays were carried out at room temperature (−22 °C). 1.7 \( \mu l \) of Buffer I was added to a cuvette followed by 2 \( \mu l \) of ACMA solution (0.3 mg/ml in methanol), 200 \( \mu l \) of the reconstituted ATPase preparation, and, except for the experiment shown in Fig. 1, 2 \( \mu l \) of 10 mM Na\(_3\)VO\(_4\) and 2 \( \mu l \) of 10 mM TEMED (N,N,N',N'-tetramethylethylenediamine; \( \Delta_{\text{pH}} \), transmembrane electrical potential difference; \( \Delta \varphi \), transmembrane electrochemical protonic potential difference; \( \Delta \varphi \), transmembrane pH difference; \( \text{CCCP} \), carbonyl cyanide m-chlorophenylhydrazine).

3 The absolute requirement for an estimation of the reconstitution efficiency in considerations of subunit composition is best explained by an example. Consider a highly purified ATPase preparation that contains 99% by mass of a 100,000-dalton hydrolytic moiety and 1% by mass of a 10,000-dalton proteolipid. In this case, 1 mg of the preparation contains about 6 \( \times \) 10\(^{10}\) molecules of the hydrolytic moiety and about 6 \( \times \) 10\(^9\) molecules of the proteolipid. Reconstitution of the enzyme’s transport function with an efficiency of 100% virtually rules out the participation of the proteolipid in the transport process because there are roughly 10 times more functional transport molecules than there are proteolipid molecules. On the other hand, if the reconstitution efficiency is only 10%, no conclusion as to the role of the proteolipid can be drawn because the number of functional transport molecules and the number of proteolipid molecules is roughly equivalent, leaving open the possibility that 10% of the molecules of the hydrolytic moiety reconstitute in 1 stoichiometry with all of the proteolipid molecules.

4 The abbreviations used are: MES, 3-(N-morpholino)propanesulfonic acid; ACMA, 9-amino-6-chloro-2-methoxyacridine; SDS, sodium dodecyl sulfate; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of SDS; TEMED, N,N,N',N'-tetramethylethylenediamine; \( \Delta_{\text{pH}} \), transmembrane electrochemical protonic potential difference; \( \Delta \varphi \), transmembrane electrical potential difference, \( \Delta \varphi \), transmembrane pH difference; \( \text{CCCP} \), carbonyl cyanide m-chlorophenylhydrazine.

5 The approximate amino acid composition of yeast extract was kindly provided by Difco.
Fructose, ~-U”C-amino-acid mixture, ~3-“C]tryptophan, and molecular weight range 2,512-16,949 polypeptide standards were from Gallard-Schlesinger Chemical Mfg. Corp. Somatostatin, L-tyrosylglycylcholate/trichloroacetic acid method of Bensadoun and Weinstein studies have been identified previously (9, 12, 27) after precipitation of the protein by the deoxy-

Determination of Protein—Protein was estimated by the method of Lowry et al. (27) after precipitation of the protein by the deoxy-

Materials—The sources of most of the materials employed in these studies have been identified previously (9, 12, 13, 20). D-[U-14C] Fructose, l-2-14C-amino-acid mixture, L-[3-“C]cyclophan, and l-“C)methionine were from New England Nuclear. NCS tissue solubilizer, 2.5% (w/v) dithiothreitol, and 0.025% (v/v) mercuric methanol, and 0.025 M H3P04 (pH 6.8 with Tris), and the resulting mixtures were added to 10 min followed by

RESULTS AND DISCUSSION

Fig. 1 shows the changes in fluorescence of the substituted aminocarboxylic acids, ACMA, that occur upon the addition of MgATP and several other agents to purified ATPase-bearing liposomes prepared as described under “Experimental Procedures.” While the precise mechanism by which aminocarboxylic acid fluorescence changes are induced is the subject of some controversy (29–31), it is generally agreed that these molecules are useful qualitative indicators of an interior acid pH gradient (ΔpH) in vesicular systems (29, 31). Fig. 1 trace A shows that the addition of MgATP to the proteoliposomes causes an instantaneous decrease in ACMA fluorescence, which is probably an artifact, followed by a marked, time-dependent additional quenching of much of the remaining fluorescence. Upon the addition of the ATPase inhibitor vanadate, the time-dependent quenching is totally reversed. These results indicate that at least some of the reconstituted H+-ATPase molecules are capable of catalyzing ATP-dependent proton translocation into the interior of the proteoliposomes, thus generating an inside acid ΔpH. This conclusion is corroborated in trace B which shows that nigericin, which dissipates pH gradients via an electroneutral H+·K+ exchange (K+ is present in the buffer), also reverses the fluorescence quenching, and in trace C, which shows that the quenching is also reversed by the protonophore, CCCP. The CCCP response is complicated by the fact that CCCP itself partially quenches the ACMA fluorescence (data not shown). However, reversal is complete as evidenced by the fact that nigericin does not bring about further reversal of the fluorescence quenching. Trace D shows an experiment similar to the others, but with valinomycin added after MgATP instead of before it as in traces A–C, and E. This trace demonstrates that the great majority of the ATP hydrolysis-dependent fluorescence quenching response is not seen in the absence of valinomycin. Trace E is included to show that dimethyl sulfoxide, the ionophore vehicle used in these experiments, does not affect

There are at least two feasible explanations for the valinomycin requirement. First, because proton translocation catalyzed by the ATPase is electrogenic (7), valinomycin may be required to prevent membrane potential (Δψ) generation by catalyzing countermovement of K+· thus allowing electrically unimpeded proton translocation and maximum ΔpH generation, which is reflected as maximum ACMA fluorescence quenching. Such an explanation was suggested by Dufour et al. (22) on the basis of similar results obtained with the reconstituted yeast plasma membrane ATPase. While this explanation may be correct, it should be kept in mind that it assumes, without evidence, that Δψ predominates the transmembrane electrochemical protontic potential difference (Δp) in these proteoliposomes and is totally reversed. This assumption is not valid, as would be the case if the proteoliposomes are unexpectedly permeable to anions or cations or are extremely small, then the valinomycin requirement cannot be explained in this way. An alternative or additional explanation, which does not require assumptions as to the values of Δψ and Δp, centers upon the probe response. Because electroactive binding of the ACMA molecules to the surface of the proteoliposomes appears to be necessary for the fluorescence quenching response (30), it may be that valinomycin is needed to dissipate an inside positive membrane potential (of unspecified magnitude relative to ΔpH) that would otherwise induce binding of the cationic ACMA molecules. Clearly, more information, beyond that shown in these experiments, will need to be obtained before firm conclusions as to the role of the valinomycin effect can be drawn. Fortunately, for the purposes of the present argument, such information is unnecessary.
**Neurospora Plasma Membrane H+*-ATPase**

**Table 1**

*Effects of ionophores on ATP hydrolysis catalyzed by the reconstituted H+*-ATPase*

See "Experimental Procedures" for details of the ATPase assay. Entries are the averages of duplicate determinations. Where indicated, the following additions were included in the assay mixtures: dimethyl sulfoxide, 1 µl of dimethyl sulfoxide; valinomycin, 0.5 µl of 0.5 mg/ml of valinomycin in dimethyl sulfoxide; CCCP, 1 µl of 50 mM CCCP in dimethyl sulfoxide; nigericin, 1 µl of 5 mg/ml of nigericin in dimethyl sulfoxide.

| Addition                           | Specific ATPase activity | µmol of P, released/min/mg protein |
|------------------------------------|--------------------------|-----------------------------------|
| None                               | 10.8                     |                                    |
| Dimethyl sulfoxide                 | 10.7                     |                                    |
| Valinomycin                        | 15.2                     |                                    |
| CCCP                               | 27.0                     |                                    |
| Nigericin                          | 30.3                     |                                    |
| Valinomycin plus CCCP              | 30.8                     |                                    |
| Valinomycin plus nigericin         | 32.0                     |                                    |
| CCCP plus nigericin                | 24.4                     |                                    |

µmol of P, released per min/mg of protein, and this value is augmented to a small extent in the presence of valinomycin. The specific ATP hydrolytic activity is increased markedly to 27 in the presence of CCCP and even more markedly to 30.3 in the presence of nigericin. The effects of valinomycin plus CCCP or valinomycin plus nigericin are only roughly additive, and the combination of nigericin plus CCCP does no more than either ionophore alone. Although not shown, the ATPase can be activated to levels similar to those shown in Table 1 by substituting small amounts of Folch fraction I lipids from bovine brain for the asolectin liposomes in an otherwise similar assay. ATPase activated in this manner is not stimulated by CCCP or nigericin, which means that neither of these ionophores per se affect the ATPase activity. Our interpretation of these results is as follows. The properly reconstituted ATPase molecules catalyze ATP hydrolysis-driven proton translocation into the liposomes, which leads to the generation of ΔΨ and ΔpH of unspecified relative magnitudes. The ΔΨ thus generated inhibits further proton translocation and hence ATP hydrolysis. The addition of valinomycin elicits K+ efflux from the liposomes, which dissipates ΔΨ and allows additional proton translocation until a ΔpH roughly equivalent to the dissipated ΔΨ is generated. This results in a transient small increase in ATP hydrolysis. The low intraliposomal pH generated in the presence of valinomycin could also increase the proton permeability of the liposomes, which would also stimulate ATP hydrolysis. In any case, the effects of valinomycin are small. The addition of CCCP causes dissipation of most of the ΔΨ, which allows proton translocation and ATP hydrolysis to proceed at a much higher rate. And finally, the addition of nigericin elicits an H*/K+ exchange that results in the dissipation of ΔpH, which again allows proton translocation and ATP hydrolysis to proceed much faster.

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6 Presumably the ATPase activated by Folch fraction I lipids does not translocate protons into a sealed space, either because no ATPase-bearing proteoliposomes are formed with these lipids or because the proteoliposomes formed are leaky to protons.

7 The marked stimulation by nigericin alone, and the lack of a large additional effect of valinomycin when added with nigericin may suggest that the ΔpH component predominates the protonic potential difference, for the reasons mentioned in Footnote 5. Alternatively, regardless of the relative magnitudes of ΔΨ and ΔpH, these results would also be expected if ΔΨ and ΔpH are not equipotent with respect to their effectiveness in reversing the ATPase reaction, with ΔpH being more effective than ΔΨ. That is to say, because intraliposomal protons are a product of the ATPase reaction, it stands to reason...
that they are capable of proton translocation. This is probably reconstituted preparation are inhibited by AP, which indicates specific ATPase activity in the control samples is thus probable. This means that at least two-thirds of the total population of hydrolytically active ATPase molecules present in the reconstituted proteoliposome preparation are capable of generating a ΔpH of a magnitude sufficient to cause inhibition of ATP hydrolysis, the data presented in Table I provide an estimate of the fraction of the total population of hydrolytically active ATPase molecules that are involved in such activity. If the specific ATPase activity measured in the presence of valinomycin and nigericin is taken as 100%, then the maximally inhibited (control) specific activity is 33-34%. This means that at least two-thirds of the hydrolytically active ATPase molecules in the reconstituted preparation are inhibited by ΔpH, which indicates that they are capable of proton translocation. This is probably a minimum estimate because, as mentioned above, the reconstituted proteoliposomes are somewhat leaky to protons. The specific ATPase activity in the control samples is thus probably higher than it would be in less proton-permeable liposomes.

The data presented thus far provide a measure of the fraction of catalytically active ATPase molecules in the reconstituted proteoliposomes that are capable of proton translocation, but do not contribute any information as to the percentage of the total population of ATPase molecules that are catalytically active. Fig. 2 presents information bearing upon this point. We have previously reported evidence that the H⁺-ATPase undergoes several conformational changes during its catalytic cycle (13). This conclusion was based upon the characteristics of protection of the ATPase against tryptic degradation by a variety of ATPase ligands. Of the several ligands employed in those experiments, the most effective proved to be the ATPase inhibitor vanadate in the presence of Mg²⁺, and vanadate protection was further enhanced in the presence of Mg²⁺. In the absence of any ligand, the hydrolytic moiety of the ATPase (M₆ ~ 105,000) is rapidly degraded to small fragments, but in the presence of Mg²⁺, vanadate, and ATP, the M₆ ~ 105,000 ATPase is rapidly degraded to an enzymatically active M₆ ~ 95,000 form, but further degradation (via an M₆ ~ 88,000 form) occurs only slowly. As elaborated upon in that report, the evidence strongly suggested that vanadate, which is most likely a transition state analogue of the enzyme dephosphorylation reaction (36, 37), binds to the ATPase and "locks" it in the conformation that it normally assumes in the transition state that occurs during the enzyme dephosphorylation reaction. Fig. 2 shows that similar results are obtained with the purified, reconstituted H⁺-ATPase. In the absence of any ligand, tryptic degradation is restricted to the generation of a M₆ ~ 95,000 form and several slightly smaller forms (Fig. 2A). Fig. 2B shows densitometer tracings of the ATPase areas of the three lanes shown in Fig. 2A. The scans were performed on a track roughly ⅜ of the distance from the left-hand to the right-hand edge of each lane. Computer-assisted integration of these tracings, and correction for the fact that the control well is approximately 12% wider than the others, indicates that 91% of the ATPase is degraded by trypsin in the absence of ligands, and virtually 100% remains in the high molecular weight region when the trypsin treatment is carried out in the presence of Mg²⁺, vanadate, and ATP. If the densities are corrected for mass loss in the clipped forms, the sum is greater than 100%, which strengthens the conclusion that the ATPase is completely protected except for minor nicking. After subtraction of 9% of the total, which represents unreacted ATPase, these numbers indicate that at least 91% of all of the ATPase molecules present in the reconstituted ATPase preparation in a conformation that presumably represents the conformation that the enzyme assumes in the transition state of the enzyme dephosphorylation reaction. Because the ability of any enzyme to firmly bind to the transition state configuration(s) of the reaction(s) that it catalyzes is the essence of enzymic catalysis (38-41), we conclude that at least 91% of the ATPase molecules in the reconstituted proteoliposomes are catalytically active. To the extent that this is true, and to the extent that the above conclusion that a minimum of two-thirds of the catalytically active ATPase molecules translocate protons is true, the data presented thus far demonstrate that the efficiency of the ATPase reconstitution procedure is 61% or greater.

The foregoing data and conclusions set specific limits on the quantity of any polypeptide other than the M₆ ~ 105,000

A vanadate, a potential ATPase ligand, was present during the trypsin treatment of sample 3 (see "Experimental Procedures") but, as was shown previously (13), this compound does not protect well against tryptic degradation of the ATPase in the absence of Mg²⁺. This presumably means that vanadate binds to the ATPase only weakly or not at all in the absence of Mg²⁺.
hydrolytic moiety that must be present in the ATPase preparation in order to qualify as a subunit. With 61% reconstitution efficiency, for every mole of the hydrolytic moiety present in the ATPase preparation used for the reconstitution, there must be 0.61 mol of any subunit, assuming a subunit stoichiometry of 1:1. For a 10,000 dalton subunit, this amounts to a mass ratio (subunit/hydrolytic moiety) of about 0.058. By the same argument, any subunits of higher molecular weight require a higher mass ratio. Fig. 3 shows the composition of the H+-ATPase preparation used for all of the reconstitution experiments described in this communication, as indicated by SDS-PAGE. The ATPase preparation was made from cells grown in the presence of radioactive carbon sources so that any 

Neurospora proteins present in the ATPase preparation could be detected, regardless of whether or not they could be stained by protein stains. About 2,515 cpm are present in the amount of 10,000 dalton hydrolytic moiety applied to the gel, and, thus, at a mass ratio of 0.058, 146 cpm of an essential 10,000 dalton subunit would need be present. More than this amount would need be present for higher molecular weight subunits. Clearly, there are no components present in anywhere near sufficient amounts in the 10,000-dalton and higher molecular weight range. The argument holds down to about 2,500 daltons where about 37 cpm would be needed, but are not present. Below this value, the situation becomes less clear because very few counts/min are required for an essential subunit, and more than an adequate amount of radioactive material is present in the relatively broad band that migrates near the tracking dye. Because a phospholipid standard (phosphatidylcholine) also migrates as a similarly broad band in about the same position, it is quite possible that this radioactive material is lipid, in which case, the hydrolytic moiety is the only polypeptide component of the H+-ATPase. However, the involvement of a very small oligopeptide subunit that migrates in the phospholipid region cannot be excluded by the present analysis, and thus, further investigation will be required before it can be stated with certainty whether or not the hydrolytic moiety alone is capable of efficient proton translocation. In any case, two reasonably firm conclusions can be drawn from the data presented in this communication. First, it is clear that no conventional protein or proteolipid subunits of the type that have been reported to be associated with certain other ATPases in the aspartylphosphoryl-enzyme intermediate family of transport ATPases and no peptides similar to those that constitute the F_1 or F_0 sectors of the F_0F_1 ATPase/ATP synthases are involved in the mechanism of proton translocation catalyzed by the Neurospora plasma membrane H+-ATPase. And second, if this enzyme contains any subunits at all other than the hydrolytic moiety, they must be very small.

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REFERENCES

1. Fillingame, R. H. (1980) Annu. Rev. Biochem. 49, 1079-1113
2. Cantley, L. C. (1981) Curr. Top. Bioenerg. 11, 201-237
3. Tada, M., Yamamoto, T., and Tonomura, Y. (1978) Physiol. Rev. 58, 1-79
4. Sachs, G. (1977) Fed. Proc. Physiol. Sci. Pharmacol. 36, 133-162
5. Goffeu, A., and Stayman, C. W. (1981) Biochim. Biophys. Acta 639, 197-209
6. Scarborough, A. G. (1982) in Plasmalemma and Tonoplast: Their Functions in the Plant Cell (Marne, D., Marre, E., and Hertel, R., eds) pp. 431-436, Elsevier Biomedical Press, Amsterdam
7. Scarborough, A. G. (1982) Biochemistry 21, 2931-2937
8. Racker, E., and Eytan, E. (1975) J. Biol. Chem. 250, 7533-7534
9. Knowles, A., Zimmink, P., Alfonso, M., Zimmink, A., and Racker, E. (1980) J. Membr. Biol. 65, 235-239
10. Forbush, B. J., Kaplan, J. H., and Hoffman, J. F. (1978) Biochemistry 17, 3657-3676
11. Dame, J. B., and Scarborough, A. G. (1961) J. Biol. Chem. 236, 1042-1046
12. Scarbrough, A. G. (1982) Annu. N. Y. Acad. Sci. 402, 99-115
13. Racker, E., and Eytan, E. (1975) J. Biol. Chem. 250, 7535-7534
14. Cappel, R. J., Goffeu, A., and Tsong, T. Y. (1982) J. Biol. Chem. 257, 5055-5071
15. Oakley, B. J., Kirsch, D. R., and Morris, N. R. (1980) Biochem. 105, 361-363
16. Metz, P., and Kadenbach, B. (1980) Eur. J. Biochem. 105, 499-507
17. Zaimin, M., and Harikharasubramanian, V. (1970) Biochem. 35, 296-297
18. Patterson, M. S., and Greene, R. C. (1965) Biochem. 37, 854-857
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-276
20. Mensadoun, A., and Weinstein, D. (1976) Biochem. 70, 241-250
21. Schuldiner, S., Rottenberg, H., and Avron, M. (1972) Eur. J. Biochem. 25, 94-70
22. Krasnyhov, R. (1977) in Structure and Function of Energy-Transducing Membranes (van Dam, K., and van Gelder, B. F., eds) pp. 235-256, Elsevier/North-Holland Biomedical Press, Amsterdam
23. Elema, R. P., Michels, P. A. M., and Konings, W. N. (1978) Eur. J. Biochem. 95, 381-387
24. Mitchell, P. (1969) Therm. Eng. Biochem. 25, 159-216
25. Maloney, P. C. (1982) J. Membr. Biol. 67, 1-13
26. Khan, S., and Berg, H. C. (1983) J. Biol. Chem. 258, 6709-6712
27. Villalobos, A. (1983) J. Biol. Chem. 258, 1824-1826
28. Mason, J. G. (1966) Trad. Biochem. Sci. 5, 92-94
29. Cantley, L. C., Jr., Cantley, L. G., and Josephson, L. (1978) J. Biol. Chem. 253, 7361-7368
30. Pauling, L. (1986) Chem. Eng. News 24, 1375-1377
31. Jencks, W. P. (1965) in Current Aspects of Biochemical Energies (Kaplan, N. G., and Kennedy, E. P., eds) pp. 273-298, Academic Press, New York
32. Wollenden, R. (1969) Nature (Lond.) 223, 704-706
33. Lienhard, G. E. (1973) Science (Wash. D. C.) 180, 149-154