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Rat Brain Has the $\alpha 3$ Form of the (Na^+,K^+)ATPase†

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ABSTRACT: Multiple forms of the catalytic subunit of the (Na^+,K^+)ATPase have been identified in rat brain. While two of them ($\alpha 1$ and $\alpha 2$) have been well characterized, the third form ($\alpha 3$) of these catalytic subunits only recently has been described by cDNA cloning; the corresponding polypeptide has not been isolated. In this paper it is shown that rat brain contains the $\alpha 3$ chain. The catalytic subunits of the (Na^+,K^+)ATPase from rat brain axolemma were purified by SDS-PAGE and subjected to formic acid cleavage. Amino acid sequence analysis of the resulting fragments revealed that axolemma has the $\alpha 3$ form of the catalytic subunit. In addition, $\alpha 3$-specific antiserum was raised in rabbits immunized with a synthetic peptide. Immunoblotting with this antiserum revealed that the $\alpha 3$ form of the (Na^+,K^+)ATPase is present also in whole brain microsomes. In SDS-PAGE, the mobilities of the three catalytic subunits of brain (Na^+,K^+)ATPase follow the order $\alpha 1 > \alpha 2 > \alpha 3$. Determination of the ouabain-inhibitable ATPase activity indicates that if the $\alpha 3$ form of the (Na^+,K^+)ATPase is able to hydrolyze ATP, it is present in a form of the enzyme with a high affinity for this cardiac glycoside and is similar to the $\alpha 2$ form in this respect.

(Na^+,K^+)ATPase [sodium and potassium ion activated ATP phosphohydrolase (EC 3.6.1.3)] is the enzyme in the plasma membrane of animal cells that establishes the sodium and potassium ion gradients across the plasma membrane at the expense of ATP. The enzyme is composed of two subunits, a large catalytic subunit of M_r 112000 (M_r 100000 by SDS-PAGE) and a smaller glycoprotein subunit of M_r 50000 of unknown function in a 1:1 stoichiometry (Cantley, 1986; Jorgensen, 1982). Biochemical studies revealed that there are two forms of the large subunit, which are called $\alpha 1$ and $\alpha 2$ chains (Sweadner, 1979; Schellenberg et al., 1981; Matsuda et al., 1984; Lytton et al., 1985). [In this paper, the α, $\alpha+$,

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and αIII chains are called α1, α2, and α3 chains, respectively, according to the suggestion of Orlowski and Lingrel (1988).] The α1 chain is present in kidney cells and is the one best studied. The α2 chain, first identified in brain axolemma, travels as a slightly larger polypeptide than the α1 chain on SDS gel electrophoresis (Sweadner, 1979). In addition, the α2 chain can be distinguished from the α1 chain by its higher affinity for the cardiac glycoside ouabain (Sweadner, 1979), greater resistance to trypsin digestion (Sweadner, 1979), and greater sensitivity to the antimetabolite pyrithiamin (Matsuda et al., 1984), as well as a greater number of NEM-accessible sulfhydryl groups (Sweadner, 1979). Results from this laboratory have shown that adipocytes and skeletal muscle cells contain both the α1 and α2 forms of the (Na^+,K^+)ATPase, while liver cells contain only the α1 form, and that the α2 form of the enzyme is required for activation of the pump by insulin (Lytton et al., 1985; Lytton, 1985b).

It has been reported recently that rat brain contains three different transcripts encoding the catalytic subunits of the (Na^+,K^+)ATPase (Shull et al., 1986). Amino acid sequences derived from two of these cDNA sequences match those of the α1 and α2 chains (Lytton, 1985a). The protein corresponding to the third type of transcript, which was designated α3 (Shull et al., 1986), has not been identified.

In this paper we show that the α3 chain is present in rat brain and that, if the α3 chain is present in a form of the enzyme that hydrolyzes ATP, this enzyme has a high affinity for ouabain, in contrast to the form of the enzyme with the α1 chain.

Experimental Procedures

Materials. Male CD rats (100–125 g) were obtained from Charles River Laboratories. Reagents for Laemmli SDS–PAGE were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). Prestained molecular weight markers were purchased from Bethesda Research Laboratory (Gaithersburg, MD). Nonfat dry milk powder was from the local Star Market. Horseradish peroxidase conjugated goat anti-rabbit antiserum and color developing reagent (4-chloro-1-naphthol) were obtained from Bio-Rad (Rockville, NY). Na_2ATP, NaATP, ATP, Tris base, NADH, phosphoenolpyruvate, dithiothreitol, lactate dehydrogenase, pyruvate kinase, and ouabain were from Sigma Chemical Co. (St. Louis, MO). Rabbit antiserum 620 was made against the α subunit of the rat kidney (Na^+,K^+)ATPase and was kindly provided by Jonathan Lytton (Harvard University). Antiserum 38 was raised against an α3-specific synthetic peptide [residues 481–497, SIHETED-PNDNRYLLVM(C)], which was obtained from Biosearch (San Rafael, CA). The peptide was coupled to keyhole limpet hemocyanin (Green et al., 1982). All other chemicals used were of analytical grade or better.

Membrane Preparation. Brain stems were dissected from the whole brain and used for axolemma preparation according to the sucrose density gradient centrifugation method (DeVries et al., 1978). The remaining parts of the brain were then used to prepare whole brain microsomes (Gill et al., 1981). Kidney microsomes prepared as previously described (Jorgensen, 1974) were provided by Michael Ho (Harvard University). Muscle plasma membranes were prepared from adult rat hindlimb (Wardzala & Jeanrenaud, 1981).

Electrophoretic and Immunological Blotting Procedures. SDS–polyacrylamide gels were prepared according to the method of Laemmli (1970). Samples were treated with Laemmli buffer (2% SDS, 10% glycerol, 1% 2-mercaptoethanol, 65 mM Tris-HCl, pH 6.8), incubated at room temperature for 15 min, and loaded onto a gel without prior boiling. For the blotting studies, proteins were then electrotransferred onto nitrocellulose membranes (Towbin et al., 1979). The nitrocellulose membrane then either was stained with Amido Black and destained in 50% methanol and 10% acetic acid to visualize the proteins or was incubated with 1% nonfat milk in 10 mM Tris-HCl and 150 mM NaCl, pH 7.4 (TBS). The nitrocellulose membrane was subsequently incubated with antiserum 620 (1:1500 dilution) or antiserum 38 (1:350 dilution) in 1% milk–TBS overnight at 4 °C. After removal of the unbound antibody, the nitrocellulose membrane was incubated with goat anti-rabbit antibody conjugated with horseradish peroxidase (1:2000 dilution) in 1% milk–TBS for 2 h at room temperature. The membrane was washed with 0.1% Tween 20 in TBS and developed in 0.5 mg/mL 4-chloro-1-naphthol in TBS containing 0.02% hydrogen peroxide.

Formic Acid Hydrolysis and Amino Acid Sequence Analysis. Brain axolemma was treated in Laemmli buffer, separated by SDS–PAGE, and stained briefly with Coomassie Brilliant Blue R-250. The region corresponding to M_r 100000 was then cut out of the gel, and the polypeptides were electroeluted (Hunkapiller et al., 1983). The eluted protein was dialyzed against 1% NH_4HCO_3, lyophilized, and treated with formic acid (Landon, 1977). The protein, at a concentration of 0.1 mg/mL, was dissolved in 88% formic acid and incubated at 37 °C for 20 h. The preparation was diluted with 10 volumes of distilled water and lyophilized. This protein mixture was then separated by 10% SDS–polyacrylamide gel electrophoresis and briefly stained with Coomassie Brilliant Blue R-250. The formic acid cleaved fragments were dissected from the gel, electroeluted as described above, dialyzed, and lyophilized. Amino-terminal analysis was carried out on approximately 0.05–0.1 nmol of purified polypeptide with an Applied Biosystem 470A instrument by Nike Brown in Dr. Ellis Reinherz's laboratory at Dana Farber Cancer Institute, Boston, MA.

Ouabain-Inhibitable ATPase Assay. The ATPase activity of the enzyme was measured with the coupled assay (Josephson & Cantley, 1977) in the presence of various concentrations of ouabain. The membrane preparations were incubated with ouabain in reaction buffer for 30 min at 37 °C before ATP was added to initiate the enzymic reaction. ATPase activity was monitored at 340 nm on the Beckman DU50 spectrophotometer with the Kinetics Soft-Pac. The data were then subjected to a nonlinear least-squares analysis by using the RS/1 program of BBN Research System (Cambridge, MA) on an AT&T PC 6300+ computer.

Results

Formic Acid Cleavage of the Large Subunits of the (Na^+,K^+)ATPase. Several investigators (Sweadner, 1979; Schellenberg et al., 1981; Matsuda et al., 1984) have described the presence of two large polypeptides of the (Na^+,K^+)ATPase in brain tissue. These have been identified as the α1 and α2 chains on the basis of their affinity for ouabain (Sweadner, 1979) and of their partial amino acid sequence (Lytton, 1985a). The cloning of the cDNA for the large subunits (Shull et al., 1986; Young & Lingrel 1987) can be interpreted, however, to suggest that the α3 subunit should also be present in rat brain tissue. The question is why the α3 subunit has not been identified so far. One possibility is that the mRNA transcript for α3 is not translated. Another possibility is that the properties of the α3 chain resemble either those of the α1 chain or those of the α2 chain and thus could not be distinguished from them.

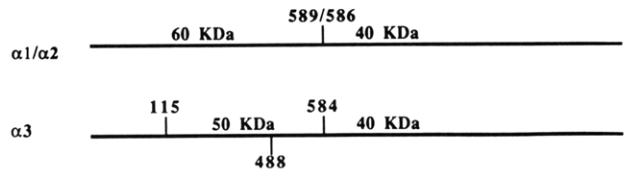

FIGURE 1: Schematic diagram of formic acid cleavage sites in the α subunits. Residue numbers above the polypeptide chains indicate the cleavage sites, while the number below indicates the potential cleavage site.

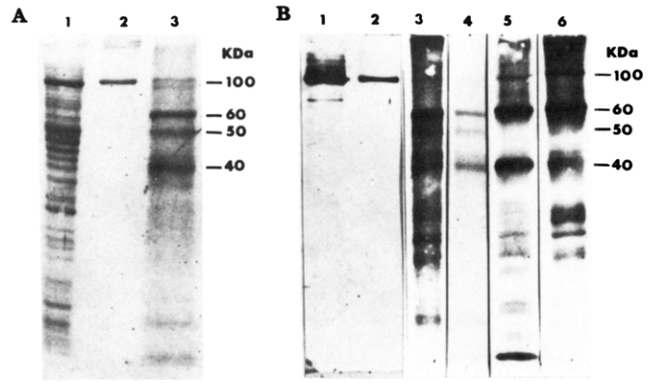

FIGURE 2: SDS–PAGE and immunoblotting of the purified catalytic subunit and of microsomes with and without formic acid treatment. A total of 12–15 μg of axolemmal microsomes, 10 μg of formic acid treated axolemmal, skeletal muscle, and kidney microsomes, and 1–2 μg of purified catalytic subunit (with and without formic acid treatment) were separated by using10% Laemmli gels and transferred to nitrocellulose as described under Experimental Procedures. (A) Amido Black stained protein pattern of axolemmal membrane (lane 1) and purified subunits before (lane 2) and after (lane 3) formic acid treatment. (B) Immunoblotting profile using antiserum 620. Crude axolemmal membrane (lanes 1 and 3), purified catalytic subunit (lanes 2 and 4), skeletal muscle microsomes (lane 5), and kidney microsomes (lane 6), without (lanes 1 and 2) and with (lanes 3–6) formic acid treatment.

In the course of work on the structure of the large subunit of the (Na^+,K^+)ATPase, we found that formic acid treatment (Landon, 1977) of the α1 chain produces two fragments, one of 60 kDa and the other of 40 kDa, as a result of cleavage of a single Asp–Pro bond between residues 588 and 589. Inspection of the amino acid sequences of the α2 and α3 chains (Shull et al., 1986) (Figure 1) revealed that the α2 chain also has a single Asp–Pro bond, residues 585–586. The α3 chain, however, has three Asp–Pro bonds, between residues 114 and 115, 487 and 488, and 583 and 584. Treatment with formic acid in this case should produce the usual 40-kDa fragment, and it should also cleave the 60-kDa fragment into smaller pieces. The results of formic acid treatment of the purified large subunit from axolemma, of crude axolemmal microsomes, of skeletal muscle microsomes, and of kidney microsomes are shown in Figure 2. In panel A are the results of SDS–PAGE stained with Amido Black. Lane A3 shows that formic acid cleavage of the isolated large subunit of rat brain axolemma (lane A2) produces three fragments of M_r 60 000, 50 000, and 40 000. There is almost complete loss of the original polypeptide with M_r 100 000. In panel B are shown the results of SDS–PAGE examined by immunoblotting with an antibody raised against the rat kidney α1 chain. Lanes B1 and B2 show the patterns obtained with crude axolemmal membranes and with the purified large subunit before formic acid treatment, while lanes B3 and B4 show the appearance of the three fragments of 60, 50, and 40 kDa after formic acid treatment. Lanes B5 and B6 show the patterns obtained with skeletal muscle and kidney microsomes, respectively, after formic acid treatment. While the 60- and 40-kDa fragments are present, the 50-kDa fragment is conspicuously absent. Since the kidney (Na^+,K^+)ATPase has only the α1 chain while skeletal muscle has both α1 and α2 chains, but neither microsome gives rise to the 50-kDa fragment upon formic acid treatment, we conclude that the 50-kDa fragment must arise from a large polypeptide of the (Na^+,K^+)ATPase that is different from α1 and α2, and is presumably α3.

Table I: Amino Acid Sequence Analysis of Formic Acid Cleaved α Subunits Purified from Rat Axolemma

sequence cycle no.	50 kDa		40 kDa	
	amino acid	pmol	amino acid	pmol
1				
2				
3	Gly	9.62	Arg	6.30
4	Asp	6.90	Ala	18.06
5	Asn	7.30	Ala	20.28
6	Leu	7.50	Val	9.24
7	Tyr	11.07	Pro	7.99
8	Leu	13.75	Asp	5.09
9	Gly	2.50	Ala	12.92
10	Ile	8.65	Val	6.96
11	Val	7.06	Gly	3.33
12	Leu	6.11	Lys	11.92
13	Ala	9.33		
14	Ala	10.67	Arg	4.38
15	Val	9.83	Ser	2.50
16	Val	11.67	Ala	2.29
17	Ile	13.17	Gly	2.22
18	Ile	14.50	Ile	6.05

Amino Acid Sequences of the Formic Acid Generated Fragments. To determine whether or not the 50-kDa fragment is part of the α3 polypeptide, it was eluted from the acrylamide gel and subjected to amino acid sequence analysis. The results of this analysis are shown in Table I, which reports the picomoles of amino acid at each cycle of the Edman degradation. The first two cycles of the degradation were noisy and could not be interpreted unambiguously. The amino acid sequence GDNLYLGIVLAAVVII corresponds exactly and uniquely with the sequence of the α3 chain between residues 117 and 132 (Shull et al., 1986). This sequence is not present in the α1 and α2 polypeptides.

The 40-kDa fragment was also eluted from the gel and subjected to amino acid sequence analysis. The amino acid sequence RAAVPDAVGKXRSAGI is present in all three catalytic polypeptides, residues 591–606 for α1, residues 588–603 for α2, and residues 586–601 for α3. This is the predicted fragment resulting from the cleavage of the Asp–Pro bond present in all three catalytic chains between residues 583 and 589.

These results indicate that rat brain axolemma has the α3 subunit.

Recognition of the α3 Polypeptide by Immunoblotting. In order to demonstrate in another way the presence of the α3 chain in rat brain tissue, antibodies to the peptide SIHETEDPNDNRYLLVM(C), which corresponds to residues 481–497 of the α3 chain,[1] were raised in rabbits by coupling to keyhole limpet hemocyanin. This is antiserum 38. In addition, we used antiserum 620 obtained by immunizing a rabbit with the α1 chain of rat kidney. Antiserum 620 contains antibodies that recognize the α1 chain very well and the α2 chain less well. The results of immunoblots with these antisera on microsomes from several tissues fractionated by SDS–PAGE are shown in Figure 3. In lanes 1 and 2, antiserum

[1] The amino acid sequences of the corresponding regions of the α1 and α2 chains are SIHKNPNASEPKHLLVM and SIHEREDSPQSHVLVM, respectively. The net charge of these peptides could be 3, 0, and −2 for the α1 and α2, and α3 chains, respectively.

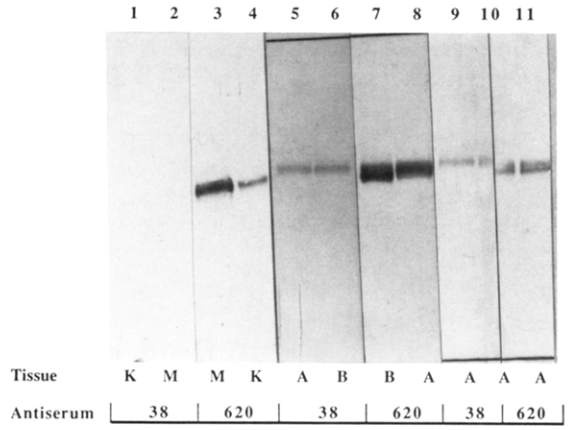

FIGURE 3: Immunoblotting analysis of the catalytic subunits in axolemmal, total brain, skeletal muscle, and kidney microsomes. A total of 0.2 μg of kidney microsomes (lanes 1 and 4), 15 μg of skeletal muscle microsomes (lanes 2 and 3), 2 μg (lanes 5 and 8) and 0.5 μg (lanes 9–11) of axolemmal microsomes, and 2 μg of brain microsomes (lanes 6 and 7) were separated by 5.5% Laemmli gels. After transfer of the separated polypeptides onto nitrocellulose, a line was drawn near the edge of the nitrocellulose. The nitrocellulose sheet was then cut into halves. Each half was subsequently incubated with antiserum 620 (lanes 3, 4, 7, 8, and 11 and the second half of lane 10) or antiserum 38 (lanes 1, 2, 5, 6, and 9 and the first half of lane 10). The drawn line provides a reference to accurately line up these two blots after development. Tissue abbreviations: K, kidney; M, skeletal muscle; B, total brain; A, axolemma.

38 is used against kidney and skeletal muscle microsomes; in lanes 3 and 4, antiserum 620 is used against skeletal muscle and kidney microsomes, respectively. With the latter antiserum, there is one band (α1) in the kidney microsomes (lane 4) and two bands (α1 and α2) in the skeletal muscle microsomes (lane 3). The α2 band is less intense than the α1 band, because of its reactivity with antiserum 620. However, neither microsome has a band that reacts with antiserum 38 (lanes 1 and 2). We conclude that antiserum 38 does not react with either the α1 or the α2 chain.

Lanes 5 and 6 show the results obtained with antiserum 38 used with axolemma and brain microsomes, respectively; lanes 7 and 8 show the effect of antiserum 620 on brain and axolemma microsomes, respectively. With the antiserum 620, there are two distinct bands in brain microsomes (lane 7), which are the α2 and α1 chains (Sweadner, 1979). Axolemma, however, has one principal band, α2, and a small amount of the α1 chain (lane 8). With antiserum 38, there is a distinct band of similar intensity, in both axolemma (lane 5) and brain microsomes (lane 6). The mobility of this band, relative to those of the bands stained with antiserum 620, is slightly slower than that of the α2 band, which is slower than the α1 band. This point is made more obvious in lanes 9–11, all of which contain samples of axolemma. Lane 9 and half of lane 10 are treated with antiserum 38, while the other half of lane 10 and lane 11 are treated with antiserum 620. Here the smaller mobility of the band staining with antiserum 38, relative to those staining with antiserum 620, is apparent.

We conclude that brain microsomes and axolemma both contain the α3 subunit. We also conclude that in the electrophoresis system used here the mobility of the catalytic chains is α1 > α2 > α3.

Ouabain Affinity of the α3 Chain of the (Na^+,K^+)*ATPase.* Examination of the pattern shown in Figure 2, lane A3, allows for an estimate of the relative amount of the α3 chain in axolemmal membranes. The estimate is based on the view that the band at 50 kDa is derived from the α3 chain, while the band at 40 kDa is contributed by all forms of the catalytic subunit (α1, α2, and α3). The relative intensity of the stain at the 50-kDa position is approximately 25% of the intensity of the stain at the 40-kDa position, as determined by densitometry. On the basis of this estimate, the following predictions can be made about the effect of ouabain on the activity of the (Na^+,K^+)ATPase of axolemmal membranes. If the enzyme with the α3 chain has properties similar to those of the enzyme with the α1 chain, at least 25% of the (Na^+,K^+)ATPase activity should have a low ouabain affinity. On the other hand, if the α3 enzyme behaves as the α2 enzyme does, axolemma should contain (Na^+,K^+)ATPase activity with only high affinity for ouabain. The third possibility is that the α3 chain is not present in an active enzyme and does not hydrolyze ATP. In this case, it would not be measured at all. The fourth possibility is that the form of the (Na^+,K^+)ATPase with the α3 chain dose not bind ouabain but hydrolyzes ATP. This possibility is unlikely because only 6% of the total ATPase activity of the axolemmal membrane is ouabain independent [total activity, 3.3 μmol min^{-1} (mg of protein)$^{-1}$; 2 mM ouabain-inhibitable activity, 3.1 μmol min^{-1} (mg of protein)$^{-1}$; 2 mM ouabain-independent activity, 0.2 μmol min^{-1} (mg of protein)$^{-1}$].

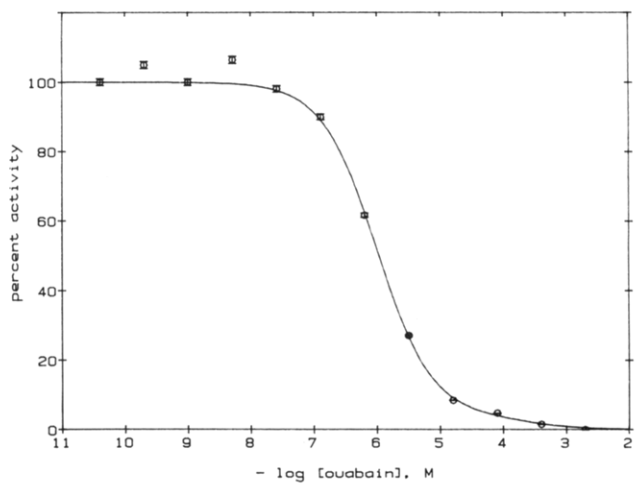

FIGURE 4: Ouabain inhibition of the rat axolemmal (Na^+,K^+)ATPase. The maximal activity is 3.1 ± 0.2 μmol min^{-1} (mg of protein)$^{-1}$; it corresponds to (Na^+,K^+)ATPase activity in the absence of ouabain minus the activity in the presence of 2 mM ouabain. Each data point represents the average of quadruplicate experiments. The curve is the best fit of these data points to a two-component inhibition model determined by nonlinear least-squares analysis. Low-affinity component, V_{max} = 3.78 ± 3.5%, K_m = 2.48 × 10^{-4} ± 1.0 × 10^{-4} M ouabain; high-affinity component, V_{max} = 96.3 ± 3.5%, K_m = 1.0 × 10^{-6} ± 1.4 × 10^{-7} M ouabain. Regression coefficient r^2 = 0.999.

When the (Na^+,K^+)ATPase activity of the axolemma used in this study was measured, the results shown in Figure 4 were obtained. More than 96% of the activity behaves as a component with a high affinity for ouabain (1.0 × 10^{-6} M). The small amount of the low-affinity component (<4%) is due to the α1 chain, which can be seen as a minor band in Figure 3, lane 8. This result is similar to that obtained by Sweadner (1979), who found one component of (Na^+,K^+)ATPase activity in rat axolemma with a high affinity for ouabain ($K_{0.5}$ = 2 × 10^{-7} M). The conclusion is that, if the form of the (Na^+,K^+)ATPase with the α3 chain can hydrolyze ATP, this enzyme has a high affinity for ouabain.

Discussion

The data presented here indicate that rat brain contains the α3 form of (Na^+,K^+)ATPase. Although the presence of two forms of the catalytic subunit of the (Na^+,K^+)ATPase, α1 and α2, in rat brain was described 10 years ago (Sweadner, 1979), the existence of a third transcript for the α3 form was only

demonstrated in 1986 (Shull et al., 1986), and the protein corresponding to this transcript has not been identified so far.

The first inkling that the α3 polypeptide may be present in axolemma was the observation that the formic acid treatment of the catalytic subunits purified from rat axolemma and of crude brain and axolemma microsomes generates a distinct 50-kDa fragment. This 50-kDa fragment was not observed in similarly treated kidney and skeletal muscle microsomes. Since kidney contains only the α1 form and skeletal muscle contains both the α1 and α2 forms of the (Na^+,K^+)ATPase (Lytton et al., 1985), it appears that this 50-kDa fragment is derived from a previously unidentified catalytic subunit, possibly α3, of the (Na^+,K^+)ATPase.

Unambiguous proof that the 50-kDa fragment is part of the α3 chain was obtained by determination of the amino acid sequence of this fragment. The sequence obtained matches only with that of residues 117–132 of the α3 chain; this sequence is not present in the α1 or the α2 chains (Shull et al., 1986). The 50-kDa polypeptide represents, therefore, the fragment between residues 115 and 583 obtained by cleavage of Asp–Pro bonds of residues 114 and 115 and residues 583 and 584, respectively. It is possible that some cleavage may have taken place also at the Asp–Pro bond of residues 487 and 488, generating fragments of 487 and 372 amino acids. These must be present in low amounts, because they are not visible as distinct bands on SDS–PAGE. If these fragments were to overlap with bands at 50 and 40 kDa, their amount would have to be sufficiently small not to interfere with the determination of specific amino acid sequences for the material in these two bands. The resistance of the Asp–Pro bond between residues 487 and 488 to cleavage by formic acid is not explained.

One may wonder why the α3 chain has not been identified in brain tissue before now. The obvious reasons are that its mobility on SDS–PAGE and the high affinity for ouabain of the enzyme containing the α3 chain resemble the properties of the α2 chain. There was no way, therefore, to distinguish these two catalytic subunits. The minimum estimate (from the data in Figure 2A, lane 3) for the amount of the α3 chain in brain axolemma is 25% of the total catalytic chain. Only by using a specific antibody against the α3 chain and by careful comparison of the position of immunoblotted bands, Figure 3, lanes 9–11, can one see that the mobility of the α3 chain is slightly slower than that of the α2 chain which is clearly slower than that of the α1 chain. It is likely that in many gel systems the α2 and α3 chains travel with a similar mobility.

With regard to the question of the ouabain affinity of the (Na^+,K^+)ATPase containing the α3 chain, it is interesting to note that the amino acid sequence of the H1–H2 region of the α3 chain is more similar to those of the rat α2 chain and of the sheep kidney and pig kidney α chains than it is to the sequence of the rat α1 chain. This observation was made by Shull et al. (1986) and led them to suggest that the H1–H2 loop of the polypeptide may be involved in determining the affinity for ouabain. Since (Na^+,K^+)ATPases containing rat α2, sheep kidney α, and pig kidney α all have a high affinity for ouabain, one would deduce that the enzyme with rat α3 should also have a high affinity for ouabain. This supposition is supported but not proven by the results obtained here.

The functional properties of the (Na^+,K^+)ATPase containing the α3 chain remain to be found. One property of the pumps containing the α2 chain is that their affinity for Na^+ can be regulated in rat adipocytes (Lytton, 1985b). A possibility, raised by a reviewer, is that the enzyme with α3 chains might have a different affinity for ATP compared to the enzyme with α2 chains.

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Registry No. ATPase, 9000-83-3; ouabain, 630-60-4.

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