Properties of the Two Polypeptides of Sodium- and Potassium-dependent Adenosine Triphosphatase*

(Received for publication, July 21, 1972)

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SUMMARY

The (Na⁺ + K⁺)-dependent adenosine triphosphatase from canine renal medulla is composed of lipids and of two polypeptide chains. The larger one has a molecular weight of 135,000, NH₂-terminal glycine, and has a high content of non-polar amino acids. The smaller polypeptide chain has NH₂-terminal alanine and it is a sialoglycoprotein. The two polypeptides are present in a mass ratio of 1.7:1 for the ratio of large to small chain. This mass ratio probably corresponds to a molar ratio of one large chain to two small chains. The two chains are close to one another in the purified enzyme because they can be covalently cross-linked by dimethyl suberimidate. Finally, the large polypeptide chain, which has all of the properties of a membrane-bound enzyme, is soluble in simple aqueous solvents in the absence of detergents and lipids, although it no longer has enzymatic activity.

The membrane-bound enzyme complex responsible for the active transport of sodium and potassium ions across the plasma membrane of eucaryotic cells is known as (Na⁺ + K⁺)-adenosine triphosphatase. Previous reports have described the isolation of this enzyme from canine renal medulla and it has been shown that the purified enzyme contains two polypeptide chains (1). The larger of the two polypeptides is specifically phosphorylated during the turnover of the enzyme (2). There is also one cardiac glycoside binding site for each large polypeptide chain (3). The function of the small polypeptide chain and its relationship to the quaternary structure of soluble enzymes (5). These results suggest that the complete enzyme is some complex of these two proteins. Therefore, the chemical properties of each polypeptide were studied. The larger polypeptide chain has a molecular weight of 135,000 and contains a high percentage of non-polar amino acids, while the smaller one is a membrane-bound sialoglycoprotein.

The larger polypeptide is soluble in a dilute buffer in the absence of lipids, detergents or denaturating agents, although it has no enzymatic activity under these conditions.

EXPERIMENTAL PROCEDURES

Materials

Sodium dodecyl sulfate was purchased from Sigma Chemical; [³⁵S]sodium dodecyl sulfate, from New England Nuclear; [2-³H]iodoacetic acid (80 mCi per mmole), from New England Nuclear; guanidinium chloride, from Hioe; Sepharose 4B, from Pharmacia. Polypeptide standards, listed with their molecular weights (6), were: bovine carbonic anhydrase (Mann Research), 29,000; muscle aldolase (Worthington), 40,000; beef liver catalase (Worthington), 60,000; bovine serum albumin (Pentex), 68,000; human transferrin (L. Waxman, Harvard University), 76,000; phosphorylase a (Worthington), 94,000; β-galactosidase (Boehringer), 130,000; myosin (G. Guidotti, Harvard University), 220,000. The canine renal medulla (Na⁺ + K⁺)-adenosine triphosphatase preparations employed in these experiments have been described (1, 3). XA14 enzyme, prepared by passing supernatant enzyme over a column of the resin XA14 (kindly provided by Dr. Ishaiahu Schechter, Harvard University) to remove bound deoxycholate, was used for the cross-linking studies. The supernatant enzyme was used for all of the other experiments. The enzyme was always assayed for (Na⁺ + K⁺)-adenosine triphosphatase activity, and only preparations of the usual specific activity were used (approximately 11 and 6 μmoles of Pi per mg per min for the XA14 and the supernatant enzyme, respectively) (1, 3).

Dimethyl suberimidate was prepared from suberonitrile according to the procedure of Davies and Stark (5).

Methods

Chemical Analyses—Amino acid analyses were done on a Beckman Spinco model 120C amino acid analyzer by the method of Davies and Stark (5).
of Spackman et al. (7). Spectra were recorded on a Cary 15 recording spectrophotometer. Protein concentrations were determined by the method of Lowry or by amino acid analysis, as previously described (1). Neutral sugars were assayed by the method of Dubois (8) with galactose and mannose as standards. Sialic acid was measured by the thiobarbituric acid method of Warren (9). Glucosamine and galactosamine were determined by chromatography on the short column of the amino acid analyzer with the pH 4.25 citrate buffer. The protein samples were hydrolyzed in 3 N HCl at 110° for 16, 21, 30, and 46 hours in sealed evacuated glass tubes with β-alanine as an internal standard. The results were extrapolated to zero time. Organic phosphate was measured by the method of Ames and Dubin (10).

Extraction of lipid from the protein was done with chloroform-methanol according to the procedure of Weinstein et al. (11). NH₂-terminal amino acids were determined by the dansyl method as described by Hartley (12) and applied by Weiner et al. (13).

Gel Electrophoresis—Acrylamide gels, run at pH 7, were prepared according to Weber and Osborn (6) with 10% acrylamide and 0.11% methylenebisacrylamide. Acrylamide gels run at pH 8.5 in a borate-acetate buffer were prepared according to Davies and Stark (5). In both systems the buffer contained 0.1% sodium dodecyl sulfate. Urea-SDS acrylamide gels were made according to Feit et al. (14) with 8 M urea and 0.1% SDS; the reservoir buffer contained 0.01% SDS.

The samples for the pH 7.0 gels were prepared by adding 2-mercaptoethanol and 20% SDS to a final concentration 0.1 M and 5 to 10 times in excess of the protein concentration, respectively. The sample was then heated to 100° for 3 min to prevent proteolytic degradation (15). Samples from the cross-linking experiments were added directly to the borate-acetate gels. The gels were stained for protein with Coomassie brilliant blue as outlined by Weber and Osborn (6) and for carbohydrate by the periodic acid-Schiff procedure described by Gossman and Neville (16). The stained gels were scanned on a Zeiss PMQ II spectrophotometer fitted with a Berg linear transport sample chamber (17).

Cross-linking Experiments—(Na⁺ + K⁺)-adenosine triphosphatase (0.3 mg per ml) was dialedyzed against 0.2 M N-ethylmorpholine acetate, pH 8.5. To 0.15-ml aliquots of this solution, various amounts of dimethyl suberimidate (10 mg per ml) in the same buffer were added. The reaction proceeded for 3 hours at room temperature; then 5 μl of 20% sodium dodecyl sulfate were added to each tube and the samples were lyophilized. The dry powders was dissolved in 1% 2-mercaptoethanol (50 μl) and from which the pH was adjusted to 8.0. After 4 hours the samples were subjected to electrophoresis on the borate-acetate SDS gels.

Gel Filtration—Gel filtration was done on columns of Sepharose 4B equilibrated either with 0.04 M Tris sulfate, pH 8.0, and 0.2% SDS (2.5 cm × 90 cm) or with 0.1 M guanidinium chloride and 0.02% bovine serum albumin (1.5 cm × 45 cm). The serum albumin was included to prevent the adsorption of small protein samples to the column. The columns were prepared as described by Rosenberg and Guidotti (18). Samples of the enzyme and of the standard proteins to be applied to the SDS column were prepared in the same way as those for acrylamide gel electrophoresis at pH 7.0. The SDS column was eluted at 5 ml per hour and 30-min fractions were collected.

The samples (20 to 50 μg of the polypeptide chains of the enzyme) for the guanidinium chloride column were dissolved in 6 M guanidinium chloride and 0.1 M dithiothreitol, and they were carboxymethylated with 1 mm (2-HIjodoacetate (80 μ Ci per mmole) for 10 min and then more thoroughly with 10 mm cold iodoacetate for 20 min at pH 8 and 20°. The standard proteins were carboxymethylated only with cold iodoacetate. The column was eluted at 2.5 g per hour and 30-min fractions were collected. Elution patterns were obtained by measuring the absorbance at 220 nm or the radioactivity of the fractions. Radioactive counting was done on 0.1- or 0.2-ml aliquots in 4 ml of Aquasol (New England Nuclear). The distribution coefficients, Kₚ, of the protein peaks and of the standards of known molecular weight were calculated from the elution position of the protein peaks according to Fish et al. (19, 20). The distribution coefficients of the standard proteins increased slowly with time on the guanidinium chloride column. To correct for this change, muscle phosphorylase and muscle aldolase were added to each sample applied to the column. The positions of these standards served as a reference for the behavior of the column.

Ultracentrifugation—Ultracentrifugation studies were done at 20° in Beckman Spineco model E ultracentrifuges equipped either with an ultraviolet scanning apparatus or with Rayleigh interference optics. Photographic plates were measured on a Gaertner microcomparator. Sedimentation velocity experiments were done in standard double sector centerpieces and recorded on the ultraviolet scanning recorder. Sedimentation equilibrium experiments were done with an AN-J rotor by the meniscus depletion method of Yphantis (21) in standard double sector centerpieces loaded with 0.1 ml of solution. The partial specific volume of the protein was calculated from the amino acid composition by the method of Cohn and Edsall (22). The solution density was calculated with the values in the International Critical Tables.

Removal of SDS from Polypeptide Chains—The bound SDS was removed from each polypeptide chain by the method of Weber and Kutter (23). [3S]SDS was added to each initial sample in order to carefully monitor the removal of the detergent. In all cases, less than 1 mole of detergent remained per mole of protein after the treatment with Dowex 1. The proteins were immediately dialyzed into 6 M guanidinium chloride, 4 mM dithiothreitol, 50 mM Tris acetate, pH 7.8, and stored under N₂.

RESULTS

Characterization of Polypeptide Chains—Purified (Na⁺ + K⁺)-adenosine triphosphatase contains two polypeptide chains (1), which appear to have molecular weights of 84,000 and 57,000 as measured by SDS gel electrophoresis (Fig. 1A and Table 1). This result suggested that the polypeptides could be separated by gel filtration on Sepharose 4B in solvents containing SDS. A representative elution profile from an SDS column is shown in Fig. 2.

The first two peaks of absorbance are the two polypeptide chains of the enzyme. The third peak of optical density is the membrane lipid, since it contains organic phosphate and no protein components as judged by gel electrophoresis. Also included in Fig. 2 are results from an experiment in which the protein was denatured with [3S]SDS. No radioactivity remains associated with the protein components, which demonstrates that the detergent bound to protein is in free exchange with that of the medium. On the basis of the absorbance at 220 nm, there

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1 The method of hydrolysis of protein samples and of analysis for amino sugars was devised by L. Waxman.

2 The abbreviations used are: dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; SDS, sodium dodecyl sulfate.
Fig. 1. Polyacrylamide gel electrophoresis of (Na\textsuperscript{+} + K\textsuperscript{+})-adenosine triphosphatase and of the separated polypeptide chains. A, SDS gel at pH 7.0 of supernatant enzyme; B, SDS gel at pH 7.0 of large chain purified by gel filtration; C, SDS-urea gel at pH 8.5 of large chain purified by gel filtration; D, SDS gel at pH 7.0 of small chain purified by gel filtration.

Table I

<table>
<thead>
<tr>
<th>Technique (Reference)</th>
<th>Molecular weights</th>
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<tbody>
<tr>
<td></td>
<td>Large chain</td>
</tr>
<tr>
<td>SDS gel electrophoresis (6, 34)</td>
<td>54,000 (1)</td>
</tr>
<tr>
<td>SDS gel filtration (19)</td>
<td>127,000</td>
</tr>
<tr>
<td>Guanidinium chloride gel filtration (20)</td>
<td>139,000</td>
</tr>
</tbody>
</table>

are 1.7 g of the large polypeptide per g of the small chain in the supernatant enzyme added to the column.

The polypeptide components were pooled separately, taking care to avoid the lipid component, and concentrated by vacuum dialysis. The smaller polypeptide was usually rechromatographed to remove contaminating quantities of the large polypeptide and lipid. Representative SDS gels of the purified polypeptides are shown in Fig. 1, B and D. The larger polypeptide chain is homogeneous as judged by electrophoresis in SDS-acrylamide gels (Fig. 1C), a technique which separates proteins on the basis of charge as well as molecular weight (24). The large polypeptide chain yielded only dansyl-glycine and the small chain only dansyl-alanine as NH\textsubscript{2}-terminal amino acids.

The apparent molecular weight of the polypeptide chains was also determined by their elution position on the SDS column. The standard curve for the SDS column and the elution positions of each chain are shown in Fig. 3. The apparent molecular weight of the larger of the two polypeptides is 127,000 and that of the smaller is 45,000 (Table I).

This was a surprising result since the molecular weights obtained by SDS polyacrylamide gel electrophoresis are rather different from those deduced from the SDS column. It therefore seemed important to use a third method for determination of molecular weights. The isolated polypeptide chains were stripped of SDS, transferred into 6 M guanidinium chloride, and applied to a column of Sepharose 4B in guanidinium chloride.

Fig. 2. Separation of the polypeptide chains by gel filtration in SDS. Supernatant enzyme (3 mg) was denatured in SDS and added to a Sepharose 4B column (90 cm × 2.5 cm) equilibrated with 0.04 M Tris sulfate, pH 8.0, 0.2% SDS. The column was run at 5 ml per hour and 30-min fractions were collected. $A_{280}$ is plotted as a function of elution volume (O—O). Also presented are the results of organic phosphate analysis ($\bullet$—$\bullet$). In another experiment protein was denatured with $\text{[P]SDS}$, and selected fractions were analyzed for radioactivity (×—×).

Fig. 3. Calibration of the Sepharose 4B column equilibrated with SDS. Polypeptides of known molecular weight were denatured with SDS and applied to the Sepharose 4B column equilibrated with 0.04 M Tris sulfate, pH 8.0, 0.2% SDS. Distribution coefficients, $K_D$ (19), were calculated from the elution positions of these standards. The logarithm of the molecular weight of each standard is plotted as a function of the logarithm of its $K_D$. The standards are: a, myosin; b, muscle phosphorylase; c, bovine serum albumin; d, catalase; e, bovine carbonic anhydrase. Arrows mark the logarithms of the distribution coefficients of the large and small polypeptide chains of the (Na\textsuperscript{+} + K\textsuperscript{+})-adenosine triphosphatase.

The majority (90%) of the large chain moved as a single component but a small fraction (10%) eluted at the position of the void volume. The small chain slowly aggregated during storage in 6 M guanidinium chloride, and on the gel filtration column a large fraction (80%) eluted as the monomer and the rest as the dimer. After prolonged storage the majority of the protein travelled as a broad peak with an apparent molecular weight of 2 to 3 × 10\textsuperscript{5}. The calibration curve for this column is shown in Fig. 4 plotted in the form recommended by Fish et al. (20).
The apparent molecular weights of the two polypeptides are 139,000 and 35,000 (Table I).

The variation in the molecular weights of polypeptide chains as measured by the different methods is unusual (19, 25) but it has been observed before, notably with the sialoglycoprotein from the membranes of human erythrocytes (molecular weight as measured by the different methods is unusual (19, 25) but it has been attributed to the presence of carbohydrate chloride in the region 240 to 320 nm is typical for proteins in this solvent (31), and the absorbance in this region can be accounted for by the contributions from tyrosyl and tryptophanyl residues.

**Solution Properties of Large Polypeptide—(Na⁺ + K⁺)-adenosine triphosphatase**. An aliquot (60 µg) of the small polypeptide chain purified by gel filtration (Fig. 2) was added to two SDS gels, run at pH 7.0. One was stained for protein with Coomassie brilliant blue, the other for carbohydrate with periodic acid-Schiff. The stained gels were scanned at 300 nm and 360 nm, respectively. The scans were aligned by electrophoretic mobility and tracings of them are presented. Curve a is a scan of the gel which was stained for protein; curve b, for carbohydrate.

The elution pattern from the SDS column of supernatant enzyme after lipid extraction with chloroform-methanol (done to remove glycolipid) is shown in Fig. 5. Most of the lipid (as judged by the absorbance at 220 nm) is missing (see Fig. 2), and both neutral sugars and glucosamine co-chromatograph with the smaller polypeptide chain. SDS gels run at pH 7.0 of the smaller polypeptide (20 µg) were stained for carbohydrate by the periodic acid-Schiff procedure (16) and a positive reaction was obtained. Scans of gels, stained with Coomassie brilliant blue and periodic acid-Schiff, are presented in Fig. 6. The large polypeptide chain did not stain for carbohydrate. Control gels on which bovine serum albumin (150 µg) and human transferrin (20 µg) were run showed a positive periodic acid-Schiff reaction only at the position of transferrin. These results demonstrate that the smaller polypeptide is a membrane-bound glycoprotein.

These conclusions were confirmed by the results of the amino acid and carbohydrate analyses on the separated chains shown in Table II. The small polypeptide chain contains 13% by weight carbohydrate.

The spectrum of the large polypeptide chain in 6 M guanidinium chloride in the region 240 to 320 nm is typical for proteins in this solvent (31), and the absorbance in this region can be accounted for by the contributions from tyrosyl and tryptophanyl residues.

**Solution Properties of Large Polypeptide—(Na⁺ + K⁺)-adenosine triphosphatase**. An aliquot (60 µg) of the small polypeptide chain purified by gel filtration (Fig. 2) was added to two SDS gels, run at pH 7.0. One was stained for protein with Coomassie brilliant blue, the other for carbohydrate with periodic acid-Schiff. The stained gels were scanned at 300 nm and 360 nm, respectively. The scans were aligned by electrophoretic mobility and tracings of them are presented. Curve a is a scan of the gel which was stained for protein; curve b, for carbohydrate.

A preparation of the larger polypeptide from which SDS had

![Fig. 4. Calibration of the Sepharose 4B column equilibrated with 6 M guanidinium chloride. Polypeptides (2 mg) of known molecular weight were denatured with 6 M guanidinium chloride, carboxymethylated, and applied to a Sepharose 4B column (45 cm x 1.5 cm) equilibrated with 6 M guanidinium chloride, 0.2 mg per ml of bovine serum albumin. Distribution coefficients, Kc, (20) were calculated from the elution positions of these standards determined by A280. The logarithm of the molecular weight of each standard is plotted as a function of the logarithm of its distribution coefficients of the large and small polypeptide chains determined by AZZ+. The logarithm of the molecular weight of the (Na⁺ + K⁺)-adenosine triphosphatase was determined by AZZ+ and electron microscopy of the larger polypeptide from which SDS had been removed was dissolved in 2% SDS and added to the Sepharose 4B column (90 cm x 2.5 cm) equilibrated with 0.04 M Tris sulfate, pH 8.0, 0.2% SDS. The column was run at 4 ml per hour, and 30-min fractions were collected. A280 is plotted as a function of elution volume (O—O). Also presented are the results of neutral sugar assays, A492 (O—O), and the glucosamine concentration (X—X) of selected fractions.](http://www.jbc.org/)
TABLE II
Amino acid and carbohydrate composition of the two polypeptide chains

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Large chain</th>
<th>Small chain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mole/100 mole amino acid</td>
<td>mole/100 mole amino acid</td>
</tr>
<tr>
<td>Lysine</td>
<td>3.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.8</td>
<td>1.27</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Serine</td>
<td>6.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>10.0</td>
<td>10.8</td>
</tr>
<tr>
<td>Proline</td>
<td>4.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>7.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Cysteic acid b</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Valine</td>
<td>7.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Methionine sulfone c</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>7.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Leucine</td>
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</tr>
<tr>
<td>Tyrosine</td>
<td>2.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.6</td>
<td>2.5</td>
</tr>
<tr>
<td>% Hydrophobic (29)</td>
<td>47%</td>
<td>44%</td>
</tr>
<tr>
<td>Glucosamine</td>
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</tr>
<tr>
<td>Galactosamine</td>
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<tr>
<td>Neutral sugar d</td>
<td>2.2 to 3.7</td>
<td></td>
</tr>
<tr>
<td>Sialic acid</td>
<td>1.7</td>
<td></td>
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</table>

* Results of analyses on duplicate samples after hydrolysis for 24, 48, and 72 hours. The values for serine and threonine are extrapolated to zero time. The values for valine, isoleucine, and leucine are those obtained after 72 hours of hydrolysis.

b Determined by performic acid oxidation (30).

c Determined spectrophotometrically (31).

d Upper and lower limits are values obtained using galactose or mannose as standards, respectively.

been removed and which was in 6 M guanidinium chloride was dialyzed into 25 mM imidazolium chloride, pH 7.0, 0.1 M KCl, 2 mM EDTA, 5 mM dithiothreitol. The final protein concentration was 0.35 mg per ml. The solution remained transparent and contained no (Na+ + K+)-adenosine triphosphatase activity. In the analytical ultracentrifuge, the protein sedimented as a single component. The boundary, however, was more diffuse than that of a globular protein. The sedimentation coefficient of this protein at several concentrations as well as sedimentation coefficients of two other preparations of dialyzed large polypeptide are presented in Table III. The sedimentation coefficient observed did not vary with concentration but did vary between preparations. Preparations I and II were taken from the same stock solution in 6 M guanidinium chloride, but Preparation II was concentrated before the guanidinium chloride was dialyzed away. The sedimentation coefficients varied between 8.7 S and 10.4 S.

When each of these preparations was examined by sedimentation equilibrium they behaved identically. A representative result is shown in Fig. 7. The slope of the line drawn in the figure as well as those from other data sets correspond to a molecular weight of 265,000 ± 20,000. In all cases the curve deviated from a straight line above a fringe displacement of 200 μ indicating aggregation at these higher concentrations of protein.

These results suggest that the large polypeptide chain is soluble in aqueous solvents in the absence of lipids and detergents and that it aggregates to form dimers. Whether or not there is a unique conformation and aggregate of the large chain in these circumstances is not clear.

Cross-linking of the Two Polypeptides—Since purified (Na+ + K+)-adenosine triphosphatase has two polypeptide chains and the larger of the two is certainly part of the enzyme (2), I wished to determine whether or not these two polypeptides are close to each other in the enzyme, which is a lipid-protein complex. Attempts to cross-link the two polypeptides to one another were carried out with dimethyl suberimidate, and the results of these experiments are shown in Fig. 8. Following treatment with the...
A.
Direction of electrophoresis

B.
Transmittance

C.
Distance

Fig. 8. Cross-linking of the two polypeptides. Samples of 
(Na\(^+\) + K\(^+\))-adenosine triphosphatase were treated with several 
concentrations of dimethyl suberimidate at pH 8.5. After incubation 
they were denatured with SDS and applied to 5\% polyacrylamide gels containing SDS and the borate-acetate buffer. The gels were stained and scans of the stained gels are presented. A, control; B, 0.3 mg per ml of dimethyl suberimidate; C, 0.6 mg per ml of dimethyl suberimidate in the reaction mixture.

TABLE IV
Cross-linking of (Na\(^+\) + K\(^+\))-adenosine triphosphatase

<table>
<thead>
<tr>
<th>Dimethyl suberimidate concentration (mg/ml)</th>
<th>Relative areas or absorbance peaks*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Suberimidate complex</td>
</tr>
<tr>
<td>0</td>
<td>0.65</td>
</tr>
<tr>
<td>0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>0.6</td>
<td>0.24</td>
</tr>
<tr>
<td>0.9</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* Scaled so that the total area on the control gel is 1.00.

The amino acid composition of the suberimidate complex was then compared with the calculated amino acid composition of mixtures of the two polypeptide chains of the enzyme in various mass ratios. The comparison was made calculating the variances of the logarithms of the ratios of the micromoles of each amino acid as described by Cook et al. (33). The results are presented in Table V. The variance is at a minimum when the amino acid composition of the suberimidate complex is compared to a mixture of the large and small polypeptide in a mass ratio of 3:1, respectively. The amino acid composition of neither chain alone compares very favorably with that of the complex. When samples were run on 10\% acrylamide SDS gels (borate-acetate buffer) calibrated with standards of known molecular weight, the apparent molecular weight of the complex was 164,000 and of the large chain and small chain 110,000 and 66,000, respectively. These results demonstrate that one large and one small polypeptide chain of (Na\(^+\) + K\(^+\))-adenosine triphosphatase can be cross-linked under conditions at which no cross-linking of other components can be observed. This suggests that the two polypeptides are intimately associated with each other on the membrane.

DISCUSSION

The two polypeptides present in purified (Na\(^+\) + K\(^+\))-adenosine triphosphatase appear to be well defined and unique protein molecules. Only one NH\(_2\)-terminal amino acid is found in purified preparations of each chain, and the larger chain is homogeneous by gel electrophoresis in SDS-urea systems.

The various techniques used to estimate the molecular weight of the larger polypeptide give results which do not agree very closely (Table I). The linear relationship between the logarithm of the molecular weight and the electrophoretic mobility of polypeptides on SDS gels (6, 34) is thought to be due to the binding of equal amounts (on a mass basis) of SDS to all of the polypeptides (35). If the large polypeptide, as a result of its unusual amino acid composition, binds more SDS than do other proteins of the same molecular weight, the electrophoretic mobility of the protein-SDS complex would be greater and its apparent molecular weight would be less than its actual molecular weight. On the other hand, additional amounts of bound SDS might not affect the structure of the SDS-protein complex (36), and thus the elution position of this complex on SDS columns should be unaffected by an increase in the amount of bound SDS. Since the results from gel filtration in guanidinium chloride and in SDS agree closely, as in fact has been shown for many proteins (19), the large polypeptide probably has a molecular weight of 135,000. This value should be the truer estimate of the molecu-
lar weight of the larger polypeptide chain of (Na+ + K+)-adenosine triphosphatase.

The assignment of a molecular weight to the smaller polypeptide of (Na+ + K+)-adenosine triphosphatase is complicated by the fact that it is a glycoprotein. It has been proposed that the observed electrophoretic mobility of the membrane-bound sialoglycoprotein from the red cell is less than that of a polypeptide of the same molecular weight (28). On the other hand, when molecular weights of proteins are estimated by gel filtration in either SDS or guanidinium chloride, the values obtained should be lower limits of the actual molecular weight because the rigid rod (36) or random coil (37), respectively, formed under these conditions are structures of maximum Stokes radius (unless the structure assumed by this protein in these solvents is an extended rod). As a result of these considerations, the molecular weight of the small chain should be between 35,000 and 57,000.

These results coincide with estimations of the molecular weight of the enzyme by radiation inactivation (38, 39). The apparent molecular weight of the whole enzyme, from each of these experiments, is about 250,000. If the molecular weight of the large chain is 135,000 and there are 1.6 to 1.7 g of large chain per g of small chain protein, the total molecular weight of the enzyme would be 220,000 according to the studies reported here.

Moreover, the radiation inactivation experiments indicated that both the potassium-dependent nitrophenyl phosphatase (38) and the sodium-dependent phosphorylation reaction (39) were catalyzed by a protein whose molecular weight was 0.58 that of the whole enzyme or 140,000. It is striking that the large chain, which is the polypeptide specifically phosphorylated during the turnover of the enzyme (2), has a molecular weight of 135,000 by the studies reported here.

When the large polypeptide chain is dialyzed out of 6 M guanidinium chloride it forms a dimer whose sedimentation coefficient is 9 to 10 S and whose f/fmin (40) is 1.6. This would be 220,000 according to the studies reported here.

The observed electrophoretic mobility of the membrane-bound sialoglycoprotein from the red cell is less than that of a polypeptide of the same molecular weight (28). On the other hand, when molecular weights of proteins are estimated by gel filtration in either SDS or guanidinium chloride, the values obtained should be lower limits of the actual molecular weight because the rigid rod (36) or random coil (37), respectively, formed under these conditions are structures of maximum Stokes radius (unless the structure assumed by this protein in these solvents is an extended rod). As a result of these considerations, the molecular weight of the small chain should be between 35,000 and 57,000.

The small polypeptide chain present in the purified enzyme is a membrane-bound glycoprotein whose major amino sugar is glucosamine (Fig. 5, Table II), in contrast to the situation in the membrane of the human erythrocyte (41). However this result agrees with the observation that more than 90% of the amino sugars in glycoproteins bound to the plasma membrane of rat liver cells is glucosamine (42). In regard to this glycoprotein, one wonders whether or not all of the carbohydrate of the plasma membrane of animal cells is located on the external surface of the cell, as seems to occur in the membrane of the red blood cell (43-45). If this is the case for other plasma membranes, one of the roles of the small polypeptide chain may be to dictate the intrinsic asymmetry displayed by the enzyme (46-47).

The results from the cross-linking experiment suggest that (Na+ + K+)-adenosine triphosphatase is some complex of the large and small polypeptide chains present in the purified preparation. Since the molecular weight of the large chain is about 135,000 its diameter would be 70 A if it were a sphere. As the width of the non-polar center of the lipid bilayer is 25 to 40 A (48, 49), the large chain of the enzyme, if it had this shape, could span the membrane with 15 A to spare on each side. The fact that almost 50% of its amino acids are hydrophobic makes this possibility even more appealing. If the small chain is a part of the enzyme and if its molecular weight is about 40,000 then there would be two small chains for each large chain, i.e. the enzyme might be represented by the structure CYβ. This would be very unusual as stoichiometries of this type have only rarely been described. The fact that the two polypeptides are always in a particular ratio, however, may say nothing about their actual relationship in the enzyme. By far the most interesting feature of the smaller polypeptide is that it is a sialoglycoprotein, for this implies that rotation about an axis parallel to the membrane is unlikely. This polypeptide could serve as a fixed feature of the smaller polypeptide is that it is a sialoglycoprotein, for this implies that rotation about an axis parallel to the membrane is unlikely. This polypeptide could serve as a fixed feature of the smaller polypeptide.

Acknowledgment—I wish to thank Guido Guidotti.

REFERENCES
Properties of the Two Polypeptides of Sodium- and Potassium-dependent Adenosine Triphosphatase

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