In any case, these experiments have demonstrated very significant metabolic activity in the substratum adhesion sites of these fibroblastic cells, particularly with regard to the conversion of heparan sulfate proteoglycan to free chains. These chains would only be univalent or at most bivalent for binding the adhesive glycoprotein fibronectin, and valency of binding is a most important determinant of adhesive bond formation (Rollins et al., 1982). It would therefore be expected that catabolism of this proteoglycan would weaken close contact adhesion processes and be an important parameter in cellular movement. A better understanding of these dynamic processes will be required to clarify the mechanisms for the making and breaking of adhesive contacts of fibroblasts with this model extracellular matrix.

Registry No. Hyaluronic acid, 9004-61-9; chondroitin sulfate, 9007-28-7; heparan sulfate, 9050-30-0.

References

Carlson, D. M. (1968) *J. Biol. Chem. 243*, 616–626.
Cathcart, M. K., & Culp, L. A. (1979) *Biochim. Biophys. Acta 556*, 331–343.
Chen, W. T. (1977) *J. Cell Biol. 75*, 411a.
Chen, W. T. (1981) *J. Cell Biol. 90*, 187–200.
Coster, L., Carlstedt, I., & Malmstrom, A. (1979) *Biochem. J. 183*, 669–677.
Culp, L. A. (1976) *Biochemistry 15*, 4095–4104.
Culp, L. A., Rollins, B. J., Buniel, J., & Hitri, S. (1978) *J. Cell Biol. 79*, 788–801.
Culp, L. A., Murray, B. A., & Rollins, B. J. (1979) *J. Supramol. Struct. 11*, 401–427.
David, G., & Bernfield, M. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 786–790.
David, G., & Bernfield, M. R. (1981) *J. Cell Biol. 91*, 281–286.
Faltz, L. L., Reddi, A. H., Hascall, G. K., Martin, D., Pita, J. C., & Hascall, V. C. (1979) *J. Biol. Chem. 254*, 1375–1380.
Garner, J. A., & Culp, L. A. (1981) *Biochemistry 20*, 7350–7359.
Izzard, C. S., & Lochner, L. R. (1976) *J. Cell Sci. 21*, 129–159.
Izzard, C. S., & Lochner, L. R. (1980) *J. Cell Sci. 42*, 81–116.
Kjellen, L., Peterson, I., & Höök, M. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5371–5375.
Lark, M. W., & Culp, L. A. (1982) *J. Biol. Chem. 257*, 14073–14080.
Laterra, J., & Culp, L. A. (1982) *J. Biol. Chem. 257*, 719–726.
Laterra, J., Ansbacher, R., & Culp, L. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6662–6666.
Laterra, J., Norton, E. K., Izzard, C. S., & Culp, L. A. (1983a) *Exp. Cell Res.* (in press).
Laterra, J., Silbert, J. E., & Culp, L. A. (1983b) *J. Cell Biol. 96*, 112–123.
Lindahl, V., Backstrom, G., Jansson, L., & Hallen, A. (1973) *J. Biol. Chem. 248*, 7234–7241.
Miketo, L. J., & Culp, L. A. (1983) *Arch. Biochem. Biophys.* (in press).
Ogata, S., & Lloyd, K. O. (1982) *Anal. Biochem. 119*, 351–359.
Revel, J. P., Hoch, P., & Ho, D. (1974) *Exp. Cell Res. 78*, 1–14.
Robinson, H. C., Horner, A. A., Höök, M., Ogren, S., & Lindahl, U. (1978) *J. Biol. Chem. 253*, 6687–6693.
Rollins, B. J., & Culp, L. A. (1979a) *Biochemistry 18*, 141–148.
Rollins, B. J., & Culp, L. A. (1979b) *Biochemistry 18*, 5621–5629.
Rollins, B. J., Cathcart, M. K., & Culp, L. A. (1982) in *The Glycoconjugates* (Horowitz, M., Ed.) Vol. III, pp 289–329, Academic Press, New York.
Rosen, J. J., & Culp, L. A. (1977) *Exp. Cell Res. 107*, 139–149.
Spiro, R. (1972) *Methods Enzymol. 28*, 2–43.
Yamada, K. M., Kennedy, D. W., Kimata, K., & Pratt, R. M. (1980) *J. Biol. Chem. 255*, 6055–6063.

Hydrophobic Labeling of (Na^+,K^+)-ATPase: Further Evidence That the β Subunit Is Embedded in the Membrane Bilayer†

M. Girardet, K. Geering, B. C. Rossier, J. P. Kraehenbuhl, and C. Bron*

ABSTRACT: *O*-Hexanoyl-3,5-diiodo-*N*-(4-azido-2-nitrophenyl)tyramine has been used after photochemical conversion into the reactive nitrene to label (Na^+,K^+)-ATPase from *Bufo marinus* toad kidney. Immunochemical evidence indicates that the reagent labels both subunits of the enzyme in partially purified form as well as in microsomal membranes. These results support the view that the glycoprotein subunit, like the catalytic subunit, possesses hydrophobic domains by which it is integrated into the plasma membrane.

Sodium- and potassium-dependent adenosine triphosphatase [(Na^+,K^+)-ATPase], which mediates the active transport of Na^+ and K^+ ions across the plasma membrane of animal cells, is composed of two subunits. The larger polypeptide (α subunit) spans the membrane and requires phospholipids for expression of the catalytic activity (Kyte, 1975; Roelofsen & Schatzmann, 1977). In contrast, the relationship of the glycosylated polypeptide (β subunit) with the lipid bilayer and the function of the β subunit are not known. In order to understand the assembly and the function of these subunits at the molecular level, it is important to know their topographical distribution and their orientation in the membrane as well as their modes of attachment with the lipid bilayer.

† From the Department of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland (M.G., J.P.K., and C.B.), and the Department of Pharmacology, University of Lausanne, CH-1011 Lausanne, Switzerland (K.G. and B.C.R.). *Received September 2, 1982.* This work was supported by Grants 3.643-0.80, 3.672-0.80, and 3.646-0.80 from the Swiss National Foundation for Scientific Research and by Grant 156 from the Fritz Hoffman-La Roche Foundation.

0006-2960/83/0422-2296$01.50/0

One approach to such questions consists of the use of lipophilic affinity labeling reagents which are generated photochemically within the membrane and hence allow the labeling and characterization of regions of membrane polypeptide embedded in the lipid bilayer (Chowdhry & Westheimer, 1979). Thus, a hydrophobic domain of the α subunit of purified (Na^+,K^+)-ATPase has been clearly identified by the use of lipophilic nitrenes or carbenes (Karlish et al., 1977; Farley et al., 1980; Jørgensen et al., 1982). In addition, the β subunit appears to possess a hydrophobic domain since when (Na^+,K^+)-ATPase preparations were treated with photoreactive phosphatidylcholine analogues both 95- and 45-dalton polypeptides of the purified enzyme were labeled (Montecucco et al., 1981). However, this does not provide rigorous proof for the tight association of the β subunit with cellular membranes since the labeling was performed in the presence of detergents required for the purification of the enzyme. Also, there is a need to prove that the labeled polypeptide was indeed the β subunit such as can be achieved by using antibodies specific for each subunit.

In this paper, we describe the labeling of *Bufo marinus* (Na^+,K^+)-ATPase with the highly lipophilic probe *O*-hexanoyl-3,5-diiodo-*N*-(4-azido-2-nitrophenyl)tyramine (Owen et al., 1980) and the subsequent immunochemical identification of the enzyme subunits from both purified and membrane-bound enzyme preparations. Using these two methods in conjunction, we present evidence that the β subunit has nonpolar portions by which it is inserted into the lipid bilayer. This confirms and extends our recent data showing by means of specific antibodies that the glycosylated polypeptide as well as the catalytic subunit are transmembranous polypeptides (Girardet et al., 1981).

Materials and Methods

All chemicals and buffer components were of analytical grade and were purchased from Merck (Darmstadt). (4-Azido-2-nitrophenyl)tyramine (NAP-tyramine) was a kind gift from Dr. M. M. Green (National Institute for Medical Research, Mill Hill, London). Protein A–Sepharose and agarose LM were obtained from Pharmacia Fine chemicals (Uppsala, Sweden). Carrier-free $Na^{125}I$ was purchased from the Radiochemical Amersham International Center (490–520 MBq of $^{125}I/\mu g$). Hexanoyldiiodo-*N*-(4-azido-2-nitrophenyl)tyramine prepared by iodination and alkylation of NAP-tyramine as described previously (Owen et al., 1980). The specific activity of the product was 200 Ci/mmol.

Preparation of microsomes and partially purified (Na^+,K^+)-APTase from kidneys of *Bufo marinus* toads (C. P. Chase, Miami, FL) has been described previously in detail (Girardet et al., 1981; Geering & Rossier, 1979). The specific (Na^+,K^+)-APTase activity was 50 μmol (mg of protein)$^{-1}$ h^{-1} for the microsomal fraction and 1000 μmol (mg of protein)$^{-1}$ h^{-1} for the partially purified enzyme.

Electrophoretic analysis was carried out according to Maizel (1971) on 5–13% polyacrylamide gradient slab gels. Samples of proteins were treated with sample buffer [50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.0, 5% β-mercaptoethanol, 5% sodium dodecyl sulfate ($NaDodSO_4$), and 10% sucrose] at 100 °C for 5 min before being layered (50 μL) onto the gel. The slabs were stained with Coomassie Brilliant Blue R-250, dried, and radioautographed with Kodak X-OMAT R X-ray films. Scanning of both gels and autoradiographies were performed with a Zeiss linear transport scanner at 515 nm.

Antisera Specific for α and β Subunits. The production, the properties, and the specificity of the antibodies against α or β subunits of (Na^+,K^+)-ATPase, as well as the immunochemical identification of these polypeptides by indirect immunoprecipitation and $NaDodSO_4$–polyacrylamide crossed immunoelectrophoresis, have been described in detail elsewhere (Girardet et al., 1981; Geering et al., 1982a). A typical preparation of partially purified enzyme and its isolated subunits used as immunogens is shown in Figure 1A. As shown in Figure 1B, the antiserum raised against purified glycosylated polypeptide specifically precipitated the 60-dalton component. No reaction could be detected against the α subunit.

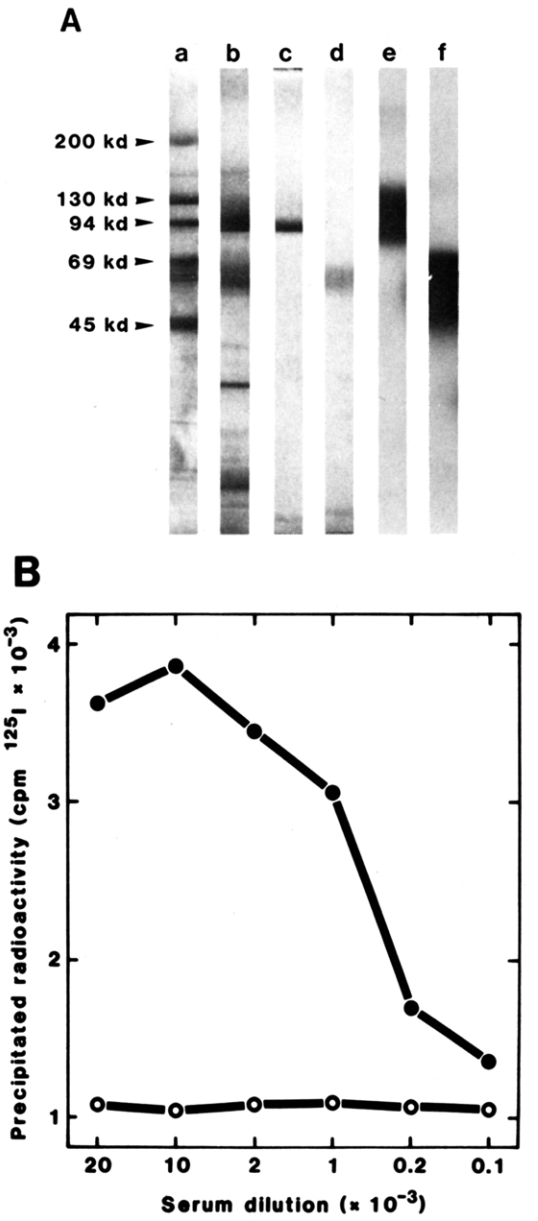

FIGURE 1: (A) $NaDodSO_4$–polyacrylamide gel electrophoresis (5–13%) stained with Coomassie Brilliant Blue (lanes a–d). (Lane a) Molecular weight markers: myosin (200 kdalton); β-galactosidase (130 kdalton); phosphorylase *b* (94 kdalton); transferrin (78 kdalton); bovin serum albumin (69 kdalton); ovalbumin (45 kdalton). (Lane b) Partially purified (Na^+,K^+)-ATPase from toad kidney membranes (50 μg of protein). (Lane c) Purified α subunit. (Lane d) Purified β subunit. (Lane e) Autoradiography of purified radioiodinated α subunit. (Lane f) Autoradiography of purified radioiodinated β subunit. (B) Immunoprecipitation with anti-β-subunit antiserum of purified radioiodinated β subunit (●) and purified radioiodinated α subunit (○).

Labeling Procedure. Nitrene labeling of microsomes or purified (Na^+,K^+)-ATPase was carried out by adding 20 μCi of hexanoyldiiodo-*N*-(4-azido-2-nitrophenyl)tyramine in 20

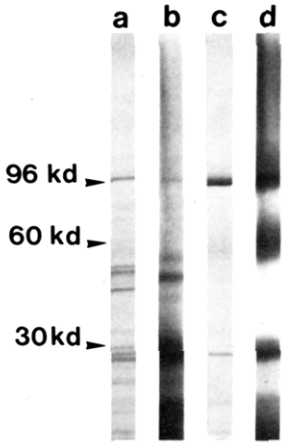

FIGURE 2: $NaDodSO_4$–polyacrylamide gel electrophoresis (5–13%). Coomassie Blue staining of kidney microsomes (10 μg) (lane a) and purified (Na^+,K^+)-ATPase (5 μg) (lane c). Autoradiography of the same samples labeled with nitrene: kidney microsomes (lane b); purified (Na^+,K^+)-ATPase (lane d).

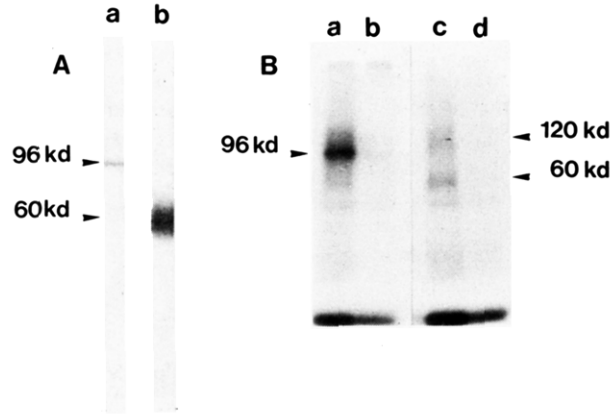

FIGURE 3: Autoradiography of 5–13% $NaDodSO_4$–polyacrylamide gel electrophoresis of immunoprecipitates of nitrene-labeled (Na^+,K^+)-ATPase. (A) Nitrene-labeled purified enzyme; indirect immunoprecipitation with anti-α-subunit antiserum (lane a) or with anti-β-subunit antiserum (lane b). (B) Nitrene-labeled kidney microsomes: indirect immunoprecipitation with anti-α-subunit antiserum (lane a) or with anti-β-subunit antiserum (lane c). Control immunoprecipitation with preimmune anti-α-subunit or anti-β-subunit antisera (lanes b and d).

μL of ethanol to a 1-mL suspension of microsomes (1.5 mg of protein) or purified enzyme (0.15 mg or protein) in 33 mM Tris–histidine buffer supplemented with 5 mM ethylenediamineteraacetic acid (EDTA) at pH 7.4 contained in siliconized glass tubes. The suspension was maintained for 30 min on ice in the dark and was then irradiated for 5 min at 0 °C by using a UV mercury lamp of 1000 W (ACE-Hanovia, ACE Glass Inc., Vineland, NJ) with a uranyl acetate filter (679 A36) with a cutoff at 310 nm. In control experiments, 15 mM reduced glutathione was added as a water-soluble scavenger before photoactivation. After photoactivation, the particulate microsomal fractions or partially purified enzyme preparations were recovered by centrifugation at $27000g_{av}$ for 30 min at 4 °C. The bound radioactivity was determined by counting the pellet prior to solubilization in 1 mL of 33 mM Tris–histidine buffer, pH 7.4, containing 5% Triton X-100. Proteins were then separated from the bulk of the lipids by precipitation with 9 volumes of absolute ethanol overnight at −20 °C. Prior to $NaDodSO_4$–polyacrylamide gel electrophoretic analysis, the protein pellet was dissolved and treated in 50 μL of sample buffer as described above. For indirect immunoprecipitation and crossed immunoelectrophoresis, the protein pellet was dissolved in 1 mL of 33 mM Tris–histidine buffer, pH 7.4, containing 4% $NaDodSO_4$ and 10 mM β-mercaptoethanol and was heated for 5 min at 100 °C in order to dissociate α and β subunits. Nine milliliters of 33 mM Tris–histidine buffer, pH 7.4, supplemented with 0.5% Triton X-100 was then added. Aliquots (800 μL) of the resultant solution were used for immunoprecipitation.

Results

Labeling of Kidney Microsomes or Partially Purified Enzyme. When suspensions of microsomes or partially purified enzymes were labeled with 20 μCi (specific activity 200 Ci/mmol) of iodinated lipophilic nitrene under the experimental conditions described under Materials and Methods, 27–29% of the radioactivity was recovered in the pellet after centrifugation. Of this activity, 29% in the case of microsomes and 34% in purified enzyme preparations were bound to proteins as assessed by precipitation with ethanol at −20 °C. That is, the remaining two-thirds of the membrane-bound radioactivity was probably associated with lipid. The overall incorporation of label into the membranes was not modified when the photoactivation was performed in the presence of 15 mM glutathione.

The selectivity of the lipophilic probe for a restricted number of proteins was indicated by the labeling pattern obtained after $NaDodSO_4$–polyacrylamide gel electrophoretic analysis of labeled microsomes (Figure 2a,b).

In fact, among the numerous Coomassie Blue stainable bands of this fraction, only bands with apparent molecular weights of 96 000, 53 000, 47 000, and 30 000 and a region with molecular weights less than 10 000 were detected with the lipophilic probe.

Moreover, the sensitivity of the probe was emphasized by the fact that none of the labeled bands was coincident with prominent Coomassie Blue stained bands with the exception of the 96 000-dalton polypeptide and a group of less than 30 000-dalton polypeptides.

At least three major polypeptides of the microsomes with 42 000, 48 000, and 50 000 apparent molecular weights were not labeled. Labeling of the purified enzyme preparations (Figure 2c,d) revealed both the 96 000-dalton α subunit and the 60 000-dalton β subunit. The latter glycosylated peptide was weakly stained by Coomassie Blue and could be detected upon overloading the gels as shown in Figure 1. An additional peptide of slightly less than 30 000 daltons which always copurified with the holoenzyme (Geering & Rossier, 1979) was also heavily labeled. In addition, about 25% of the lipophilic probe was bound to polypeptides of less than 10 000 daltons as assessed by scanning of the autoradiograms. These possibly represent contaminants and/or degradation products of the enzyme preparation.

Immunochemical Identification of Labeled (Na^+,K^+)-ATPase. Nitrene-labeled microsomes or purified enzyme preparations were submitted to indirect immunoprecipitation by using antisera specific for each subunit. Results of these experiments are depicted in Figure 3. Both α and β subunits precipitated from purified enzyme (Figure 3A), or microsomes (Figure 3B) were labeled by the hydrophobic probe. However, in the latter case, the α subunit seemed to react more readily with the nitrene than the β subunit, a result which is consistent with the labeling pattern of microsomes where only the 96 000-dalton subunit can be detected (Figure 2b).

In order to ascertain whether the reaction of the nitrene occurred essentially with protein functional groups in a hydrophobic environment, we performed the labeling of microsomal fractions and purified enzyme in the presence of 15 mM

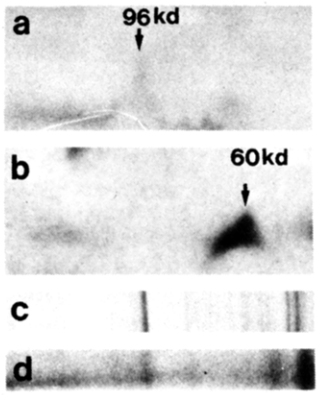

FIGURE 4: Autoradiograms of two-dimensional crossed immunoelectrophoresis of nitrene-labeled kidney microsomes: anti-α-subunit antiserum (panel a); anti-β-subunit antiserum (panel b). Nitrene-labeled kidney microsomes run in the first dimension of a 5–13% $NaDodSO_4$–polyacrylamide gel and used as a source of antigen: Coomassie Blue staining (panel c); autoradiography after nitrene labeling (panel d).

reduced glutathione. Immunoprecipitation from both types of preparations revealed that the total radioactivity inserted into the β subunit was identical in the presence and absence of this water-soluble scavenger.

The specific immunoprecipitation of the β subunit also revealed a 120 000-dalton band. This probably represents a dimer of the polypeptide, since the immunoprecipitation of this band can be inhibited by addition of a purified 60 000-dalton glycosylated polypeptide (Figure 1A) to the solubilized microsomes (K. Geering et al., unpublished results).

In addition, when labeled microsomes were used as a source of antigens, a faint 45 000–50 000-dalton radioactive band always coprecipitated with antisera against α or β subunits. The same material was found, although in barely detectable amounts, when preimmune sera were used as controls (Figure 3B). This probably represents radioactive material entrapped nonspecifically to an extent which depends upon the amount of immunoprecipitate formed. In control experiments, no labeling was obtained when the nitrene was inactivated by illumination prior to reaction with the purified enzyme.

Further demonstration of the labeling of both subunits of (Na^+,K^+)-ATPase was clearly provided by the autoradiography of two-dimensional $NaDodSO_4$–polyacrylamide crossed immunoelectrophoresis of nitrene-labeled microsomal fractions (Figure 4). However, due to the experimental conditions required for this type of method, the partial degradation of the α subunit could not be prevented, thus leading to the reduction of the amount of native polypeptide immunoprecipitated and as a consequence an apparent decrease in the radioactivity inserted into the α subunit as compared to the β subunit.

Discussion

The results presented here demonstrate that both subunits of the (Na^+,K^+)-ATPase are accessible to a hydrophobic radioactive probe when inserted into membranes or in purified enzyme vesicles. An important consideration in the interpretation of the data is whether the antibodies used for the identification of both α and β subunits of (Na^+,K^+)-ATPase are indeed specific for these polypeptides. Both reagents have been raised by immunization with two of the major components of purified enzyme preparations (Figure 1A) (Geering & Rossier, 1979; Girardet et al., 1981). Whereas the specificity of the anti-α-subunit antiserum could be clearly demonstrated by its reactivity with the phosphorylated catalytic polypeptide (Girardet et al., 1981; Geering et al., 1982b), it is conceivable that antibodies raised against the 60 000-dalton band could be directed against degradation products of the 96 000-dalton subunit known to be particularly susceptible to protease. This can be ruled out since upon absorption with purified α subunit the antiserum reacts exclusively with the 60 000-dalton polypeptide (Figure 1B) (Girardet et al., 1981). Moreover, the following results provide further evidence that these antibodies are indeed directed against the glycosylated β subunit and not against a contaminant comigrating with the 60 000-dalton band of purified enzyme. When toad bladder cells are biosynthetically labeled for 15–60 min, this antiserum precipitates a 42 000-dalton polypeptide which converts upon prolonged chase periods into a 60 000-dalton component. This is typical of the maturation of a nonglycosylated or core-glycosylated polypeptide to a fully glycosylated component. Moreover, the biosynthesis rates both of this polypeptide and of the α subunit are identical. In addition, in all cases studied, the synthesis of these subunits is induced in parallel by aldosterone (Geering et al., 1982a,c).

As anticipated from its known transmembranous orientation, the catalytic α subunit of (Na^+,K^+)-ATPase was labeled by the highly lipophilic probe hexanoyldiiodo-*N*-(4-azido-2-nitrophenyl)tyramine. In fact, immunochemical identification of the α subunit from both purified enzyme preparations and kidney microsomes revealed strong labeling of this polypeptide, thus confirming results obtained with other photogenerated reagents (Karlish et al., 1977; Farley et al., 1980; Montecucco et al., 1981; Jørgensen et al., 1982).

Extensive labeling was also found on the apparent M_r 30 000 peptide which always copurifies with (Na^+,K^+)-ATPase. The exact structural and functional relationship of this component with the holoenzyme is not yet clear. However, its strong reactivity with the nitrene confirms its tight association with the membrane phospholipids as previously reported (Geering & Rossier, 1979). Moreover, the possibility that this component represents a proteolytic fragment of the NH_2-terminal region of the catalytic subunit cannot be ruled out. In fact, such a segment of lower molecular weight (M_r 12 000) has been shown to interact strongly with the membrane and to be labeled with the lipophilic adamantylidiene (Castro & Farley, 1979; Farley et al., 1980).

Our recent immunochemical studies strongly suggested that the β subunit of (Na^+,K^+)-ATPase spans the lipid bilayer (Girardet et al., 1981). Further evidence in support of such a transmembranous orientation of the glycosylated polypeptide is provided by the reactivity of this ATPase subunit with the lipophilic nitrene when either purified enzyme or microsomal fraction was labeled. Moreover, the same result was obtained when epithelial cell suspensions from the toad bladder were labeled (M. Girardet et al., unpublished results).

Similar photogenerated nitrenes (Karlish et al., 1977) or carbenes (Farley et al., 1980) have been used to label (Na^+,K^+)-ATPase subunits from purified enzyme preparations only. In these earlier experiments, the β subunit was always only barely labeled. The extensive labeling of the β subunit observed here is probably a result of the marked hydrophobicity of the lipophilic reagent used (Owen et al., 1980). In order to exclude that the labeling resulted from a reaction of the nitrene with protein functional groups in a hydrophilic environment, we labeled microsomes or partially purified enzyme in the presence of 15 mM reduced glutathione. The presence of this water-soluble scavenging reagent did not modify the incorporation of label into the β subunit, thus indicating that the labeled domains of this polypeptide are

buried in the lipid matrix. This confirms the results recently obtained with photoreactive phosphatidylcholine derivatives that suggested the existence of hydrophobic domains of the glycoprotein subunit of purified (Na^+,K^+)-ATPase from pig kidney and from the electric organ of *Electrophorus electricus* (Montecucco et al., 1981).

In the present study, it is interesting to note the unequal reactivity of both subunits in microsomes. In fact, as suggested by the labeling pattern of microsomes (Figure 2b), the nitrene seemed to insert more readily in the α than in the β subunit. This was confirmed when the subunits were directly precipitated from labeled microsomes under conditions preventing the proteolytic degradation of the particularly labile α subunit (Figure 3B). The following explanations can account for these results. When both subunits are embedded in the membrane, the larger intramembranous segment of the catalytic subunit might have more nitrene reactive groups than the glycosylated polypeptide. Alternatively, the α subunit might shield the β subunit from reaction with the lipophilic probe. Both these possibilities are supported by the model of the path of the α-subunit polypeptide chain with multiple traverses across the bilayer (Jørgensen et al., 1982). These explanations are also consistent with the fact that both subunits are probably tightly associated in the membrane since drastic dissociating conditions are required in order to achieve independent immunoprecipitation of each subunit (Girardet et al., 1981). A similar unequal distribution of the same lipophilic probe has been observed between the two polypeptides of HLA-DRw antigens (Owen et al., 1980) and between glycophorin and band 3, two transmembranous proteins of erythrocytes (C. Bron and M. J. Crumpton, unpublished results).

In contrast, comparable heavy labeling of both α and β subunits was obtained when purified enzyme vesicles were reacted with the nitrene (Figure 2d). This could be a consequence of the higher nitrene/protein ratio used in the labeling of these preparations as compared to microsomes. Alternatively, the purification of the enzyme is known to result in the destruction of its oligomeric structure (Hansen et al., 1979). This might render the glycosylated subunit more accessible to the lipophilic probe.

In conclusion, the high specific activity of the radioactive probe used in this study in conjunction with specific immunochemical methods enabled us to identify clearly the glycoprotein labeled from within the membrane of kidney microsomes. This indicates that this protein is indeed embedded in the lipid bilayer and might therefore play a role in the transmembrane organization of the monovalent cation channel or carrier.

Acknowledgments

We thank Drs. R. Rodewald and M. J. Crumpton for helpful discussions and for revising the manuscript, M. C. Knecht for secretarial assistance, and Z. Friewald for photographic and graphic work.

Registry No. ATPase, 9000-83-3.

References

Castro, J., & Farley, R. A. (1979) *J. Biol. Chem. 254*, 2221–2228.

Chowdhry, V., & Westheimer, F. H. (1979) *Annu. Rev. Biochem. 48*, 293–325.

Farley, R. A., Goldman, D. W., & Bayley, H. (1980) *J. Biol. Chem. 255*, 860–864.

Geering, K., & Rossier, B. C. (1979) *Biochim. Biophys. Acta 566*, 157–170.

Geering, K., Girardet, M., Bron, C., Kraehenbühl, J. P., & Rossier, B. C. (1982a) *J. Biol. Chem. 257*, 10338–10343.

Geering, K., Girardet, M., Bron, C., Kraehenbühl, J. P., & Rossier, B. C. (1982b) *Prog. Clin. Biol. Res. 91*, 537–542.

Geering, K., Girardet, M., Kraehenbühl, J. P., & Rossier, B. C. (1982c) *J. Cell Biol. 95*, 379a (abstract).

Girardet, M., Geering, K., Frantes, J. M., Geser, D., Rossier, B. C., Kraehenbuhl, J. P., & Bron, C. (1981) *Biochemistry 20*, 6684–6691.

Hansen, O., Jensen, J., Møorby, J. G., & Ottolenghi, P. (1979) *Nature (London) 280*, 410–411.

Jørgensen, P. L., Karlish, S. J. D., & Gitler, C. (1982) *J. Biol. Chem. 257*, 7435–7442.

Karlish, S. J. D., Jørgensen, P. L., & Gitler, C. (1977) *Nature (London) 269*, 715–717.

Kyte, J. (1975) *J. Biol. Chem. 250*, 7443–7449.

Maizel, J. V., Jr. (1971) *Methods Virol. 5*, 179–246.

Montecucco, C., Bisson, R., Gache, C., & Johannsson, A. (1981) *FEBS Lett. 128*, 17–21.

Owen, M. J., Knott, J. C. A., & Crumpton, M. J. (1980) *Biochemistry 19*, 3092–3099.

Roelofsen, B., & Schatzmann, H. J. (1977) *Biochim. Biophys. Acta 464*, 17–36.