Journal of Neurochemistry
34(6):1745–1752, June. Raven Press, New York
© 1980 International Society for Neurochemistry

0022-3042/80/0601-1745/$02.00/0

Goldfish Brain (Na^+,K^+)-ATPase: Purification of the Catalytic Polypeptide and Production of Specific Antibodies

Michal Schwartz, George J. Siegel, Nelson Chen, and Bernard W. Agranoff

Departments of Biological Chemistry, Neurology and Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48109, U.S.A.

Abstract: The denatured catalytic polypeptide of (Na^+,K^+)-ATPase of goldfish brain was purified and identified as the ^{32}P-labeled phosphoprotein. The protein served as immunogen for the preparation of rabbit antisera for immunohistochemical application to goldfish tissue sections, using the peroxidase-antiperoxidase indirect method. Labeling in brain cross-sections appears primarily in fibers of the optic nerve layer of the tectum. In optic nerve cross sections, labeling is restricted to fiber bundles. **Key words:** Goldfish—(Na^+,K^+)-ATPase—Immunocytochemistry—Visual system—Optic nerve.

(Na^+,K^+)-ATPase is a membrane enzyme that couples ATP hydrolysis to active transport of Na^+ and K^+ ions (Dahl and Hokin, 1974; Skou, 1975; Glynn and Karlish, 1975; Schwartz et al., 1975). In nervous system development, its occurence parallels the appearance of excitability, as has been shown by the increase in ATPase concentration in brain microsomal fractions during the period of development (Bertoni and Siegel, 1978; 1979). This suggests the usefulness of the enzyme as a developmental and functional marker. A number of histochemical methods for its localization are available (Guth and Albers, 1974; Stahl and Broderson 1976*a,b*), including, most recently, immunohistochemical procedures (Wood et al., 1977). For the preparation of antibodies, the immunogen has been derived from non-neural tissues, since purest enzyme preparations have in the past been most readily obtained from organs other than the brain. Antiserum to a purified (Na^+,K^+)-ATPase from eel electroplax, however, has been shown to react with brain of a related species, the knifefish (Wood et al., 1977). We have found that this antibody preparation also reacts with the brain of the goldfish (Schwartz et al., 1979), a species that has served as a useful model for studies on regeneration. The recovery of vision following optic nerve section in the goldfish has been studied morphologically (Grafstein, 1975) and electrophysiologically, (Jacobson and Gaze, 1965; Gaze, 1970) as well as biochemically (Burrell et al., 1977). Furthermore, cultures of retinal explants (Landreth and Agranoff, 1979) have been demonstrated to exhibit extensive outgrowth of neurites from ganglion cells *in vitro*, providing a system for direct examination of the axolemmal ATPase in the absence of other cell types. In the course of these studies, a simple procedure for the purification of the denatured catalytic polypeptide of goldfish brain (Na^+,K^+)-ATPase was developed. We also report here the efficacy of the polypeptide as an immunogen in the rabbit and initial immunocytochemical studies with the antibody in goldfish brain and optic nerve preparations.

MATERIALS AND METHODS

Goldfish (*Carrasius auratus*), 6–7 cm in body length, were obtained from Ozark Fisheries, Stoutland, Missouri. Rabbits, 6–8 months old and weighing 2–3 kg each, were used for immunization. Normal goat serum and

Received October 24, 1979; accepted December 21, 1979.
Address reprint requests to B. W. Agranoff, Neuroscience Laboratory Building, The University of Michigan, 1103 East Huron, Ann Arbor, Michigan 48109, U.S.A.

Abbreviations used: M_r, Molecular weight; NGS, Normal goat serum; PAP, peroxidase-antiperoxidase; PB, Phosphate buffer; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

peroxidase-rabbit antiperoxidase soluble complex were obtained from Sternberger-Myer Immunocytochemicals Inc., and goat anti-rabbit IgG was a product of Miles Laboratories, Elkhart, Indiana.

(Na^+,K^+)-ATPase

Goldfish brain microsomes enriched in (Na^+,K^+)-ATPase were prepared by a modification of the method of Nakao et al. (1965), previously adapted for rat brain (Bertoni and Siegel, 1978). Samples were stored in liquid nitrogen for periods up to several months. Protein was measured by the method of Lowry et al. (1951). Assays of (Na^+,K^+)-ATPase activity were carried out according to the method of Siegel and Albers (1967). Microsomal fraction (2–5 μg protein) was incubated at 26°C for 10 min in a medium containing 75 mM-imidazole-HCl buffer (pH 7.4), 3 mM-$MgCl_2$, and 3 mM-Tris-[γ-^{32}P]ATP, with or without the addition of 80 mM-NaCl and 10 mM-KCl. Steady state levels of phosphorylation in 45 s at 2°C were obtained as described previously (Siegel and Fogt, 1976) in media containing 80–100 μg of microsomal protein, 3 mM-$MgCl_2$, 1 mM-Tris-[γ-^{32}P]ATP, and 75 mM-imidazole-HCl (7.4), with or without 100 mM-NaCl or 50 mM-KCl.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

For batch preparation of catalytic protein, SDS-PAGE was performed as described by Hokin et al. (1973) except that slabs 165 × 190 × 3 mm were used. NaI-extracted microsomal suspensions (270 μl containing 2–4 mg of protein) were added to equal volumes of solubilizing medium (10% glycerol, 5% mercaptoethanol, 3% SDS, and 62.5 mM-Tris-HCl buffer, pH 8.8), and the combined mixture was applied immediately to the slab gel. After electrophoresis at 4°C, gels were fixed and stained with Coomassie blue. Na^+-dependent, acid-stable, nonexchangeable phosphoprotein labeled with ^{32}P as described in Methods was subjected to SDS-PAGE (Siegel and Fogt, 1976). Cylindrical gels were halved, wrapped in Mylar (Saran Wrap), frozen, applied to Kodak XRP-1 film in a cassette, and exposed at −20°C for 5 days. Some gels were sliced for scintillation counting (Siegel and Fogt, 1976). A single phosphoprotein band was detected and had the same R_f value by either method. Samples phosphorylated in media containing 50 mM-K^+ instead of Na^+ yielded no labeled bands. Slabs as well as cylindrical gels in many experiments characteristically exhibited, under these conditions, a very dense single-staining band in the neighborhood of the labeled phosphoprotein. By inspection, this was always the most dense band on the gel, in agreement with densitometric data from rat brain samples prepared in the same way (Bertoni and Siegel, 1978; 1979).

Isoelectric Focusing

Isoelectric focusing was performed according to a published method (Danno, 1977) with minor modifications. The gels contained, in final concentration: 4.9% (w/v) acrylamide, 0.13% (w/v) *N,N'*-methylenebisacrylamide (both from Eastman Kodak), 8 M-urea, 0.075% (v/v) TEMED, 6% (v/v) ampholines (Biorad pH 3–10), and 0.5% (w/v) Triton X-100. The Triton (Sigma) was first treated with mixed-bed ion exchange resin, and the pH adjusted to 8.5 with NH_4OH (Banga et al., 1978). After polymerization, the gels were covered with a 5-mm layer of 4% (w/v) sucrose, and then the samples were applied over the gel surface beneath the sucrose layer. Finally, the sucrose layer was covered with 0.02 M-NaOH. The lower reservoir contained 0.01 M-H_3PO_4 and the upper contained 0.02 M-NaOH. Focusing was started with constant current (10 mA) until the voltage rose to 250–350 mV, at which time the setting was switched to constant voltage. Focusing was carried out in a cold room (4°C) overnight. For protein staining, gels were soaked in 500 ml of 50% aqueous methanol for 36 h with two changes. Gels were then fixed and stained in water-methanol-acetic acid (5:5:1) and with 0.01% Coomassie blue overnight. Destaining was accomplished by soaking the gels in water-methanol-acetic acid (5:5:1).

Preparation of Rabbit Antisera

The catalytic protein was extracted into 10% SDS from the excised band of SDS-acrylamide gel. The extracted protein was then dialyzed against water at room temperature and lyophilized. This protein was used as the immunogen. Samples were subjected to isoelectric focusing and repeat SDS-PAGE.

Rabbits were bled prior to the primary inoculation to obtain preimmune sera. The first inoculum consisted of approximately 100 μg of goldfish brain (Na^+,K^+)-ATPase catalytic protein emulsified in complete Freund's adjuvant (CFA). Three weeks later a booster injection was given with the same amount of antigen emulsified in incomplete Freund's adjuvant. All sera were stored at −20°C.

Preparation of Tissue Sections

Optic nerves and brains were removed from adult goldfish, quickly frozen, and sectioned in a cryostat. Sections were dried on a hot plate at 70°C and then fixed for 1 h in AFA, a mixture of 80% aqueous ethanol, formalin, and glacial acetic acid (90:5:5, by vol.).

Binding of Anti-Goldfish (Na^+,K^+)-ATPase Catalytic Protein

Detection of antigenic determinants in goldfish brain and optic nerve was accomplished with the peroxidase-antiperoxidase (PAP) method of Sternberger (1976). Fixed tissue sections were preincubated with normal goat serum (NGS) diluted 1:4 in 0.12 M-sodium phosphate buffer, pH 7.2 (PB). After preincubation, the samples were incubated for 1 h with the primary antisera diluted in PB. After this incubation, they were rinsed for 2 h with PB containing 1% NGS and then incubated for 30 min with goat anti-rabbit IgG (320 μg/ml). The samples were rinsed for 1 h and then further incubated for 30 min with a solution of 1% NGS containing a soluble complex of peroxidase-rabbit antiperoxidase (3:2) diluted 1:40. After this last incubation the samples were rinsed for 30 min in

PB and for an additional 30 min in 0.05 M-Tris-HCl buffer (pH 7.6). The bound peroxidase was then detected by its enzymatic reaction with 3,3'-diaminobenzidine (60 mg/100 ml) in the presence of 0.06% hydrogen peroxide.

RESULTS

Table 1 shows the typical yield for a NaI-extracted microsomal preparation from goldfish brain. Although the (Na^+,K^+)-ATPase activity is low, a small amount of starting tissue (9 g) provided quantities of pure catalytic protein ample for analytic and immunological studies, as is shown below. Moreover, the initial volume can be increased more than 10-fold without inconvenience.

A Coomassie blue-stained SDS-polyacrylamide gel containing the NaI-extracted microsomal fraction is shown in Fig. 1, A. The densest staining band (R_f 0.26) corresponds to the Na^+-dependent phosphoprotein demonstrated in the autoradiograph (Fig. 1, B). The band corresponding to the catalytic protein was excised from the slab with a razor blade. After extraction, dialysis, and lyophilization of the protein as described in Methods, samples were subjected to isoelectric focusing and a repetition of SDS-PAGE. The samples contained only one Coomassie blue band by both methods (Fig. 2, A and B).

If the catalytic protein is about 5% of the microsomal protein in these NaI-extracted preparations,

TABLE 1. *Yield of microsomal (Na^+,K^+)-ATPase from goldfish brain*

	ATPase ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	Phosphoprotein ($nmol \cdot mg^{-1}$)
Δ Mg^{2+}	0.26	0.14
Δ (Na^+,K^+)	0.58	—
Δ Na^+	—	0.22

One hundred goldfish brains, 9.2 g wet weight, were homogenized; the NaI-extracted microsomal fraction yielded 21.4 mg protein.

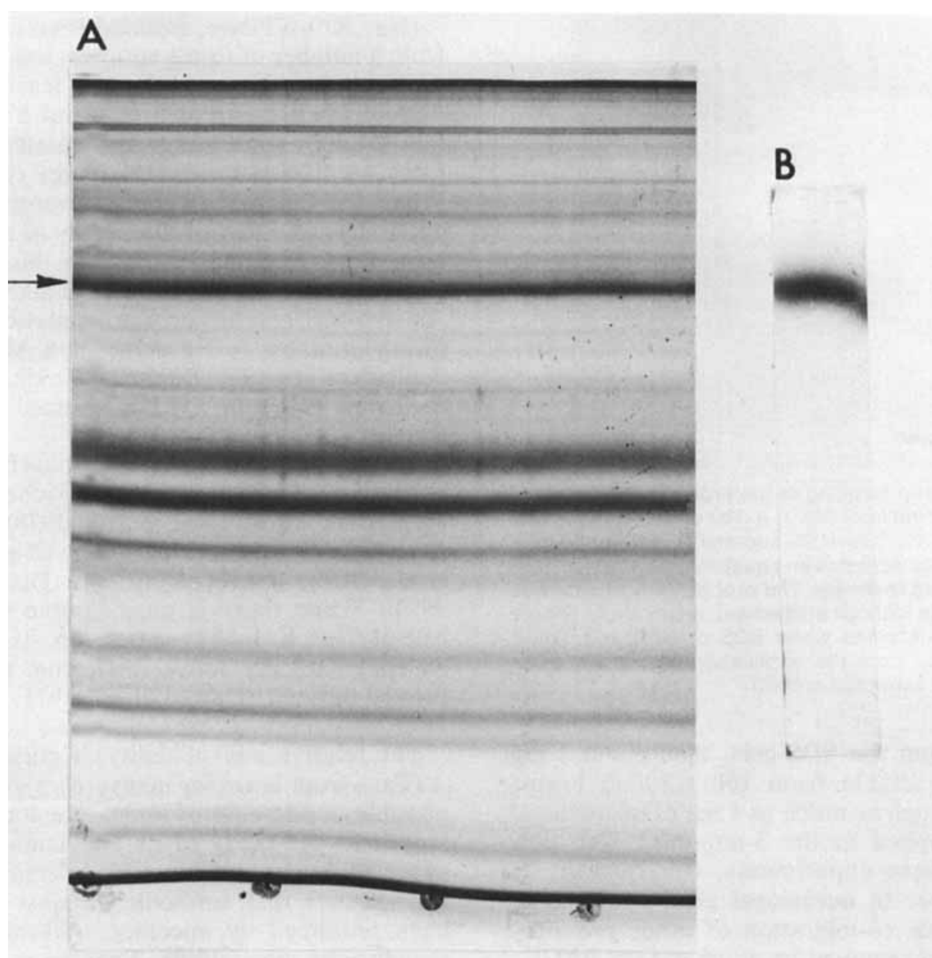


FIG. 1. **(A)** SDS-PAGE of goldfish brain NaI-extracted microsomal fraction. The applied sample contained 2.15 mg of microsomal protein. The slab gel is stained with Coomasie blue. R_f of the catalytic polypeptide (densest staining band in 100,000 M_r region) is 0.26 (arrow). **(B)** Phosphorylation and SDS-PAGE of NaI-extracted microsomal pellet from goldfish brain. The cylindrical gel was exposed to Kodak XRP-1 film for 5 days at −20°C.

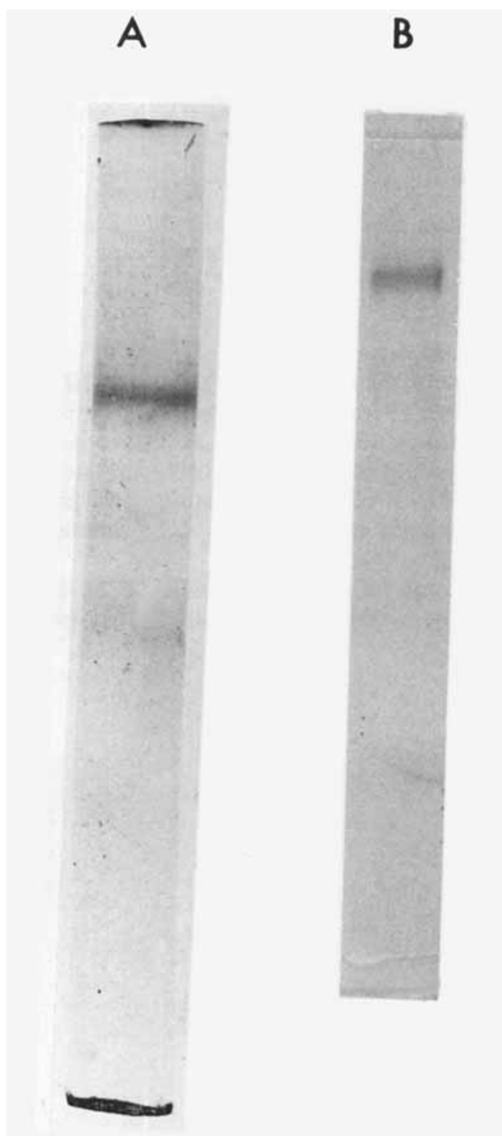


FIG. 2. (A) Isoelectric focusing of the protein extracted from the excised strip of gel from Fig. 1, A. The protein sample was first treated with 1.5% SDS in 5% sucrose at 23°C for 20 min, and this solution was added to an equal volume of 20% Triton X-100 for application to the gel. The pI of the polypeptide was 6.1 and 5.9 with and without SDS added, respectively. Single bands were also obtained when SDS or SDS and Triton X-100 were omitted from the application media. **(B)** SDS-PAGE of the same extracted protein.

as estimated from the SDS-gels, then about 1 mg should be extractable from 100 goldfish brains (Table 1). Although as much as 4 mg of microsomal protein was applied to the 3-mm-thick slab gels employed in these experiments, this quantity is nearly excessive. In occasional gels, overloading led to detectable co-migration of other proteins, which were then removed by another SDS-PAGE.

Figure 3 (A and B) demonstrates the immunocytochemical reaction of a tissue section through the goldfish tectum with the antisera followed by the PAP method. In the optic tectum, labeling was seen mainly in stratum opticum (SO), in part of the stratum fibrosum et griseum superficiale (SFGS), and in the innermost layer of the optic tectum, the stratum periventriculare (SPV) (Meek and Schellart, 1978; Fig. 3, B). Ventral to the tectum, the lamina commissuralis tecti (TC) and tractus spinotectalis (TST) (Kappers et al., 1960) were labeled. Reaction product was also seen in the cells of the midbrain tegmentum (MT) (Peter and Gill, 1975) and in the fiber layer of the cerebellum (C) (Fig. 3, A).

Cross sections as well as longitudinal sections of optic nerve exhibited high activity with the catalytic protein antisera (Fig. 4). Positive reactions were achieved with sera diluted as much as 1:160. It should be noted that the binding of the antisera was confined to axonal bundles only. No binding was detected in the epineurium or other surrounding tissue.

DISCUSSION

(Na^+,K^+)-ATPase, isolated from cell membranes from a number of tissue sources, has been shown in each instance to consist of at least one catalytic protein (84,000–110,000 M_r) and a sialoglycoprotein (44,000–60,000 M_r), as indicated by migration rates on SDS-polyacrylamide gel electrophoresis. While the function of the glycoprotein is unclear, antibodies to either of the chains of the eel enzyme have been reported to produce (Na^+,K^+)-ATPase inhibition (Jean et al., 1975; Jean and Albers, 1976). An aspartyl residue on the catalytic unit is phosphorylated by [γ-^{32}P]ATP in a Mg^{++} + Na^+)-dependent reaction (Dahl and Hokin, 1974) and, as indicated, this property can be used to identify the protein.

Tissues that have served as useful sources for enzyme purification include the kidney (Kyte, 1971; Jorgensen, 1974; Lane et al., 1979), shark rectal gland (Hokin et al., 1973), duck salt gland (Hopkins et al., 1976), and eel electroplax (Dixon and Hokin, 1974). While there is considerable interest in the role of (Na^+,K^+)-ATPase in brain, its purification in an enzymatically active form from this tissue has proved difficult (Uesugi et al., 1971; Klodos et al., 1975; Sweadner, 1978).

The relative unavailability of purified (Na^+,K^+)-ATPase from brain for many years discouraged the possible application of immunohistochemical techniques to the study of its localization with brain, although Askari and Rao (1972) demonstrated in tissue extracts that antibodies against the brain had been produced by injecting rat brain microsomal suspensions into rabbits. Enzymatic histochemical approaches have been applied to the studies of the brain enzyme (Na^+,K^+)-ATPase. Using *p*-nitrophenylphosphate as substrate, the enzyme has been

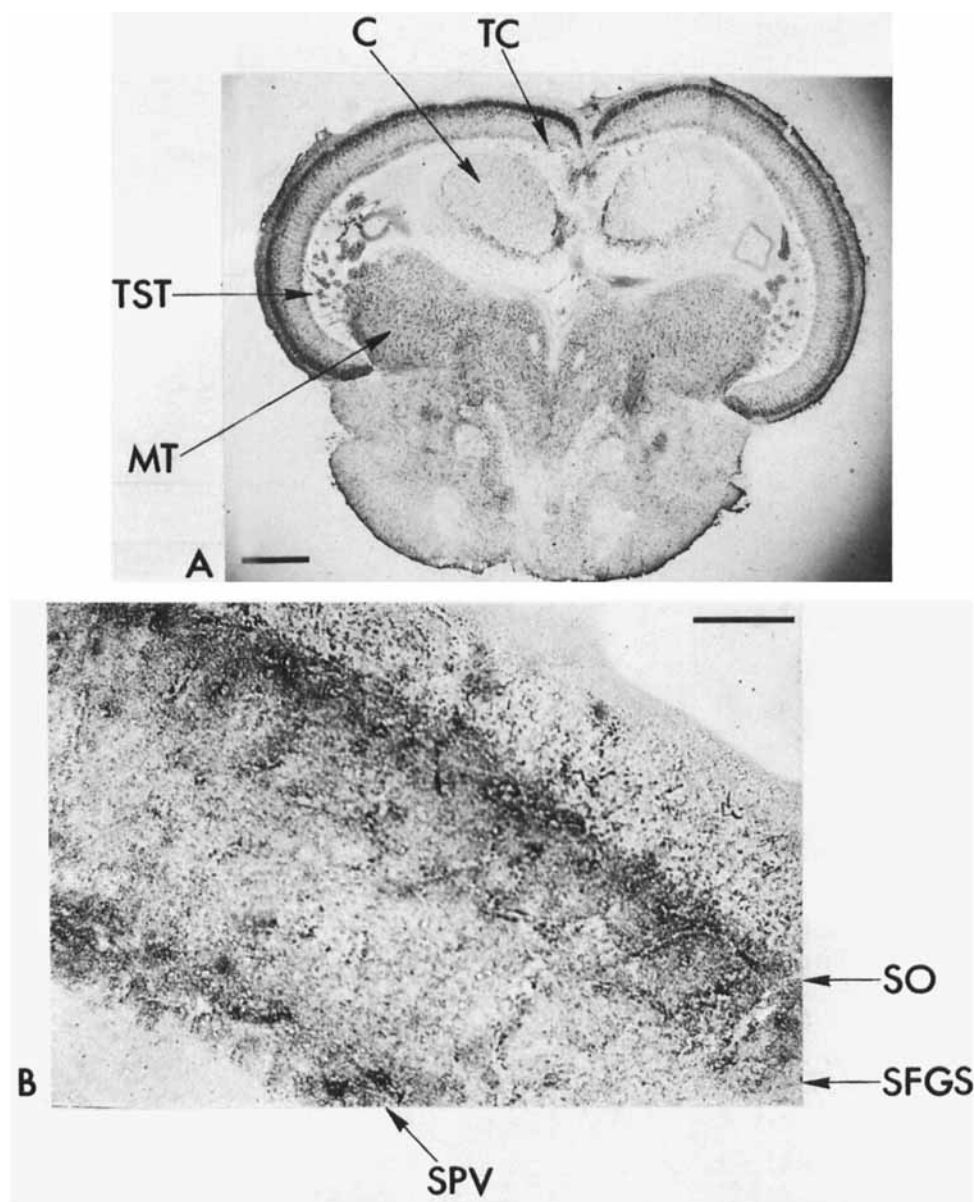
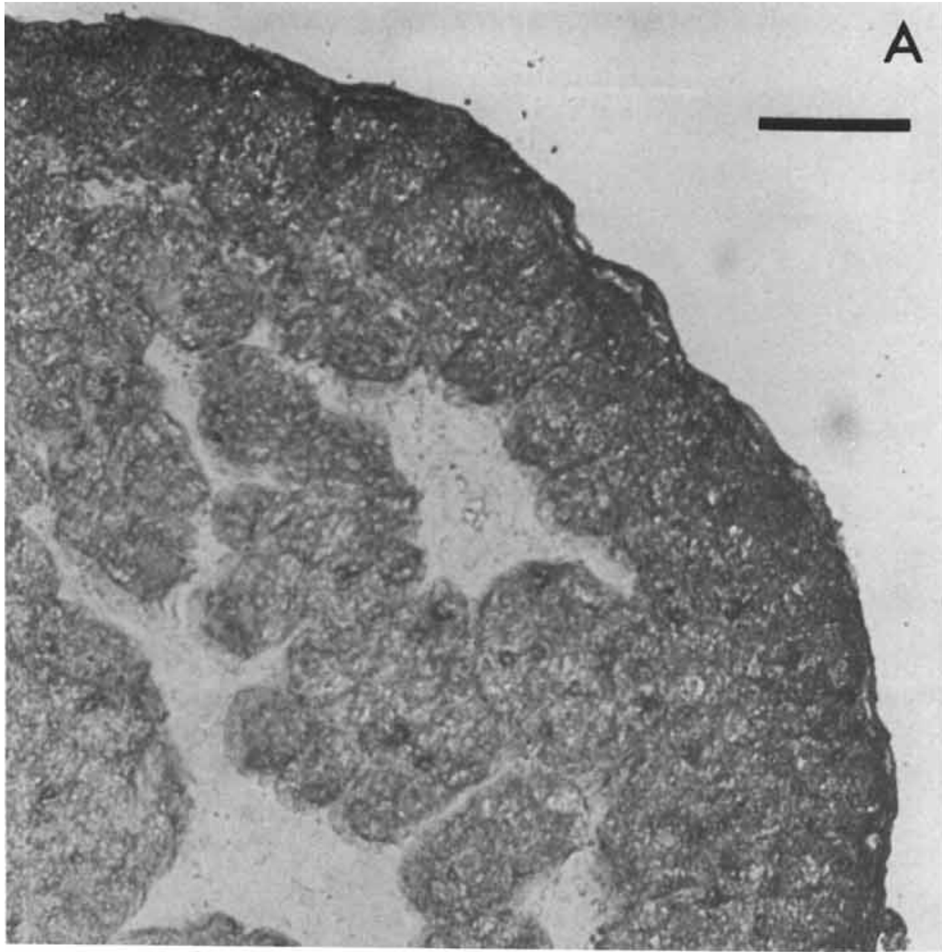
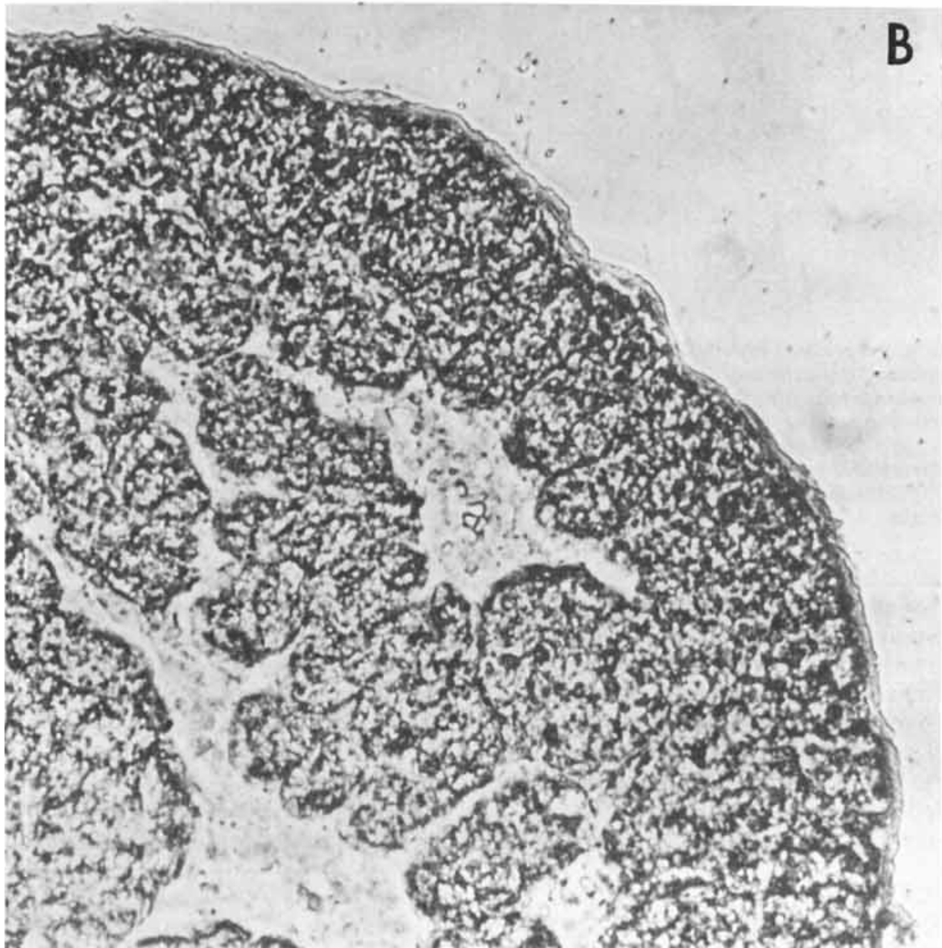


FIG. 3. Binding of rabbit antisera (diluted 1:40) directed against (Na^+,K^+)-ATPase catalytic polypeptide. **(A)** Photomicrograph of goldfish brain section (10 μm frozen section) through the tectum. In addition to the tectal layers, labeling is seen mainly in cerebellum (C), midbrain tegmentum (MT), lamina commissuralis tecti (TC), and tractus spinotectalis (TST). Space bar = 0.24 mm. Artifactual labeling seen at the edges of the section is attributed to the cryoform embedding agent.

(B) Photomicrograph (bright field) of a region of the optic tectum (between the arrows pointing to C and TC). Labeling is seen in the following layers: stratum opticum (SO), stratum fibrosum et griseum superficiale (SFGS), and stratum periventriculare (SPV). Brain sections treated similarly but with preimmune sera substituted for immune sera showed no detectable labeling. Space bar = 21.7 μm.

localized in brain at the light microscopic (Guth and Albers, 1974; Stahl and Broderson, 1976*a,b*) as well as at the electron microscopic level (Broderson et al., 1978). In these studies, reaction product was found mainly over axons, fine neuronal and glial processes, and neuropil, but not over neuronal or glial cell bodies. Wood et al. (1977) found that antibody to purified denatured electroplax (Na^+,K^+)-ATPase reacted with membrane protein in the brain of the knifefish, a species closely related to the eel, the immunogen source. In an electron microscopic study, immunoreactive sites were observed on plasma membrane, spread over glia and neuronal perikarya, dendrites, glial processes, and outermost lamellae of mylein. In regions containing myelinated fibers, the common reaction products along the axolemma were observed only at the nodes of Ranvier, and not at internodal segments. We recently completed con-


A


B

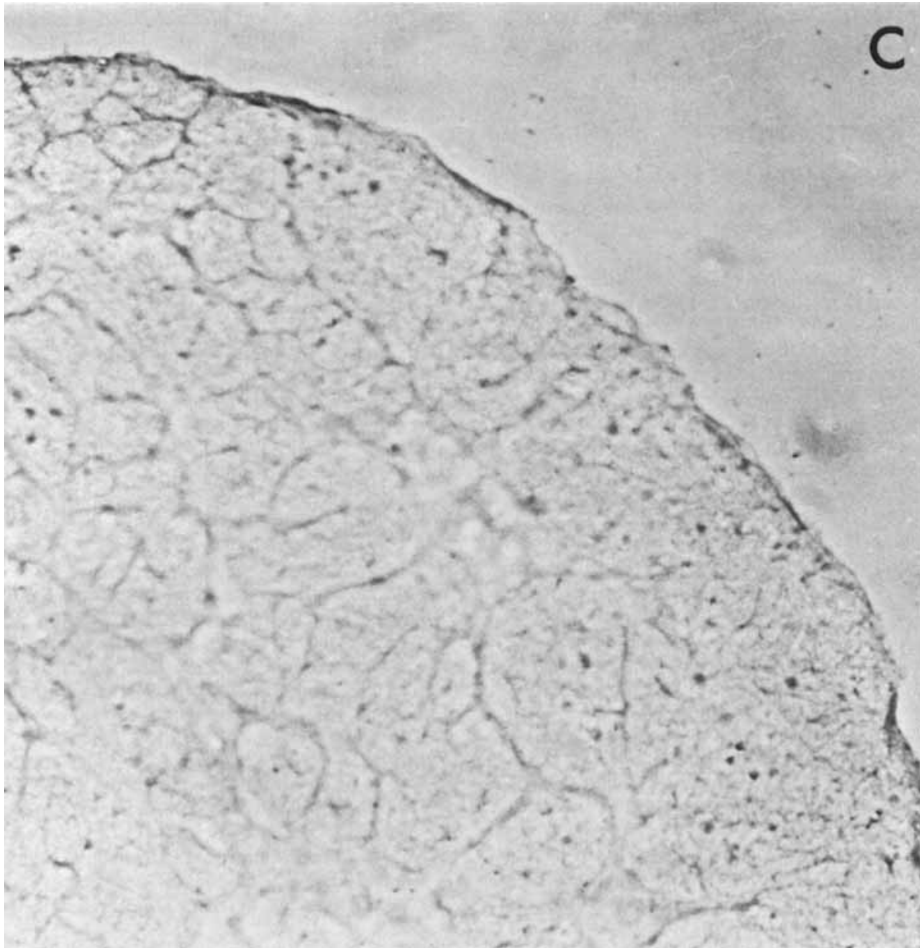


FIG. 4. Localization of (Na^+,K^+)-ATPase antigenic sites in adult goldfish optic nerve. Cross sections (10 μm) were treated according to the peroxidase-antiperoxidase procedure described in Methods. **(A)** Bright field micrograph of section treated with antisera directed to the goldfish brain (Na^+,K^+)-ATPase catalytic polypeptide. **(B)** The same section viewed by phase contrast. **(C)** Phase contrast micrograph of a control section treated as A and B but incubated with preimmune serum obtained from the rabbit used for production of the antiserum. Space bar for A, B, and C = 20 μm.

firmatory studies demonstrating the nodal distribution of (Na^+,K^+)-ATPase in the goldfish optic nerve, using the eel antiserum (Schwartz et al., 1979). These interesting results suggest concentration of (Na^+, K^+)-ATPase at the node of Ranvier, although additional studies are needed to rule out the possibility that the observed distribution reflects variable penetrability of the immunoreagents. In the course of these studies, a more specific antiserum has been obtained by using goldfish brain rather than electroplax enzyme as the immunogen.

A native immunogen might be advantageous for a number of applications, such as the study of membrane conformation in the region of the active site. The use of denatured enzyme (Jean et al., 1975; Jean and Albers, 1976; present study) may have other advantages in that additional antigenic determinants are expressed. While enzyme active sites are characterized by a high degree of specificity, membrane-bound proteins may share common sequences elsewhere in the peptide chain. If such common regions become exposed upon denaturation, and in addition are highly immunogenic, the possibility arises that antisera to the denatured proteins will be directed to classes of, in addition to specific, proteins. At the light microscopic level, it is clear that the antibody preparation in the present study acts as an adequate nerve fiber marker, as might have been anticipated from the known physiological role of (Na^+,K^+)-ATPase. Current studies are under way at the electron microscopic level using the specific antibody to goldfish brain (Na^+,K^+)-ATPase catalytic unit presented here.

ACKNOWLEDGMENT

This research was supported by grants NSF PCM75-05979 and NINCDS-13743. Dr. M. Schwartz was supported by a fellowship from the

Weizmann Institute of Science, Rehovot, Israel. We thank Prof. Roger E. Davis for assistance in interpreting the histological observations.

REFERENCES

Askari A. and Rao S. N. (1972) (Na^+,K^+)-ATPase complex: Effects of anticomplex antibody on the partial reactions catalysed by the complex. *Biochem. Biophys. Res. Commun.* **49**, 1323–1328.

Banga J. P., Anderton B. H., and Roitt I. M. (1978) Separation of membrane proteins in an undenatured form by isoelectric focusing. *Anal. Biochem.* **89**, 348–354.

Bertoni J. B. and Siegel G. J. (1978) Development of (Na^+-K^+)-ATPase in rat cerebrum: Correlation with Na^+-dependent phosphorylation and K^+-paranitrophenylphosphatase. *J. Neurochem.* **31**, 1501–1511.

Bertoni J. B. and Siegel G. J. (1979) Development of (Na^+-K^+)-ATPase in rat hindbrain—increments in parallel with Na^+-dependent phosphorylation and K^+-paranitrophenylphosphatase. *J. Neurochem.* **32**, 573–580.

Broderson S. H., Patton D. L. and Stahl W. L. (1978) Fine structural localization of potassium stimulated *p*-nitrophenylphosphatase activity in dendrites of cerebral cortex. *J. Cell Biol.* **88**, R 13–R 17.

Burrell H. R., Heacock A. M., Water R. D., and Agranoff B. W. (1978) Increased tubulin messenger RNA in the goldfish retina during optic nerve regeneration. *Brain Res.* **168**, 628–632.

Dahl J. L. and Hokin L. E. (1974) Sodium-potassium adenosinetriphosphatase. *Annu. Rev. Biochem.* **43**, 327–356.

Danno G. (1977) Isoelectric focusing of proteins separated by SDS-polyacrylamide gel electrophoresis. *Anal. Biochem.* **83**, 189–193.

Dixon J. F. and Hokin L. E. (1974) Studies on characterization of sodium potassium transport adenosinetriphosphatase. 16. Purification and properties of enzyme from electric organ of *Electrophorus electricus*. *Arch. Biochem. Biophys.* **163**, 749–758.

Gaze R. M. (1970) *The Formation of Nerve Connections.* Academic Press, London.

Glynn I. M. and Karlish S. J. D. (1975) The sodium pump. *Annu. Rev. Physiol.* **37**, 13–55.

Grafstein B. (1975) The nerve cell body response to axotomy. *Exp. Neurobiol.* **48**, 32–51.

Guth L. and Albers R. W. (1974) Histochemical demonstration of (Na^+,K^+)-activated adenosine triphosphatase. *J. Histochem. Cytochem.* **22**, 320–326.

Hokin L. E., Dahl J. L., Deupree J. D., Dixon J. F., Hackney J. F., and Perdue J. F. (1973) Studies on the characterization of sodium-potassium transport adenosine triphosphatase. X. Purification of the enzyme from rectal gland of *Squalus acanthias*. *J. Biol. Chem.* **248**, 2593–2605.

Hopkins B. E., Wagner H., Jr., and Smith T. W. (1976) Sodium and potassium-activated adenosine triphosphatase of the nasal salt gland of the duck (*Anas platyrhynchos*). *J. Biol. Chem.* **251**, 4365–4371.

Jacobson M. and Gaze R. M. (1965) Selection of appropriate tectal connections by regenerating optic nerve fibers in adult goldfish. *Exp. Neurol.* **13**, 418–430.

Jean D. H. and Albers R. W. (1976) Immunochemical studies on the large polypeptide of *Electrophorus* electroplax (Na^+ + K^+)-ATPase. *Biochem. Biophys. Acta* **452**, 219–226.

Jean D. H., Albers R. W., and Koval G. J. (1975) Sodium-potassium activated adenosine triphosphatase of Electrophorus electric organ. 10. Immunochemical properties of the lubrol-solubilized enzyme and its constituents. *J. Biol. Chem.* **250**, 1035–1040.

Jorgensen P. L. (1974) Purification and characterization of (Na^+ + K^+)-ATPase. IV. Estimation of the purity and of the molecular weight and polypeptide content per enzyme unit in preparation from the outer medulla of rabbit kidney. *Biochem. Biophys. Acta* **356**, 53–67.

Kappers C. U. A., Huber G. C., and Crosby E. C. (1960) *The Comparative Anatomy of the Nervous System of Vertebrates Including Man*, Vol. II. Hafner Publishing Company, New York.

Klodos I., Ottolenghi P., and Boldyrev A. (1975) Large scale preparation of (Na^+,K^+)-ATPase from ox brain. *Anal. Biochem.* **67**, 397–403.

Kyte J. (1971) Purification of the sodium- and potassium-dependent adenosine triphosphatase from canine renal medulla. *J. Biol. Chem.* **246**, 4157–4165.

Landreth G. E. and Agranoff B. W. (1979) Explant culture of adult goldfish retina: A model for the study of CNS regeneration. *Brain Res.* **161**, 39–53.

Lane L. K., Potter J. D., and Collins J. H. (1979) Large scale purification of Na,K-ATPase and its protein subunits from lamb kidney medulla. *Prep. Biochem.* **9**, 157–170.

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Meek J. and Schellart N. A. M. (1978) A Golgi study of goldfish optic tectum. *J. Comp. Neurol.* **182**, 89–122.

Nakao T., Tashuma Y., Nagano K., and Nakao M. (1965) Highly specific sodium-potassium activated adenosine triphosphatase from various tissues of rabbit. *Biochem. Biophys. Res. Commun.* **19**, 755–758.

Peter R. E. and Gill V. E. (1975) A stereotaxic atlas and technique for forebrain nuclei of the goldfish, *Carrasius auratus*. *J. Comp. Neurol.* **159**, 69–102.

Schwartz A., Lindenmayer G. E., and Allen J. C. (1975) The sodium potassium adenosine triphosphatase pharmacological, physiological and biochemical aspects. *Pharmacol. Rev.* **27**, 3–134.

Schwartz M., Feldman E. L., Jean D. H., Siegel G. J., and Agranoff B. W. (1979) Na-K-ATPase in the regenerating goldfish visual system. *Trans. Am. Soc. Neurochem.* **10**, 233.

Siegel G. J. and Albers R. W. (1967) Sodium potassium activated adenosine triphosphatase of Electrophorus electric organ. IV. Modification of response to sodium and potassium by arsenite plus 2,3-dimercaptopropanol. *J. Biol. Chem.* **193**, 265–275.

Siegel G. J. and Fogt S. K. (1976) Lead ion activates phosphorylation of electroplax (Na^+,K^+)-ATPase in the absence of sodium ion. *Arch. Biochem. Biophys.* **174**, 744–746.

Skou J-C. (1975) The (Na^+,K^+) activated enzyme system and its relationship to transport of sodium and potassium. *Quart. Rev. Biophys.* **7**, 401–434.

Stahl W. L. and Broderson S. H. (1976*a*) Histochemical localization of potassium stimulated *p*-nitrophenyl phosphatase activity in somatosensory cortex of the rat. *J. Histochem. Cytochem.* **24**, 731–739.

Stahl W. L. and Broderson S. H. (1976*b*) Localization of (Na^+,K^+)ATPase in the brain. *Fed. Proc.* **35**, 1260–1265.

Sternberger L. A. (1976) *Immunocytochemistry*, Prentice-Hall, Englewood Cliffs, New Jersey.

Sweadner K. J. (1978) Purification from brain of an intrinsic membrane protein fraction enriched in (Na^+ + K^+)-ATPase. *Biochem. Biophys. Acta* **508**, 486–499.

Uesugi S., Dulak N. C., Dixon J. F., Hexum T. D., Dahl J. L., Perdue J. F., and Hokin L. E. (1969) Studies on the characterization of the sodium potassium transport adenosine triphosphatase. IV. Large scale purification and properties of a lubrol solubilized bovine brain enzyme. *J. Biol. Chem.* **25**, 531–543.

Wood J. B., Jean D. H., Whitaker J. N., McLaughlin N. J., and Albers R. W. (1977) Immunocytochemical localization of sodium potassium ATPase in knifefish brain. *J. Neurocytol.* **6**, 571–581.