RELEASE OF RNA FROM GOLDFISH BRAIN NUCLEI BY SODIUM DODECYL SULFATE*

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Intracranial injections of antimetabolites which block protein or RNA synthesis have been shown to prevent formation of memory of shock avoidance in the goldfish (Agranoff et al., 1967). Correlative studies carried out in this laboratory have shown the presence in the goldfish brain of RNA molecules which become labeled soon after intracranial injection of tritiated uridine (Casola and Agranoff, 1967). Rapidly labeled RNA (sedimenting 10S -.>45S) have been found in pure nuclear fractions from goldfish as well as in rat (Jacob et al, 1966), rabbit (Vesco and Giuditta, 1967) and mouse (Kimberlin, 1967) brain. While the 45S region is heavily labeled following a pulse of labeled precursor, the optical density profile of brain RNA extracted from a purified nuclear fraction in all animals tested has failed to show a discrete peak in the corresponding sucrose density region. Furthermore the amount of material sedimenting in the gradient at approximately 18S largely exceeded the amount of 18S ribosomal RNA expected within the nuclei.

Most of the work described above was done with RNA preparations extracted from brain nuclei by the use of cold and/or hot phenol. The work to be reported in this publication is an attempt to ascertain to

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what degree the various types of nucleic acids can be extracted from brain nuclei without the use of phenol. The results show that a relatively mild treatment with sodium dodecyl sulfate (SDS) of DNase-digested nuclei appears sufficient to extract most of the nuclear RNA, including the 45S ribosomal RNA precursor. The pattern of radioactivity following an intracranial injection with a radioactive precursor now reveals that most of the label sediments at 45S, indicating the presence of active synthesis of ribosomal RNA in the goldfish brain.

MATERIALS AND METHODS

Isolation of nuclei. Brains from 15 goldfish were homogenized by hand with 15 ml of 0.32 M sucrose in 0.001 M potassium phosphate buffer (pH 6.5) and 0.002 M $CaCl_2$ (sol. A). The homogenate was filtered through 8 layers of cheesecloth and centrifuged for 10 min at 850 x g. The sediment was washed twice more by gentle homogenization with 10 ml of sol. A and centrifuged as above. The resulting crude nuclear pellet was suspended by gentle homogenization in 5 ml of sol. A and mixed with 26 ml of 2.39 M sucrose in 0.001 M potassium phosphate buffer (pH 6.5) and 0.002 M $CaCl_2$. After centrifugation in a Spinco SW25 rotor at 25,000 rpm for 45 min, the nuclei were obtained as a pellet while cytoplasmic debris remained at the top of the tube.

Release of RNA from nuclei. The pure nuclei were lysed with 1.5 ml of 0.5 M NaCl in 0.01 M Tris-HCl buffer (pH 7.4) and 0.01 M $MgCl_2$. After digesting the nucleohistone gel with approximately 100 μg of electrophoretically pure DNase (20° for 2 min, or until viscous clumps disappear), the preparation was made 0.5% with respect to SDS and layered directly over 30 ml of a 10-50% sucrose gradient (in 0.1 M NaCl, 0.01 M Tris buffer (pH 7.4), and 0.001 M EDTA). The gradients were centrifuged at 23,000 rpm for 17 hr at 4° in the SW25 rotor.

The contents of each tube were then analyzed for materials absorbing at 254 mμ by pumping through an absorbance-recording fraction collector (LKB) equipped with a flow cell (0.4 cm path length). After addition of

100 μg of bovine albumin the TCA-precipitable material of each tube was collected on nitrocellulose filters (Gelman, 0.4 mμ) and counted for radioactivity by scintillation techniques.

RESULTS AND DISCUSSION

The data of Fig. 1 show a typical sucrose density gradient analysis of nuclear RNA released by SDS. Under the conditions employed here (10-50%

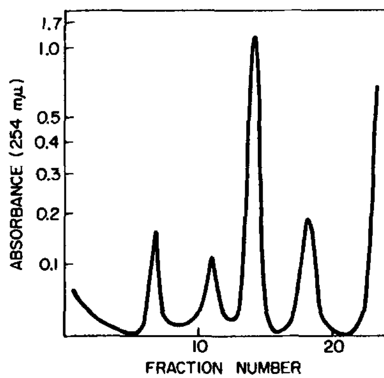

Fig. 1. Sedimentation analysis of goldfish brain nuclear RNA extracted with SDS (see text for details). The direction of sedimentation in the 10-50% sucrose gradient is from right to left. The three lighter peaks correspond to 45S, 32S and 18S.

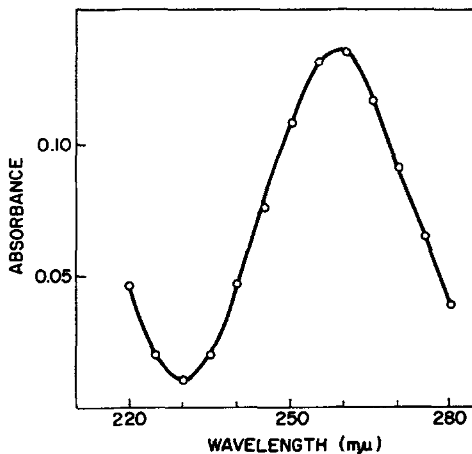

Fig. 2. Absorbance spectrum of contents of tube number 14 (Fig. 1).

sucrose gradient) a sharp band is seen sedimenting in the lower third of the tube (fraction 7). This band is most probably composed of nuclear and endoplasmic reticulum membranes. The optical density profile of the remainder of the gradient, nuclear RNA, shows a fast sedimenting peak corresponding to the 45S ribosomal RNA precursor (Penman, 1966). Two more slowly sedimenting peaks with approximate sedimentation values of 32S and 18S can be seen in fraction 14 and 18 of the gradient. The amount of 18S is very small compared to the large peak of optical density of 32S RNA. The absorbance spectrum of the material sedimenting at 32S shows the characteristics of pure, undegraded RNA (Fig. 2). Following a 20 min pulse of ^{3}H-uridine injected intracranially into 15 goldfish, a large fraction of the radioactivity coincides with the observed 45S optical density peak (Fig. 3). In addition, material of low specific radioactivity (<8 S) is found as well as label distributed throughout the gradient in a polydisperse fashion.

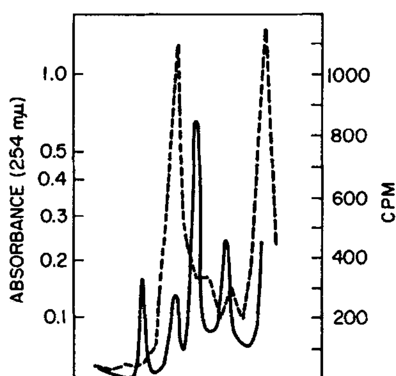

Fig. 3. Pattern of RNA labeling by uridine-5-^{3}H in goldfish brain nuclei. Fifteen goldfish were injected intracranially with 10 μc of uridine-5-^{3}H (8.0 c/mmole) and sacrificed 20 min later. RNA from the pooled brains was extracted as described in the text. Solid line, absorbance at 254 mμ; dashed line, TCA-insoluble radioactivity. The radioactivity present in the pellet (1513 cpm) may be due either to the presence of unbroken nuclei or to some polydisperse RNA uncompletely released by SDS. Background = 14 cpm.

The release of RNA from brain nuclei by SDS reported here provides a fast and useful method, which brings about the release of the major components of RNA normally present in brain nuclei. The physiological presence of 18S RNA in nuclei has been questioned (Penman, 1966), and the present study supports the idea that it is absent. The relatively small amount found in our goldfish brain nuclear preparation may be accounted for, partly by endoplasmic reticulum contamination and partly by the physiological presence of small amounts of 18S RNA being transported from the nuclear site of synthesis into the cytoplasm. The large peak of radioactivity sedimenting at 45S after a short pulse with labeled uridine indicates active synthesis of ribosomal RNA in the goldfish brain. That RNA molecules other than ribosomal precursor are labeled during this brief pulse is suggested by the presence of polydisperse radioactivity throughout the gradient as has also been observed in rat and rabbit brain. This rapidly-labeled polydisperse RNA may at least in part represent precursors to cytoplasmic messenger RNA.

REFERENCES

Agranoff, B. W., Davis, R. E., Casola, L., and Lim, R., Science, 158, 1600 (1967).

Casola, L. and Agranoff, B. W., Abstracts 1st International Meeting of Neurochemistry, Strasbourg (1967).

Jacob, M., Stevenin, J., Jund, R., and Mandel, P., J. Neurochem. 13, 619, (1966).

Kimberlin, R. H., J. Neurochem. 14, 123 (1967).

Penman, S., J. Mol. Biol. 17, 117 (1966).

Vesco, C. and Giuditta, A., Biochim. Biophys. Acta 142, 385 (1967).