AN ORGAN SPECIFIC RIBONUCLEOPROTEIN FROM GOAT BRAIN*

N.C. Sharma and G.P. Talwar

Department of Biochemistry, All-India Institute of Medical Sciences, New Delhi-16, India

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Summary -- An organ specific fast migrating protein isolated from goat brain by preparative electrophoresis on polyacrylamide gel columns of the fraction remaining unprecipitated by 75% saturation with ammonium sulphate has been found to be a ribonucleoprotein. This conclusion has been derived from a) absorption characteristics of the protein, b) shifts in absorption maxima after ribonuclease treatment, c) chemical tests showing the presence of ribose, purines and pyrimidines.

In recent years a number of organ specific proteins have been isolated and identified in the brain (Moore, 1965; Moore and Perez, 1968, Rajam and Bogoch, 1966; MacPherson and Liakopoulou, 1966; Warecka, 1970). Amongst these the earliest discovered was an acidic fast migrating protein (S.100) in beef brain. This protein is mainly of glial origin (Cicero, et al; 1970; Benda, 1968). Antiserum against this protein fails to detect its presence in other organs of the body. Furthermore, there is an immunological cross-reaction between the proteins extracted from the brains of a wide variety of vertebrates, suggesting the conservation of at least a part of the molecular structure intact through various stages of evolution. S.100 protein from beef brain has $S_{20,W}$ of 2.02 (Vincendon et al., 1967), molecular weight of 21,300 and is

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composed of probably three subunits (Dannies and Levine, 1969). Its physiological role is not precisely known eventhough it has been implicated as an important functional protein in several studies (Perez and Moore, 1968, Singh and Talwar, 1969, Calissano et al., 1969; Hydén and Lange, 1970; Talwar, 1970; Talwar and Singh, 1971). Data to be presented in this communication suggest that a protein from goat brain analogous in its migration characteristics to the previously described S.100 is a conjugated protein and contains RNA moeity as a part of the molecule.

Goat brain was brought to the laboratory from slaughter house under chilled conditions. It was homogenised with 2 volumes (W/V) of 20mM Tris - HCl, pH 7.4 containing 1 mM EDTA and centrifuged at 11,900 g for 60 min. The supernatant was brought to 75% saturation with ammonium sulphate maintaining the pH at 7.4. The precipitate was discarded, the supernatant dialysed against 2 mM Tris - HCl of pH 7.4 and lyophilized. 100 mg of the preparation was dissolved in 1 ml of water, redialysed and loaded on preparative 10% polyacrylamide electrophoretic column. The protein eluting as a homogeneous peak ahead of all other proteins was collected. The protein at this stage was homogeneous as judged by its migration as a single band during electrophoresis at three pH values in 7.5% analytical polyacrylamide gels(Fig.1).

The protein gives a broad absorption band between 250 and 275 mμ (Fig. 2), suggesting the possibility that it may be a conjugated protein. Chemical examination indicated the absence of hexoses, sialic acid, deoxypentose, cholesterol and lipids. The absence of sialic acid was also confirmed by the fact that digestion of the protein with neuraminidase did not

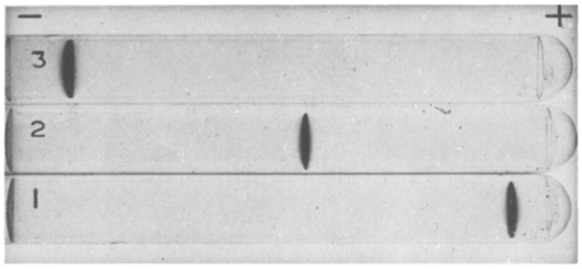

Fig. 1 Electrophoretic migration of purified goat brain protein at different pH values, 1) pH 9.5, 2) pH 7.0, 3) pH 4.3, on analytical polyacrylamide (7.5%) gels.

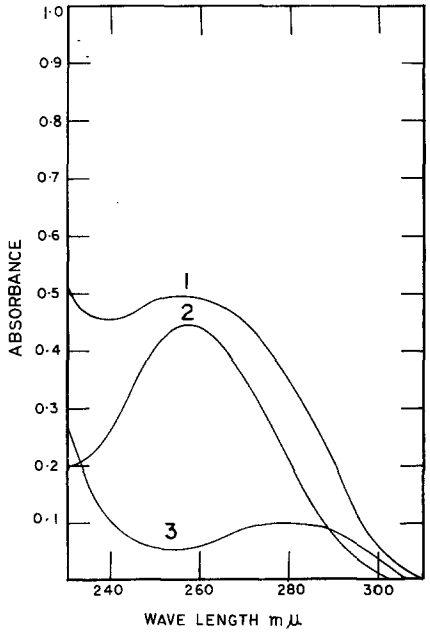

Fig. 2 Absorption spectrum in aqueous solution of 1) goat brain protein, 136.2 μg/ml; 2) yeast RNA, 26.3 μg/ml; and 3) goat brain protein using RNA solution (2) as blank.

alter the charge on the molecule as indicated by its migration characteristics on electrophoresis in polyacrylamide gels. Tests were positive for ribose and protein.

When the absorption spectrum of the protein was studied against a solution of RNA, the absorption curve shifted towards a maxima around 280 mμ (Fig. 2). Treatment of the protein with

ribonuclease also caused similar shift in the absorption characteristics (Fig. 3).

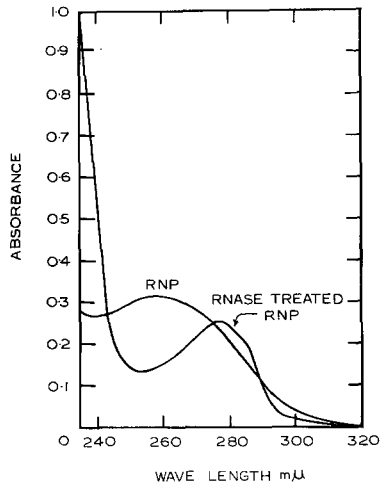

Fig. 3 Absorption spectrum of goat brain protein (RNP) before and after treatment with ribonuclease (RNase). 10 mg of RNP was treated with 5 mg of 5 x crystalline RNase (Nutritional Biochemicals Corporation, Ohio) for two hours at 37°C in 1 ml of 0.05 M Tris-HCl, pH 7.46. RNase was preincubated at 80°C for 10 min. to eliminate other hydrolytic enzymes present as possible contaminants. RNase and the protein were separated from the enzyme digest on Sephadex G-75 column (2.35 x 49.0 cms) using 0.05 M Tris-HCl, pH 7.46 containing 0.1 M KCl as the equilibration buffer. The absorption spectrum has been taken before and after the enzyme treatment.

The presence of RNA in the protein was confirmed by separation and identification of the four bases adenine, cytosine, guanine, and uracil in the hydrolysate obtained with 70% perchloric acid. Table 1 gives the base composition of the RNA moeity of the protein. The presence of the four bases has also been confirmed by ion exchange resin chromatography of the alkaline hydrolysates of the protein by the method of Katz and Comb (1963). The presence of RNA moeity in this protein has been consistently found in all batches of the protein preparations.

Preliminary experiments show that the type of RNA

Table 1

Analysis of the bases present in the purified protein preparation after hydrolysis with 70% $HClO_4$.

Base	Moles*
Uracil	13.0
Adenine	19.1
Guanine	32.6
Cytosine	35.3

* Data are expressed as moles per 100 moles of total bases recovered.

Purified goat brain protein was hydrolysed with 70% $HClO_4$ by the method of Marshak and Vogel (1951). After removal of $HClO_4$ as potassium salt, the hydrolysate was chromatographed on paper as described by Wyatt (1951). The spots were eluted and estimated spectrophotometrically by the method of Vischer and Chargaff (1948).

present in this complex is a short chain polynucleotide. Though more rigorous criteria to ascertain specificity of combination between the RNA and protein moeity remain to be applied to exclude a random association of RNA with the protein the acidic character of the protein as evidenced by its high electrophoretic mobility and rich content of glutamic and aspartic acids would not indicate a simple ionic combination. The liaison between RNA moeity and the protein can only be speculated at the moment. As it withstands electrophoretic preparations at pH 9.0, the aminoacyl linkage sensitive to

alkaline pH may not be involved in the conjugation.

In view of the conjugated character of the protein, an experiment was performed to test whether the RNA moeity contributed to organ specific antigenic determinants. Anti - serum against this protein from goat brain was raised in rabbits. Native and ribonuclease treated protein gave identical precipitin lines (Fig. 4), suggesting that the antigenic determinants were present primarily on the proteinic moeity. The anti-serum gave reactions with extracts from brains of monkey, rat and chick and from human astrocytomas in culture, but no reaction with similar extracts from liver, lung, kidney, spleen, serum, red blood cells and heart.

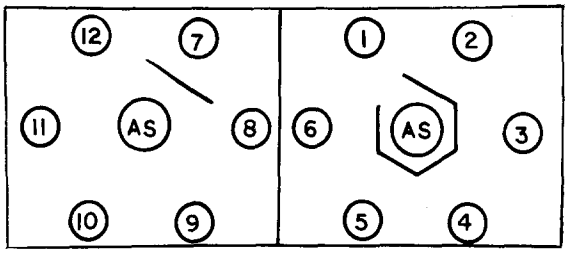

Fig. 4 Immuno-precipitin reaction of anti goat brain protein serum (AS) with 1) normal rat serum, 2) pure goat brain protein, 3) ribonuclease treated goat brain protein, 4) chick brain extract, 5) pure monkey brain analogous protein, 6) extract of human astrocytomas in culture. Extracts from rat organs, 7) brain, 8) kidney, 9) lungs, 10) liver, 11) spleen and 12) heart.

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