Effect of Polyamines on the Stability of Brain-Cortex Ribosomes

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1. Ribosomes isolated from the cortex tissue of goat brain contain very small amounts of spermidine and spermine. Ribosomes isolated from spermidine-treated slices have a higher spermidine content.
2. The polyamines partially prevent the temperature-dependent breakdown of ribosomes into acid-soluble nucleotides.
3. The 'melting' temperature of ribosomes rises slightly when the ribosomes are heated slowly in the presence of polyamines.
4. The pH-dependent breakdown of ribosomes into protein, RNA and acid-soluble nucleotide is markedly decreased by polyamines present in media in which ribosomes are suspended.
5. The breakdown of ribosomes in the presence of high concentrations of salts and EDTA is partially checked by the concurrent presence of polyamines.
6. Spermidine and spermine make ribosomes less susceptible to enzymic digestion by crystalline trypsin and ribonuclease.

Though ribosomes from various animals, plants and bacteria have been studied in some detail, little has been reported about the stability of brain-cortex ribosomes, especially under different physiological and experimental conditions (reviewed by Datta, 1966). According to our previous studies the stability of brain-cortex ribosomes is affected by temperature and the pH of the medium (Datta & Ghosh, 1964). Moreover, brain-cortex ribosomes become increasingly unstable in the presence of high concentrations of salts, EDTA, fluoride, phosphate, surface-active agents and urea. Studies by various investigators (Mangiantini, Tecee, Toschi & Trentalance, 1965; Barros & Giudice, 1968; Moruzzi, Barbiroli & Caldarera, 1968; Stevens & Morrison, 1968; Weller, Raina & Johnstone, 1968) have indicated that spermidine, spermine and other polyamines are generally effective in preserving ribosomes from different sources. In view of these findings the stability of brain-cortex ribosomes was re-examined on the basis of their degradation into smaller components in the presence of spermidine, spermine and other diamines. The results presented in this paper indicate that polyamines are highly effective in decreasing the degradation of brain-cortex ribosomes.

MATERIALS AND METHODS

Treatment of brain-cortex slices with spermidine. The cortex portion of brain tissue of freshly slaughtered goats was cut into thin slices (0.5 mm. thick) in the cold with a

Stadie-Riggs slicer. Approx. 10 g. wet wt. of cortex slices was suspended in 100 ml. of Krebs-Ringer buffer (0.15 M-NaCl, 3 mM-KCl, 2 mM-CaCl₂ and 0.2 M-NaHCO₃) containing 0.5 M-glucose, pH 7.0, previously saturated with O₂ + CO₂ (95:5). The respiring conditions of these slices were periodically checked by separate manometric experiments carried out under similar conditions. After incubation in the presence of 50 mM-spermidine at 37° for 30 min. the slices were collected by centrifugation and washed four times with 100 ml. portions of Krebs-Ringer buffer containing glucose before the ribosomes were prepared by the procedure outlined in the next section.

Preparation of brain-cortex ribosomes. Ribosomes were prepared from the brain-cortex tissue of freshly slaughtered goats by using deoxycholate as described by Datta & Ghosh (1963a). Ribosomes were further purified by layering over a discontinuous sucrose gradient consisting of 9 ml. of 2 M-sucrose and 11 ml. of 1 M-sucrose (previously sterilized by autoclaving) and by centrifuging for 15 hr. at 60000g_{av.} in a Spinco SW25 rotor to collect a pellet of free ribosomes. The purity of the ribosome preparation was judged as described by Datta & Ghosh (1963a).

Extraction of ribosomal RNA. Ribosomal RNA was extracted by the method of Kirby (1956) as described by Littauer & Eisenberg (1959). The precipitated RNA was dissolved in water and further purified by passage through a column (2 cm. x 30 cm.) of Sephadex G-25 that had been equilibrated with water as described by Martin (1966).

Chemical determinations. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), with crystalline bovine serum albumin as a standard. RNA was determined by the orcinol method of Meijbaum (1939) and also by a u.v.-spectrophotometric procedure (Hotchkiss, 1957). The concentrations of acid-soluble nucleotides were determined spectrophotometrically on the basis of E_{260} 33 for a solution containing a nucleotide mixture obtained

from complete hydrolysis of 1mg. of yeast RNA/ml. Nitrogen was determined by the method of Ma & Zuazaga (1942). The organic phosphorus compounds were digested according to the procedure of King (1932) and the inorganic phosphorus was determined by the method of Lowry, Roberts, Wu, Hixon & Crawford (1954). Ribosomes and ribosomal RNA were extracted three times with 0.5M-HClO₄. From the combined extracts polyamines were separated by paper electrophoresis, and spermidine and spermine were determined by the Amido Black method (Raina, 1963; Janne, Raina & Siimes, 1964).

Assays of ribonuclease and trypsin activities. Ribonuclease activity was measured by the principle of McDonall (1955) as described by Datta, Bhattacharyya & Ghosh (1964). Trypsin digestion was carried out by the procedure of Anson (1938).

Determination of stability of ribosomes. The stability of ribosomes was determined on the basis of degradation into smaller components, such as protein, RNA and acid-soluble nucleotides, released into the medium of suspension. Ribosomes suspended in a suitable buffer were incubated at 37° under different conditions as described in the Results section. At the end of the incubation the suspensions were cooled in ice and centrifuged at 105000g in a Spinco model L ultracentrifuge for 3 hr. The supernatants were very carefully collected. The protein and nucleic acid contents of the supernatant were determined in the 5%-trichloroacetic acid-precipitated residues of the supernatants and the acid-soluble nucleotides were determined in the trichloroacetic acid supernatant.

Thermal denaturation profiles. A dilute suspension of ribosomes (E_{260} 0.3–0.4) in MC-sucrose buffer, pH 7.0, was heated in 1cm. cuvettes for 15 min. at different temperatures in a thermostatically controlled heating chamber (sensitivity $\pm 0.5^\circ$) attached to a Beckman model DU spectrophotometer. The E_{260} values were measured over a range of temperatures between 30° (room temperature) and 85° during heating. The percentage increase in E_{260} or thermal hyperchromicity between 30° and 85° was calculated on the basis of the E_{260} value at 30°.

Buffers. Magnesium-cacodylate buffer, pH 7.0 (5mm), was prepared as described by Gilchrist & Bock (1958). MC-sucrose buffer comprises 5mm-magnesium-cacodylate buffer containing 0.25M-sucrose, pH 7.0. Acetate-veronal buffers, pH 2.5–9.6 (28mm), were prepared as described by Michaelis (1931).

Materials. Spermidine trihydrochloride, spermine tetrahydrochloride, putrescine dihydrochloride and cadaverine

dihydrochloride were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. They were neutralized before use.

Crystalline pancreatic ribonuclease and crystalline ($\times 2$) trypsin were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Trypsin was checked for the absence of ribonuclease activity by the procedure described by Piatigorsky (1968).

RESULTS

Polyamine contents of brain-cortex ribosomes. The isolated microsomes and ribosomes of normal cortex tissue of goat brain contain very small amounts of polyamines, mostly spermidine and spermine. The polyamines in the isolated microsomes are apparently associated with ribosomes, since after deoxycholate treatment of microsomes the polyamines are still sedimentable in the ribosomes and remain so during the purification procedures. Repeated washing up to three times with MC-sucrose buffers at pH 7.0 and at pH 5.0 did not appreciably decrease the polyamine contents of the ribosomes. However, when the purified ribosomes were treated with phenol to extract RNA the most of the polyamines were removed from RNA. Table 1 shows the spermidine and spermine contents of purified ribosomes and ribosomal RNA of normal brain-cortex tissue. The molar ratio of spermidine and spermine in the isolated ribosomes is about 2.0. When the normal brain-cortex slices were preincubated for 1 hr. in the presence of 50mm-spermidine the concentration of polyamines found in the isolated ribosome was increased about twofold (Table 1). Repeated washing up to three times with MC-sucrose buffers at pH 5.0 and 7.0 did not appreciably remove the polyamines attached to the ribosomes of the spermine-treated brain-cortex slices. Moreover, when the isolated ribosomes were incubated at 37° in the presence of 1mm-spermidine or -spermine for 15 min. and then collected by centrifugation at 105000g and washed twice with MC-sucrose buffer, the polyamine content of these ribosomes appeared to be about 30–40% higher than that of ribosomes

Table 1. *Polyamine contents of goat brain-cortex ribosomes and ribosomal RNA*

Isolation of ribosomes and ribosomal RNA and determination of polyamines were as described in the Methods and Materials section. Average values of three different experiments are given.

Source	Fraction	Polyamines (μ moles/100mg. of ribosomes)		Polyamine N/RNA P molar ratio
		Spermidine	Spermine	
Normal brain-cortex tissue	Ribosomes	11.9	6.0	0.05
	Ribosomal RNA	< 1.0	< 1.0	< 0.001
Spermidine-treated brain-cortex tissue slices	Ribosomes	30.0	6.0	0.10
	Ribosomal RNA	< 1.0	< 1.0	< 0.01

Table 2. *Effect of temperature on the stability of brain-cortex ribosomes in the presence of polyamines*

Brain-cortex ribosomes (30 mg.) were suspended in 5 ml. of MC-sucrose buffer, pH 7.0, containing 1 mM-polyamine. The suspensions were heated in a water bath for exactly 5 min., cooled in ice immediately and centrifuged at 105 000g for 3 hr. The supernatants were collected carefully, and each was treated with an equal volume of ice-cold 10% (w/v) trichloroacetic acid for 1 hr. and then centrifuged. The acid-soluble nucleotides of the supernatants were determined spectrophotometrically. The release of acid-soluble nucleotides is expressed as a percentage of RNA initially present in ribosomes before heating. Average values of determinations with five different ribosomal preparations are given.

Temp.	Release of acid-soluble nucleotides (mg./5 ml.)			
	No polyamine	With spermidine	With spermine	With putrescine
0°	< 0.01	< 0.01	< 0.01	< 0.01
30	< 0.01	< 0.01	< 0.01	< 0.01
45	0.01	< 0.01	0.01	0.01
60	0.05	0.02	0.02	0.02
80	0.13	0.04	0.05	0.07
90	0.24	0.08	0.09	0.10

similarly incubated and treated in the absence of polyamines. These results seem to indicate that endogenous and exogenous polyamines are attached to ribosomes of brain-cortex tissue.

Effect of polyamines on the temperature-dependent stability of ribosomes. Similar to ribosomal particles from other sources, brain-cortex ribosomes are susceptible to degradation by heat as revealed by release of acid-soluble nucleotides (Datta & Ghosh, 1964). To check whether spermidine and other polyamines have any effect on the thermal degradation, brain-cortex ribosomes were heated at various temperatures in the presence and absence of spermidine and other polyamines. The release of acid-soluble nucleotides during incubation at different temperatures for 5 min. is presented in Table 2. The results indicate that the stability of brain-cortex ribosomes is temperature-dependent and that the degradation into acid-soluble nucleotides occurs with rise of temperature. When the ribosomes were heated in the presence of 1 mM-spermidine the rate of degradation as measured by the release of acid-soluble nucleotides was much decreased. Similarly decreased degradation of ribosomes occurred in the presence of spermine, cadaverine and putrescine. It was also observed that, like acid-soluble nucleotides, protein and RNA were released, in trace amounts, from the ribosomes when they were heated alone. The release of these components, however, decreased or ceased when the ribosomes were heated in the

Fig. 1. Thermal hyperchromicity (heat-denaturation) profiles of brain-cortex ribosomes in the presence of polyamines. The conditions are described in the Materials and Methods section. —, No polyamine; — —, with spermine; — · —, with spermidine.

presence of 1 mM-spermidine. It may be mentioned here that ribosomes are quite stable at low temperature and practically no degradation of ribosomes occurred on storage at -10 to -20° for a week. Repeated freezing and thawing up to five times in quick succession caused no appreciable release of acid-soluble nucleotides. It may be further mentioned that the released acid-soluble nucleotides were found, by paper chromatography (Magasanik, Vischer, Doniger, Elson & Chargaff, 1950; Elson, Gustaffson & Chargaff, 1954), to be mostly 2'- and 3'-mononucleotides of both purines and pyridines with traces of di- and tri-nucleotides.

Hyperchromicity profiles. Further confirmation of the increased thermal stability of brain-cortex ribosomes in the presence of spermidine and spermine was obtained by studying the thermal hyperchromicity (heat-denaturation) profiles of ribosomes (Fig. 1). When heated in MC-sucrose buffer, the 'melting' temperature, T_m , for brain-cortex ribosomes was 63° . When ribosomes were heated in the presence of 1 mM-spermidine and -spermine the corresponding values were 70° and 67° . The increased values in the presence of spermidine and spermine are indicative of enhanced stability of brain-cortex ribosomes in the presence of these polyamines.

Effect of polyamines on the pH-dependent stability

Table 3. *Effect of pH on the stability of brain-cortex ribosomes in the presence of spermidine*

Brain-cortex ribosomes (30 mg.) were suspended in 5 ml. of acetate-veronal buffer (14 mM) at different pH values as indicated and incubated for 3 hr. at 37°. The supernatants were collected by centrifugation at 105 000g for 3 hr. in the cold, and portions were used for determination of protein, RNA and acid-soluble nucleotides. The concentration of spermidine used was 1 mM. Average values of determination with six different ribosomal preparations are given.

pH	Components released in 3 hr. (mg./5 ml.)					
	Protein		RNA		Acid-soluble nucleotides	
	No spermidine	With spermidine	No spermidine	With spermidine	No spermidine	With spermidine
3.5	0.48	0.27	0.11	0.06	0.01	0.01
5.0	0.49	0.30	0.14	0.07	0.02	0.01
6.0	0.45	0.32	0.14	0.08	0.03	0.01
7.0	0.47	0.30	0.14	0.08	0.03	0.01
8.0	0.50	0.34	0.16	0.08	0.04	0.02
9.0	0.65	0.40	0.21	0.09	0.08	0.02
9.5	0.80	0.46	0.23	0.10	0.12	0.03

Table 4. *Effect of high concentrations of salts and EDTA on the stability of brain-cortex ribosomes in the presence of spermidine*

Brain-cortex ribosomes (30 mg.) were suspended in 5 ml. of 5 mM-magnesium-cacodylate buffer, pH 7.0, in which the substances indicated were dissolved with subsequent adjustment of pH where needed. The suspensions were incubated at 37° for 3 hr. and the supernatants were collected by centrifugation at 105 000g for 3 hr. in the cold. The concentration of spermidine used was 1 mM. Average values of determinations with six different ribosomal preparations are given.

Suspension medium		Components released in 3 hr. (mg./5 ml.)					
		Protein		RNA		Acid-soluble nucleotides	
		No spermidine	With spermidine	No spermidine	With spermidine	No spermidine	With spermidine
Control (MC-sucrose buffer)		0.44	0.30	0.14	0.08	0.03	0.02
NaCl	0.1M	1.50	0.80	0.30	0.17	0.16	0.09
	0.5M	2.10	0.99	0.66	0.39	0.30	0.19
MgCl ₂	0.01M	0.50	0.30	0.15	0.09	0.03	0.02
	0.05M	0.63	0.37	0.28	0.12	0.09	0.05
	0.50M	1.60	0.55	0.51	0.18	0.29	0.09
EDTA	0.01M	0.67	0.40	0.37	0.19	0.01	0.01
	0.10M	0.93	0.49	0.63	0.29	0.30	0.02

of ribosomes. In view of the degradation of ribosomes in response to changes of pH of the suspension medium the breakdown of brain-cortex ribosomes at different pH values was studied. Ribosomes were suspended in acetate-veronal buffer (containing 5 mM-magnesium chloride) of various pH values and the release of protein, RNA and acid-soluble nucleotides in the suspension medium was measured with or without spermidine (1 mM) in the medium. The results (Table 3) indicate that brain-cortex ribosomes were fairly stable at different pH values up to 8.0, though the release of protein, total RNA

and acid-soluble nucleotides became greater the higher the pH of the medium. However, the release of all these components was remarkably decreased at all pH values when spermidine was present in the suspension medium of the ribosomes. Similar effects were also noted with spermine, putrescine and cadaverine (1 mM).

Effect of polyamines on the stability of ribosomes in the presence of salts and EDTA. The results (Table 4) indicate that the higher the concentrations of added Na⁺ and Mg²⁺ the greater was the breakdown of brain ribosomes into smaller components. A similar

Fig. 2. Digestion of brain-cortex ribosomes by trypsin in the presence of polyamines. $\circ$, No polyamine; $\bullet$, spermine; Δ , spermidine. The incubation system contained 5mg. equivalent of ribosomal protein, 1mg. of crystalline trypsin and veronal buffer, pH 8.0 (14mm), in a total volume of 5ml. Incubation was carried out at 37°. The concentration of polyamine was 0.2mm.

effect was also observed with other univalent and bivalent metal ions. The presence of spermidine (1mm) along with high concentrations of Na^+ and Mg^{2+} decreased the extent of the breakdown of ribosomes. An increased release of components of ribosomes also took place in the presence of EDTA. The addition of spermidine, however, caused a decreased release of these components. Similar effects were also noted with spermine, putrescine and cadaverine (1mm).

Digestion of ribosomes by trypsin in the presence of polyamines. The susceptibility of ribosomes to enzymic digestion was usually determined by incubating ribosomes with trypsin and measuring the extent of digestion of the ribosomal protein constituent. Results are expressed as percentage of total constituent protein (or ribosomes) digested by crystalline trypsin (Fig. 2). As the digestion of ribosomal particles by crystalline trypsin proceeded, the turbidity of the ribosomal suspension decreased visibly. However, a small residue of acid-insoluble protein fraction amounting to about a quarter of the ribosomal protein initially present was left undigested, indicating that the digestion of the protein moiety was not complete in 2hr. of incubation. Incubation for a longer time or with more trypsin failed to bring about the complete digestion of ribosomal protein. It is also evident that ribosomes treated with trypsin were more slowly digested in the presence of spermidine (0.2mm) than in its absence. A 50% digestion of ribosomal protein occurred in 68min. when spermidine was present during tryptic digestion, whereas a similar extent of digestion occurred in 45min. in the absence of spermidine. The retarding effect of

Fig. 3. Digestion by trypsin of brain-cortex ribosomes preincubated in the presence of polyamines. $\circ$, No polyamine; $\bullet$, spermine; Δ , spermidine. The incubation system was as stated in the text and in Fig. 2.

spermidine on the digestion was dependent on the concentration of spermidine and was also observed with spermine, but to a smaller extent. The concentration of spermidine used was not inhibitory to trypsin, since 0.2mm-spermidine did not inhibit the digestion of purified bovine haemoglobin and albumin (fraction V) by crystalline trypsin. As a further check, ribosomes were preincubated in the presence of 1mm-spermidine and -spermine separately for 10min., collected by centrifugation, washed twice with MC-sucrose buffer and then subjected to digestion by trypsin. The control was a ribosomal preparation similarly preincubated without spermidine or spermine and treated identically. Fig. 3 shows that ribosomes preincubated with spermidine and spermine were less susceptible to tryptic digestion than were the control ribosomes preincubated without spermidine or spermine as described above.

Digestion of ribosomes by ribonuclease in the presence of polyamines. The susceptibility of ribosomes to enzymic digestion was also determined by incubating ribosomes with ribonuclease and measuring the extent of digestion of the RNA constituent of ribosomes. Results are expressed as percentage of total constituent RNA (of ribosomes) digested by crystalline pancreatic ribonuclease (Fig. 4). During the digestion of ribosomes by ribonuclease, the turbidity of the ribosome suspension was visibly decreased and no acid-insoluble RNA was left, indicating that the digestion of the RNA moiety proceeded to near completion in about 2hr. in the absence of any polyamine. The results also indicate that ribosomes treated with ribonuclease were digested much more slowly in the presence of spermidine (0.2mm) than in its absence. This effect was dependent on the concentration of

Fig. 4. Digestion of brain-cortex ribosomes by ribonuclease in the presence of polyamines. ○, No polyamine; ●, spermine; △, spermidine. The incubation system contained 5mg. equivalent of ribosomal RNA, 0.5mg. of crystalline pancreatic ribonuclease and veronal buffer, pH 7.5 (14mM), in a total volume of 5ml. Incubation was carried out at 37°. The concentration of polyamine was 0.2mM.

Fig. 5. Digestion by ribonuclease of brain-cortex ribosomes preincubated in the presence of polyamines. ○, No polyamine; ●, spermine; △, spermidine. The incubation system was as stated in the text and in Fig. 4.

spermidine and was also observed with spermine. Moreover, the digestion of the RNA moiety did not proceed to near completion in about 2hr. in the presence of polyamines. The concentration of spermidine or spermine used was not inhibitory to ribonuclease, since at 0.2mM concentration neither of them inhibited the digestion of purified yeast RNA by crystalline pancreatic ribonuclease. Next the ribosomes were preincubated in the presence of 1mM-spermidine and -spermine separately for 10min., collected by centrifugation, washed twice with MC-sucrose buffer and then subjected to digestion by pancreatic ribonuclease. The control was a ribosomal preparation similarly preincubated without spermidine and spermine and treated identically. Fig. 5 shows that the ribosomes preincubated with spermidine and spermine were less susceptible to ribonuclease digestion than were the control ribosomes preincubated without polyamine as stated above.

These digestion experiments with trypsin and ribonuclease indicate that spermidine and spermine make ribosomes less susceptible to enzymic digestion.

DISCUSSION

Though the functions of polyamines in cellular metabolism are unknown, spermidine and spermine are present in large amounts in tissues such as pancreas, spleen, liver and thymus and in small amounts in brain (Rosenthal & Talor, 1956; Janne *et al.* 1964). According to these investigators the ratio of spermidine to spermine is about 2 in adult

rat brain tissue. In the present study, spermidine and spermine were found to be present in isolated brain microsomes, and to be sedimentable, after deoxycholate treatment of microsomes, in the ribosomes (Table 1). The ratio of spermidine to spermine in the washed and purified ribosomes is about 2, and this ratio does not change on repeated washing of ribosomes with buffer. Similar observations were made by Raina & Telaranta (1967) that polyamines in isolated subcellular particles are associated with RNA of rat liver and, after deoxycholate treatment of microsomes, the polyamines are still sedimentable in ribosomes. The association of polyamines with ribosomes was found to be doubled when the brain-cortex slices are incubated in the presence of spermidine and glucose (Table 1). These observations, together with the binding of spermidine by the isolated ribosomes from the medium and the failure of repeated washing of ribosomes with acidic buffer (pH 5.0) to remove polyamines, indicate that the endogenous and exogenous polyamines are attached to ribosomes of brain-cortex ribosomes. While characterizing the ribosomes of *Tetrahymena pyriformis*, Weller *et al.* (1968) observed the molar ratio of polyamine N to RNA P to be 0.24. With brain-cortex ribosomes this ratio was found to be 0.05. In the absence of any known values for this ratio for ribosomes from other mammalian tissues, it is highly unwise to accept the lower ratio found with brain-cortex ribosomes as a characteristic of brain tissue, which is apparently not rich in polyamines. Further work is desirable to examine whether the molar ratio of polyamine N to RNA P in ribosomes (or in other RNA-containing subcellular particles) bears any significance as to the tissue specificity or species specificity. Brain-cortex ribosomes are quite active in protein synthesis. Preliminary

studies have indicated that spermidine stimulates the protein-synthesizing activity of these ribosomes (R. K. Datta, unpublished work).

From the results in Table 2 and Fig. 1 polyamines increase the thermal stability of brain-cortex ribosomes. Similarly the increased 'melting' temperature of ribosomal RNA in the presence of polyamines (R. K. Datta & J. J. Ghosh, unpublished work) suggests that an association of polyamines with RNA may help to maintain the structural integrity of RNA-containing cellular macromolecules. The demonstration that polyamines bind, as with ribosomes of other tissues, with ribosomes in the brain-cortex tissue (Table 1) and also with isolated ribosomes suggests that polyamines have physiological functions in preserving the structures of brain-cortex ribosomes and possibly RNA. Various studies (Silman, Artman & Engelberg, 1965; Raina & Cohen, 1966; Cohen, Hoffner, Jensen, Moore & Raina, 1967) have indicated that some polyamines can preserve ribosomes from different bacterial, plant and animal sources, which otherwise tend to break down into smaller components. Results presented in this paper point to the same stabilizing effect of polyamines on brain-cortex ribosomes. It is also apparent from the present study that, on an equimolar basis, spermidine and spermine have higher stabilizing effects than have cadaverine and putrescine.

Ribosomes (70S) from spermine-grown cells of *Aerobacter aerogenes* are more stable with respect to dissociation in the presence of a suboptimum concentration of Mg^{2+} (McGivern & Hersh, 1967). An idea is gaining ground that the role of spermine is similar to Mg^{2+} in the formation and preservation of ribonucleoprotein particles (Siekevitz, 1961). Further studies are desirable to look into the molecular mechanism by which polyamines or Mg^{2+} confer stability to the ribosomal particles and by which polyamines attached to the ribosomes or present *in vivo* affect the protein-synthesizing functions of ribosomes of liver and brain tissue.

It has long been known that in the different functional activities of brain there are marked alterations in the distribution and in the staining properties of basophilic Nissl granules (Krogh, 1950; Geiger, 1956; Hyden & Pigon, 1960). Electron-microscopic examination has revealed that the Nissl granules are ribonucleoprotein particles arranged in a specific manner on the endoplasmic reticulum (Palay & Palade, 1955). The chromatolytic changes (staining with galloxyanin-chrome alum) that these RNA-rich Nissl granules undergo during stimulation or stress has focussed attention on factors affecting the stability of these ribonucleoprotein particles in the Nissl granules. As an approximation to Nissl granules and as model macromolecules of known chemical composition

and biochemical functions we studied cerebral ribosomes and determined the factors that affect the stability of these particles (Datta & Ghosh, 1964). Previous studies (Datta & Ghosh, 1963b, 1965) have demonstrated that, when brain-cortex slices are treated with anti-depressant and convulsant drugs, the ribosomal particles become more susceptible to breakdown, releasing protein, RNA and acid-soluble nucleotides. During the action of the hallucinogenic drug mescaline on brain-cortex slices similar changes in the stability of ribosomes are found to occur (R. K. Datta & J. J. Ghosh, unpublished work). In this later work, evidence was obtained that the mescaline-induced breakdown of ribosomes of the drug-treated brain slices can be partially checked in a suspension medium if spermine is present therein.

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REFERENCES

- Anson, M. L. (1938). *J. gen. Physiol.* **22**, 79.
- Barros, C. & Giudice, G. (1968). *Exp. Cell Res.* **50**, 671.
- Cohen, S. S., Hoffner, N., Jensen, M., Moore, M. & Raina, A. (1967). *Proc. nat. Acad. Sci., Wash.*, **57**, 721.
- Datta, R. K. (1966). *Brain Res.* **2**, 301.
- Datta, R. K., Bhattacharyya, D. & Ghosh, J. J. (1964). *J. Neurochem.* **11**, 87.
- Datta, R. K. & Ghosh, J. J. (1963a). *J. Neurochem.* **10**, 363.
- Datta, R. K. & Ghosh, J. J. (1963b). *Biochem. Pharmacol.* **12**, 1355.
- Datta, R. K. & Ghosh, J. J. (1964). *J. Neurochem.* **11**, 595.
- Datta, R. K. & Ghosh, J. J. (1965). *J. Pharmacol.* **150**, 449.
- Elson, D., Gustaffson, T. & Chargaff, E. (1954). *J. biol. Chem.* **209**, 285.
- Geiger, R. S. (1956). *Arch. Neurol. Psychiat.* **75**, 442.
- Gilchrist, W. C. & Bock, R. M. (1958). In *Microsomal Particles and Protein Synthesis*, p. 1. Ed. by Roberts, R. B. London: Pergamon Press Ltd.
- Hotchkiss, R. D. (1957). In *Methods in Enzymology*, vol. 3, p. 708. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Hyden, H. & Pigon, A. (1960). *J. Neurochem.* **6**, 57.
- Janne, J., Raina, A. & Siimes, M. (1964). *Acta physiol. scand.* **62**, 352.
- King, E. J. (1932). *Biochem. J.* **26**, 292.
- Kirby, K. S. (1956). *Biochem. J.* **64**, 405.
- Krogh, E. (1950). *Acta physiol. scand.* **20**, 263.
- Littauer, U. Z. & Eisenberg, H. (1959). *Biochim. biophys. Acta*, **32**, 320.
- Lowry, O. H., Roberts, N. R., Wu, M. L., Hixon, W. S. & Crawford, E. J. (1954). *J. biol. Chem.* **207**, 19.

- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Ma, T. S. & Zuazaga, G. (1942). *Industr. Engng Chem. (annu. Ed.)*, **14**, 280.
- McDonall, M. R. (1955). In *Methods in Enzymology*, vol. 2, p. 427. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- McGivern, T. & Hersh, R. T. (1967). *Exp. Cell Res.* **46**, 268.
- Magasanik, B., Vischer, E., Doniger, B., Elson, D. & Chargaff, E. (1950). *J. biol. Chem.* **186**, 37.
- Mangiantini, M. T., Tecee, G., Toschi, G. & Trentalance, A. (1965). *Biochim. biophys. Acta*, **103**, 252.
- Martin, S. J. (1966). *Biochem. J.* **101**, 721.
- Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
- Michaelis, L. (1931). *Biochem. Z.* **234**, 139.
- Moruzzi, G., Barbiroli, B. & Caldarera, C. M. (1968). *Biochem. J.* **107**, 609.
- Palay, S. L. & Palade, G. E. (1955). *J. biophys. biochem. Cytol.* **1**, 69.
- Piatigorsky, J. (1968). *Biochim. biophys. Acta*, **166**, 142.
- Raina, A. (1963). *Acta physiol. scand.* **60** (Suppl.), 218.
- Raina, A. & Cohen, S. S. (1966). *Proc. nat. Acad. Sci., Wash.*, **55**, 1587.
- Raina, A. & Telaranta, T. (1967). *Biochim. biophys. Acta*, **138**, 200.
- Rosenthal, S. M. & Talor, C. W. (1956). *J. Pharmacol.* **116**, 131.
- Siekevitz, P. (1961). In *Biological Structure and Function*, vol. 1, p. 239. Ed. by Goodwin, T. W. & Lindberg, O. London: Academic Press (Inc.) Ltd.
- Silman, N., Artman, M. & Engelberg, H. (1965). *Biochim. biophys. Acta*, **103**, 231.
- Stevens, L. & Morrison, M. R. (1968). *Biochem. J.* **108**, 633.
- Weller, D. L., Raina, A. & Johnstone, D. B. (1968). *Biochim. biophys. Acta*, **157**, 558.