ACTA NEUROBIOL. EXP. 1971, 31: 389-398

CHARACTERISTIC OF MITOCHONDRIAL NUCLEIC ACIDS OBTAINED FROM CALF BRAIN GRAY AND WHITE MATTER

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The brain mitochondrial system is of a special interest because of the high concentration of mitochondria in the synaptic regions of neurons. Klee and Sokoloff (1965) have demonstrated the occurrence of protein synthesis in brain mitochondria. Campbell et al. (1966) have isolated two distinct cell-free protein synthesizing systems from immature rat brain, one localized in purified ribosomes, the other in mitochondria. The participation of the mitochondrial synthesizing system in synaptosomal protein synthesis is still discussed (Barondes 1966, 1968, Morgan and Austin 1968). Recent studies lend support to the view that mitochondria posses a remarkable degree of autonomy; they appear to contain some of the information and apparatus for macromolecular biosynthesis and possibly for their own biogenesis (Nass 1967, Karol and Simpson 1968, Meyer and Simpson 1969).

In our previous work (Borkowski et al. 1967, 1968) we have demonstrated the difference between nucleic acids obtained from the whole brain and liver mitochondria. It is evident that the tissue of the central nervous system is morphologically heterogeneous. Therefore in this communication the experiments performed on two anatomically distinct parts of an adult brain have been described.

MATERIAL AND METHODS

The experiments were carried out on freshly obtained calf brains from the slaughter-house. The cerebral hemisphere with superficial blood vessels and meninges removed was cut vertically into slices. The gray

matter was obtained mainly from the cerebral cortex, whereas white matter was principally isolated from corpus callosum. Both parts of the analysed brain tissue were homogenized in 0.44 M sucrose containing 5.0×10^{-4} M EDTA and 0.01 M Tris (pH — 7.2).

The homogenates were centrifuged according to the method described by Lövtrup and Zelander (1962). Owing to this procedure the finally obtained mitochondrial pellet from the gray matter was morphologically pure. Mitochondria isolated in the same manner from the white matter were contaminated by myelin fragments. Therefore an additional purification of these mitochondria was necessary. The crude mitochondrial pellet was homogenized in 20 volumes of 0.6 M sucrose-tris solution and centrifuged at 20,000 × G for 2 hr in a Sorval refrigerated centrifuge. In this conditions the homogenate was divided into two parts, the supernatant composed of myelin fragments exclusively and sediment which was identified as pure mitochondria. The morphological purity of isolated mitochondria was controlled by means of electron micrography and biochemical tests as described previously (Borkowski et al. 1968).

Nucleic acid concentration in the whole mitochondrial fraction was determined colorimetrically in reaction with orcinol (Mejbaum 1939) and diphenylamine (Burton 1956, Borkowski and Sikorska 1964) after alcaline hydrolysis (Schmidt and Tannhauser 1945, Borkowski 1962). Nucleotide composition of RNA and base composition of DNA was determined by means of a micromethode previously described (Borkowski et al. 1969, 1970).

Extraction of nucleic acids was carried out using the following procedure. The isolated mitochondrial sediments were homogenized in 80 ml of ice-cold 0.14 M NaCl containing 0.01 M Tris (pH 7.2) by means of a glass-teflon Potter-Elvehjem homogenizer. Then SDS was added at concentration 1% and an equal volume of freshly prepared neutralized 90% phenol solution. The suspension was further extracted during 60 min at 0°C. After centrifugation, the aqueous phase was collected. The phenol phase and interphase were extracted once more with a 0.5 M NaCl solution in Tris pH 8.2 for 15 min at room temperature. Aqueous phases were purified of phenol by an ether extraction. Sodium acetate was added to the ether free solution to a concentration of 2%. The nucleic acids were precipitated by addition of 2 volumes of 96% ethanol. After beeing kept at −20°C for 24 hr the nucleic acids were separated by cold centrifugation. The sediments were collected and prepared to chromatography.

Chromatography on methylated albumine kieselguhr (MAK) column described by Mandell and Hershey (1960) was adapted for separation of different kinds of nucleic acids. The nucleic acids were eluted from the

column by a continuous gradient of NaCl and 3 ml fractions were collected. Fractions of nucleic acids remaining on the column after elution by NaCl were liberated from the column by the addition of a solution of 1.5 M NaCl in ammonia (Smith and Burton 1966).

RESULTS

Quantitative analysis performed on the whole mitochondrial fraction revealed some differences between mitochondria isolated from the gray and white matter (Table I).

TABLE I

Concentrations of nucleic acids in brain mitochondria
These values were calculated from 10 experiments with standard deviation

Mitochondria obtained from	Mitochondrial protein in mg/g tissue	μg P/mg of mitochondrial protein	
		RNA	DNA
Gray matter	10.0±2.83	0.648±0.213	0.0158±0.0021
White matter	4.3±1.59	0.888±0.166	0.0246±0.0015

It can be seen that the quantity of mitochondria isolated from the gray matter was two times larger that of the white matter. The concentration of DNA in mitochondria from the white matter was almost twice higher than that of mitochondria from the gray matter. RNA concentration was also higher but this value was not statistically significant.

When nucleic acids were extracted by means of the phenol method, almost 70-80% of total mitochondrial nucleic acids were obtained in a pure form. Chromatography analysis of mitochondrial nucleic acids on a MAK column was performed and a typical diagram is presented in Fig. 1.

In both samples of mitochondrial nucleic acids we have distinguished five peaks of absorbance curve. All fractions corresponding to one peak were precipitated by ethanol and subsequently separately analyzed. Fraction I is almost exclusively composed of products of nucleic acids degradation. Fraction II which was eluted from the column with 0.4 M NaCl corresponds to s-RNA.

The next fraction (III) was identified as DNA and Fraction IV as r-RNA. The last fraction (V) was obtained by elution with 1.5 M NaCl in ammonia solution.

The concentration of different kinds of RNA in both preparations of mitochondrial nucleic acids is presented in Table II.

The values in Table II were calculated from six individual experi-

ments. In these values, absorption of the DNA fraction was not considered.

The data showed that on the average 30% of total extractable mitochondrial nucleic acids was composed of s-RNA. The r-RNA was pre-

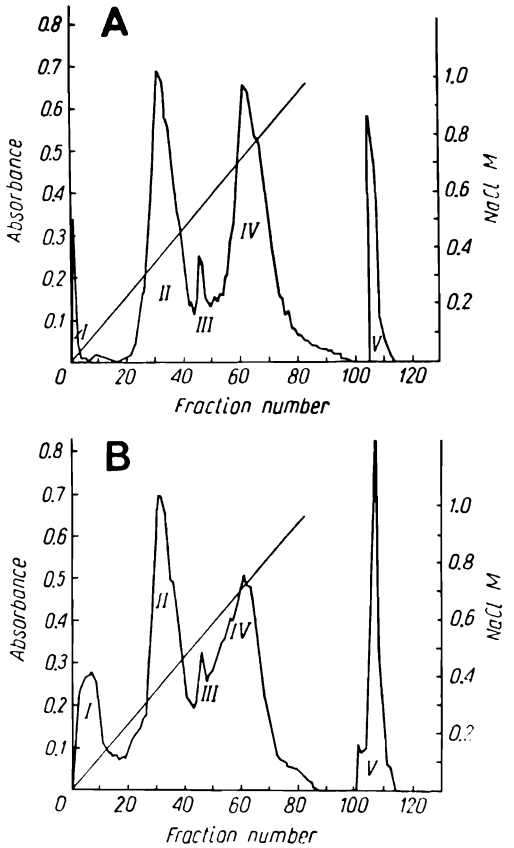

Fig. 1. Chromatogram of nucleic acids extracted from mitochondria of *A*, gray brain matter, *B*, white brain matter, on a column of methylated albumin kieselguhr. About 0.5–1.0 mg of nucleic acids are loaded on the column in 0.01 M Tris-HCl buffer pH 7.1 in 0.14 M NaCl. Elution is carried out by means of a linear gradient from 0.14 M to 1.5 M NaCl in Tris buffer at 20° C. 3 ml fractions are collected.

sent in this preparation in 40%. It is known that r-RNA is more fragile to the action of nucleolytic enzymes. The variable concentration of Fraction I, which is composed mainly of RNA degradation products, depends mainly on r-RNA degradation. In our experiments Fraction V was also considered to be a part of r-RNA. Therefore we have found out two values of the s-RNA/r-RNA ratio. It is interesting to note that nucleic acids isolated from the cerebral mitochondria showed a relatively high concentration of s-RNA. The difference between both nucleic acids samples analysed was not statistically significant.

In the two main fractions of nucleic acids obtained after chromatography on a MAK column the analysis of nucleotide composition was

TABLE II

Repartition of different kinds of mitochondrial nucleic acids after chromatography on methylated albumine kiselguhr

Mitochondria obtained from	Recovery of nucleic acids after chromatography on MAK as per cent of total optical density				s-RNA / r-RNA	s-RNA / r-RNA + + "NH_3-RNA"
	I Degradation products	II s-RNA	IV r-RNA	V "NH_3-RNA"		
Gray matter	6.94 (0–11.6)	33.8 (27.5–41.9)	41.16 (38.1–55.7)	15.1 (8.0–22)	0.806 (0.5–1.0)	0.598 (0.4–0.9)
White matter	9.6 (0–18.7)	28.6 (24.7–36.5)	38.2 (34.4–50.5)	20.5 (13.0–25.9)	0.702 (0.46–0.9)	0.483 (0.31–0.71)

TABLE III

Nucleotide composition of different kinds of mitochondrial nucleic acids obtained from white and gray brain matter

Mitochondria obtained from	Nucleic acids	Nucleotide composition in mol per cent				Pu / Pi	GC / AU
		UMP	GMP	AMP	CMP		
Gray matter	s-RNA	19.8	31.7	21.0	27.5	1.112	1.450
	r-RNA	17.6	33.7	19.5	29.1	1.135	1.685
White matter	s-RNA	18.8	29.6	21.2	30.4	1.020	1.500
	r-RNA	18.5	37.1	17.1	27.4	1.180	1.820

performed. The data presented in Table III shows, that the nucleotide composition of both s-RNA analysed was very similar.

Their GC/AU ratio was typical for s-RNA isolated from other mammalian tissues. In our experiments both preparations of r-RNA were characterized by a high concentration of guanylic and cytydilic nucleotides.

Small differences observed in the GC/AU ratio between the two mitochondrial r-RNA's was not statistically significant. We have also shown that the nucleotide composition of the last fractions eluted from the MAK column was similar to r-RNA nucleotide composition.

The quantity of DNA in the preparations of mitochondrial nucleic acids analysed was very small. Therefore in view of the next analysis it was necessary to collect DNA fractions from several experiments. The base composition of mitochondrial DNA compared to nuclear DNA is presented in Table IV.

TABLE IV

Base composition of mitochondrial and nuclear DNA obtained from calf brain

DNA preparation	Base composition in mol per cent				$\frac{G+C}{A+T}$	$\frac{G+A}{C+T}$
	T	G	A	C		
Mitochondrial	19.75	31.45	29.50	19.90	1.055	1.555
Nuclear	27.40	23.30	29.30	20.00	0.784	1.105

The values presented in Table IV are calculated from three separately performed experiments. We have shown some differences between the base composition of nuclear and mitochondrial DNA.

Mitochondrial DNA was characterized by a high concentration of adenylic and guanylic nucleotides. It is interesting that the purine/pirymidine ratio of mitochondrial DNA was higher than that of nuclear DNA.

DISCUSSION

In a biochemical study of subcellular structures the purity of the fractions analysed is very important. Whittaker and his associates (Eichberg et al. 1964, Whittaker et al. 1964) have demonstrated that crude mitochondrial preparations obtained from the whole brain showed several other structures besides mitochondria.

In our study, the method of Lövtrup and Zelander (1962) performed for the isolation of mitochondria appeared to be satisfactory in application to the celebral cortex.

Mitochondria from the white matter isolated by means of this method

were contaminated by myeline fragments. Therefore in this case it was necessary to apply some additional purification. Finally we could obtain a morphologically pure mitochondrial fraction (confirmed by electron-micrography).

It is generally known that brain white matter is mainly composed of axon fibers, glial cells and myeline. The mitochondria from the white matter prepared by us originated from axonal and glial cells. The concentration of mitochondria in these parts of the brain was small in comparison to the mitochondria concentration in the gray matter.

We have shown differences in the concentrations of nucleic acids in both types of the mitochondria analysed. The mitochondria isolated from the white matter showed a higher RNA and DNA concentration. The concentration of nucleic acids showed by us in brain mitochondria was within the same range of values as that obtained from any other tissues (Nass et al. 1965, O'Brien and Kalf 1967). The existence of DNA and different RNA fractions in mitochondria isolated mainly from the liver was described by several authors (Nass et al. 1965, Dubin and Brown 1967, Bartkowiak and Wierzbicki 1968, Leffler et al. 1969).

In cerebral mitochondria obtained from the gray and white matter we have demonstrated two main types of RNA: s-RNA and r-RNA. The elution profile from MAK column and the nucleotide composition of both fractions confirmed the observation that cerebral mitochondria posses s-RNA and r-RNA. The nucleotide composition of s-RNA may be compared with the results obtained by other authors for s-RNA isolated from mammalian tissues (Bartkowiak and Wierzbicki 1968). The r-RNA was characterized by its high concentration of guanylic and cytidylic nucleotides and its composition was similar to the nucleotide composition of 28 S r-RNA isolated by Hirsh (1966) from whole rat and rabbit brain.

The small differences showed by us in RNA nucleotide composition in both types of the mitochondria analysed was not statistically significant. The fraction eluted from MAK column by high NaCl concentration in ammonia may be considered as a part of r-RNA because of its characteristic nucleotide composition.

In the paper published previously we have demonstrated that the DNA present in the mitochondria was not of nuclear origin (Borkowski et al. 1967, 1968). This was also demonstrated by several other authors (Nass et al. 1965). The base composition of DNA isolated from brain mitochondria compared with the base composition of the nuclear DNA confirmed that mitochondrial DNA was not of nuclear origin. The difference between the base composition of mitochondrial and nuclear DNA was also shown by other authors (Kazakowa and Markosian 1966, Nass 1967).

It has to be stressed that both types of the analysed brain mitochondria were characterized by their high s-RNA concentration. About 30–40% of total extractable mitochondrial nucleic acids were identified as s-RNA. In the next paper our results concerning the biological activity of mitochondrial s-RNA shall be presented.

SUMMARY

The quantity of mitochondria isolated from the gray matter was two times larger than from the white matter.

The mitochondrial DNA concentration from the white matter was almost twice higher than that from the gray matter mitochondria. The ribonucleic acids were represented in both mitochondrial fractions by two main types: s-RNA and r-RNA. This was confirmed by the elution profile from a MAK column and nucleotide composition. In both mitochondrial fractions analysed we have found high concentrations of s-RNA. The analysis of their base composition revealed a difference between mitochondrial and nuclear DNA.

The author wishes to acknowledge the skilful technical assistance of Mgr Irena Snieżyńska and Mgr Alicja Paszkowska. This investigation was partially supported by Polish Academy of Sciences, Biochemical Committee.

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Received 15 January 1971