PROTEIN SYNTHESIS IN ISOLATED RAT BRAIN MITOCHONDRIA

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Summary

Protein synthesis in a rat brain mitochondrial preparation purified by centrifugation through a ficoll gradient is inhibited by chloramphenicol and oxytetracycline but not by cycloheximide. This establishes that rat brain mitochondrial protein synthesis is similar to liver mitochondria in its sensitivity to these antibiotics. The possible nature of the cycloheximide-sensitive protein synthesis found with other brain mitochondrial preparations is discussed.

Introduction

Recent studies have suggested that protein synthesis in brain mitochondrial preparations is different from that in other mitochondria in its sensitivity towards certain antibiotics. For example, cycloheximide inhibits protein synthesis in brain mitochondrial fractions (1-7) while the same antibiotic has no effect upon mitochondria from other sources (8-10). On the other hand, chloramphenicol inhibits protein synthesis in mitochondria isolated from brain (2,3,5-7,11) as well as other tissues (12-14). A rat brain mitochondrial preparation produced only insoluble protein via the chloramphenicol sensitive protein synthesizing site whereas the cycloheximide sensitive site produced both soluble and insoluble proteins (6). Therefore, attempts have been made to separate these two protein synthesizing sites asso ciated with isolated brain mitochondrial preparations to determine whether these two sites reside in the same or in different subcellular components. In this communication isolation of rat brain mitochondrial fractions using a discontinuous ficoll gradient is reported. Protein synthesis in these preparations is inhibited by chloramphenicol and oxytetracycline but not by cycloheximide. The cycloheximide sensitive protein synthesizing component

is recovered in non-mitochondrial fractions obtained during ficoll gradient purification.

Methods

Female Sprague/Dawley rats weighing approximately 150 gm were used in this investigation. The brains were homogenized (0-4°) is a tissue grinder with 10 times (v/w) of a medium containing 0.3 M mannitol-0.001 M EDTA pH 7.2-7.4 (ME). The whole homogenate was subjected to two centrifugations at 770 g for 15 and 10 min. The supernatant was centrifuged at 12,500 g for 15 min to sediment the mitochondria. This crude mitochondrial fraction was resuspended in ME medium and was layered on a discontinuous ficoll gradient composed of 5 ml each of 2%, 8%, 12%, 16%, and 20% ficoll in ME medium. The gradient was prepared at room temperature and was allowed to stand at room temperature for 60-75 min and then in ice-water bath for 30-45 min. The gradient with the crude mitochondrial preparation at the top was spun at 63,000 g (av) for 1 hr using SW 25.1 rotor. Six fractions (A-F) were obtained. The fraction A is the topmost layer and F is the pellet. These fractions were recovered and diluted with fresh ME medium and resedimented. This method of fractionation of crude mitochondrial preparation is similar to that described by Abdel-Latif (15) and Moore and Jobsis (16). Prevention of and checking for bacterial contamination was performed as previously described (6). Protein synthesis was measured by measuring C^{14}-leucine incorporated into protein in a medium with the following final composition: 0.1 M mannitol, 50 mM KCl, 0.33 mM EDTA, 10 mM succinate, 20 mM potassium phosphate, 2 mM ADP, 5 mM $MgCl_2$, 50 ug synthetic amino acid mixture/ml and 0.6 uc of L-leucine - $^{14}C(U)$/ml. Proteins were precipitated with trichloroacetic acid, processed and counted as previously described (6).

For electron microscopy the washed pellets were fixed in 2.5% gluteraldehyde After 2 hrs or more fixation the pellets were washed with 3 or more changes of 0.1 cacodylate buffer, cut into approximately 1 mm^2 blocks and fixed for an additional hour in 1% OsO_4 in 0.1 M cacodylate. The fixed blockes were

dehydrated in alcohol and imbedded in Epon 812 (Shell Chemical Co.) according to the methods of Luft (17). Sections were cut with glass knives on an LKB ultramicrotome and collected on formvar coated copper grids. Sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I electron microscope.

Results

Figure 1 shows an electron micrograph of the pellet (Fraction F) obtained from the ficoll gradient and washed once with ME medium. It consists almost exclusively of mitochondria. Most of the mitochondria are in the "condensed" form although a few are present in the "orthodox" form. This mitochondrial fraction is also characterized by having highest cytochrome oxidase and very little esterase activity in comparison to the other fractions.

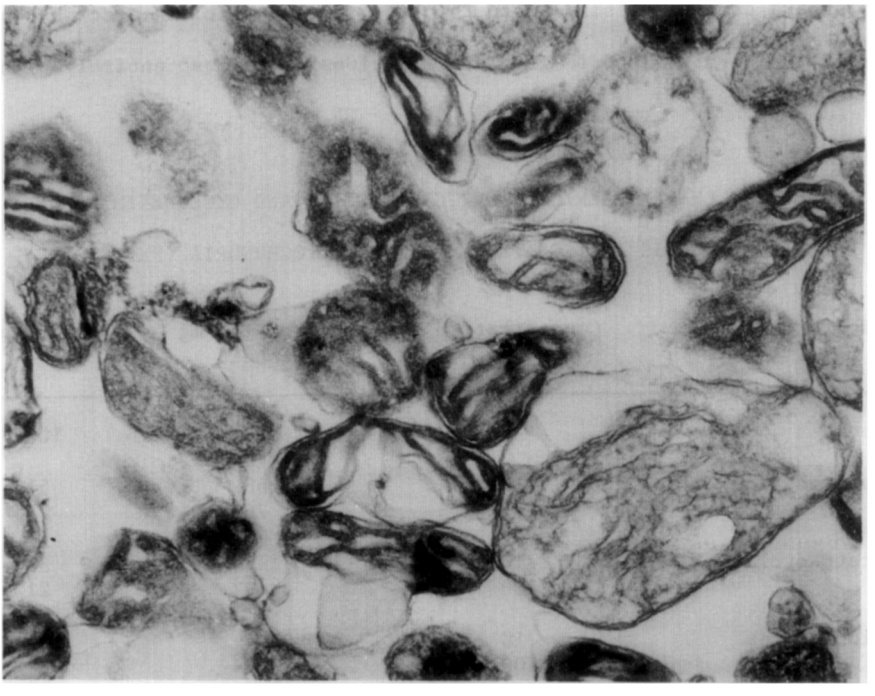

Figure 1. Electron micrograph of Fraction F. Magnification 30,000 X.

The effect of chloramphenicol, oxytetracycline and cycloheximide on the incorporation of ^{14}C leucine into protein by the isolated rat brain mitochondrial preparation (Fraction F) is shown in Table I. Both chloramphenicol and oxytetracycline strongly inhibited protein synthesis. On the other hand, cycloheximide did not significantly inhibit protein synthesis even at a concentration as high as 500 ug/ml. The extents of inhibition by chloramphenicol and oxytetracycline on protein synthesis by this brain mitochondrial preparation are comparable to those found with mitochondria isolated from other sources (12-14)

The rate of protein synthesis in Fraction A is very low. The protein synthesis in Fractions B and C is strongly inhibited by cycloheximide and to a small extent by chloramphenicol. Fractions D and E are more sensitive to chloramphenicol and less to cycloheximide. Electron micrographs of fractions B and C show the present of synaptosomes, vesicles as well as some unidentified particles and structures similar to the "very rare particles" described by Morgan (7). The mitochondria in these fractions are almost entirely within the

TABLE I

EFFECT OF CHLORAMPHENICOL, OXYTETRACYCLINE AND CYCLOHEXIMIDE ON PROTEIN SYNTHESIS IN ISOLATED RAT BRAIN MITOCHONDRIA (FRACTION F)

Inhibitor	Concentration ug/ml	counts/min/mg protein	% control
None	-	330	100
Chloramphenicol	10	168	51
	100	76	23
Oxytetracycline	10	125	38
	100	69	21
Cycloheximide	10	304	92
	100	294	89
	500	290	88

The incubations were carried out in the medium described in the text with approximately 1 mg of Fraction F protein per ml. Incubation time was 30 min at 30°.

synaptosomes. Moreover, the specific activities of cytochrome oxidase are very low in these fractions. Fraction D is apparently a mixture of free mitochondria and synaptosomes. Fractions E is mostly free mitochondria as judged by electron microscopy but it is apparently less pure than Fraction F.

Discussion

Previous work (6) has shown that protein synthesis in a rat brain mitochondrial preparation purified by centrifugation through a sucrose gradient was inhibited partially by both chloramphenicol and cycloheximide. The results presented in this paper clearly show that the purification of the brain mitochondrial preparation by the ficoll procedure separated the cycloheximide and chloramphenicol sensitive protein synthesizing sites. The cycloheximide sensitive protein synthesizing site is located in a subcellular component different from that containing the chloramphenicol sensitive site. The highest chloramphenicol sensitivity is found in Fraction F which is richest in mitochondria.

In general, protein synthesis in isolated mitochondria is strongly inhibited by chloramphenicol and oxytetracycline (12-14), but is insensitive to cycloheximide (8-10). Table I shows that the protein synthesis in Fraction F is strongly inhibited by chloramphenicol and oxytetracycline but is virtually unaffected by cycloheximide. Thus, rat brain mitochondrial protein synthesis is identical to liver mitochondria in its sensitivity to the three antibiotics tested.

Since the cycloheximide sensitive protein synthesizing site is located in Fractions B and C it suggests that the cycloheximide sensitive site found with other brain mitochondrial preparations is due to contamination with non-mitochondrial components. The nature of the subcellular component containing this cycloheximide sensitive site is not yet clear. However, it is reasonable to believe that this site is enclosed by a membrane (6). The synaptosomes are apparently the most likely candidates because of their sensitivity to cycloheximide (4,18). It is possible that some of the synaptosomes may show substantial protein synthesizing capacity and may sediment with the mitochondria

during certain isolation procedures. While this work was in progress Morgan (7) proposed that the cycloheximide sensitive protein synthesis associated with brain mitochondrial preparations might be due to the "very rare particles" containing cytoplasmic ribosomes. Although this is an attractive hypothesis the other possibility as mentioned above should also be considered. One point is clear, however, the cycloheximide sensitive component is certainly due to cytoplasmic ribosomes enclosed within a membrane.

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References

1. Gordon, M.W. and Deanin, G.G., J. Biol. Chem., 243,4222 (1968).
2. Cunningham, R.D. and Bridgers, W.F., Biochem. Biophys. Res. Commun., 38,99 (1970).
3. Bosmann, H.B. and Hemsworth, B.A., J. Biol. Chem., 245,363 (1970).
4. Deanin, G.G. and Gordon, M.W., Fed. Proc., 29,871 (1970).
5. Winston, R.A. and Bosmann, H.B., Fed. Proc., 29,941 (1970).
6. Haldar, D., Biochem. Biophys. Res. Commun., 40,129 (1970).
7. Morgan, I.G., Febs Letters, 10,273 (1970).
8. Beattie, D.S., Basford, R.E. and Koritz, S.B., Biochemistry, 6,3099 (1967).
9. Ashwell, M. and Work, T.S., Biochem. Biophys. Res. Commun., 32,1006 (1968).
10. Lamb, A.J., Clark-Walker, G.D. and Linnane, A.W., Biochim. Biophys. Acta, 161,415 (1968).
11. Bachelard, H.S., Biochem. J., 100,131 (1966).
12. Kroon, A.M., In "Handbook of Molecular Cytology", Ed. A. Lima de Feria, North-Holland, Amsterdam, 1969, p. 943.
13. Wheeldon, L. and Lehninger, A.L., Biochemistry, 5,3533 (1966).
14. Haldar, D. and Freeman, K.B., Fed. Proc., 27,771 (1968).
15. Abdel-Latif, A.A., Biochim. Biophys. Acta, 121,403 (1966).
16. Moore, C.L. and Jobsis, F.F., Arch. Biochem. Biophys., 138,295 (1970).
17. Luft, J.H., J. Biophys. Biochem. Cytol., 9,409 (1961).
18. Morgan, I.G. and Austin, L., J. Neurochem., 15,41 (1968).