PROTEIN SYNTHESIS IN MAMMALIAN BRAIN MITOCHONDRIA

D. Haldar
Department of Biochemistry
St. Jude Children's Research Hospital
Memphis, Tennessee 38101

Received May 26, 1970

SUMMARY

Protein synthesis by purified brain mitochondrial preparations from rat (RBM), mouse and guinea pig is inhibited partially by both chloramphenicol (CAP) and cycloheximide (CH). Ribonuclease has no inhibitory action on protein synthesis by RBM. Both soluble and insoluble proteins isolated from RBM labeled *in vitro* have been found to be labeled. The synthesis of the soluble proteins is strongly inhibited by CH but is virtually insensitive to CAP. The synthesis of insoluble proteins is inhibited by both CAP and CH. These results suggest the presence of two sites in the protein synthesizing system of RBM.

INTRODUCTION

In general the protein synthetic machinery of mitochondria resembles that of bacteria in that it is inhibited by chloramphenicol (9, 11, 18), but is insensitive to cycloheximide (1, 4, 12). However, the action of these two antibiotics on the protein synthesis in isolated mammalian brain mitochondria is different. The protein synthesis in brain mitochondria isolated from the rat is inhibited by acetoxycycloheximide (7). Using a mitochondrial fraction from mouse brain Cunningham and Bridgers (6) also found that the protein synthesis is inhibited by CH. In this respect, the protein synthesis in brain mitochondria resembles that in the cytoplasmic ribosomal system (5, 15-17).

The action of CAP on the protein synthesis in isolated mammalian brain mitochondria is controversial. For example, Bachelard (2) reported the protein synthesis in isolated mitochondria from cerebral cortex of guinea pig and rabbit was inhibited by CAP. But the high level of bacterial contamination found in these studies raised the question of how much of the CAP inhibition was due to contaminating bacteria. Conversely, Gordon and Deanin (7) reported that the protein synthesis by isolated mitochondria from rat cerebral cortex is resistant to CAP. Cunningham and Bridgers (6), working with mitochondrial preparations from mouse brain and liver, found that the inhibitory action of CAP is observed only in K^+-rich media but not in Na^+-rich media. However, it is interesting to note that Gordon and Deanin (7) did not observe the inhibitory action of CAP on

the protein synthesis by isolated rat brain mitochondria incubated in the K^+-rich medium of Bachelard (2). The discrepancies observed may be explained by the differences in species of the animals used by different investigators. To resolve the above discrepancies and to extend the findings, the effects of CAP and CH on protein synthesis by isolated mammalian brain mitochondria from different species have been studied. Furthermore, the product of protein synthesized in isolated brain mitochondria also has been characterized.

METHODS

Sprague Dawley rats weighing approximately 150 gm, adult BALB/C mice (22-25 gm), and adult guinea pigs (300-400 gm) were used. All the animals were female and were fed *ad libitum*. They were killed by decapitation. The brains were homogenized (0-4°) in tissue homogenizer with 10 volumes of a medium containing 0.3M sucrose - 0.002M EDTA pH 7.2 (SE). The crude mitochondrial fraction was prepared following essentially the method of Roodyn *et al.* (14) with two washings of the mitochondrial pellets at 8000g. The crude fraction was purified by the method of Gray and Whittaker (8). The pellet obtained by this procedure was washed once with SE medium and finally resuspended in the same medium. Protein synthesis was measured by studying the amino acid incorporation into protein in the "improved medium A" of Haldar and Freeman (10) at 30°. This medium had the following final composition: 0.1M sucrose, 50mM KCl, 0.67 mM EDTA, 10 mM succinate, 20 mM potassium phosphate, 2 mM ADP, 5 mM $MgCl_2$,50 μg synthetic amino acid mixture/ml, and 0.6 μc of L-Leucine-^{14}C (U)/ml. All equipment, the isolation medium and the individual solutions of the incubation medium excepting ADP were autoclaved before use. Bacterial contamination of all mitochondrial preparations was checked by plating aliquots (2 x 0.05 ml) on blood agar plates and incubating at 37° for 48 hours. The soluble mitochondrial proteins were prepared by the method of Beattie *et al.* (3) involving two water extractions of the mitochondrial pellet followed by two extractions with 0.9% KCl at 30°. Proteins were precipitated with trichloroacetic acid (final concentration 5%) and processed essentially as previously described (10), and finally counted in a Nuclear-Chicago gas flow counter with an efficiency of 17%. Estimation of protein was carried out by the method of Lowry *et al.* (13) with bovine plasma albumin as standard.

RESULTS AND DISCUSSION

The action of CAP and CH on the incorporation of ^{14}C leucine into protein by RBM is shown in Table I. Both antibiotics inhibited protein synthesis. When the antibiotics were added together, the extent of inhibition was more than that obtained with either alone. In order to check whether the inhibitory action of CAP was due to bacterial contamination in the mitochondrial fraction, all

TABLE I

EFFECT OF CHLORAMPHENICOL AND CYCLOHEXIMIDE ON PROTEIN SYNTHESIS BY ISOLATED RAT BRAIN MITOCHONDRIA

Inhibitor	Concentration μg/ml	Expt. 1 cpm/mg protein	Expt. 1 % control	Expt. 2 cpm/mg protein	Expt. 2 % control
None	-	145	100	128	100
CAP	10	-	-	96	75
	25	100	69	86	67
	100	-	-	56	44
CH	10	-	-	96	75
	25	70	48	56	44
	100	-	-	64	50
CAP + CH	25 + 25	30	21	-	-
RNAse	100	-	-	138	108

The incubations were carried out in the medium described in the text with approximately 1 mg of mitochondrial protein per ml. Uniformly labeled L-Leucine-^{14}C (Specific Activity, 50 μc/μmole) was added to a final concentration of 0.6 μc/ml. Incubation time was 30 min.

preparations were plated and incubated. Bacterial contamination was found to be negligible. No experiment has been reported in this article where the bacterial colonies exceeded 100/mg incubated protein. Thus, it is clear that the inhibitory action of chloramphenicol observed in these experiments was not due to its action on contaminating bacteria.

When CAP and CH were tested on brain mitochondrial preparations from other mammalian species such as mouse and guniea pig, both antibiotics inhibited protein synthesis as shown in Table II. This indicates that the susceptibility to the inhibitory actions of CAP and CH is a common property of the protein synthesis associated with isolated mammalian brain mitochondria.

It is very unlikely that the inhibitory action of CH observed in these experiments is due to

TABLE II

EFFECT OF CHLORAMPHENCIOL AND CYCLOHEXIMIDE ON PROTEIN SYNTHESIS BY ISOLATED BRAIN MITOCHONDRIA FROM MOUSE AND GUINEA PIG

Inhibitor	Concentration (μg/ml)	Mouse		Guinea Pig	
		cpm/mg protein	% control	cpm/mg protein	% control
None	-	152	100	76	100
CAP	100	83	55	37	49
CH	100	77	51	52	68

The conditions of the incubations were the same as described for Table I.

cytoplasmic ribosomal contamination since ribonuclease (100 μg/ml) did not result in inhibition of protein synthesis of the mitochondrial preparations (Table I). Secondly, repeated washings of the mitochondrial fractions with SE did not significantly reduce their sensitivity to CH. Similar results were obtained by other workers (6, 7). However, the possibility that the cytoplasmic ribosomes are protected from ribonuclease action by some sort of membrane and that the ribosome-membrane structure sedimented with the mitochondria under the isolation procedure employed cannot be ruled out.

To determine the type of protein synthesized in RBM, the organelles labeled *in vitro* were fractionated into soluble and insoluble proteins and the specific activities determined. Not only the insoluble proteins but also the soluble proteins contained significant amount of label (Table III). The effects of CAP and CH on the *in vitro* synthesis of the soluble and insoluble proteins also are documented in the same table. It was observed that CAP inhibited the synthesis of insoluble proteins while CH inhibited the synthesis of the soluble proteins and also probably that of a limited amount of insoluble proteins.

These results suggest the presence of two protein synthesizing sites in this *in vitro* system. One site synthesizes insoluble proteins and is inhibited by CAP, whereas the other site synthesizes both soluble and insoluble proteins and is inhibited by CH. Both of these protein synthesizing sites may be present in the same organelle. Alternatively, there may be two types of organelles each

TABLE III

ACTION OF CHLORAMPHENICOL AND CYCLOHEXIMIDE ON SYNTHESIS OF SOLUBLE AND INSOLUBLE PROTEINS BY ISOLATED RAT BRAIN MITOCHONDRIA

Inhibitor	Concentration (μg/ml)	Whole Mitochondria cpm/mg protein	Whole Mitochondria % control	Soluble Protein cpm/mg protein	Soluble Protein % control	Insoluble Protein cpm/mg protein	Insoluble Protein % control
None	-	740	100	455	100	835	100
CAP	100	340	46	436	96	242	29
CH	100	429	58	177	39	659	79

The incubations were carried out for 40 min. as described for Table I. The specific activity of the L-Leucine-^{14}C (U) was 250 μc/μmole. The reactions were terminated by chilling the flasks in ice and the mitochondria then were recovered by sedimenting in the cold at 10,000 g for 10 min.

containing one type of protein synthesizing site. If there are two types of organelles, one type synthesizes only insoluble protein and is inhibited by CAP and thus mimics the *in vitro* protein synthesis in liver mitochondria. The other type of organelle synthesizes both soluble and insoluble proteins and is susceptible to CH. Experiments are in progress to decide between the above possibilities and to determine whether the CH sensitive protein synthesis is truly mitochondrial.

ACKNOWLEDGEMENT

I thank Mrs. Susan Dodson and Mrs. Connie Gowder for their excellent technical assistance. The studies were supported in part by Public Health Service Grants AM-07375 (to Dr. Bruce Sells) and CA-08480.

REFERENCES

1. Ashwell, M. and Work, T.S. (1968) Biochem. Biophys. Res. Commun., *32*, 1006.
2. Bachelard, H.S. (1966) Biochem. J., *100*, 131.
3. Beattie, D.S., Basford, R.E. and Koritz, S.B. (1966) Biochemistry, *5*, 926.

4. Beattie, D.S., Basford, R.E. and Koritz, S.B. (1967) Biochemistry, *6*, 3099.
5. Colombo, B., Felicetti, L. and Baglioni, C. (1966) Biochim. Biophys. Acta, *119*, 109.
6. Cunningham, R.D. and Bridgers, W.F. (1970) Biochem. Biophys. Res. Commun., *38*, 99.
7. Gordon, M.W. and Deanin, G.G. (1968) J. Biol. Chem., *243*, 4222.
8. Gray, E.G. and Whittaker, V.P. (1962) J. Anat. (London), *96*,79.
9. Haldar, D. and Freeman, K.B. (1968) Federation Proc., *28*, 771.
10. Haldar, D. and Freeman, K.B. (1969) Biochem. J., *111*, 653.
11. Kroon, A.M. (1963) Biochim. Biophys. Acta, *76*, 165.
12. Lamb, A.J., Clark-Walker, G.D. and Linnane, A.W. (1968) Biochim. Biophys. Acta, *161*, 415.
13. Lowry, O.H., Roseborough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem., *193*, 265.
14. Roodyn, D.B., Reis, P.J. and Work, T.S. (1961) Biochem. J., *80*, 9.
15. Siegel, M.R. and Sisler, H.D. (1965) Biochim. Biophys. Acta, *103*, 558.
16. Vazquez, D. and Monro, R.E. (1967) Biochim. Biophys. Acta, *142*, 155.
17. Wettestein, F.O., Noll, H. and Penman, S. (1964) Biochim. Biophys. Acta, *87*, 525.
18. Wheeldon, L and Lehninger, A.L. (1966) Biochemistry, *5*, 3533.