Biochemical Pharmacology, Vol. 22, pp. 659–660. Pergamon Press, 1973. Printed in Great Britain.

SHORT COMMUNICATION

Effect of rifampicin on mitochondrial protein synthesis in rat liver

(*Received* 28 *June* 1972; *accepted* 25 *September* 1972)

PROTEIN synthesis by isolated mitochondria from different sources has been studied widely.[1–4] Synthetic mRNA viz. Poly U can be incorporated into apparently intact mitochondria and stimulate phenylalanine incorporation there.[5–7] Actinomycin D, an inhibitor of DNA dependent RNA synthesis, has already been found to inhibit mitochondrial protein synthesis in *vigna sinensis*.[8] Some members of rifamycin group have been reported to inhibit cell-free protein synthesis in different systems.[9,10] But the inhibition of RNA synthesis is the primary action of the rifamycins and their effect on protein and DNA synthesis is only a consequency of the inhibition of RNA synthesis.[11,12] Very little work has been done to see the effect of rifamycin on the protein synthesis in eukaryotic system particularly in mitochondria. The present paper is mainly concerned with the effects of rifampicin, a member of rifamycins, poly U and actinomycin D on the *in vitro* incorporation of L-phenylalanine-U-^{14}C into proteins by isolated mitochondria from rat liver.

Mitochondria from rat liver were prepared according to the method described by Chakrabarti *et al.*[1] Rat liver was homogenized with Potter–Elvehjem homogenizer in 8 vol. of ice-cold medium A (0·25 M sucrose; 0·05 M Tris–HCl buffer, pH 7·4; 0·025 M potassium phosphate buffer, pH 7·4 and 0·025 M KCl). The homogenate was centrifuged at 1000 *g* for 10 min at 0°. The supernatant fluid was centrifuged at 10,000 *g* for 40 min, the sediment was suspended in medium A and then centrifuged at 1000 *g* for 10 min at 0°. The supernatant fluid was finally centrifuged at 10,000 *g* to obtain the mitochondrial fraction. The 10,000 *g* pellet thus separated was washed twice with cold medium A.

Mitochondria were practically devoid of glucose-6-phosphatase activity. The RNA of the mitochondrial fraction was extracted according to Schneider[13] and the liberated ribose obtained after the hydrolysis of RNA with 5% TCA was determined according to Dische, as described by Ashwell.[14] The level of RNA in the mitochondria was not reduced by exogenous RNase (Sigma, five times crystallized protease-free). Protein content of the mitochondrial fraction was determined by the biuret method.[15] RNA–protein ratio of the mitochondrial fraction was found to be 0·11 which was not affected due to the treatment of mitochondrial fraction with RNase. The phase contrast microscopic study indicated that mitochondria were free from nuclear or whole cell material. Amino acid incorporating activity of the mitochondria was found resistant to RNase which clearly rules out the doubt about the intactness of mitochondria.[7,16]

TABLE 1. EFFECT OF RIFAMPICIN, POLY-U AND ACTINOMYCIN D ON THE INCORPORATION OF L-PHENYLALANINE-U-^{14}C INTO PROTEINS BY ISOLATED MITOCHONDRIA FROM RAT LIVER

System	Incorporation (counts/min/mg protein)
Complete	4560 ± 420
Complete + poly U (50 μg)*	8420 ± 370
Complete + poly U (50 μg) + act. D (20 μg)*	7520 ± 300
Complete + poly U (50 μg) + rifampicin (75 μg)*	450 ± 60
Complete + act. D (20 μg)†	1150 ± 170
Complete + act. D (20 μg) + poly U (50 μg)†	8840 ± 320
Complete + rifampicin (75 μg)‡	380 ± 80
Complete + rifampicin (75 μg) + poly U (50 μg)‡	460 ± 80

* Mitochondria preincubated with poly U (50 μg) for 10 min at 30°.
† Mitochondria preincubated with act. D (20 μg) for 10 min at 30°.
‡ Mitochondria preincubated with rifampicin (75 μg) for 10 min at 30°.
Results are the averages of five experiments with ±S.D.

The complete incubation system contained 1 μm of ATP, 3 μm phosphoenolpyrurate, 10 μg of pyrurate kinase, 5 μm of $MgCl_2$, 250 μm of sucrose, 50 μm of Tris–HCl buffer (pH 7·4), 20 μm of potassium phosphate buffer (pH 7·4), L-phenylalanine-U-^{14}C (counts/min $1{\cdot}15 \times 10^5$), spec. act. 25 mCi/mmole) and 4–4·5 mg of mitochondrial protein. The total volume of the incubation mixture was 1 ml. The incubation was carried out aerobically for 2 hr at 37° with constant shaking. The incubation was stopped by the addition of 0·3 ml of 30% TCA.

For the measurement of radioactivity, protein was processed according to the method of Stachiewicz and Quastel[17] as reported by Das *et al.*[2] The radioactivity was determined in a gas-flow counter (Nuclear Chicago).

The data given in Table 1 indicate that labelled phenylalanine incorporation into proteins by isolated mitochondria from rat liver is inhibited by both actinomycin D and rifampicin, a member of rifamycins. But the inhibitory effect is more pronounced with the latter. When mitochondria were preincubated with actinomycin D, synthetic mRNA viz. Poly U can stimulate labelled phenylalanine incorporation into mitochondrial protein. But poly-U failed to stimulate phenylalanine incorporation when isolated mitochondria were pretreated with rifampicin. It has already been reported that both actinomycin D[18] and rifampicin[19,20] inhibit mitochondrial RNA synthesis—the former combines with DNA[21] but the latter with the β-subunit of RNA polymerase.[22] As rifampicin but not actinomycin D inhibits the poly U-dependent stimulation of ^{14}C-phenylalanine incorporation into mitochondrial proteins of rat liver, rifampicin must deprogramme the protein synthetic sites and also prevent poly-U attachment. Further studies are required to elucidate the actual mechanism by which rifampicin inhibits mitochondrial protein synthesis in eukaryotic systems.

Department of Biochemistry,
University of Calcutta,
35, *Ballygunge Circular Road,*
Calcutta 19, *India*

DIPAK K. DUBE
SYAMALIMA CHAKRABARTI
ADITI SARKAR
PRABIR. BHATTACHARYA
BISHENDU B. GOSWAMI
SAILESH C. ROY

REFERENCES

1. S. CHAKRABARTI, D. K. DUBE and S. C. ROY, *Biochem. J.* **128,** 461 (1972).
2. H. K. DAS, S. K. CHATTERJEE and S. C. ROY, *J. biol. Chem.* **239,** 1126 (1964).
3. M. A. ASHWELL and T. S. WORK, *Ann. Rev. Biochem.* **39,** 251 (1970).
4. A. M. KROON, *Biochim. biophys. Acta* **69,** 184 (1963).
5. S. C. ROY, D. K. DUBE, S. BISHAYEE, C. LAHIRI and S. CHAKRABARTI, *Tenth Int. Natn. Cancer Con.* Houston, Abst. p. 28 (1970).
6. D. K. DUBE, S. CHAKRABARTI and S. C. ROY, *Cancer* **29,** 1575 (1972).
7. R. F. SWANSON, *Nature* **231,** 31 (1971).
8. S. K. CHATTERJEE, H. K. DAS and S. C. ROY, *Biochim. biophys. Acta* **114,** 349 (1966).
9. C. CALVORI, L. FROMTALI, L. LEONI and G. TECCE, *Nature* **207,** 417 (1965).
10. L. FRONTALI and G. TECCEE, in *Antibiotics* (Eds. D. GOTTLIEB and P. D. SHAW) Vol. 1, p. 415 (1967).
11. G. C. LANCINI and G. SAROTORI, *Experientia* **24,** 1105 (1968).
12. P. REED and J. SPEYER, *J. Bacteriol.* **104,** 376 (1970).
13. W. C. SCHNEIDER, *J. biol. Chem.* **161,** 293 (1945).
14. G. ASHWELL, *Methods in Enzymology*, Vol 3, p. 8. Academic Press, New York (1955).
15. A. G. GRONALL, C. S. BARDERWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1949).
16. D. K. DUBE, S. CHAKRABARTI and S. C. ROY, *Sci. Culture, India* **38,** 139 (1972).
17. E. STACHIEWICZ and T. H. QUASTEL, *Can. J. Biochem. Physiol.* **37,** 687 (1959).
18. S. YANG and R. S. CRIDDLE, *Biochemistry* **9,** 3063 (1970).
19. J. G. GAMBLE and R. H. MCCLUER, *J. Molec. Biol.* **54,** 557 (1970).
20. ZH. G. SHMERLING, *Biochem. biophys. Res. Commun.* **37,** 965 (1969).
21. E. REICH, *Cancer Res.* **23,** 1428 (1963).
22. W. K. ZILLIG, D. ZECHEL, D. RABUSSAY, M. SCHACHNER, V. S. SETHI, P. PALM, A. HELL and W. SEIFERT, *Cold Spring Harbor Symp. Quant. Biol.* **35,** 47 (1970).