THE EFFECTS OF THE RIFAMYCIN ANTIBIOTICS ON ALGAE

M.RODRIGUEZ-LÓPEZ, M.L.MUÑOZ
Instituto Marañon, Velázquez 144, Madrid 6

and

D.VAZQUEZ
Instituto de Biología Celular, Velázquez 144, Madrid 6, Spain

Received 14 May 1970
Revised version received 24 June 1970

1. Introduction

It is well known that blue-green algae and green algae clearly differ in their protein synthesis machinery, blue-green algae having 70 S ribosomes similar to those of bacteria in their sedimentation coefficient and sensitivity towards antibiotics [1–4] whereas the green algae cytoplasm contains 80 S ribosomes similar to other eukaryotic ribosomes in their sensitivity to antibiotics [1–7]. It appears that a similar situation might occur in the mechanism of RNA synthesis as it is widely accepted that the rifamycins are inhibitors of RNA polymerase from bacteria, mitochondria and chloroplasts but are not active on RNA polymerase from eukaryotic cells [8–14]. We have studied the effects of the rifamycin antibiotics on a blue-green and a green algae and the results obtained are reported in this work.

2. Materials and methods

2.1. *Algae and growth conditions*

The work was carried out with *Anacystis montana* (1405/3) and *Chlorella pyrenoidosa* (211/8a) from the Culture Collection of Algae and Protozoa (The Botany School, University of Cambridge, England). Cultures were free from bacteria. *A. montana* was grown in medium D [15] and *C. pyrenoidosa* in a medium previously described [16]. The cells were grown exponentially at 24° passing air containing 2% CO_2 through the cultures exposed to the light. Growth was estimated by measuring absorbancy at 600 nm in a Beckman D.U. spectrophotometer.

2.2. *Net synthesis and uptake of ^{14}C-rifampicin by intact bacteria*

To estimate net synthesis, total nucleic acids were extracted in 0.5 N perchloric acid at 70° and absorbancy of the extract measured at 260 nm [17]. DNA was estimated in the same extract by the diphenylamine method of Burton [18] and total protein was estimated by the method of Lowry et al. [19] in the residual pellet.

Uptake of ^{14}C-rifampicin (specific activity 693 μCi/mmole) (Lepetit Laboratories) by the cells was estimated as previously described using other antibiotics and organisms [20].

2.3. *Incorporation of ^{3}H-thymidine, ^{3}H-uridine and ^{14}C-leucine by intact cells*

^{3}H-Thymidine (515 mCi/mmole), ^{3}H-uridine (6.7 mCi/mmole) and ^{14}C-leucine (22.2 mCi/mmole) were used to study incorporation in the DNA, RNA and protein fractions, respectively. 5 ml Aliquots of *A. montana* cultures (0.1 mg dry wt/ml) were treated with the required antibiotic while growing exponentially and adding either 5 μCi ^{3}H-thymidine/ml or 0.2 μCi ^{13}H-uridine/ml or 0.1 μCi ^{14}C-leucine/ml. 1 ml samples were extracted with 5% trichloroacetic acid

at 0° to remove the pool after 5, 15, 30 and 60 min of incubation. The precipitates were collected by filtration through glass filters (Whatman GF/A) washed with 5% cold trichloroacetic acid and finally 1% acetic acid before measuring the radioactivity incorporated.

2.4. *Incorporation of ^{14}C-UTP in a cell-free system*

A. montana extracts were prepared by alumina grinding and used without further purification after separation of cell debris and membranes. The system for incorporation of ^{14}C-UTP was taken from that previously described by other workers [21]. 0.1 μCi ^{14}C-UTP (specific activity 5 mCi/mmole were added per tube.

2.5. *Incorporation of ^{14}C-phenylalanine in a cell-free system*

A. montana and *C. pyrenoidosa* ribosomes were prepared as previously described [3]. *Escherichia coli* MRE 600 and *Saccharomyces cerevisiae* supernatant fractions were used respectively in *A. montana* and *C. pyrenoidosa* cell-free systems to catalyse incorporation of ^{14}C-phenylalanine following methods essentially similar to those previously described [22]. 28 pmoles of ^{14}C-Phe-tRNA were added per tube.

3. Results

Rifamycin B (12 μM), rifamycin S (6 μM) and rifampicin (12 μM) completely blocked growth of *A. montana* observed after 24, 48 and 72 hr. Under the same conditions, these antibiotics even at concentration 24 μM did not affect growth of *C. pyrenoidosa* but the three antibiotics at concentration 12 μM caused bleaching of this algae. The bleaching of *C. pyrenoidosa* by the rifamycin antibiotics suggest that they are active on the chloroplasts and so the lack of growth inhibition in the cell is not due to impermeability to the antibiotics. In order to confirm this point, the uptake of ^{14}C-rifamycin by *A. montana* and *C. pyrenoidosa* was studied under similar conditions and results not presented here showed that the amount of the antibiotic taken up per mg dry weight of the two algae was practically the same.

Incubations of *A. montana* cultures for 24 hr were required in order to study net synthesis of DNA, RNA and protein. Under these conditions inhibitory concentrations of the rifamycin antibiotics drastically affected protein and RNA synthesis and also inhibited DNA synthesis although to a lesser extent. More realistic estimates of the degree of inhibition require shorter periods of incubation. This was done in separate experiments to study incorporation of ^{3}H-uridine, ^{3}H-thymidine and ^{14}C-leucine in the RNA, DNA and protein fractions respectively. The results obtained showed no inhibition of the incorporation of ^{14}C-thymidine into DNA by the rifamycins but there was a clear cut inhibition on the incorporation of ^{3}H-uridine and ^{14}C-leucine in the RNA and protein fractions respectively in the presence of the rifamycin antibiotics (table 1). In all cases and mainly in short incubations (results not shown here) the inhibition of ^{3}H-uridine incorporation is higher than inhibition of ^{14}C-leucine incorporation.

The effect of the rifamycin antibiotics on RNA synthesis was studied in *A. montana* and *C. pyrenoidosa* cell-free systems by following the incorporation of ^{14}C-UTP and the results obtained are presented in table 2. These results clearly show that the rifamycins inhibit ^{14}C-UTP incorporation by *A. montana* extracts but have no significant effect on *C. pyrenoidosa* extracts.

The effect of the rifamycin antibiotics on protein synthesis was also tested in cell-free systems by studying poly-U directed ^{14}C-phenylalanine incorporation using either *A. montana* ribosomes and *E. coli* supernatant or *C. pyrenoidosa* ribosomes and *S. cerevisiae* supernatant. No significant inhibition by the rifamycins was observed in these systems (table 3). Protein synthesis inhibitors of 70 S ribosomes affected the *A. montana* system as expected but not the *C. pyrenoidosa* system showing that this is not contaminated with chloroplast ribosomes. As would be expected incorporation by *C. pyrenoidosa* ribosomes is blocked by anisomycin and cycloheximide, two specific inhibitors of protein synthesis by eukaryotic systems.

4. Discussion

Results presented in this work show that the rifamycin antibiotics block growth of the blue-green algae *A. montana* but do not affect directly growth of the green algae *C. pyrenoidosa*. However the rifamycins caused bleaching of *C. pyrenoidosa* most

Table 1
Effects of the rifamycins on ^{3}H-thymidine, ^{3}H-uridine and ^{14}C-leucine incorporation on the DNA, RNA and protein fractions respectively of *A. montana*.

Antibiotic	(μM)	^{3}H-Thymidine incorporation (% control)	^{3}H-Uridine incorporation (% control)	^{14}C-Leucine incorporation (% control)
Rifamycin B	26	107	69	82
	65	93	59	66
	130	84	48	53
Rifamycin S	26	90	53	67
	65	104	41	54
	130	91	16	24
Rifampicin	26	96	56	66
	65	100	35	52
	130	79	12	21

Experimental procedures were as indicated in Materials and methods. Incubation times were 60 min for ^{3}H-thymidine and 30 min for ^{3}H-uridine and ^{14}C-leucine. Incorporation in the control experiments without antibiotics was 11.5 pM for ^{3}H-thymidine, 133 pM for ^{3}H-uridine and 3260 pM for ^{14}C-leucine.

Table 2
Incorporation of ^{14}C-UTP in cell-free systems.

Antibiotic	(μM)	*A. montana* ^{14}C-UTP incorporation		*C. pyrenoidosa* ^{14}C-UTP incorporation	
		pmoles	% control	pmoles	% control
Control without antibiotic		445	100	99	100
+ Rifamycin B	130	104	23	83	84
+ Rifamycin S	130	62	14	86	87
+ Rifampicin	130	100	22	84	85

Experimental conditions were as indicated in Materials and methods.

probably due to a direct effect of the antibiotics on the chloroplasts.

The inhibitory effect of the rifamycins on blue-green algae growth appears to be due to a direct effect of the antibiotics on RNA synthesis as it was shown in intact cells and confirmed in a cell-free system. Significant inhibition of protein synthesis was also found in intact cells but was not confirmed in a cell-free system. Inhibition of protein synthesis by the rifamycins has also been reported using intact bacteria and some bacterial cell-free systems [23] but inhibition of the RNA polymerase by these antibiotics is always more relevant to their mode of action [8–14]. It appears that *A. montana* RNA polymerase is not so sensitive to the rifamycin antibiotics as the bacterial enzyme. In fact it has been observed by other workers in purified preparations of RNA polymerase from *Anacystic nidulans* that the activity of the enzyme is not completely inhibited even at high concentrations of rifampicin [24].

The results reported in this work support the proposition that in blue-green algae RNA polymerase is the main target of the rifamycin antibiotics. It is concluded that RNA polymerase of blue-green algae has in common with the enzyme of bacteria, mitochondria and chloroplasts their sensitivity to the rifamycins.

Table 3
Poly U-directed incorporation of ^{14}C-phenylalanine in cell-free systems.

Antibiotic	(μM)	*A. montana* ^{14}C-phenylalanine incorporation		*C. pyrenoidosa* ^{14}C-phenylalanine incorporation	
		pmoles	% control	pmoles	% control
Control without antibiotic		8.0	100	1.04	100
+ Rifamycin B	130	6.6	83	0.96	92
+ Rifamycin S	130	6.7	84	1.02	98
+ Rifampicin	130	6.7	84	0.94	90
+ Lincomycin	100	0.2	2	1.01	97
+ Chloramphenicol	100	5.4	67	–	–
+ Streptogramin A	100	0.0	0	–	–
+ Anisomycin	100	–	–	0.02	2
+ Cycloheximide	100	–	–	0.13	12

Experimental conditions were as indicated in Materials and methods. Control experiments were carried out without poly U and figures obtained for incorporation deducted.

On the other hand RNA polymerase from green algae like the enzyme from other eukaryotic cells is not sensitive towards the rifamycins. In analogy to species specificity of ribosomes, it appears that RNA polymerases in prokaryotic systems have a number of features in common which differ from those of the enzyme in eukaryotic systems.

Acknowledgements

This work has been supported by a grant from Sociedad Española de Industrias Químicas Farmaceúticas, Division Farmaceútica Lepetit. We thank Mr. J.Avila and Mr. E.Battaner for their help in the experiments shown in tables 2 and 3 and Miss V.Azorin for except techncal assistance.

References

[1] M.M.Taylor and R.Storck, Proc. Natl. Acad. Sci. U.S. 52 (1964) 1875.
[2] L.A.Anderson and R.M.Smillie, Biochem. Biophys. Res. Commun. 23 (1966) 535.
[3] M.Rodriguez-López and V.Vazquez, Life Sci. 7 (1968) 327.
[4] M.Rodriguez-López, M.L.Celma, R.Fernández-Muñoz and D.Vazquez, Atti VII Simposio Internazionale di Agrochimica (1968) p. 63.
[5] R.Sager, I.B.Weinstein and I.Ashkenazy, Science 140 (1963) 304.
[6] J.Eisenstadt and G.Brawerman, Biochim. Biophys. Acta 80 (1964) 463.
[7] R.Sager and M.G.Hamilton, Science 157 (1967) 709.
[8] G.Hartmann, K.O.Honikel, F.Knüsel and S.Nüesh, Biochim. Biophys. Acta 157 (1967) 215.
[9] W.Wehrli, J.Nüesh, F.Knüsel and M.Staehelin, Biochim. Biophys. Acta 157 (1968) 215.
[10] H.Umezawa, M.Staoshi, H.Tamazaki and K.Nitta, J. Antibiotics 21 (1968) 234.
[11] J.M.Wilhelm, N.L.Oleinick and J.W.Corcoran, Biochim. Biophys. Acta 166 (1968) 268.
[12] E.Wintersberger and U.Wintersberger, FEBS Letters 6 (1970) 58.
[13] Zh.G.Shmerling, Biochem. Biophys. Res. Commun. 37 (1969) 965.
[14] S.J.Surzycki, Proc. Natl. Acad. Sci. U.S. 63 (1969) 1327.
[15] W.A.Kratz and J.Myers, Am. J.Botany 42 (1955) 282.
[16] M.Rodriguez-López, J. Gen. Microbiol. 43 (1966) 139.
[17] D.Vazquez, J. Gen. Microbiol. 42 (1966) 93.
[18] K.Burton, Biochem. J. 62 (1956) 315.
[19] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[20] D.Vazquez, Nature 203 (1964) 257.
[21] Ch.Babinet, Biochem. Biophys. Res. Commun. 26 (1967) 639.
[22] (a) D.Vazquez, Biochim. Biophys. Acta 114 (1966) 289; (b) C.F.Heredia and H.O.Halvorson, Biochemistry 5 (1966) 946.
[23] L.Frontali and G.Tecce, in: Antibiotics, Vol. 1, Mechanism of Action, eds. D.Gottlieb and P.Shaw (Springer, Berlin, 1967).
[24] K.Von der Helm, Ph.D.Thesis, University of Munich (1969).