INTERACTION OF ACTINOMYCIN D WITH YEAST RIBOSOMAL RNA

Liliana W. WALTSCHEWA
Department of Molecular Genetics, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Received 18 December 1979

1. Introduction

Saccharomyces cerevisiae cells are resistant to actinomycin D. (AM). This resistance is most likely due to poor permeability, since a mutant characterized by increased permeability to different antibiotics [1] is sensitive to AM. Antibiotic at 10–20 μg/ml caused a complete arrest of RNA synthesis, but a post-transcriptional degradation of rRNA precursors (pre-rRNA) was also observed [2]. An AM-sensitive mutant of *Saccharomyces cerevisiae* has been isolated [3]. AM at 1 μg/ml inhibited the synthesis of rRNA by 70% in this mutant, while residual rRNA synthesized was processed normally. Since abnormal rRNA processing in the presence of AM was found also in other eukaryotic systems [4], we felt that this effect of the antibiotic might be due to a direct interaction of AM with pre-rRNA species.

The binding of AM to RNA has not been reported, probably because of the low binding affinity and the lack of sensitivity in the methods used [5]. In an extensive study of 5 different techniques, equilibrium dialysis was considered to be most reliable assay for studying the binding of AM [6]. Therefore, we have studied the interaction of AM with yeast rRNA using equilibrium dialysis.

2. Experimental

Total RNA was extracted from *Saccharomyces cerevisiae* VY1160 and extensively deproteinized as in [2]. rRNA was freed of DNA fragments, 'ds' RNA and tRNA by repeated precipitation with 2 M LiCl. Highly purified 37 S pre-rRNA, 25 S and 18 S rRNA species were obtained by repeated preparative and analytical sucrose density centrifugation. f2'ds'RNA (a gift from Dr J. Doskocil, Institute of Organic Chemistry and Biochemistry, Prague) represents RNA from a replicative form of f2 sus1 1 phage. Equilibrium dialyses were performed in plexiglass cells, as in [7]. The solvent for all experiments was 0.2 M NaCl + 0.04 M $MgCl_2$ + 1 mM sodium phosphate (pH 7.4). RNA at 1.5–3.0 mg/ml and [^{3}H]actinomycin D (Amersham, 12 Ci/mmol) at 4.13×10^{-7}–2.08×10^{-6} M were used in the different experiments. Equilibrium was achieved at 4°C in the dark after 72 h. Samples were withdrawn in triplicate from each chamber and counted. Scatchard plots were obtained from the data and the K_a values and no. 6 ligand binding were determined. An experimental error of 10% was estimated in the evaluation of the K_a values.

3. Results and discussion

The results presented in table 1 show that interaction between AM and highly purified yeast rRNA fractions could be detected. However, the K_a values for RNA are >1 order of magnitude lower when compared with the constants obtained for DNA ([6]; table 1, this study). Therefore, several control experiments were performed to exclude possible artefacts. Treatment of rRNA fractions with DNase I (Worthington, RNase free, 20 μg/ml, for 10 min at 37°C) does not change the K_a values, thus excluding the possibility of interference by DNA fragments in the binding of AM to rRNA samples. Since AM was found to interact with ds phage RNA (table 1), the rRNA fractions used were thoroughly purified to remove ds RNA species, known to exist in most yeast strains [8]. Control experiments with parallel electrophoresis of ds RNA purified from a killer strain failed to detect the presence of ds RNA species in the rRNA fractions used (data not shown). Heating in low salt solutions (15 min, 90°C) leads to a

Table 1
Association constants of actinomycin D binding to yeast rRNA

	Not treated	Association constants [$\times 10^6$ (M^{-1})]		Binding sites
		DNase treated	Heat denatured	
37 S pre-rRNA	0.092	0.090	0.000	17
25 S rRNA	0.054	0.055	0.000	9
18 S rRNA	0.051	0.052	0.000	7
f2'ds'RNA	0.123	0.120	–	–
DNA salmon sperm	2.100	–	–	–

Experimental details given in the text

significant destruction of the secondary structure in the rRNA molecules. Binding of AM to rRNA was not established with thermally denatured RNA fractions, suggesting the involvement of the specific secondary structure in the complex formation.

These results suggest that AM binds to yeast rRNA. However, this binding is much weaker than the binding of AM to DNA and could be detected only with highly sensitive methods. Since RNA in solution has an A-conformation, the interaction of AM with rRNA should have a molecular basis different from that suggested for the intercalation complex of AM with DNA for which a B-conformation of the helix is a prerequisite [5]. Further experiments are necessary to clarify the nature of binding between AM and RNA.

A K_a of 0.092×10^6 (M^{-1}) and a total of 17 binding sites were found for 37 S pre-rRNA which are relatively higher than the values obtained for the mature 25 S and 18 S rRNA. Specific secondary structures were visualized in several regions of eukaryotic pre-rRNA molecules [9,10]. Since the interaction between AM and rRNA depends upon the secondary structure of RNA, one can suggest a non-random binding of AM to 37 S pre-rRNA. The binding of several AM molecules to small parts of the 37 S pre-rRNA could change the structure of the ribonucleoprotein particles and promotes degradation, rather than maturation of pre-rRNA, as observed in [2]. Abnormal processing of rRNA was also found in yeast cells treated with the intercalating agent ethidium bromide [11].

Acknowledgements

The author is greatly indebted to Professor A. A. Hadjiolov, Sofia and to Professor V. A. Erdmann, West-Berlin, for their advice and critical reading of the manuscript.

References

[1] Venkov, P. V., Stateva, L. I. and Hadjiolov, A. A. (1977) Biochim. Biophys. Acta 475, 245–253.
[2] Waltschewa, L. W., Venkov, P. V., Stojanova, B. B. and Hadjiolov, A. A. (1976) Arch. Biochem. Biophys. 176, 630–637.
[3] Gorenstein, C., Atkinson, K. D. and Falke, E. V. (1978) J. Bacteriol. 136, 142–147.
[4] Reichman, M., Karlan, D. and Penman, S. (1973) Biochim. Biophys. Acta 229, 173–175.
[5] Hollstein, U. (1974) Chem. Rev. 74, 625–652.
[6] Wells, R. D. and Larson, J. E. (1970) J. Mol. Biol. 49, 319–342.
[7] Pongs, O., Bald, R. and Reinwald, E. (1973) Eur. J. Biochem. 32, 117–125.
[8] Petras, D. E. and Bruenn, J. A. (1976) Int. J. Biochem. 7, 173–179.
[9] Wellauer, P. K. and Dawid, I. B. (1974) J. Mol. Biol. 89, 379–395.
[10] Dabeva, M. D., Dudov, K. P., Hadjiolov, A. A., Emanuilov, I. and Todorov, B. (1976) Biochem. J. 160, 495–503.
[11] Waltschewa, L. W. (1979) Curr. Microbiol. vol. 3, no 1.