EFFECTS OF INTERCALATING AGENTS ON THE STRUCTURE OF THE RIBOSOME

T. Suryanarayana* and D. P. Burma

Molecular Biology Unit, Department of Biochemistry,
Institute of Medical Sciences, Banaras Hindu University
Varanasi 221005, U.P., India

Received June 3,1975

SUMMARY

Ribosomes treated with an intercalating dye (ethidium bromide or acridine orange) are degraded more rapidly by RNase I than untreated ribosomes. Spermidine or spermine counteracts to great extent the effect produced by the intercalating dye. It appears that the intercalation leads to the 'loosening' of the structure of the ribosome which is also reflected in the decreased inhibitory capacity of the treated ribosomes towards RNase I. Intercalation appears to decrease the affinity of rRNA towards proteins causing the structure of the ribosome to become unfolded.

INTRODUCTION

The intercalating dyes like ethidium bromide and acridine orange have been extensively used to study the structure of polynucleotides (1,2). Acridine orange binds to RNA in yeast ribosomes (3); ethidium bromide binds to free rRNA as well as to rRNA in ribosomes (4). In this laboratory the mechanism of artificial association of RNase I with 30S subunit (5) is being investigated in detail (6). Because the hydrolytic attack of RNase I on the ribosome depends on the conformation of the ribosome (7) this association can be utilised to study the structural topography of ribosomes. In the present communication the effects of

* Senior Research Fellow under a project financed by the Indian Council of Medical Research.

intercalating agents on the structure of the ribosomes as monitored by RNase I will be described.

MATERIALS AND METHODS

Poly(A) was the product of Miles Laboratories, U.S.A. Spermidine, spermine, bovine serum albumin and ethidium bromide were purchased from Sigma Chemical Co., U.S.A. Acridine orange was the product of British Drug House, England.

E.coli ribosomes and S.typhimurium RNase I were prepared as described earlier (6). Ribosomes were treated with ethidium bromide or acridine orange according to the method of Stevens and Pascoe (4).

The degradation of ethidium bromide-or acridine orange-treated and untreated ribosomes by RNase I was followed in Zeiss PMQ II spectrophotometer. The reaction was carried out in the cuvette at 25°C in a total volume of 1 ml containing 0.1 M Tris-HCl pH 7, 1 A_{260} unit of treated or untreated ribosome, 4 units of RNase I (6) and desired amount of Mg^{++}. A blank mixture without the enzyme was used to adjust the initial absorbancy of the reaction mixture to zero.

The inhibition of RNase I-catalysed hydrolysis of poly(A) by treated and untreated ribosomes was measured according to the method described by Datta and Burma (6).

RESULTS AND DISCUSSION

Degradation of ethidium bromide-and acridine orange-treated ribosomes by RNase I: Ethidium bromide treatment seems to produce a large amount of distortion in the ribosomal structure (Fig.1A). At a Mg^{++} concentration of 1.35 mM the untreated ribosomes are completely protected against RNase I, whereas the treated ribosomes are degraded at a reasonably fast rate. When Mg^{++} concentration is lowered to 0.35 mM the treated ribosomes are degraded at a much faster rate whereas the untreated ribosomes are degraded slowly and incompletely. Similar differences are also observed at other Mg^{++} concentrations. Even at a very high Mg^{++} concentration (5 mM) the treated ribosomes are not completely protected against RNase I. Similar results were obtained following treatment with acridine orange (Fig.1B). These results suggest that the distortion

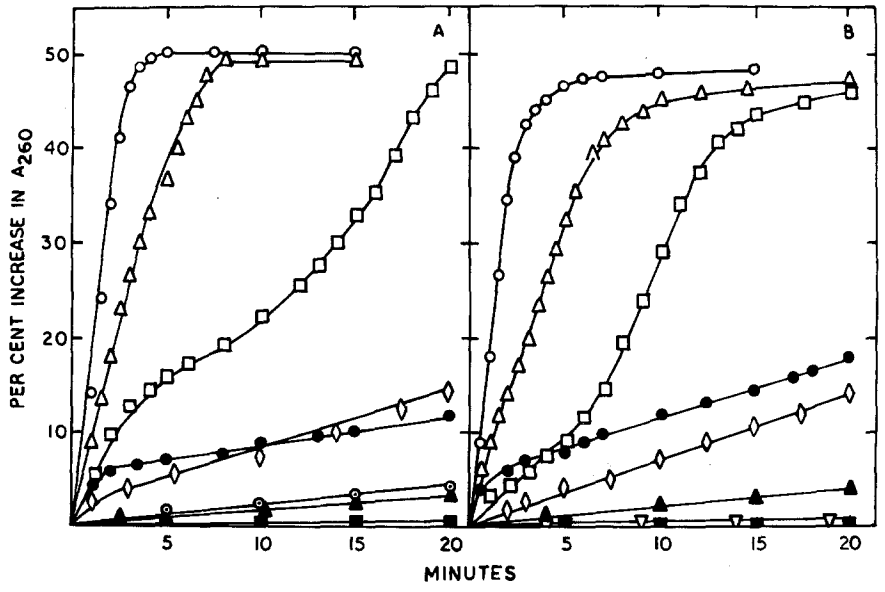

Fig.1. Degradation of ethidium bromide (A) and acridine orange (B) treated ribosomes by RNase I at different Mg^{++} concentrations. The treatment of ribosomes with ethidium bromide and acridine orange and the assay were carried out as described under 'Materials and Methods'. Closed symbols and open symbols represent untreated and treated ribosomes respectively. Mg^{++} concentrations (mM) are A.●○ 0.35; ▲△ 0.7;■□ 1.35;■◇ 2.35;■⊙ 5.0 B.●○ 0.35;▲△ 0.7;■□ 1.0; ■◇ 1.35;■▽ 2.35.

produced by the intercalating agent leads to the unfolding of the overall structure of the ribosome and makes it amenable to the attack of RNase I.

RNase I catalysed degradation of treated ribosome in presence of polycationic amines: Polycationic amines have profound influence on the structure and function of the ribosome (4,7-10). It is well known that polycationic amines such as spermidine and spermine cause the overall structure of the ribosome to become tightened and thus mimick the effect of Mg^{++}. Spermidine or spermine was found to decrease the rates of

degradation of both ethidium bromide-treated and untreated ribosomes (Fig.2). Similar results were obtained with ribosomes treated with acridine orange (data not shown). Thus the presence of a polycationic agent reverses the distortion produced in the ribosome by the intercalating agent.

Inhibition of RNase I by ribosomes treated with the intercalating agents: Ribosomes have the ability to inhibit the hydrolysis of a polynucleotide as catalysed by RNase I and this inhibition is dependent on the concentration of Mg^{++}(6). This inhibitory capacity which is dependent on the overall structure of the

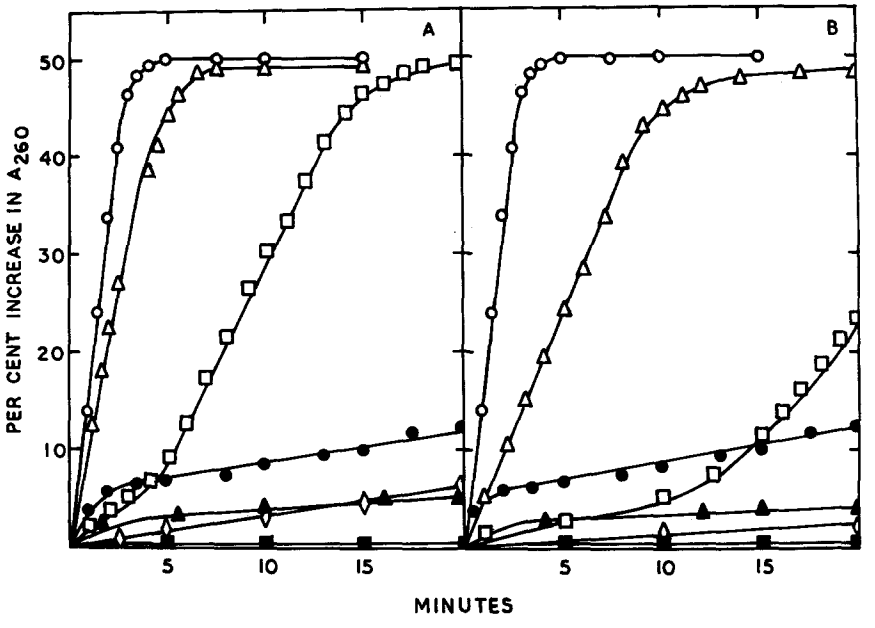

Fig.2. Degradation of ethidium bromide treated ribosomes in presence of spermidine (A) and spermine (B). The assay was carried out as described under 'Materials and Methods' excepting that indicated amount of spermidine or spermine was present where mentioned. The concentration of Mg^{++} during assay was invariably 0.35 mM. Closed symbols and open symbols represent untreated and treated ribosomes respectively. Spermidine concentrations (mM) are A.●○ nil;▲△ 0.05;■□ 0.25;■ ◇ 0.5. B.● ○ nil;▲△0.01; ■□0.05; ■ ◇ 0.125.

ribosome, is also profoundly affected by treatment with the intercalating dyes (Fig.3). For example, at a Mg^{++} concentration of 0.8 mM the ethidium bromide-or acridine orange-treated ribosomes are poor inhibitors of RNase I whereas considerable inhibition, dependent on the concentration of ribosomes, is observed with the untreated ribosomes. At 5 mM Mg^{++} the difference in the inhibitory capacities of the treated and untreated ribosomes becomes smaller but there is definitely less inhibition produced by the treated ribosomes.

Studies carried out in this laboratory with the ribosomes treated with the thiol reacting reagents like N-ethyl-

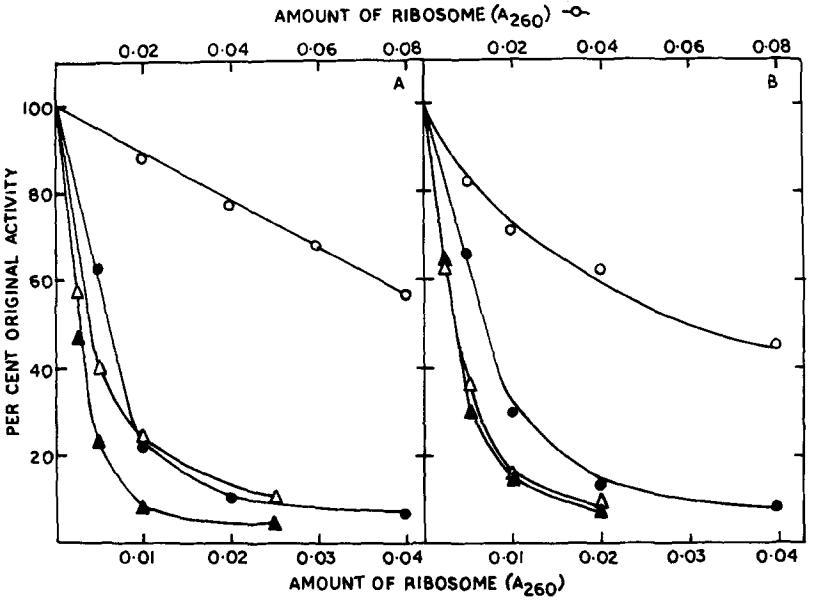

Fig.3. Inhibition of RNase I by ribosomes treated with ethidium bromide (A) and acridine orange (B). The treatment of ribosomes with ethidium bromide and acridine orange and inhibition assay have been described in the text. Closed symbols and open symbols represent untreated and treated ribosome respectively. ● ○ assayed at 0.8 mM Mg^{++} ▲ △ assayed at 5.0 mM Mg^{++}.

maleimide, parachloromercuribenzoate, 5,5'-dithiobis(2-nitrobenzoic acid), indicate similar unfolding of the structure of the ribosome. Apparently the distortion produced in the structure of either rRNAs or proteins present in the ribosome leads to the weakening of the interaction between nucleic acids and proteins and thus the 'loosening' of the overall structure of the ribosome. RNase I appears to be a useful tool for monitoring such conformational change.

REFERENCES

(1) Waring, J., Nature, 219, 1320(1968).
(2) Carlberg-Bacq,C.M., in Fundamentals of Biochemical Pharmacology (Z.M.Bacq,R.Capek,R.Paoletti, and J.Renson, Eds.) Pergamon Press, p.476 (1971).
(3) Morgan,R.S., and D.G.Rhoads, Biochim.Biophys.Acta, 102, 311 (1965).
(4) Stevens,L., and G.Pascoe, Biochem.J., 128, 279(1972).
(5) Neu,H.C. and L.A.Heppel, Proc.Nat.Acad.Sci., U.S.A., 51, 1267 (1964).
(6) Datta, A.K., and D.P.Burma, J.Biol.Chem., 247,6795(1972).
(7) Suryanarayana,T., Ph.D.Dissertation submitted to Banaras Hindu University, Varanasi, India (1975).
(8) Bretthauer,R.K., L.Marcus,J.Chaloupka,H.O.Halvorson, and R.M.Bock, Biochemistry, 2, 1079 (1963).
(9) Takeda,Y., Biochim.Biophys.Acta, 182, 258(1969).
(10) Spirin,A.S., in 'Molecular mechanism of antibiotic action on protein synthesis and membranes' (E.Munoz, F.Garcia-Ferrandiz, and D.Vazquez, Eds.) Elsevier Scientific Publishing Co., Amsterdam, p.11 (1972).