ON THE MECHANISM OF THE INHIBITING EFFECT OF AURIN TRICARBOXYLIC ACID ON THE BINDING OF RIBOSOMES TO RIBONUCLEIC ACIDS

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1. Introduction

Aurin tricarboxylic acid (ATA) in low concentrations has been shown to inhibit protein chain initiation in bacterial [1, 2] as well as in animal systems [3], by preventing the binding of the small ribosomal subunit to messenger RNA (mRNA) [4, 5]. The effect of ATA is not restricted to natural messengers. Thus, ATA inhibits also the translation of synthetic polyribonucleotides [6]. Interestingly, chain initiation on messengers which lack the physiological signals AUG or GUG appears to be preferentially inhibited.

In attempts to throw light on the mechanism of action of ATA, its effect on the binding of ribosomes and of cytoplasmic proteins to complementary nuclear RNA (cRNA) and to ribosomal RNA (rRNA) has been studied. Data are presented suggesting that the inhibiting effect of ATA on the binding of ribosomes to nucleic acids is due to an interaction with the protein moiety of the ribosomes.

2. Materials and methods

Ribosomes were isolated from rat livers by treating the postmitochondrial supernatant with 1% Triton X-100 and sedimenting the ribosomes through 1 M sucrose as previously described [7]. The pellet was resuspended in buffer A (0.15 M KCl, 1 mM $MgCl_2$ and 10 mM triethanolamine, pH 7.5). Sucrose gradient centrifugation showed that the suspension contained polysomes, monosomes as well as ribosomal subunits. In the following the preparation is referred to as ribosomes.

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RNA was extracted from ribosomes with phenol as described by Cartouzou et al. [8]. The ribosomal RNA was layered onto a 0.5–1.0 M sucrose gradient in buffer A and centrifuged at 198,000 *g* for 10 hr. 28 S RNA was precipitated from the appropriate part of the gradient by adding 2 volumes of ethanol and dissolved in buffer A.

Complementary RNA (cRNA) was isolated from purified rat liver nuclei by fractionated phenol extraction [9, 10]. The fraction extracted between 55° and 65° was used as the preparation of cRNA. Labelled cRNA was obtained by injecting the animals with 1 mCi ^{3}H-orotic acid 30 min prior to sacrifice.

Binding of labelled RNA to ribosomes was studied by adding both components to 1 ml of buffer A and incubating at 0° for 15 min. The samples were filtered on millipore filters (HAWT) and washed with 35 ml of buffer A. The radioactivity retained on the filters was counted as previously described [7].

The cytoplasmic proteins used were obtained by centrifuging a postmitochondrial supernatant at 101,000 *g* for 3 hr. The upper half of the supernatant was carefully pipetted off. The protein content was measured by the method of Lowry et al. [11].

5-^{3}H-orotic acid (specific activity 22 Ci/mM) and ^{14}C-orotic acid (spec. act. 60 mCi/mM) were obtained from the Radiochemical Centre, Amersham. Aurin tricarboxylic acid was obtained from Sigma Chem. Co., St. Louis, Mo., USA.

3. Results and discussion

Ribonucleoprotein complexes like ribosomes bind to nitrocellulose filters, whereas pure RNA fails to do

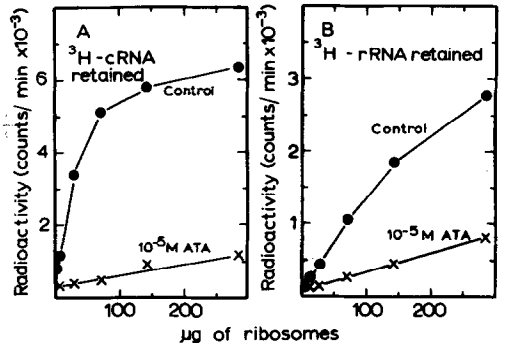

Fig. 1. Inhibiting effect of ATA on the binding of cRNA (A) and of rRNA (B) to ribosomes. Different amounts of ribosomes were incubated for 15 min at 0° with 3 µg of ^{3}H-labelled cRNA (9000 cpm) or with 14 µg of ^{3}H-labelled 28 S rRNA in the absence (control) or in the presence of 10^{-5} M ATA. The samples were then filtered on millipore filters and the radioactivity retained on the filters was measured.

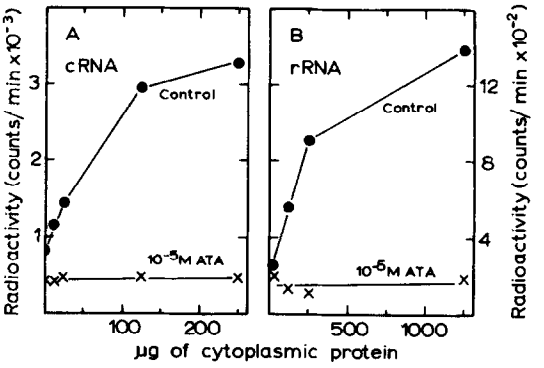

Fig. 2. Effect of ATA on the binding of proteins to RNA. Increasing amounts of a postribosomal supernatant from rat liver were added (A) to ^{3}H-labelled cRNA (3800 cpm) and (B) to ^{3}H-labelled ribosomal RNA (5800 cpm) in 1 ml of buffer A in the absence and presence of 10^{-5} M ATA. The samples were incubated and filtered and the radioactivity on the filters measured as in fig. 1.

so [12]. The binding of labelled RNA to ribosomes can therefore conveniently be studied by measuring the retention of radioactivity on such filters [13]. From the results in fig. 1 it appears that when increasing amounts of ribosomes were incubated with labelled cRNA (fig. 1A) and rRNA (fig. 1B), increasing amounts of the labelled nucleic acids were retained on the filters. In the presence of 10^{-5} M ATA, a concentration which prevents the initiation of protein synthesis while it fails to inhibit the completion of already initiated peptide chains [3], low amounts of ribonucleic acids were retained on the filters. Since it was found in control experiments (data not shown) that the binding of labelled ribosomes to the filters was not affected by the ATA concentration used, the results demonstrate that ATA inhibits effectively the binding of ribosomes to cRNA and rRNA.

In order to study the mechanism of ATA its effect on artificial protein-RNA complexes was investigated. Advantage was taken of the fact that when ribonucleic acids are incubated with cytoplasmic proteins, complexes are formed which are adsorbed to nitrocellulose filters [14]. In fig. 2 it is shown that when increasing amounts of cytoplasmic proteins from rat liver supernatant were added to labelled cRNA (fig. 2A) and to rRNA (fig. 2B) increasing amounts of the labelled nucleic acids were retained on the filters. In the presence of 10^{-5} M ATA the retention of radioactivity was virtually abolished. Clearly, ATA inhibits effecti-

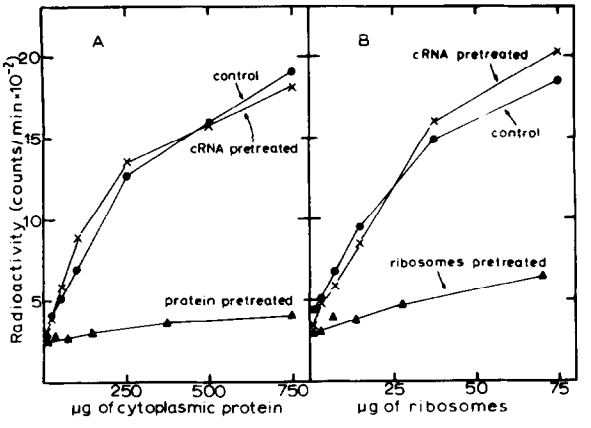

Fig. 3. Binding of protein or ribosomes to cRNA after treatment of either of the components with ATA. The pretreatment was carried out by incubating cRNA, ribosomes or the post-ribosomal supernatant with 10^{-5} M ATA and then filtering the suspensions through a column of Sephadex G-25, equilibrated with buffer A, in order to remove unbound ATA. ^{3}H-labelled cRNA was added (A) to increasing amounts of cytoplasmic protein (postribosomal supernatant) and (B) to increasing amounts of ribosomes. The samples were incubated, filtered, and the radioactivity retained on the filters was measured as in fig. 1. (•) Control (both components untreated); (x) cRNA pretreated with ATA, (▲) Postribosomal supernatant or ribosomes pretreated with ATA.

vely the binding of cytoplasmic proteins to these nucleic acids.

The inhibiting effect of ATA on the binding of the proteins to nucleic acids could be due to an effect on the RNA, on the protein, or on both components. In order to distinguish between these possibilities, experiments were carried out where either the nucleic acids or the proteins were exposed to ATA and subsequently permitted to interact with the other untreated component. The data in fig. 3A demonstrate that when cRNA was treated with ATA and the excess ATA was removed, the cRNA retained its ability to bind cytoplasmic proteins. On the other hand, when the proteins were pretreated with ATA in a similar way, their binding to cRNA was abolished. Moreover, similar experiments with cRNA and ribosomes demonstrate (fig. 3B) that pretreatment of the ribosomes with ATA reduced strongly the binding to cRNA, whereas pre-treatment of the cRNA did not inhibit the binding of ribosomes. When cRNA was re laced by rRNA, similar results as those in fig. 3 were obtained.

The present results indicate that the inhibiting effect of ATA on the binding of ribosomes to ribonucleic acid is due to an effect on the ribosomes rather than to interaction with the RNA. The ability of ATA to modify cytoplasmic proteins in such a way that they become unable to bind to the ribonucleic acids (fig. 3A) suggests that the effect of ATA on the ribosomes may primarily be due to an interaction with their protein components.

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