AURINTRICARBOXYLIC ACID INHIBITION OF THE BINDING OF PHENYLALANYL-tRNA TO RAT LIVER RIBOSOMAL SUBUNITS

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1. Introduction

Aurintricarboxylic acid (ATA) inhibits the initiation of protein synthesis in cell-free systems from both prokaryotes and eukaryotes at concentrations which do not affect chain elongation [1–7]. We report here the effect of ATA on the poly U-dependent binding of Phe-tRNA to rat liver ribosomal subunits. ATA inhibits Phe-tRNA binding to 40 S subunits, catalysed by the rat liver cytosol initiation factor Ml (formerly called 40 S binding factor [8, 9]). Binding to recombined 40 S and 60 S subunits, catalysed by elongation factor T-I, is inhibited to a similar extent; however, non-enzymic binding to subunits is inhibited to a greater extent. This unexpected differential inhibition appears to be due to the ability of enzymically inactive protein (present in the partially purified factor preparations) to bind ATA, reducing the amount of the latter available for inhibition of the binding of Phe-tRNA to ribosomes.

2. Materials and methods

ATA was purchased from Aldrich. Bovine serum albumin (fraction V) was from Armour.

The following methods have been described before: the preparation of ribosomes [10] and ribosomal subunits [11] from rat liver; of Ml [12], transfer factors TI and TII [13]; the method of assaying cell-free protein synthesis [14] and binding of Phe-tRNA to ribosomes [8]. The binding reaction mixture (0.1 ml) contained, unless stated otherwise, Tris-HCl, pH 7.5 (1 μmole), KCl (8 μmole), $MgCl_2$ (1 μmole), GTP (0.05 μmole), β-mercaptoethanol (1 μmole), polyuridylic acid (10 μg), ^{3}H-aminoacyl-tRNA (40 μg of *E. coli* B tRNA acylated with 19 non-radioactive amino acids and ^{3}H-Phe, 5 Ci/mmole) and ribosomes and enzyme fractions as indicated in the text. Incubation was for 15 min at 30°. The efficiency of determination of radioactivity was 16%; thus 1000 cpm were equivalent to 0.57 pmole of Phe-tRNA bound to ribosomes.

3. Results and discussion

It is apparent from table 1 that ATA, at a concentration of 10^{-5} M, does inhibit Ml-catalysed binding of Phe-tRNA to 40 S subunits. Polypeptide elongation on endogenous ribosomal message (table 2) is not inhibited at 10^{-5} M ATA (and is, in fact, slightly stimulated – cf. [7]). However, it can be seen from table 1 that non-enzymic binding to either 40 S or recombined 40 S + 60 S subunits is also inhibited by 10^{-5} M ATA, as is T-I dependent binding to recombined subunits. These results are consistent with the known ability [1] of ATA to inhibit the binding of template RNA (poly U in the present work) to ribosomes. Indeed one would expect that any cell-free reaction that is dependent on the ribosomal binding of an exogenous RNA template would be inhibited

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Table 1
Effect of ATA on binding of Phe-tRNA to ribosomal subunits.

ATA (M)	Phe-tRNA bound (pmole)			
	No factor		+ M1	+ T-I
	40 S	40 + 60 S	40 S	40 + 60 S
0	0.67 (100)	1.22 (100)	1.32 (100)	1.74 (100)
10^{-7}	0.63 (93)	1.18 (96)	1.40 (105)	1.50 (86)
10^{-6}	0.15 (22)	0.21 (17)	1.34 (101)	1.54 (89)
10^{-5}	0.02 (4)	0.05 (4)	0.48 (36)	0.31 (18)

The ribosomal subunits (3.6 pmole) were incubated with the factors indicated (Ml = 100 μg, T-I = 20 μg) at different concentrations of ATA, as described in Materials and methods. Figures in parentheses are the values expressed as a percentage of the appropriate control without ATA.

Table 3
Effect of ATA on Phe-tRNA binding to 40 S ribosomal subunits at different concentrations of magnesium.

$MgCl_2$ (mM)	Phe-tRNA bound (pmole)			
	No factor		+M1	
	–ATA	+ATA	–ATA	+ATA
5	–	–	1.38 (100)	1.09 (79)
10	0.55 (100)	0.18 (32)	2.31 (100)	1.90 (82)
15	0.92 (100)	0.11 (12)	2.35 (100)	2.10 (86)
20	1.30 (100)	0.38 (19)	2.20 (100)	1.82 (83)

Ribosomal 40 S subunits (3.6 pmole) were incubated at various concentrations of magnesium, as described in Materials and methods. M1 (100 μg) and ATA (10^{-6} M) were present or absent as indicated. Figures in parentheses are the values expressed as a percentage of the appropriate control without ATA.

Table 2
Effect of ATA on endogenous protein synthesis.

ATA (M)	Phe incorporated (pmole)
0	1.08 (100)
10^{-7}	1.42 (132)
10^{-6}	1.32 (123)
10^{-5}	1.36 (126)
10^{-4}	0.88 (82)

Ribosomes (3.6 pmole) were incubated for 30 min at 37° in 0.1 ml containing (in μmole) ATP, 0.1; GTP, 0.01; creatine phosphate, 0.5; creatine phosphokinase, 20 μg; ^{3}H-amino-acyl-tRNA, 80 μg; Tris-HCl pH 7.5, 2; KCl, 12.5; $MgCl_2$, 0.5; β-mercaptoethanol, 0.6; 100,000 *g* cytosol (375 μg protein). The protein was isolated [14] and the radioactivity determined by scintillation spectrometry with an efficiency of 5%. Thus 1000 cpm was equivalent to 1.8 pmole Phe incorporated. Figures in parentheses are the values expressed as a percentage of the no-ATA control.

The incorporation of Phe represents elongation of nascent polypeptide chains (rather than reinitiation) because the 100,000 *g* cytosol does not contain M2 [15].

by ATA, whether or not the reaction is a paradigm for physiological initiation. Our results emphasise this frequently neglected point.

A more surprising result (table 1) was that non-enzymic binding was inhibited to a greater extent than factor-catalysed binding (10^{-6} M rather than 10^{-5} M ATA produced approx. 80% inhibition). The extent of inhibition, in either case, was not significantly altered by the presence of the 60 S subunit. The difference in ATA inhibition of the two reactions was not related to the use of a common magnesium concentration (10 mM) rather than their respective magnesium optima, for the extent of inhibition by 10^{-6} M ATA was independent of the magnesium concentration (table 3). It occurred to us, however, that the effect might be due to inactive protein, present in the rather low specific-activity preparation of M1 (and T-I). We therefore examined the effect of bovine serum albumin (BSA) on the inhibition of non-enzymic binding by ATA. The addition of 50 μg BSA reduced the inhibition by 10^{-6} M ATA to the level seen with factor-dependent binding (table 4). (Other proteins gave similar results.). The concentration dependence of this protein protection was

Table 4
Effect of bovine serum albumin on the ATA inhibition of non-enzymic Phe-tRNA binding to 40 S ribosomal subunits.

ATA (M)	Phe-tRNA bound (pmole)	
	No addition	BSA
0	0.53 (100)	0.61 (100)
10^{-7}	0.50 (94)	0.68 (111)
10^{-6}	0.21 (39)	0.53 (87)
10^{-5}	0.02 (4)	0.04 (7)

Ribosomal 40 S subunits (3.6 pmole) were incubated in the presence or absence of BSA (50 μg) as described in Materials and methods. Figures in parentheses are the values expressed as a percentage of the appropriate control without ATA.

examined (fig. 1); half-maximal protection against 10^{-6} M ATA being obtained with about 5 μg BSA.

These results suggest that the added protein binds ATA, reducing the amount of 'free' ATA available for inhibition of Phe-tRNA binding to ribosomes. ATA has, in fact, been shown to bind strongly to several proteins, including BSA [16–18]. The question that now arises is whether the results in table 2 still indicate that elongation is less sensitive to ATA inhibition than initiation. It appears from table 1 that the concentration of free ATA for 80% inhibition of binding is about 10^{-6} M and, as 10^{-5} M ATA causes over 60% inhibition of the binding with M1 (1 mg/ml), 1 mg protein binds about 9×10^{-6} mmole ATA. Thus 3.75 mg protein (which is the amount present per ml in the experiment of table 2) would bind about 3.4×10^{-5} mmole ATA or lower the concentration of ATA by 3.4×10^{-5} M. This could account for the lack of inhibition of elongation by 10^{-5} M ATA but not for the feeble (18%) inhibition by 10^{-4} M ATA (table 2) where the concentration of free ATA should be about 6.6×10^{-5} M. Hence these results are still consistent with preferential inhibition of initiation by ATA although, taken at face value, they exaggerate this.

It is clear, therefore, that comparison of the effect of ATA on different cell-free ribosomal reactions must take into account the protein concentration in the reaction mixture. This may be especially important in comparing the published results of different workers.

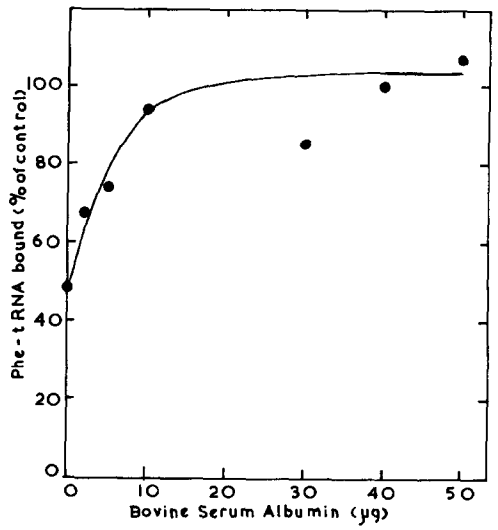

Fig. 1. Effect of bovine serum albumin concentration on the ATA inhibition of Phe-tRNA binding to 40 S ribosomal subunits. Ribosomal 40 S subunits (3.6 pmole) were incubated with 10^{-6} M ATA and the amount of BSA indicated, as described in Materials and methods. The amount of Phe-tRNA bound in the absence of ATA and bovine serum albumin was 0.81 pmole.

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