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A hydrogen exchange study of Escherichia coli 5S RNA

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ABSTRACT

Using the tritium Sephadex method, the number of exchangeable protons in *E.coli* 5S RNA and the kinetics of their exchange reactions have been measured at two different Mg^{++} concentrations (10^{-2} M and 10^{-3} M). A quantitative analysis of these results indicates the presence of two classes of protons exchanging with very different rates. The protons of the slow class, not seen in linear molecules of double helical RNA, exchange with a half-time of 0.6 hour and their exchange kinetics are independent of Mg^{++} concentration. Reduction of the Mg^{++} concentration from 10^{-2} to 10^{-3} M, however, results in a decrease in the number of these exchangeable protons from 26 to 19. Neither the total number, nor the exchange kinetics of the fast protons are affected by this change in Mg^{++} concentration. Comparison of these results with those previously obtained with tRNA, suggests the presence of a Mg^{++} dependent tertiary structure in 5S RNA. The number of exchangeable protons obtained from extrapolation of the exchange curves (120 and 126 respectively for 10^{-3} and 10^{-2} M Mg^{++} concentration) are compared to the calculated number of exchangeable protons predicted by previous proposed structural models for *E.coli* 5S RNA.

INTRODUCTION

5S RNA is a central constituent of the large ribosomal subunits of pro- and eukaryotic ribosomes. In recent years, several studies have been devoted to the elucidation of the secondary and the tertiary structure of the molecule. (for a review, see reference 1). Despite these efforts there are still doubts concerning the extent of base pairing and the structural aspects of the tertiary structure.

In this communication we report the results of a study of *E.coli* 5S RNA using the tritium-hydrogen exchange method. The comparison of these data with those obtained previously in a similar study on tRNA (2) points towards the existence of a salt-dependent tertiary structure for 5S RNA. The number of protons involved in base pairing estimated by extrapolation to zero time of the exchange curves is compared with the extent of base pairing predicted by previous models.

MATERIALS AND METHODS

E.coli 5S RNA was isolated as previously described (3). All buffers contained 0.01 M sodium cacodylate and were adjusted to pH 6.5 (20°C).

To measure as a function of time the number of unexchanged protons per 5S RNA molecule we used the tritium gel filtration method of Englander (4). The macromolecule was first incubated in the presence of tritiated water (10 mC) for 21 hours at 0°C. The free tritiated water and the labelled nucleic acid were separated by Sephadex filtration (G 25 fine grade). After passing through the Sephadex column the fractions containing nucleic acid were examined for radioactivity in an Intertechnique SL 30 liquid scintillation counter by counting a mixture of 0.5 ml of aqueous sample in 6 ml of scintillation fluid.

The nucleic acid concentration was determined by measuring the optical density at 260 nm and using an extinction coefficient ε of 7.831×10^5 $cm^{-1} \times M^{-1}$. These data taken together with the radioactivity level of the incubation solution allow the calculation of the number of unexchanged protons per molecule of 5S RNA (4). For early time points all time corrections as indicated by Englander have been performed (4).

RESULTS AND DISCUSSION

The exchange curves of 5S RNA for two different Mg^{++} concentrations (10^{-2} M and 10^{-3} M) are presented in Figure 1. The latter parts of both curves are nearly linear and parallel suggesting the presence of a homogeneous class of slow protons whose exchange rate is independent of Mg^{++} concentration ; in contrast, the number of protons of this class decreases from 26 to 19 on reducing the Mg^{++} concentration from 10^{-2} M to 10^{-3} M. Upon substraction of this slow class from the total number of unexchanged protons one is left with a class of fast protons which is heterogeneous as judged from the nonlinearity of the corresponding exchange curves (inset Fig. 1). The exchange kinetics of these protons are unaffected by the Mg^{++} concentration change. The values of the half-times and corresponding number of protons of the two classes are collected on Table 1.

Before discussing these results let us briefly recall the main features of the hydrogen exchange kinetics in long double helical RNA and DNA and also in tRNA which in addition to helical secondary structure has a superimposed tertiary structure. With the technique we have used here 5 and 3 exchangeable protons should be measured respectively for each G.C and A.U base pair involved in a double helical structure free from all tertiary structural constraints (5,6) ; these are the amino and imino protons involved in hydrogen bonds between

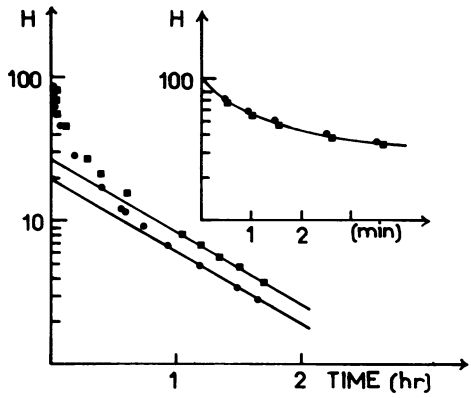

Figure 1 - Unexchanged protons per *E.coli* 5S RNA molecule were determined as a function of time by Sephadex gel filtration under the following ionic conditions : ● 0.14 M KCl/1mM $MgCl_2$; ■ 0.14 M KCl/10 mM $MgCl_2$. All solutions contained in addition 10 mM Na cacodylate pH 6.5. Temperature 0°C. Inset : Exchange of the fast protons after substraction of the slow protons.

base pairs. The exchange kinetics of these protons show some heterogeneity, with the imino protons of U and G exchanging fastest ; the slowest protons are the amino protons of guanine which exchange with a half-time of about 10 minutes. All these protons except the guanine exocyclic amino protons are dependent for exchange on transitory opening of the base pairs. Their exchange rate (in the order of minutes) as well as the number of exchanging protons have been found independent of the presence of Mg^{++} (7). We have recently re-

Table 1 - Salt concentration dependence of the size and half-time of the two hydrogen exchange classes in *E.coli* 5S RNA.

	Salt concentration		
	K^+ (M)	0.14	0.14
Class	Mg^{++} (M)	0.01	0.001
Fast	Size	100	100
	Half-time (min)	∿ 3	∿ 3
Slow	Size	26	19
	Half-time (hr)	0.6	0.6

The size of each class is expressed as hydrogens per 5S RNA molecule, estimated standard deviations on this figure is 2 %. The fast class is not homogeneous and the half-time of exchange is therefore an average value.

examined the exchange kinetics of protons in tRNA (2) and it appeared that the existence of the tertiary structure is reflected in the exchange behaviour by the presence of two additional slow classes with half-times of about 0.5 and 5 hours. The number of protons in each class is strongly dependent upon the concentration of monovalent and divalent cations whereas the rates are independent.

The fast protons seen in 5S RNA display all the characteristic features of the exchangeable protons in double helical nucleic acid. They are heterogeneous and their half-times are in the order of minutes and, within the limits of experimental errors, their exchange kinetic behaviour is unaffected by the change from 10^{-2} M to 10^{-3} M in Mg^{++} concentration. It is thus very reasonable to assign theseprotons to the exchangeable protons of base pairs located in double helical regions of the 5S RNA which are free of any tertiary structural constraints. This class of fast protons is also present in tRNA.

The slow class of 5S RNA protons which has no counterpart in double helical nucleic acid devoid of any tertiary structure is very similar to one of the slow classes found in tRNA. Both classes have nearly equal exchange half-times (0.54 hr and 0.6 hr respectively for tRNA and 5S RNA) and furthermore the number of protons of each class is strongly dependent upon Mg^{++} concentration. By analogy with the tRNA molecule, where the tertiary structure has been well-characterized and has been shown to be stabilized by Mg^{++} ions, it is reasonable to attribute these slow exchanging protons to the existence of a 5S RNA tertiary structure. The origin of the slowly exchanging protons is not yet known. Two explanations are possible : the slow protons are involved in tertiary structural hydrogen bonds or alternatively that tertiary structural constraints impedethe exchange of protons located in some double helical regions of the 5S RNA molecule.

Several structural models with varying numbers of base pairs have been proposed (1,8). It is interesting to compare the number of exchangeable protons determined in this work by extrapolation to zero time of the exchange curves of Figure 1 with the number of hydrogen bonded protons calculated for the different models counting 3, 4 and 5 protons respectively for A.U, G.U and G.C base pairs. At 10^{-3} M Mg^{++} the extrapolated value is about 120 protons per 5S RNA molecule. This can be considered as a lower limit of the number of protons involved in base pairing hydrogen bonds. The imino protons are the fastest exchanging and may escape detection by the tritium exchange method ; therefore an upper limit on the number of hydrogen bonded protons in 5S RNA can be set by adding the extrapolated value at 10^{-2} M Mg^{++} (126 protons) to

the number of imino protons of the corresponding model. The results are summarized in Table 2. One sees that the models which are in agreement with our experimental data have a number of exchangeable protons ranging from 125 to 170. According to our results several models underestimate the number of exchangeable protons; this discrepancy may result from the existence of a 5S RNA tertiary structure which besides Watson-Crick base pairing is stabilized by other known base pairing types and which have not been taken into account in these models.

In conclusion, let us summarize the main results of our study. The existence of salt-dependent protons with exchange half-time of 0.6 hour strongly suggests a salt dependent tertiary structure. The comparison of the hydrogen exchange in tRNA and 5S RNA reveals an important difference : the tRNA slow protons with an exchange half-time of 5 hours could not be found in the 5S RNA, reflecting a very specific feature of the tRNA tertiary structure.

Table 2 - Comparison of the number of exchangeable protons calculated for various structural models with those determined experimentally by the hydrogen exchange method.

Model proposed	Calculated exchangeable protons		Measured exchangeable protons	
			Upper limit	Lower limit
Brownlee *et al.* (9)	104		152	120
Boetker and Kelling (10)	164	(+)	168	120
Cantor (11)	214		178	120
Raake (12)	139	(+)	161	120
Madison (13)	170	(+)	172	120
Jordan (14)	134	(+)	160	120
Dubuy and Weissman (15)	176		172	120
	168		170	120
Monier (16)	176		172	120
	146	(+)	162	120
	147	(+)	163	120
Kearns and Wong (17)	125	(+)	155	120
	96		148	120
Fox and Woese (18)	112		154	120
Österberg *et al.* (19)	186		174	120
Weidner *et al.* (20)	112		154	120
	76		144	120
Luoma and Marshall (21)	155	(+)	167	120
Hori and Osawa (22)	112		153	120

The upper limit = 126 + imino protons of the corresponding model. The calculated exchangeable protons were obtained counting 3, 4 and 5 exchangeable protons respectively for A.U, G.U and G.C base pair. (+) Model compatible with the measured exchangeable protons.

Finally, the measured number of exchangeable protons allowed to discriminate between some of the previously proposed *E.coli* 5S RNA structural models.

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