# INFLUENCE OF TERTIARY STRUCTURE UPON THE RATE OF ISOTOPE HYDROGEN EXCHANGE IN $C_{(8)}H$ GROUPS OF tRNA PURINE RESIDUES

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## 1. Introduction

Previously we have reported [1] that the slow isotope hydrogen exchange between water and $C_{(8)}H$ groups of purine residues in synthetic polyribonucleotides is retarded as compared to this process in the corresponding ribonucleotides. It has been shown that the retardation of the hydrogen exchange is proportional to the fraction of stacked bases. Participation of all the adenine residues of poly-rA and poly-rA: poly-rU in stacking interactions results in about a 4-fold retardation of the exchange as compared to that in completely non-ordered riboadenosine monophosphate (rAMP) molecules. These data were interpreted as reflecting changes in the reactivity of $C_{(8)}H$ groups of purine residues which resulted from the formation of the secondary structure of the synthetic polynucleotide molecules.

We have sought to obtain quantitative data characterizing the effect mentioned above in the case of natural nucleic acids and to study the influence of the tertiary structure upon the exchange rate of the $C_{(8)}H$ groups in this molecule. tRNA was chosen as the model since this nucleic acid shows gradual "melting" of secondary structure with increasing temperatures and also exhibits the ability to exist in a tertiary structure at sufficiently low temperatures (in particular, in the presence of $Mg^{2+}$).

To evaluate the dependence of the exchange retardation on the degree of ordered structure in tRNA molecules, the rates of the $^1H \rightarrow {}^3H$ exchange between $C_{(8)}H$ groups of purines residues and $^3H_2O$ were compared for tRNA and a corresponding mixture of ribonucleoside monophosphates (rNMP). The exchange rates were measured at different temperatures in the presence and absence of $Mg^{2+}$.

## 2. Materials and methods

Preparations of total tRNA were used isolated from baker's yeast by a previously described method [2]. Ribonucleoside monophosphates (rNMP) were purchased from "Reanal" (Hungary).

$^1H \rightarrow {}^3H$ exchange between $C_{(8)}H$ groups of purine residues and $^3H_2O$ was measured as follows. tRNA and rNMP mixtures (18.6% rAMP; 29.4% rGMP; 24.0% rUMP; 28.0% rCMP) were incubated in the same experiment for 24 hr at a chosen temperature in buffered $^3H_2O$ solutions (0.1 M NaCl + 0.01 M Tris, pH 7.1; and 0.1 M NaCl + 0.01 M Tris, pH 7.1 + 5 mM $MgCl_2$). Specific radioactivity of the buffer solutions was 250 mCi/ml. After incubation, the tRNA and the rNMP mixtures were separated from $^3H_2O$ by repeated lyophilization. Thereafter, to remove $^3H_2O$ traces, tRNA was passed through a Biogel P-2 column whereas rNMP mixture was desiccated in a rotary evaporator 4-times. This procedure yielded preparations of tRNA and rNMP tritiated only in the $C_{(8)}H$ positions of purine residues and devoid of $^3H$-atoms in the rapidly exchangeable $-OH$, $-NH_2$, and $-NH$ groups. Lack of any changes in the specific radioactivity of all the tRNA fractions obtained by gel-filtration and in that of rNMP mixture after one additional desiccation was used as a criterion for the absence of $^3H_2O$ traces in the preparations obtained.

The purity of the rNMP mixture and the tRNA was monitored by examination of the absorbtion spectra in the wavelength region from 200 to 300 nm as measured in a "Hitachi", model-EPS-3T recording spectrophotometer. Concentrations of tRNA and rNMP were calculated from the values of the molar extinction coefficients equal to $7.4 \times 10^3$ and $10.3 \times 10^3$, respectively. Radioactivity was measured in a

model SL-40 "Intertechnique" (France) automatic liquid scintillation spectrometer. The scintillator which was used contained 1000 ml dioxane, 100 g naphthalene, 4 g PPO, 0.2 g POPOP. Specific radioactivity (SA) of rNMP mixture and of tRNA was expressed as a number of disintegrations per minute per micromole of phosphorus (dpm × mole $P^{-1}$). The SA values in the preparations were not lower than $2 \times 10^4$ dpm × mole $P^{-1}$ and the sample count rate was at least 5 times background. Error of estimation was 3–4%.

Retardation of $^1H \rightarrow {}^3H$ exchange in the $C_{(8)}H$ groups of tRNA purine residues as compared to that in rNMP was expressed, by means of a retardation coefficient:

$$K_r = \frac{SA_{rNMP\ mixture}}{SA_{tRNA}}$$

## 3. Results and discussion

Specific radioactivities of tRNA and rNMP mixture were compared after incubation with $^3H_2O$ under the same conditions at temperatures from 20° to 80°C for 24 hr. It was observed that the $^1H \rightarrow {}^3H$ exchange in $C_{(8)}H$ groups of the purine residues in tRNA proceeds more slowly than in non-ordered rNMP molecules. This is similar to that seen for poly-rA and poly-rA: poly-rU [1].

Fig. 1a shows the temperature dependence of the value of $K_r$ for tRNA incubated in the absence of $Mg^{2+}$ For comparison, the melting curve of tRNA is given in the same figure (fig. 1b). It is readily seen that the curve representing the dependence of $K_r$ on the temperature may be divided into two parts: low temperature and high temperature ones. In the low temperature region (from 20° to 40°C) a decrease of $K_r$ value is not accompanied by an increase in optical den-

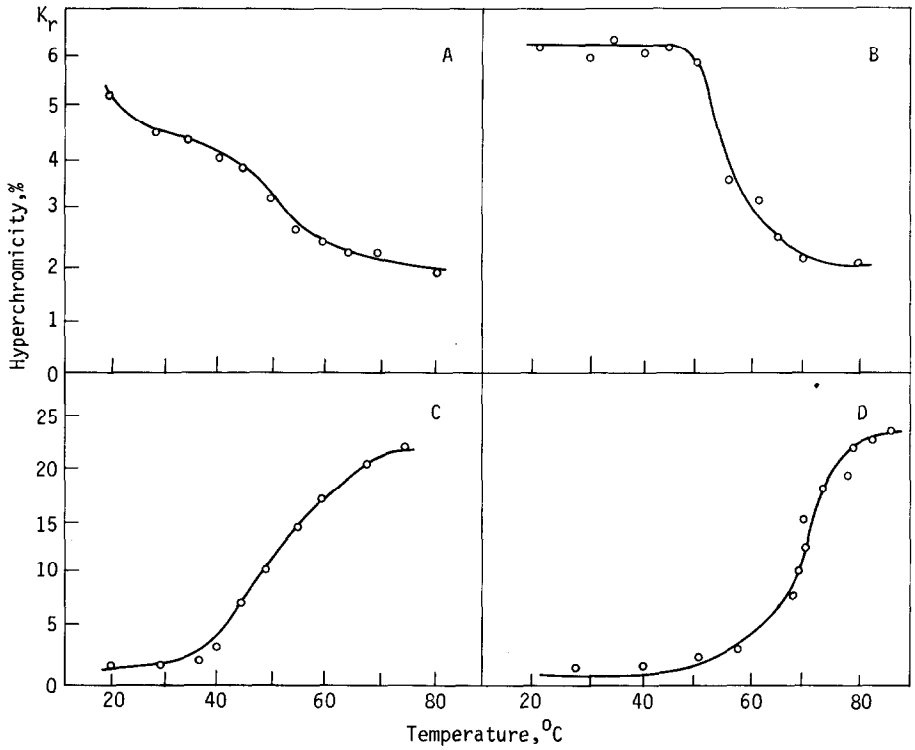


Fig. 1. Temperature dependence of $K_r$ and optical density at 260 nm ($A_{260}$) for the total yeast tRNA incubated in: (A, B) 0.1 M NaCl + 0.01 M Tris-HCl, pH 7.1; (C, D) 0.1 M NaCl + 0.01 M Tris-HCl + 5 mM $Mg^{2+}$, pH 7.1.

sity whereas in the high temperature region (from 40° to 80°C) a decrease in the $K_r$ value is accompanied by a rise in optical density.

It is known that an increase of optical density of a polymer solution may be used as a probe of the disturbance of secondary structure of the polymer. Fig. 2 shows dependence of $K_r$ for tRNA on the hyperchromicity percentage reflecting the fraction of non-ordered bases. For comparison, a respective curve obtained for the synthetic polynucleotide poly-rA [1] is given in the same figure. Investigation of hydrogen exchange in the single-stranded poly-rA and in the poly-rA: poly-rU complex devoid of tertiary structure has shown that the value of $K_r$ is directly proportional to the fraction of stacked bases. The curves for tRNA and poly-rA represented in the fig. 2 have the same slope in the range from 40° to 80°C, apparently indicating that the decrease of $K_r$ value in this temperature region reflects identical process in both polymers, viz. disturbance of stacking interactions between the bases.

Therefore, in the high temperature region the values of $K_r$ are proportional to the fraction of purine stacked bases located in helical and non-helical regions of the tRNA molecule.

It should be noted that in the low temperature region relatively small change in the optical density of tRNA solution (hyperchromicity is about 3% at 40°C)

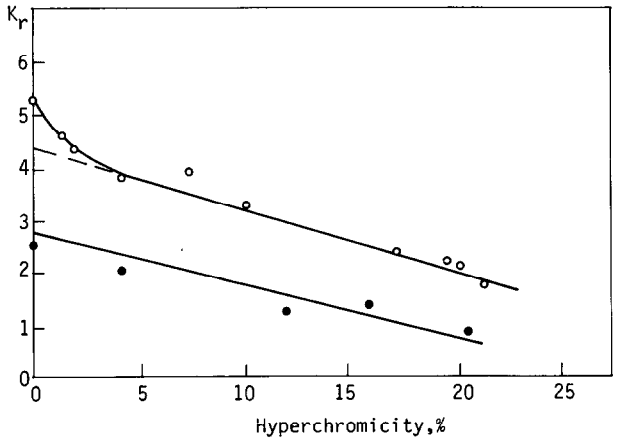


Fig. 2. Dependence of $K_r$ on hyperchromicity for the total yeast tRNA (o—o—o) incubated in 0.1 M NaCl + 0.01 M Tris-HCl, pH 7.1, and poly-rA incubated in 0.1 M NaCl + 0.05 M sodium acetate (•—•—•) [1].

is followed by a considerable decrease in the $K_r$ value (34% of the total change). Extrapolation to zero hyperchromicity (see fig. 2) shows that, if in the entire temperature range, the value of $K_r$ depended only on stacking interactions, it would be equal to 4.3 at 20°C. The obtained experimental value of $K_r$ was 5.2. This difference exceeds the error of measurement and may be accounted for by the fact that contrary to poly-rA molecules, those of tRNA form tertiary structure stable at 20°C.

Existence of a biologically inactive tertiary structure of tRNA at 20°C in 0.1 M NaCl was discovered by Fresco et al. [3]. They have shown that this structure is stable only at low temperatures and undergoes denaturation when heated from 20° to 40°C. This loss of tertiary structure is accompanied by a considerable increase in intrinsic viscosity (30%) and a decrease in sedimentation coefficient (30%) whereas the increase of optical density is rather insignificant (2–3%). Hence, the lack of proportionality between the $K_r$ value and hyperchromicity in the range from 20° to 40°C as well as a similarity in dependence of $K_r$ and $s_{20,w}$ on the temperature make it possible to suggest that the decrease of $K_r$ in this range reflects two parallel processes: denaturation of tertiary structure of tRNA molecules and unstacking of a certain fraction of bases.

According to the data obtained by Fresco et al. [3], addition of $Mg^{2+}$ facilitates formation of a "correct", biologically active tRNA structure which is more compact and stable as compared to the "wrong", biologically inactive one. Fig. 1c shows the temperature dependence of $K_r$ for tRNA incubated in the presence of $Mg^{2+}$ ions. The $K_r$ value is equal to 6.0 in this case which is 15% higher than that obtained without $Mg^{2+}$ ions. Therefore, changes in tRNA tertiary structure affect the reactivity of $C_{(8)}H$ groups of purine residues. Data concerning retardation of the isotope hydrogen exchange in $-NH_2$, $-NH$, and $-OH$ groups of tRNA occurring in the presence of $Mg^{2+}$ ions have been obtained by Englander et al. [4].

Since the loss of tertiary structure of tRNA is accompanied by a considerable decrease of $K_r$ the invariability of this value during heating to about 50°C in the presence of $Mg^{2+}$ ions indicates an increased relative stability of the biologically active, "correct" form under these conditions (see fig. 1c). Further heating of the solution to 60°C results in a sharp decrease in the $K_r$ value and is accompanied by a rather

insignificant increase in optical density (see fig. 1d). Sharp reduction of $K_r$ (about 50% of the total change) upon heating from 50° to 60°C may reflect the cooperative loss of tertiary structure which preceeds the "melting" of the secondary structure. These data are in agreement with results concerning temperature dependence of the fluorescence depolarization of $tRNA^{Phe}$ [5] which also indicate that unfolding of the tertiary structure of $tRNA^{Phe}$ stabilized by $Mg^{2+}$ ions occurs before the secondary is destroyed.

Therefore, formation of tertiary structures in tRNA leads to an additional retardation of $^1H \rightarrow {}^3H$ exchange as compared to that resulting from participation of bases in stacking interactions upon formation of secondary structure.

The reason why the formation of tertiary structure in tRNA enhances retardation of $^1H \rightarrow {}^3H$ exchange in $C_{(8)}H$ groups of purine residues, remains unclear.

It is quite possible that this may be accounted for by a decrease in accessibility of $C_{(8)}H$ groups for the molecules of water due to the compactness of the tertiary structure. A similar phenomenon is observed for hydrogen exchange between the peptide NH-groups in native proteins and water [6].

It cannot be excluded that this retardation is due to additional base interactions which occur upon the formation of the tertiary structure in tRNA molecules.

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