DENATURATION OF UGA SUPPRESSOR $tRNA^{Trp}$ FROM E.COLI

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SUMMARY

To help elucidate the structure of inactive $tRNA^{Trp}$ (E.coli), reversible denaturation has been studied in the UGA suppressor tryptophan tRNA from strain CAJ64, which has an A.U instead of G.U pair in the dihydrouridine stem, and is more stable than the wild type tRNA. The Su^+ tRNA is half-denatured at 10 mM Na^+, in the absence of magnesium, at 55°C, compared to 1M Na^+ for the wild type tRNA. Denatured Su^+ tRNA is less stable than the wild type, and ΔH_r^* in 5 mM Mg^{2+} is 33 kcal/mole compared to 74 kcal/mole. These results favour the hypothesis that guanidine-24 pairs with a cytidine in the metastable denatured form.

INTRODUCTION

$tRNA^{Trp}$ from E.coli can be reversibly denatured and has a metastable denatured form[1], the structure of which has yet to be elucidated. Considerable activation energy is required for renaturation[2] and hyperchromicity is observed[3], which suggests that changes in secondary structure are involved. A mutant species of $tRNA^{Trp}$ has also been isolated which has UGA suppressor activity and has been found to differ from the wild type by a single base change in the dihydrouridine arm ($G_{(24)}$ to A), unlike other suppressor species characterised in which the mutation has been in the anticodon[4]. The base change imparts much greater stability towards denaturation, suggesting that the dihydrouridine arm is involved in the denaturation process. In order to determine more precisely the structure of the denatured form, we have studied denaturation of the wild type and mutant species.

$G_{(24)}$ pairs with $U_{(11)}$ in the normal cloverleaf structure. There is some doubt as to whether a Watson-Crick pair will be formed in this situation[5], but in any case the effect of the mutation will be to increase the stability of the helix[6]. Three possibilities exist for base pairing of $G_{(24)}$ in the denatured state : it might pair with a cytidine, a uridine, or remain unpaired. The effect of the base substitution in the Su^+ tRNA on the thermodynamic parameters for

denaturation and renaturation would be expected to differ in each case. We have therefore investigated reversible denaturation in $tRNA^{Trp}$ (Su^+_{UGA}, CAJ64) and present our observations, which favour the hypothesis that $G_{(24)}$ pairs with a cytidine in the denatured state of wild type $tRNA^{Trp}$.

MATERIALS AND METHODS

Mixed stripped tRNA from E.coli B was obtained from General Biochemicals Inc. and was purified by gel filtration on Sephadex G-100 (7). E.coli CAJ64 : lac^- Su^+_{UGA} (λ) was obtained from Dr.J.Smith (MRC Lab. of Molecular Biology, Cambridge, U.K.) and was grown to late exponential phase in minimal medium (8) supplemented with tryptophan (2 mg/ml); tRNA was prepared by phenol extraction, differential precipitation with propan-2-ol and chromatography on DEAE-cellulose (9). Mg^{2+}-free tRNA was obtained by heating in 100 mM Tris-HCl, pH 8, 4 mM EDTA for 10 min at 50°C, and recovered by ethanol precipitation. Tryptophanyl-tRNA-synthetase was prepared by the method of Joseph and Muench (10). Tryptophan acceptance assays were performed in 0.1M Tris-HCl, pH 8, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM ATP, 2.5 μM (^{14}C)tryptophan (29.1mCi/mmole, CEA, Saclay, France), with excess enzyme (170 units/ml), and unfractionated tRNA at 30-50 A_{260} units/ml. Incubation was for 10 min at 0°C or 25°C, and tryptophanyl-tRNA was precipitated, collected, and counted by liquid scintillation methods (11). Counting efficiency was about 70 %.

RESULTS

I. Denaturation of $tRNA^{Trp}$ (Su^+); dependence on temperature

In order to find conditions appropriate for the denaturation of $tRNA^{Trp}$ (Su^+), the tRNA was freed of Mg^{2+} by heating with EDTA (see Methods). This treatment did not itself reduce the tryptophan acceptor capacity of the tRNA, assayed at either 25°C or 0°C. At lower ionic strength, however, heating to 25°C or higher produced partial denaturation, similar to, though less complete than, that observed in wild type $tRNA^{Trp}$, as shown in

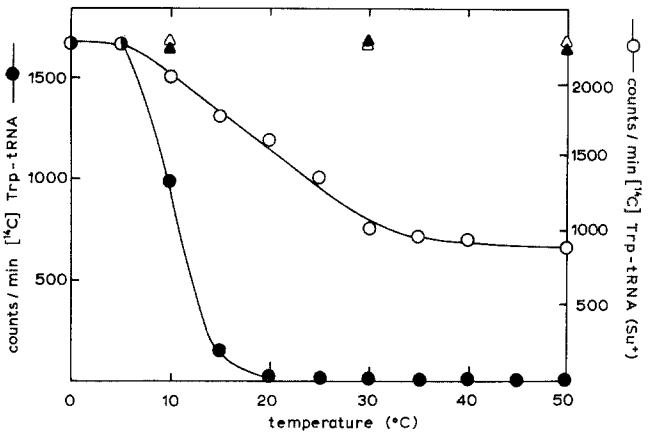

Fig. 1 : Effect of temperature on tryptophan acceptance of $tRNA^{Trp}$ and $tRNA^{Trp}(Su^+)$

Mg^{2+}-free renatured tRNA (wild type or Su^+) was incubated for 5 min at the indicated temperature in 5 mM NaCl, 1 mM EDTA-Na, pH 6.8. Aliquots containing 1 A_{260} unit were cooled quickly to 0°C and assayed immediately at 0°C for tryptophan acceptance capacity (wild type —●—; Su^+ —o—). Further samples were treated similarly but heated to 50°C for 5 min in 0.1 M Tris-HCl, pH 8, 5 mM $MgCl_2$, 50 mM NaCl, 0.5 mM EDTA before assay (wild type —▲— ; Su^+ —Δ—).

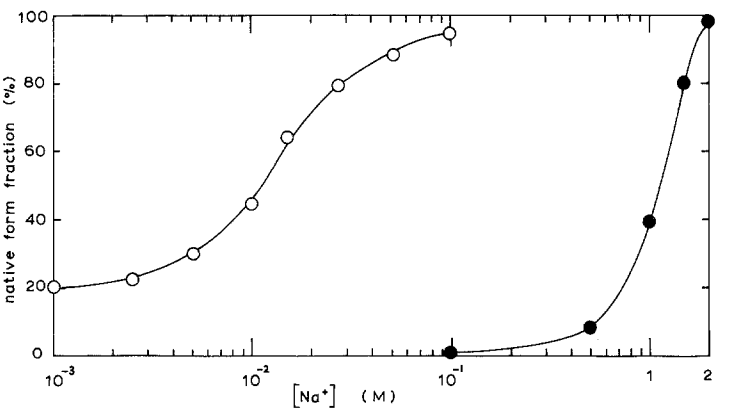

Fig. 2 : Effect of ionic strength on denaturation of $tRNA^{Trp}$

Mg^{2+}-free renatured tRNA (wild type or Su^+) was incubated for 5 min at 55°C in 0.5 mM EDTA-Na, pH 6.8, 0 to 2 M NaCl. Samples containing 1 A_{260} unit were cooled quickly to 0°C and assayed immediately for tryptophan acceptance capacity. The results are expressed as a percentage of Mg^{2+}-renatured controls, performed as described in Fig. 1. Wild type —●—; Su^+ —o—.

Fig. 1. The tRNA could be renatured completely by heating to 50°C in the presence of 5 mM Mg^{2+}.

II. Denaturation of $tRNA(Su^+)$; effect of ionic strength

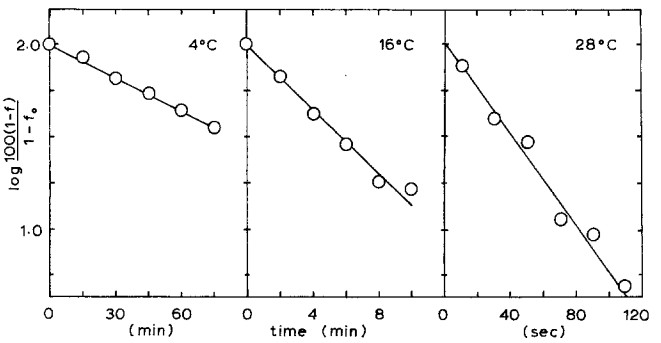

Fig. 3 : Kinetics of renaturation of $tRNA^{Trp}(Su^+)$

Denatured $tRNA^{Trp}(Su^+)$ was prepared by heating Mg^{2+}-free tRNA (Su^+) in 5 mM NaCl, 0.5 mM EDTA-Na, pH 6.8, to 50°C for 10 min. Renaturation at 4, 16, or 28°C in 100 mM Tris-HCl, pH 8, 5 mM $MgCl_2$, 50 mM NaCl, 1 mM EDTA was followed as a function of time by tryptophan acceptance assay of the fraction, f, of tRNA in the native form; f_o is the active fraction at time zero.

Figure 2 shows the fraction of acceptor activity remaining in wild type or in (Su^+) $tRNA^{Trp}$ after heating for 5 min at 55°C over a range of Na^+ concentration. Whereas wild type $tRNA^{Trp}$ is 50 % denatured at about 1 M NaCl, 55°C, a much lower concentration of NaCl, about 10 mM, is required to half denature $tRNA^{Trp}(Su^+)$. These data represent the equilibria between native and denatured forms at each NaCl concentration, unlike those presented in Fig. 1, where the equilibrium proportion of denatured tRNA has been only partially attained within 5 min at temperatures below about 25°C.

III. Renaturation of $tRNA^{Trp}(Su^+)$; kinetics and thermodynamic parameters

The thermodynamic parameters associated with renaturation of wild type and (Su^+) $tRNA^{Trp}$ are valuable in attempting to discriminate between different possible denatured structures. Kinetic data were therefore obtained for the renaturation of each species of tRNA. Denaturation was carried out at low ionic strength in the absence of Mg^{2+} and the course of renaturation at a series of temperatures was followed under conditions favouring virtually complete renaturation. The results of such experiments for $tRNA^{Trp}(Su^+)$ at three temperatures are shown in Fig. 3. Renaturation always appeared to follow first order kinetics; this was true also for the wild type $tRNA^{Trp}$ (not shown) as found previously [2, 11]. The rate constants are presented as an

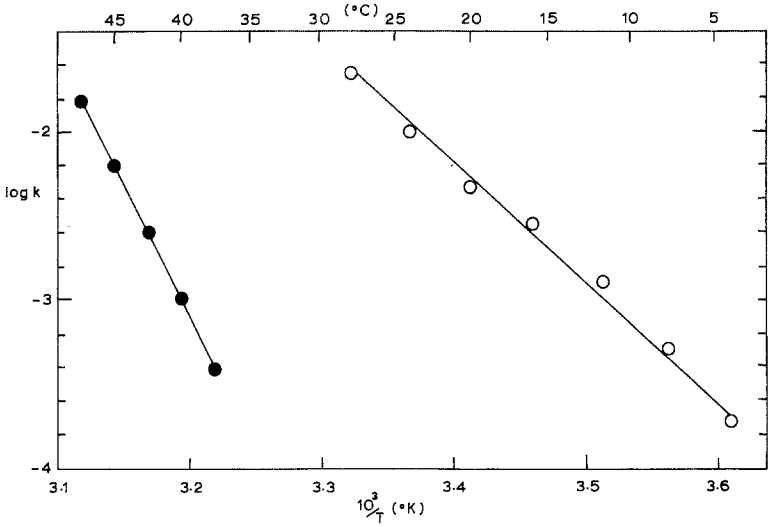

Fig.4 : Arrhenius plot of rate constants for renaturation of wild type and Su^+tRNA^{Trp}

The kinetics of renaturation were measured as described in Fig.3 for wild type (—●—) and Su^+tRNA^{Trp}(—o—). The first order rate constant, k is in sec^{-1}. The standard enthalpy change for activation of renaturation, ΔH^*_r (see text) was obtained from the relation : $\Delta H_r^{o*} = 2.303\ R\ \underline{d}\log k/\underline{d}(1/T) - RT$.

Arrhenius plot in Fig.4, which shows the considerable difference in stability of the denatured forms. The enthalpy changes for activation of renaturation, ΔH^*_r, derived from Fig.4, are 74 and 33 kcal/mole for the wild type and Su^+ species of $tRNA^{Trp}$, respectively.

DISCUSSION

Denaturation of $tRNA^{Trp}$ is thought to involve a rearrangement of secondary structure that includes the dihydrouridine stem. Our results will be considered in relation to the possible pairing states of $G_{(24)}$ in the denatured state : with U, unpaired, or with C, on the assumption that only Watson-Crick pairing is involved. Direct interpretation of activation enthalpy is uncertain, since interconversion might require transient disruption of more structure than is finally rearranged[12], and intermediate states in the overall transition may effectively reduce the activation parameters. However, kinetic studies of reversible denaturation in $tRNA^{Trp}$ support a single step process[11].

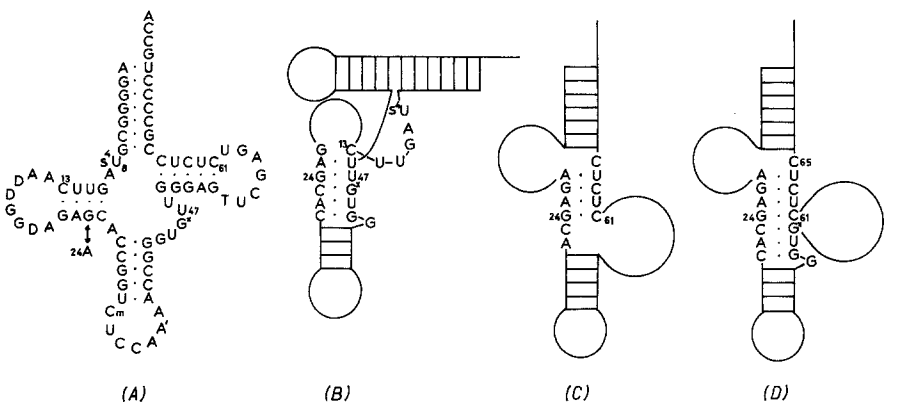

Fig. 5 : Models for secondary structure in denatured $tRNA^{Trp}$

The sequence and cloverleaf form (A) are from Hirsch[4]; structure B is derived from the representation of yeast $tRNA^{Phe}$[13]. G^x is m^7G, and A' is ms^2i^6A.

Furthermore, we are more concerned with the differences in enthalpy of the stable (or metastable) states than with the nature of the transition state.

If, in the denatured form of wild type $tRNA^{Trp}$, $G_{(24)}$ paris with a uridine, as in the cloverleaf structure which is presumed to be the basis of the native conformation, then it would be expected that the mutant species would have a denatured form similar in conformation to that of the wild type tRNA. This would not necessarily be the only denatured form, but would be the most stable, and both the native and denatured forms would be more stable than those of the wild type with respect to the transition state. Assuming that the change G to A did not introduce intermediate stages into the renaturation, ΔH_r^* would be higher than for the wild type. Using a variety of conditions for denaturation, we have been unable to demonstrate such a form.

The second possibility is that $G_{(24)}$ remains unpaired in the denatured form. Probably in this case also denatured $tRNA^{Trp}$ (Su^+) would have a conformation like the wild type, and hence a similar ΔH_r^*, although it is possible that a different denatured structure is preferred thermodynamically for $tRNA^{Trp}(Su^+)$, involving $A_{(24)}$ in an A . U pair and formed reversibly with lower ΔH_d^* and ΔH_r^*. The most simple explanation of our observations is that

$G_{(24)}$ in fact pairs with a cytidine in the denatured form. The base change $G_{(24)}$ to $A_{(24)}$ would introduce an A, C opposition into such a structure and increase its free energy and enthalpy, possibly so that other forms of denatured structure become thermodynamically preferred. This third hypothesis is clearly consistent with a much lower ΔH_r^* for $tRNA^{Trp}(Su^+)$, as we observe.

Previous experiments on photochemically cross-linked $tRNA^{Trp}$ (wild type) suggested that the cross-linked bases $s^4U_{(8)}$ and $C_{(13)}$ were either in a part of the structure substantially conserved in denaturation, or are present in the denatured form in a largely unstructured region[11]. These extremes are represented by the models in Fig. 5, all of which conserve the acceptor stem of the native form, which should be very stable. Structure B (Fig. 5) might retain much of the tertiary structure proposed from X-ray data by Kim et al.[13] for yeast $tRNA^{Phe}$, in spite of the rearrangement of the dihydrouridine stem and extra loop. The other extreme is illustrated in models C and D where the TΨC stem is also disrupted and bases 8 and 13 are in a large loop region. Our present data is opposed to model B, which contains $G_{(24)} \cdot U_{(47)}$, as a structure for denatured wild type $tRNA^{Trp}$; however, for the mutant it would contain the only possible $A_{(24)} \cdot U$ pair in which $A_{(24)}$ is part of a stable helix, apart from the original dihydrouridine stem, and is thus a possible denatured structure for $tRNA^{Trp}(Su^+)$. Denatured structures with $G_{(24)}$ unpaired that appear stable are difficult to construct, the most plausible being the original cloverleaf structure lacking the dihydrouridine stem. The magnitude of ΔH_d^* and ΔH_r^* are difficult to reconcile with a denatured form having this basis, as it seems at present unlikely that tertiary structure could contribute predominantly to these parameters. Structures C and D show the most probable forms containing a $G_{(24)} \cdot C$ pair, both with the same helix containing $G_{(24)} \cdot C_{(61)}$. Structure C conserves the original anticodon stem, whereas D proposes an alternative with more continuous stacked bases, but also with destabilizing defects.

Although the evidence on which specific structures for denatured $tRNA^{Trp}$ can be based is indirect and involves some assumptions, the models

proposed make useful predictions for other experimental approaches, such as chemical reactivity of exposed bases, accessibility to nucleases, and high resolution nuclear magnetic resonance.

ACKNOWLEDGEMENTS

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