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A novel conformational change of the anticodon region of $tRNA^{Phe}$ (yeast)

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ABSTRACT

The temperature dependence of the fluorescence of the Y-base of $tRNA^{Phe}$ (yeast) was investigated kinetically by the temperature jump method. In the range between -15 °C and +30 °C a novel conformational transition of the tRNA could be characterized. This conformational change was found in the absence of any artificial label; it is a characteristic property of $tRNA^{Phe}$ in its native structure. This transition accounts for 30 % of the total fluorescence change. Its activation enthalpy is 16 kcal/mole (67 kJ/mole), and the transition enthalpy is between -2 kcal/mole and +2 kcal/mole (±8 kJ/mole). A model is represented in which this transition can be explained by a a change in the stacking pattern of the anticodon loop. The experimental findings are discussed with respect to several hypotheses about the molecular mechanism of protein biosynthesis which postulate conformational rearrangements of the anticodon loop.

INTRODUCTION

The conformational states of many tRNAs have been widely studied during the last years. Most of these investigations, however, were concerned with the mechanisms of those denaturing processes (1, 2), which generally are not relevant in the biological role of tRNA. On the other hand, models have been proposed for the mechanism of protein synthesis involving conformational changes of the tRNA (3, 4, 5). These models generally propose a codon dependent rearrangement of the tertiary structure of the tRNA. Conformational changes of tRNA with possible biological significance have been observed by experiments on oligonucleotide binding (6) and by studying the dependence of the diffusion constants of tRNA upon Mg-ion concentration (7). In the present paper we present a direct spectroscopic evidence and a physico-chemical characterization of a conformational transition in the anticodon region of $tRNA^{Phe}$ (yeast).

MATERIALS AND METHODS

$tRNA^{Phe}$ (yeast) was prepared for us by Dr. G. Krauss of this institute from soluble RNA (baker's yeast) (Boehringer, Mannheim). The tRNA was pure as judged by its aminoacid acceptance. The specific fluorescence of the Y-base of this tRNA was about twice that of the commercial $tRNA^{Phe}$ (brewer's yeast) (Boehringer, Mannheim). The tRNA was desalted as described previously (8). The oligonucleotides were a gift from Dr. F. von der Haar, Max-Planck-Institut fuer experimentelle Medizin, Goettingen. All other chemicals were of highest available purity grade from Merck (Darmstadt).

Fluorescence temperature jump experiments were carried out in a modified version of the apparatus described in (9). The exciting light came from a 5000 W Hg/X lamp (Hanovia 932-B 39) in a Schoeffel LH 250 lamp housing. Excitation was at 313 nm and emission was above 408 nm (KV 408 Filter, Schott + Gen., Mainz).

Photodegradation of the sample due to the high intensity illumination could be excluded by checking the reproducibility of the amplitude and relaxation time of temperature jumps at the same temperature before and after each series of experiments with the same solution. The relaxation curves were evaluated by simulating them with an analog computer as described previously (8).

RESULTS

We have found, that the temperature induced quench of the fluorescence of the Y-base of $tRNA^{Phe}$ (yeast) located in the anticodon loop is characterized by two processes. A fast and unspecific quenching which is faster than the heating time of the instrument (0.01 ms) is followed by a comparatively slow quench (0.05 ms $< \tau <$ 2 ms depending on the temperature) which comprises about 30 % of the total fluorescence change. A typical fluorescence monitored temperature jump experiment is shown in fig. 1. This latter relaxation process could always be described by a single exponential function. The relaxation times were independent of concentration. Therefore we can as-

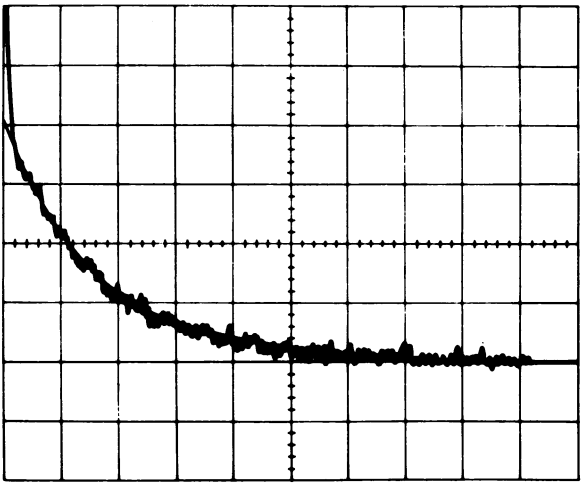

Fig. 1: Fluorescence temperature jump experiment with intact $tRNA^{Phe}$ (yeast)
3.6 µM $tRNA^{Phe}$ (yeast), 0.03 M phosphate, pH 6.0, 0.19 M K^+, 1.0 mM EDTA, 20 % Glycerol. Excitation 313 nm, emission >408 nm. One horizontal division corresponds to 0.5 ms. Temperature jump: 3.5°, final temperature 0.7 °C. The smooth line represents the calculated relaxation curve.

sume that the transition is characterized by a monomolecular "All - or - None" mechanism. The slow process could be observed under conditions where in the absence of Mg-ions the native structure of the tRNA is intact, i.e. none of the known melting effects (1, 8) had already occurred. It could also be excluded that the relaxation process observed was a consequence of the binding of magnesium to the tRNA since EDTA had no effect on either the amplitude or the time constant of the relaxation. This shows that a novel conformational change of the tRNA itself is responsible for this effect.

The temperature dependence of the relaxation time of the conformational change is shown in fig. 2. An apparent activation enthalpy of 16 kcal/mole (67 kJ/mole) is evaluated from this plot. For a monomolecular mechanism $A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$ the relaxation time is given by $1/\tau = (k_1 + k_{-1})$. Therefore the limiting values of the slope of the Arrhenius-type plot at the extreme high and low temperatures should give the activation enthal-

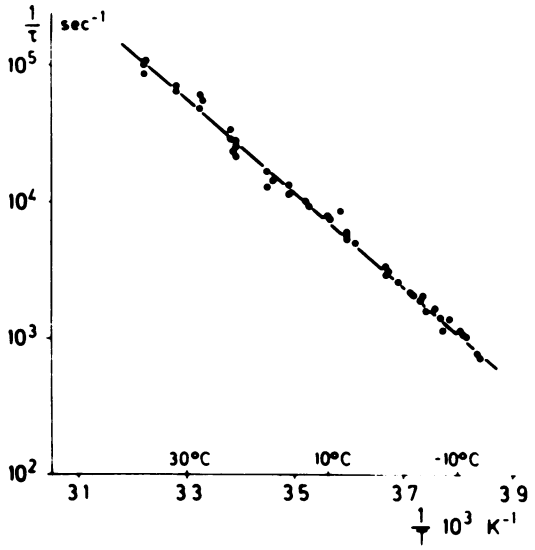

Fig. 2:
Arrhenius-type plot of log 1/τ vs. 1/T. Conditions as in fig. 1.

pies for the forward and backward reactions, respectively. A change in the slope then corresponds to the difference of the activation enthalpies, i. e. the reaction enthalpy ΔH. Since within the limits of error there is no deviation from a single straight line in the Arrhenius-type plot the reaction enthalpy has to be between -2 kcal/mole and +2 kcal/mole (±8 kJ/mole). In addition, the amplitude of the relaxation effect does not change by more than a factor of two in the temperature range investigated (-15 °C - +30 °C) indicating a very broad melting behaviour as a consequence of a small reaction enthalpy. Unfortunately a detailed analysis of the fluorescence temperature jump amplitudes is not possible. This is due to the fact that the relaxation amplitudes at different temperatures cannot be related to concentration changes because the fluorescence yield of the individual conformational states is strongly temperature dependent.

The rate of the conformational transition is slightly pH-dependent. Fig. 3 shows the variation of 1/τ with pH. The pH-dependence (log (1/τ) = 0.2 pH), however, is too weak to account for a protonation reaction; in addition no pK-value

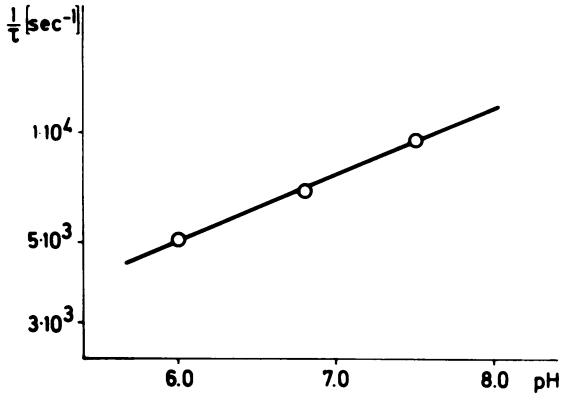

Fig. 3:
pH-dependence of the relaxation time. Conditions as in fig. 1.

for tRNA^{Phe} (yeast) is known in the pH-range investigated.

The amplitude of the relaxation effect can be enhanced by inducing a pH change concomitantly with the temperature jump. This can be achieved by using a buffer system with a large ΔH for the protonation reaction as for example Tris-HCl. Fig. 4 shows a comparison of the amplitudes in phosphate and Tris-HCl buffer. The apparent activation enthalpy for the conformational change in question in Tris-HCl buffer is about 12 kcal/mole (50 kJ/mole). This is in agreement with the change in activation enthalpy calculated from the ΔH of the buffer system and the

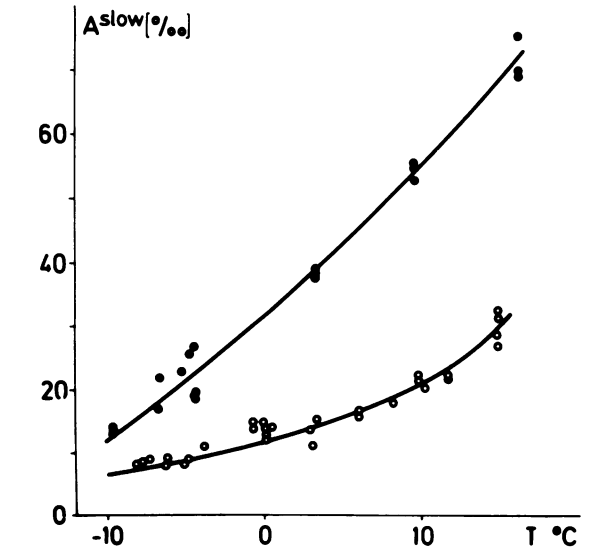

Fig. 4:
Effect of buffer on the amplitude of the fluorescence temperature jump amplitude. The amplitude is given in ‰ of the total fluorescence intensity. (●) 0.03 M Tris-HCl, (O) 0.03 M phosphate. Both experiments were at pH 6.0, 0.2 M K^+, 1.0 mM EDTA, 20 % Glycerol.

pH-dependence of the relaxation time (fig. 3).

The conformational change is not present in oligonucleotides containing the base sequence of the anticodon loop. Fig. 5 shows the time course of an RNase A digest of $tRNA^{Phe}$ monitored by UV-absorption and the amplitude of the conformational transition. The increase of UV-absorption coincides with the decrease in the amplitude of the slow fluorescence relaxation. The relaxation time, however, was not influenced within the limits of error. Since the RNase A digest contains the hexanucleotide Gm-A-A-Y-A-Ψp (10), we conclude that the conformational change is not present in this hexanucleotide. This conclusion is confirmed by the absence of any slow relaxation effect in the isolated hexanucleotide alone. On the other hand, temperature jump experiments with the dodecanucleotide A-Cm-U-Gm-A-A-Y-A-U-m5C-Ψ-Gp showed a slow fluorescence increase ($\tau \simeq 500$ µs, fluorescence increase of 6 % of the total fluorescence change; T = -4 °C) after the fast and unspecific quench. The dodecanucleotide dimerizes below +5 °C with a relaxation time in the range of 100 ms. A possible base pairing scheme of this dimer is proposed in fig. 6 (H. Grosjean, private communication). The fluorescence change should therefore be attributed to some rearrangement of the Y-base in this dimer.

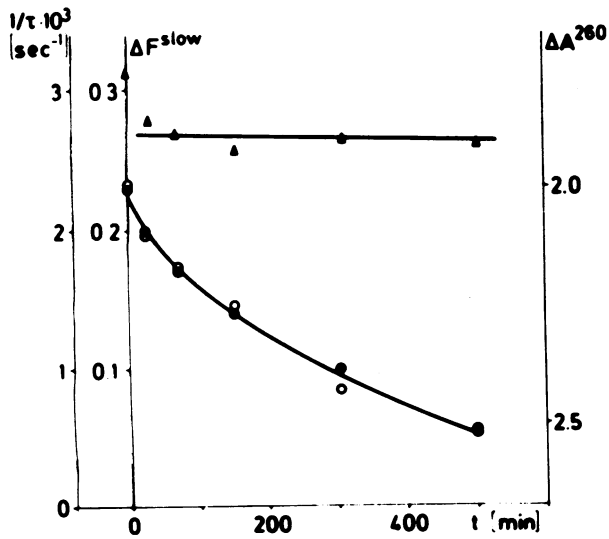

Fig. 5:
Digestion of $tRNA^{Phe}$ with RNase A at 25 °C monitored by UV-absorption (O) and the amplitude of the slow relaxation at -5 °C monitored by Y-base fluorescence (●). (▲) relaxation time.

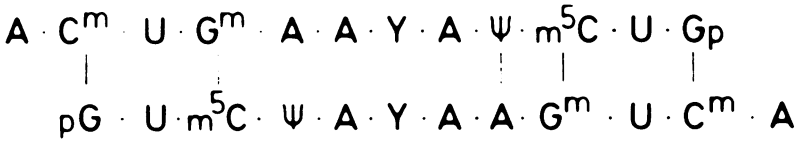

Fig. 6: Sequence of the dodecanucleotide from the anticodon loop of $tRNA^{Phe}$ with the possible base pairing scheme for a bimolecular association (H. Grosjean, private communication).

DISCUSSION

What is the nature of the conformational transition observed?

The data presented here show that the transition involves a change in the vicinity of the Y-base of the anticodon loop of $tRNA^{Phe}$. This change could only be observed in intact tRNA and not in the oligonucleotides containing all or part of the loop sequence. This implies that at least the anticodon arm if not the complete tRNA is necessary for the transition to occur.

The results indicate that the conformational change observed reflects an alternation in the stacking geometry of the anticodon loop: One of several possible models for a molecular mechanism of this reaction would be a transition from 3'-stacked anticodon as proposed by W. Fuller and A. Hodgson (11) to a 5'-stack as in the model proposed by C.R. Woese (12). These two conformations are visualized in fig. 7. The data presented above are in agreement with this speculation. The overall ΔH of a conformational change of this kind can be rather small since the number of stacking interactions in both conformations is the same. The activation enthalpy would then represent a partial unstacking of one of the states to make the formation of the other conformation possible. The measured apparent activation enthalpy of 16 kcal/mole (67 kJ/mole) shows that at least two stacking interactions have to be resolved before the transition can occur.

The pH-dependence of the relaxation amplitude can be a consequence of a different accessibility of the Y-base to the environment in its different states. In the 3'-stack the Y-base

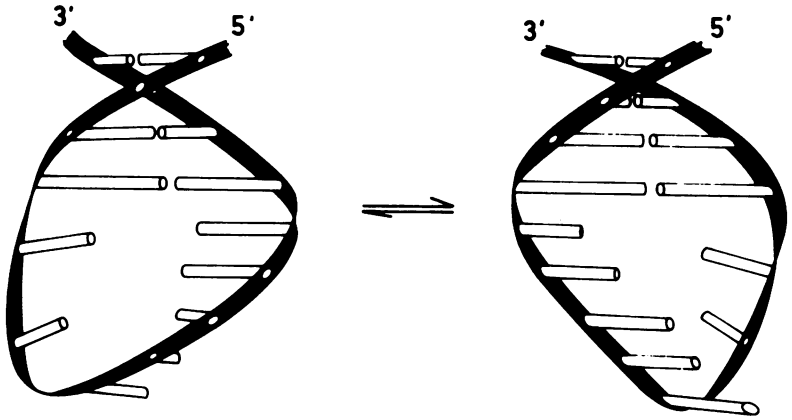

Fig. 7: Possible model for the conformational change in the anticodon loop.

is stacked completely and is therefore less influenced by the surrounding medium than in the 5'-stacked conformation (cf. fig. 7). The larger amplitude of the conformational change in the Tris buffer system can then be correlated to the direction of the transition by the following argument. The Y-base fluorescence decreases with decreasing pH (13), and according to the proposed model this effect should be more pronounced in the "free", i. e. 5'-stacked, conformation. With increasing temperature accompanied by a decrease of pH the equilibrium would be shifted towards the 5'-stacked conformation, since this state has the higher pH-dependence of the fluorescence. This means, that the low temperature state of the tRNA is the 3'-stacked ("Fuller-Hodgson") conformation, i. e. the same conformation as found in the X-ray crystallographic analysis (14, 15).

What is the functional relevance of this conformational change?

There have been several hypotheses which postulate functionally different conformations of the anticodon region and therefore require conformational changes. Some of them are concerned with the recognition process of the tRNA by the codon on the ribosome (4, 5). The model of J.A. Lake (4) assumes

that after the correct decoding of the tRNA in the receptor site of the ribosome a switch of the anticodon conformation from a 5'- to a 3'-stack brings the aminoacyl moiety into close contact with the peptidyl-tRNA. This further reaction on the ribosome would also help to increase the fidelity of the translation process.

Another hypothesis which assumes a conformational change of the anticodon loop was recently published by F.H.C. Crick et al. (16). They put forward a model for a primitive protein biosynthesis in the absence of ribosomes. In this model the aminoacyl-tRNA binds to the mRNA involving a pentanucleotide interaction. The movement of the tRNA is then accomplished by a change in the stacking geometry of the anticodon loop from 3'- to 5'-stack.

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