MULTIPLE STATES IN MACROMOLECULES
II. Entropic behaviour of tRNA degraded by polynucleotide phosphorylase

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1. Introduction

Degradation of tRNA by polynucleotide phosphorylase has been shown to be very different from the degradation of other tRNAs (for review, see [1]); this was supposedly due to the peculiar structural features of transfer RNA [2]. A theoretical model for single nucleation process in macromolecules has been described in a previous article [3] and we shall try to use it on the special case of tRNA, with as few thermodynamic parameters as possible, and then to relate them to known features of the tRNA molecule.

The characteristic degradation path of polynucleotide phosphorylase is the following reversible reaction:

$$(\mathrm{pN})_n + n\mathrm{P}_i \underset{\longleftarrow}{\overset{\mathrm{Mg}^{2+}}{\longrightarrow}} n\ \mathrm{ppN}$$

and it has been shown, for almost all polynucleotides, that the equilibrium is classical, the rate of the reaction depending on the structure of the polynucleotide. The mechanism of the phosphorolysis reaction [2, 4, 5] has been shown to be a "non-synchronous" process, i.e. the enzyme degrades the polymer molecules entirely in a sequential and non-dissociating fashion from the 3′-OH to the 5′ end. In the presence of excess inorganic phosphate, the reaction goes to completion with almost all natural and biosynthetic polynucleotides. However, the behaviour of tRNA is different since at low and moderate temperatures, the reaction does not proceed to completion [6, 7]: Thus, at a given temperature some tRNA molecules are entirely degraded, while the remaining molecules are resistant with their 3′-OH terminal adenosine intact. Therefore, when we say $n\%$ of phosphorolysis, it means that $n\%$ of the molecules have been destroyed (and not fragments of $n\%$ from all the molecules). Polynucleotide phosphorylase will thus be used here as the experimental means for separating the tRNA population [3]. It is worth emphasizing again that the reaction stops before complete phosphorolysis of the total tRNA population is achieved, not because the enzyme has become inactive, but because the limiting factor resides in the tRNA itself.

We shall sum up the experimental facts and correlate them to the single nucleation model to finally obtain values of the thermodynamic parameters involved.

2. Experimental

2.1. *Phosphorolysis at different temperatures*

Unfractionated tRNA, purified tRNA, as well as a tRNA recombined from the 5′ and 3′ half of yeast $\mathrm{tRNA}^{\mathrm{Phe}}$ [8], behave in the same way: at a given temperature, the phosphorolysis levels out at a percentage depending on the temperature, the plateau being real (no detectable phosphorolysis after a given time) at low temperatures, and increasing (pseudo-plateau) at higher temperatures (phosphorolysis proceeding at a slow rate), up to a temperature where all the tRNA is degraded; this last temperature (T_0) depends on the nature of the tRNA and on its prior thermal treatment. The equilibrium obtained is not frozen since heating from an initial temperature, T_i, to a new one, T_j, results in the phosphorolysis

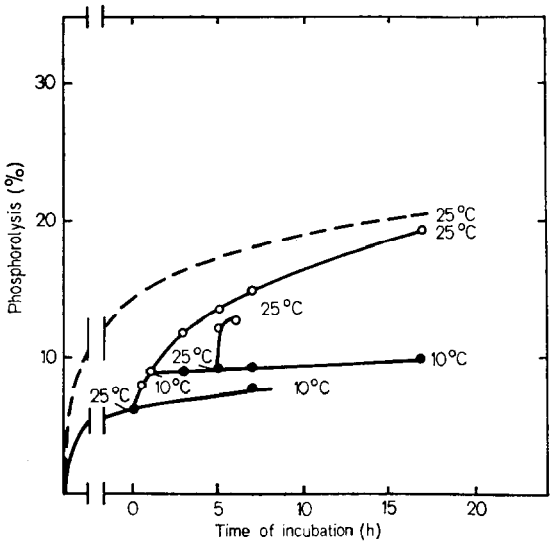

Fig. 1. Phosphorolysis of unfractionated *E. coli* tRNA at two different temperatures from [7].

immediately reaching the plateau normally obtained for T_j; contrarywise, heating at T_j (in the absence of enzyme) followed by phosphorolysis at T_i *does not raise* the plateau normally obtained at T_i (fig. 1).

The tRNA which is not degraded at a given temperature can be separated from the incubation mixture and incubated again with polynucleotide phosphorylase: no further phosphorolysis occurs (or phosphorolysis at the same rate as the increasing slope of the pseudo-plateau.

2.2. *Pre-treatment of tRNA*

a) Heating at 60° in the presence of 10 mM Mg^{2+}: this treatment is the procedure used to "renature" tRNA to an active form when it has been partially inactivated in the absence of divalent ions [9]. Both *E. coli* and yeast unfractionated tRNA thus treated have a phosphorolysis pattern similar to that obtained with untreated tRNA, though the extent of degradation is significantly reduced.

b) Heating at 60° in the presence of EDTA: some specific tRNAs, such as yeast $tRNA^{leu}_{III}$ have been trapped into a denatured form by heating at 60° in the presence of 1 mM EDTA [9]. Phosphorolysis of this particular tRNA in the denatured form goes practically to completion.

3. Results: The nucleation model and tRNA

We use here the model proposed in the preceding article [3]. Polynucleotide phosphorylase is used to separate the tRNA population, and we assume that phosphorolysis at a given temperature, T_i, will only depend on the state (0 or 1) of the accessible level *i*. Since phosphorolysis occurs after a specific association of tRNA with the enzyme, we shall suppose that it can only happen with a notable probability on a molecule having its i^{th} level in the open state, thus giving a possibility of association with polynucleotide phosphorylase with a smaller activation energy than when the level is in a closed state (with a difference Δg_i). The difference of Gibbs free energy at the i^{th} level between the open and the closed state will be a function of the previous history of the tRNA.

We shall consider tRNA behaviour at a given temperature to determine the proportions of open and closed kernels, and we shall then consider the problem of interconversion of one set of forms (having their kernel *i* closed at a temperature (T_i) into the other (kernel *i* open at the same temperature). This last interconversion corresponds to the behaviour of tRNA at the pseudo-plateau, after all the molecules which have their kernel *i* open have been phosphorolyzed: the proportion of molecules having their i^{th} kernel open is given by the intercept of the pseudo-plateau with the ordinate axis, since the corresponding distribution of open states is that of the *initial* population of the tRNA molecules.

To obtain the experimental values for the Gibbs free energy, we shall fit the experimental points at time 0 with the theoretical curve obtained from the probability law $P(T)$:

$$P(T) = \frac{e^{-\Delta gi/RT}}{1 + e^{\Delta gi/RT}}$$

To compare with the experimental curves we shall assume as a further simplification that $\Delta g_i = \Delta g$ independent of *i*. We shall see from the curves (fig. 2) that such a simplification is in agreement with the precision on the experimental points. This assumption supposes that all the differences between the open and closed states are of similar magnitude whatever the level.

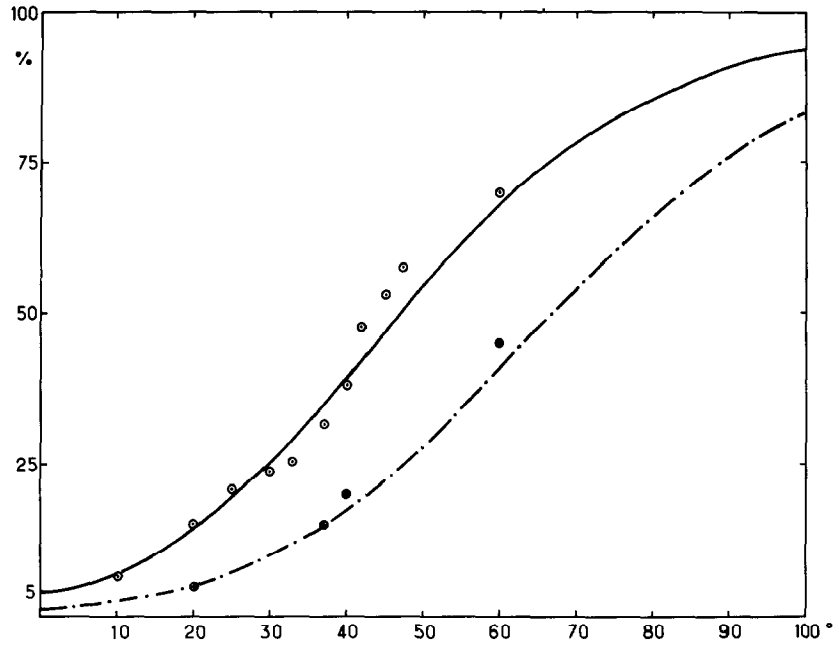

Fig. 2. Theoretical and experimental values of the initial phosphorolysis of unfractionated *E. coli* tRNA as a function of temperature. Untreated tRNA: theory (——), experiments (⊙). tRNA heated at 60° in 10 mM Mg^{2+}: theory (— · — ·), experiments (⊕).

3.1. *Untreated tRNA*

tRNA from *E. coli* strain B, purified but unfractionated gives the following values:

$$\Delta g = \Delta h - T\Delta s \qquad \Delta h = 12.2 \text{ Kcal}$$
$$\Delta s = 38.1 \text{ cal/mole} \times {}^\circ\text{K}$$

The values obtained for purified species of tRNA are similar, except that entropy seems to play a more important function; the enthalpy factor is constant within the limits of the precision of the experiments, but the entropy fluctuates (see table1).

3.2. *Denatured tRNA*

Yeast unfractionated tRNA, treated by triacetylhexadecyl ammonium in dimethyl formamide or the denatured yeast specific leucine III tRNA (one of these tRNA being trapped into an inactive conformation and stable in that state in the phosphorolysis assay medium) are phosphorolyzed almost to completion at low temperature. This can be explained, as in section 3.1 above, by a contribution of entropy: an enhancement of entropy to a value higher than 42 cal/mole × °K would explain the experimental results.

Table 1

tRNA species	Δs (cal/M × °K) (values ± 0.2)
Unfractionated *E. coli* tRNA	
untreated	38.1
heat treated*	35.9
Yeast $tRNA^{leu}_{III}$ denatured	42.0
E. coli $tRNA^{tyr}$	39.1
E. coli $tRNA^{phe}$	38.3
E. coli $tRNA^{ser}$	38.4
E. coli $tRNA^{thr}$	38.4
E. coli $tRNA^{val}$	36.0
Yeast $tRNA^{phe}$	38.6
Yeast $tRNA^{ser}$	39.2

* tRNA heated 3 min at 60° in the presence of 10 mM Mg^{2+}.

3.3. *tRNA heated at 60° in 10 mM Mg^{2+}*

The values for unfractionated *E. coli* tRNA are the following: Δh = 12.2 Kcal Δs = 35.9 cal/mole × × °K. All the phosphorolysis plateaus appear at values lower than those for the native species; we also

see that, here again, the entropy contribution alone accounts for the differences observed. The plateaus appear at values lower than for the native species by an almost constant factor.

4. Conclusion and perspectives

The entropic behaviour of tRNA which we found correlated with an enzymic degradation process which acts in the following way [2]:

1. The RNA binds to the enzyme; the binding is in favor of the molecules which have their i^{th} level closed.

2. The interaction of the tRNA molecule with the enzyme allows the unfolding of the tRNA so that its 3′-OH end is accessible to the active site of the enzyme; at this stage a part of the tRNA structure, involving entropy, has to be loosened.

3. The enzyme degrades the tRNA sequentially; the folded regions and the secondary structure of the tRNA being disrupted as the reaction goes on.

Since the enzyme degrades the tRNA from one end to the other once it has attacked the first nucleotide, steps 1 and 2 are the only limiting stage where tRNA molecules, in a statistical population, can be discriminated. In a purified tRNA population, it is only the entropy involved in step 2 which will give the values of the phosphorolysis plateau, according to the Δg involved in step 1; contrarywise, in a heterogenous population, step 1 can be a stage where competition arises between different tRNA species which have their i^{th} level empty. This may work in a way opposite to that of step 2, for instance in a case where a tRNA species which has plateaus at lower values than another, binds better to the enzyme than that other species. This is observed in the phosphorolysis of a mixture of tRNA [7].

The behaviour of polynucleotide phosphorylase allows the discrimination between inhomogeneities in a population of tRNAs of unique primary sequence: these inhomogeneities are due to differences, dependent on the entropy, in the structure of the tRNAs, and one may try to describe a feature of the tRNA which might involve such differences.

The most simple starting point (but not the only one) is since the enzyme attacks tRNA from the 3′-OH end, that the entropy is involved at the -CCA stem of the tRNA, and that the differences between various species (untreated and treated) is due to several possible states of a topological property involving this stem [7]. However, the pCpCpA terminal sequence is not directly involved as such since several purified tRNA without the CCA sequence have a behaviour similar to that of the original tRNAs (unpublished results). We shall illustrate the theoretical model with the tentative following picture: the entropy involved might be due to the fact that the -CCA stem goes through a loop in the tRNA with the clover leaf secondary structure. Experimental and theoretical evidences suggest that, if this were the case, the loop which is involved would be the so-called dihydro U loop [10–12]. In such a model, the influence of divalent ions might be to diminish the probability of the -CCA stem sliding inside the loop, so that tRNA treated with a high magnesium concentration at 60° has a lower entropy than tRNA treated with EDTA. The limiting factor in step 2 of the enzymic degradation would thus be the unfolding of the "knot"; and the observation that the -CCA of unphosphorolyzed molecules is not attacked is explained by the fact that the loop prevents the binding of the 3′-OH end to the active site of the enzyme by steric hindrance.

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