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Thermal conductivity measurements of tRNA melting process

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Abstract

In this paper we present investigations of the structural changes of the transfer ribonucleic acid (tRNA) from *Escherichia coli*. The measurements were performed by the thermal conductivity technique previously used to study DNA melting process (M. Tuliszka and F. Jaroszyk, Thermochim. Acta, 194 (1992) 67). The paper provides the results of temperature measurements of the thermal conductivity coefficient of tRNA solution in (a) 0.001 M $MgCl_2$ and (b) in 0.01 M cacodylic acid + sodium cacodylate buffer and 0.001 M EDTA. The results obtained are interpreted on the basis of results of DSC measurements obtained by other authors. The results show the possibility of application of the thermal conductivity method to the investigation of the tRNA molecule's conformational changes in solutions.

INTRODUCTION

At the turn of the 1960s the primary and secondary structure of the tRNA was recognized. The secondary structure pattern was called the "clover leaf" structure and it is valid for all known tRNAs with a few modifications. The tRNA molecule also has a three-dimensional structure [1–5] referred to as the tertiary structure. Its stability is determined by many factors. Among these factors the ionic conditions of the solutions are of great importance — especially the presence or absence of Mg^{2+} ions in solution [2, 4, 6].

From the 1960s differential scanning calorimetry (DSC) has been widely used in biochemical research. On the basis of measurements performed by DSC it is possible to observe changes of the thermodynamic quantities of a material under study occurring with temperature (collagen [7–9]; DNA [10–12]; tRNA [6, 13, 14]). Most often the change in the specific heat (C_p) which accompanies a conformational change is observed. From its temperature dependency $C_p = f(t)$ it is possible to evaluate the value of the change of enthalpy ΔH which accompanies the investigated process.

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The specific heat at constant pressure C_p and the thermal conductivity coefficient λ are interrelated by the formula

$$C_p = \frac{\lambda}{\rho\kappa} \tag{1}$$

were ρ is the density of the medium and κ is the thermal diffusivity coefficient. An important thermodynamic parameter is the thermal conductivity. In this work we undertook the investigation of the temperature dependence $\lambda = f(t)$ of the thermal conductivity λ of the tRNA solutions. We wanted to know whether this dependence may be used in investigations of the changes occurring within the tRNA molecule, and to what degree.

In the previous paper [15] the results of measurements concerning the possibility applying thermal conductivity phenomena to detection of conformational changes within the DNA molecule were presented. This paper reports the investigation of the melting process of tRNA by the same method.

In interpreting the results obtained we used the results of the investigations of the melting process of tRNA obtained by DSC. A comparison of these results provided a basis for interpretation of the temperature dependency $\lambda = f(t)$ in the temperature range proper to the thermal denaturation (melting) process. In further considerations we assumed that the difference R (defined in one of the next paragraphs) between the thermal conductivity coefficient of tRNA solution and that of the pure solvent, depends on the conformational changes in the tRNA structure.

MATERIALS

Samples of tRNA (*Esherichia coli* MRE 600) were obtained from Boehringer Mannheim GmbH. The measurements were performed in two kinds of solvents: **a**, 0.001 M $MgCl_2$; **b**, 0.01 M sodium cacodylate buffer and 0.001 M EDTA.

The weighed samples of dry tRNA were dissolved in the appropriate solvent **a** or **b**. The solution was then dialyzed at 4°C against a 20-fold larger volume of pure solvent **a** or **b**. After the dialysis (lasting 18–20 h) the solution was degassed under vacuum for 10 min. The concentration was determined by weighing the dry sample of tRNA.

The appropriate concentrations were 1.45 g l^{-1} for tRNA in solvent **a** and 0.94 g l^{-1} for tRNA in solvent **b**.

METHOD

The thermal conductivity of the tRNA solutions was determined with the a.c. version [16–18] of the transient hot wire method [19, 20]. This method

is the most accurate and fastest of all thermal conductivity measurement methods for liquids. However, it should be noted that the absolute results of thermal conductivity coefficient measurements contain a systematic error of several percent. This is because the investigated solutions are electrolytes.

First, the above method was applied to find the temperature dependences of the thermal conductivity coefficient λ_S for pure solvents **a** or **b**. The temperature dependences of the thermal conductivities $\lambda_{tRNA/S}$ of tRNA solutions in appropriate solvents were determined. On the basis of these measurements we introduced the quantity R as the difference between $\lambda_{tRNA/S}$ and λ_S, i.e.

$$R = \lambda_{tRNA/S} - \lambda_S \tag{2}$$

The temperature dependences $R = f(t)$ were subject to analysis.

RESULTS

Figure 1 shows the temperature dependences $\lambda_{tRNA/S} = f(t)$ for tRNA solution in solvent **a** (curve 1) and $\lambda_S = f(t)$ for pure solvent **a** (curve 2). In curve 1, for the temperature range from about 70 to 90°C the $\lambda_{tRNA/S} = f(t)$ dependence shows an anomaly. In the remaining temperature range the $\lambda_{tRNA/S} = f(t)$ curve is similar to that of $\lambda_S = f(t)$.

Figure 2 shows the temperature dependences $\lambda_{tRNA/S} = f(t)$ for the tRNA solution in solvent **b** (curve 1) and $\lambda_S = f(t)$ for pure solvent **b** (curve 2). Curve 1 shows an anomaly for the temperature range from about 25 to 50°C compared to curve 2. In the remaining temperature range both curves are similar.

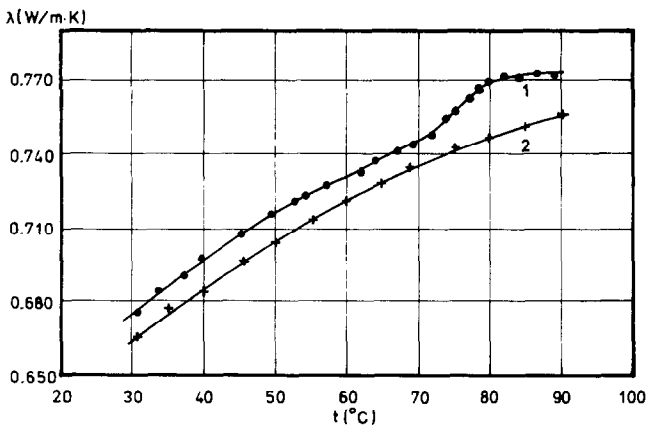

Fig. 1. Thermal conductivty λ of tRNA solution in solvent **a** (curve 1) and of pure solvent **a** (curve 2).

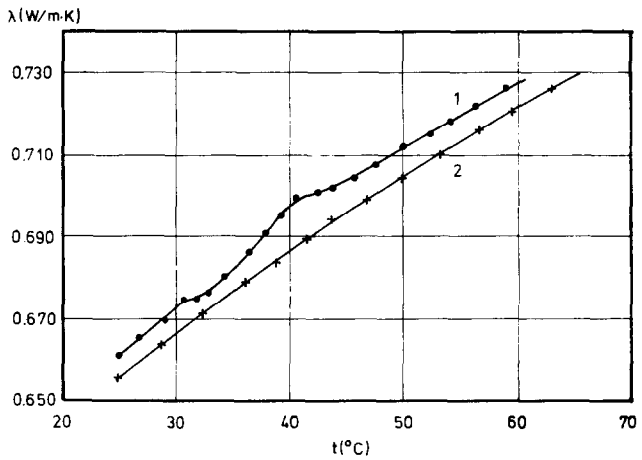

Fig. 2. Thermal conductivity λ of tRNA solution in solvent **b** (curve 1) and of pure solvent **b** (curve 2).

DISCUSSION

From the literature data it is evident that the presence of Mg^{2+} ions in solution affects the stability of a 5S ribosomal RNA [14, 21, 22] molecule and a tRNA molecule [2–4, 6, 13]. This is why the measurements of thermal conductivity of tRNA (*E. coli*) in a solvent containing Mg^{2+} ions (solvent **a**) and in a solvent without Mg^{2+} ions (solvent **b**) were carried out. Figure 3 shows the dependence $R = f(t)$ for tRNA in solvent **b** obtained in the present work. The temperature at which the first maximum in $R = f(t)$ appears is about 31°C. There is another maximum (substantially greater than the first) at about 40°C. Thus it cannot be concluded that there is only

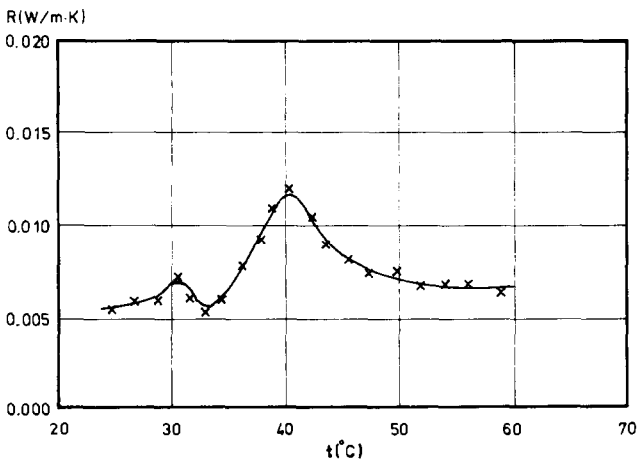

Fig. 3. Temperature dependence $R = f(t)$ in solvent **b**.

one temperature at which the melting process occurs, which is due to the fact that the melting process of tRNA is a multistage process. According to Crothers [4], in the absence of Mg^{2+} ions in solution, $tRNA^{Met}$ (*E. coli*), the first step is the degradation of the tertiary structure of this molecule (the unfolding of "clover leaf") together with the melting of the D-stem helix. This is followed by the melting of TΨC stem helix and anticodon stem helix. Finally, because of the high enthalpy change ΔH accompanying the melting process, the acceptor stem helix is melted.

The question of an interpretation of the course of the dependencies obtained $R = f(t)$ now arises. For the dependence $R = f(t)$ for tRNA in solution **b** (Fig. 3), the most probable conclusion is that the appearance of the first maximum is due to the melting process of the tertiary structure of a tRNA molecule. The spatial arrangement of the molecule changes and this should be reflected in the process of heat transportation in the considered medium. However, the melting of the tRNA acceptor stem is accompanied by a high change of entropy ΔS and enthalpy ΔH. This fact is significantly connected with the structural changes of the macromolecular arrangement and it should considerably affect the thermal conductivity of the solution under study. For this reason the second maximum on the $R = f(t)$ curve may be recognized as related to the tRNA acceptor stem melting process.

As was mentioned at the beginning of this section, the stability of the tertiary structure of the tRNA molecule in solution is strongly affected by the presence of the divalent ions in solution, especially by Mg^{2+} ions [4, 6, 13]. A similar phenomenon has also been observed in the case of 5S RNA molecules [14, 21, 22]. In the presence of Mg^{2+} ions in solution the stability of the tertiary structure increases considerably, which brings about a significant shift in the melting temperature toward higher values. Figure 4 shows the dependence $R = f(t)$ for tRNA (*E. coli*) in solvent **a** obtained in

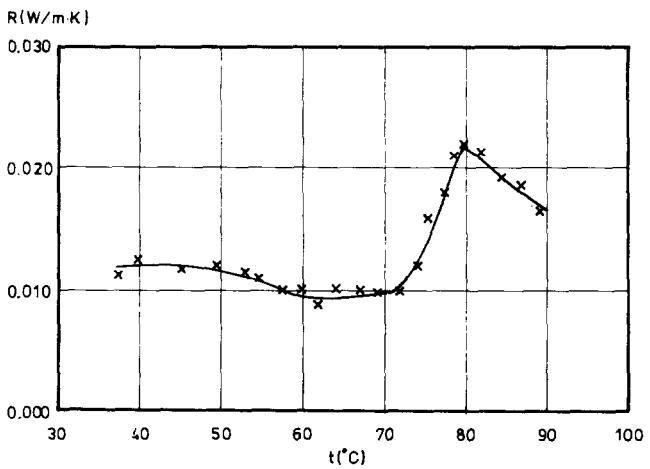

Fig. 4. Temperature dependence $R = f(t)$ in solvent **a**.

this work. This dependence shows only one maximum in the vicinity of 80°C. The anomalies at lower temperatures characteristic of the solutions of tRNA not containing Mg^{2+} ions (Fig. 3) have not been observed. Therefore, it may be concluded that the anomaly in the $R = f(t)$ dependence observed in the vicinity of 80°C reflects the melting process investigated by the thermal conductivity method. It must be pointed out that the width of the anomalous part of the curve $R = f(t)$ is greater than that of the appropriate part of the DSC curves. This is probably due to the fact that the material used (tRNA; *E. coli*; MRE 600) was a mixture of different specific tRNAs and was not specific for any particular amino acid. The DSC curves applied for the sake of comparison were obtained for specific $tRNA^{Met}$ (*E. coli*) [13].

In conclusion it can be stated that the melting process of tRNA molecules can be observed by the thermal conductivity method. However, the possibility of a quantitative analysis of this process with this technique requires further study.

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