# ABSORBANCE-TEMPERATURE PROFILE OF RIBOSOMES FROM SEEDS OF PINUS LAMBERTIANA

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Summary: The absorbance (260 nm)-temperature profile of ribosomes from seeds of Pinus lambertiana, in the temperature range of about 25 - 60° was observed to be biphasic. When r-proteins in ribosomes were dansylated by 5-dimethylamino-1-naphthalenesulfonyl chloride dispersed on celite (180 molecules of 5-dimethylamino-1-naphthalenesulfonyl chloride per one ribosome particle), each transition midpoint was lowered by 2 - 3° and the sharpness of the transition of each phase was increased. The result of the present work suggests discrete cooperative transitions of the conformation of r-RNAs in ribosomes and its control by r-proteins.

## Introduction

Conformational transitions in r-RNA within ribosomes may be of decisive importance in the stepwise process of protein biosynthesis (1,2,3). The conformational transitions in nucleic acids usually proceed cooperatively. In other words, the elementary process of the transition of an individual segment of the molecule is influenced by the state of other segments through intramolecular interactions. As the result of a cooperative interaction between the elementary steps of the transformation, large changes may be induced by small effects. On the other hand, r-proteins may be involved in the regulation of such conformational changes in ribosomes (4,5,6,7,8). In this paper, I report a polyphasic absorbance-temperature profile, which may be indicative of distinct steps of cooperative transitions in the conformation of ribosomes, instead of a simple monophasic profile. Differential detachment of r-proteins from ribosomes, or derivatization of r-proteins in ribosomes, etc., and then subsequent examination of the effects of such a treatment on the conformational transition profile of

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ribosomes may be employed as methods of studying the effect of r-proteins on the conformational transition of ribosomes. In the present work, for that purpose, r-proteins in ribosomes were dansylated by dansyl chloride (5-dimethylamino-1-naphthalensulfonyl chloride) dispersed on celite. Previously (9) we observed that this reagent derivatized r-proteins selectively leaving r-RNA unreacted in ribosomes, and that sedimentation properties of ribosomes were not altered, whereas ribosomes became more sensitive to ribonuclease, as the result of the dansylation. Also, we found that dansylation with the dansyl chloride-celite reagent which does not include any solvent that might affect the ribosome, can be carried out under conditions (buffer, temperature, reaction period, etc.) suitable for the maintenance of ribosome integrity without side effects.

## Materials and Methods

Ribosomes were prepared by the usual methods (9) from seeds of Pinus lambertiana (Forest Seeds of California, Davis, Calif.) that had been soaked in distilled water overnight.

Protein content of the ribosome preparation was estimated by the method of Lowry et al. (10), using bovine serum albumin as the standard. Dische's orcinol method (11) was used for the RNA determination, using yeast RNA as the standard.

Dansylation of the ribosome: Dansyl chloride dispersed on celite, colorimetric assay 11%, was purchased from Calbiochem and methyl-$^{14}$C-dansyl chloride in acetone was from Schwarz Bioresearch Inc. The reaction mixture (5 ml.) containing 0.5% ribosomes, 2% dansyl-Cl-celite (w/v) which corresponded to 8.0 mM dansyl chloride, 0.025 M sodium cacodylate (pH 8.8), 0.02 M KCl, and 0.005 M $MgCl_2$, was stirred gently for 2.5 h in the cold. Dansyl-Cl-celite residues were then removed from the reaction mixture by centrifugation. The supernatant was dialized overnight in the cold against a buffer composed of 0.025 M Tris-HCl (pH 7.8), 0.02 M KCl, and 0.005 M $MgCl_2$. A system containing celite instead of dansyl-Cl-celite was used as a control. The determination of the time course, the extent of the dansylation, and the test of the reactivity of ribosomal components with dansyl-Cl-celite was carried out as described previously (9).

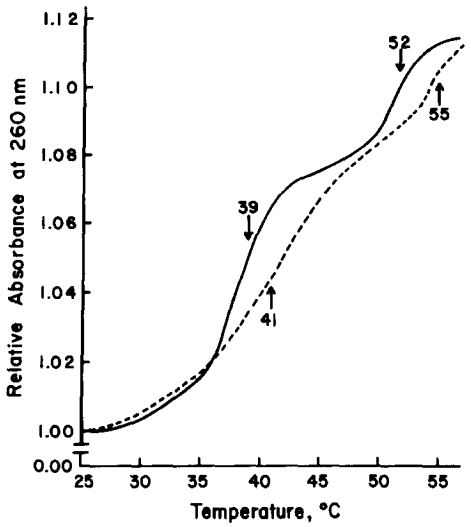


Figure 1. Absorbance-temperature profiles of undansylated ribosomes and dansylated ribosomes (180 molecules of dansyl chloride per ribosome particle) at 260 nm. Ribosomes were suspended in a buffer containing 1.5 mM $MgCl_2$, 0.025 M Tris-HCl (pH 7.8), 0.02 M KCl, and 0.006 M 2-mercaptoethanol. The initial absorbance, 0.5 - 0.6 were normalized to 1.0. The profile was scanned with Gilford 2400 Spectrophotometer. The heating was 1.63° per minute. Correction for the thermal expansion of water was not done. Numbers on the curve indicate Tm values. ——— , Dansylated ribosomes; -----, undansylated ribosomes.

In the preparation of dansylated ribosomes used in the present work, the uptake of dansyl group by ribosomes increased linearly with time and reached a plateau after 2.5 h, with about 45 mμ moles of dansyl chloride bound per mg of ribosomes, under the reaction conditions described in the text. This corresponds to 180 molecules of dansyl chlorider per ribosome particle, assuming the molecular weight of the ribosome to be $4 \times 10^6$ (12).

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Absorbance-temperature profiles at 260 nm for normal (undansylated) and dansylated ribosomes (180 molecules of dansyl chloride per ribosome particle) were tested as described in the legend of Figure 1.

## Results

Absorbance-temperature profiles at 260 nm for undansylated and dansylated ribosomes are shown at Figure 1. The absorbance change of either normal (undansylated) r-protein (cf., 13,14,15) or dansylated r-proteins at 260 nm was negligible with increasing temperature in the experimental range tested. Above about 60°, ribosomes started to be precipitated, and therefore the profiles were plotted up to below this temperature. The profiles for both normal and dansylated ribosomes were biphasic instead of simple mono-

Table 1. Transition midpoint (Tm); molar enthalpy ($\Delta H$) of transition at the Tm; ratio of cooperative lengths (n), and ratio of cooperative parameters ($\sigma$), dansylated (180 molecules of dansyl chloride per ribosome particle) to undansylated ribosomes.

| Phase of profile | Ribosomes | Tm, °C | $\Delta H$, $\frac{Kcal}{mole}$ | $\frac{n_{dansylated}}{n_{undansylated}}$ | $\frac{\sigma_{dansylated}}{\sigma_{undansylated}}$ |
|---|---|---|---|---|---|
| 1 | Dansylated | 39 | 58 | 2.5 | 0.16 |
| | Undansylated | 41 | 23 | | |
| 2 | Dansylated | 52 | 26 | 1.7 | 0.33 |
| | Undansylated | 55 | 15 | | |

phasic profiles. Furthermore, as indicated in Figure 1 and Table 1, dansylation of ribosomes lowered the transition midpoint (Tm) by 2 - 3°, and increased the slope in each phase. The effect of dansylation was not simply the removal of large amounts of proteins from ribosomes, since both undansylated and dansylated ribosomes contained the same gross composition of 42% RNA and 58% protein.

In cooperative transition, the sharpness of the transition generally increases with the "cooperative length", n. Among other things, this leads to a characteristic increase in the molar enthalpy of transition $\Delta H_{app}$. This enthalpy at the Tm in each phase was calculated for a two-state model. In this case, the apparent rate constant is: $K_{app} = K^n = \frac{\theta}{1-\theta}$, where $\theta = \frac{[I]}{[I]+[II]}$, and [I], [II] = concentration of species. If the normalized increase in absorbance during the transition can be equated with the quantity, 1 - $\theta$, based on the fact that the ordered forms are hypochromic with respect to the denatured species, then at a transition midpoint:

$$\left(\frac{d \ln K_{app}}{dT}\right)_{Tm} = \left[\frac{d}{dT}\left(\ln \frac{\theta}{1-\theta}\right)\right]_{Tm} = \frac{\Delta H_{app}}{RT^2} = \frac{n.\Delta H_U}{RT^2}$$

where $\Delta H_U$ is the molar enthalpy of transition for the elementary transition process. Thus, the van't Hoff enthalpy, $\Delta H_{app}$, was obtained from a van't Hoff plot; i.e., ln $K_{app}$ against 1/T, and the value is shown in Table 1. Also, taking the values of $\Delta H_U$ for undansylated and the dansylated ribosomes to be the same, the ratios for cooperative lengths n and cooperati-

vity parameters $\sigma$ of dansylated and undansylated ribosomes were calculated and shown in Table 1.

## Discussion

Present work with ribosomes from pine seeds shows that the absorbance-temperature profile of the ribosomes is polyphasic in accordance with discrete stepwise cooperative transitions. Presumably each discrete step of cooperative transition may reflect a two-state transition, although the validity of the assumption of the two-state transitions may require further tests as described by Lumry et al. (16). In connection with present observation, it is to be mentioned that both ribosomal precursor particles and ribosomal functions usually occur in a few discrete kinds rather than a continuous spectrum (17,18). The observation of the polyphasic behavior of a temperature-induced conformational transition of ribosomes can be visualized often only in the presence of a proper concentration of $Mg^{++}$. The first phase of transition could hardly be detected in the absence of $Mg^{++}$. However, in the presence of $Mg^{++}$, the precipitation of ribosomes occurs easily on heating, and thereby the transition profile is obscured. By the employment of techniques, such as relaxation methods or careful tests at small temperature intervals, it may be possible to detect further smaller changes in the conformational transition profile with greater sensitivity.

One can see the increased sharpness of the transition in Figure 1 and, accordingly, increased cooperative length n and decreased cooperativity parameter $\sigma$ in Table 1, upon dansylation of the ribosomes. Thus, the present observation shows that r-proteins control the cooperative transition of r-RNAs in ribosomes.

The lowering of the Tm of ribosomes by the dansylation of r-proteins within ribosomes shows the importance of r-proteins for the stability of ribosomal structure. One might tentatively ascribe the temperature-dependent increase in absorbance to the temperature-dependent helix-coil transition of r-RNAs in ribosomes, and apply the equation expressing the thermodynamic relation between Tm and electrostatic free energy due to interactions between the phosphate charges fixed on nucleic acid molecules (19),

$$Tm = (\Delta H_o + \Delta F_{dansyl}) / \Delta S_o$$

where $\Delta H_o$, $\Delta S_o$, and $\Delta F_{dansyl}$ are changes of molar enthalpy, entropy, and electrostatic free energy due to dansylation of r-proteins, respectively, in the transition of helix to coil. Since $\Delta F_{dansyl}$ is negative, Tm must be lowered with dansylation, as the electrostatic repulsions between the phosphate charges fixed on the helical segment of r-RNA increase upon dansylation of r-proteins due to the decrease of positive charges of r-proteins by dansylation. The present observation demonstrates the importance of r-proteins for the structural stability of r-RNAs in ribosomes.

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