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# The conformational changes of 5SrRNA of plant origin in presence of magnesium cations by adiabatic scanning differential calorimetry

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**Abstract**

The results of calorimetric studies of specific transfer for lupin seed and wheat germ 5SrRNA in 10 mM sodium cacodylate buffer (1 mM $Na_2EDTA$ and 20 mM NaCl, pH 7.2) alone and with different concentrations of $MgCl_2$ are reported. A dependence of the concentration of $MgCl_2$ on the temperature of specific transfer was found. Deconvolutions of the DSC curves were carried out. The DSC curve shifts, temperatures of peaks and values of enthalpies of the domains distinguished are discussed.

## INTRODUCTION

As is well known, the helix–coil transition of 5SrRNA takes place on changing pH, temperature and the ionic strength of the solution. Scanning adiabatic differential calorimetry is a convenient method of measurement for this kind of study. It was applied to a detailed study of two nucleic acids (5SrRNA) of plant origin, isolated from lupin seeds (LS) and wheat germ (WG) [1]. The investigations were carried out for LS and WG 5SrRNA dissolved in the basic buffer alone, and with the addition of various salts. In this work, calorimetric studies for 5SrRNA obtained from LS and WG in the basic solution as well as with addition of different concentrations of $MgCl_2$ are presented.

## MATERIALS AND METHODS

The 5SrRNA isolated [2,3] from LS and WG were dissolved in a basic buffer of pH 7.2 containing 10 mM sodium cacodylate, 1 mM $Na_2EDTA$

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and 20 mM NaCl. Samples of 45 $A_{260}$ units of 5SrRNA in 1.5 ml (final concentration, $2.9 \times 10^{-5}$ M) were used for the measurements. The calculated extinction coefficient [4] used was $10.29 \times 10^{-5}$ $M^{-1}$ for LS and $10.03 \times 10^{-5}$ $M^{-1}$ for WG.

The differential adiabatic scanning calorimeter DASM-1M [5] was used for the measurements. Calorimetric recordings were usually started around 288 K and continued at a rate of 1 K $min^{-1}$ up to 348 or 373 K, depending on the character of the observed phenomena. Experimental data were used for the analysis of the complex unfolding process. For this purpose, deconvolution of DSC curves, based on the methods of Freire and Biltonen [6] and Chang [7] was carried out. The first method considers a system in which the molecule is composed of $N$ identical units of residues. The residue partition function $q(N)$ can be defined as

$$q(N) = \exp\left[\int_{T_0}^{T} \left(\Delta H_{exp}/RT^2\right) dt\right]$$

where $\Delta H_{exp}$ is obtained from experimental $C_p$ data. It is an iterative method in which, after identification of the first (lowest temperature) transition and its subtraction from the DSC curve, the calculation of the new statistical sum, covering all states except the first, is made and this procedure repeated. Taking into account that the $C_p$ values of native and denatured forms are different, it is necessary to assume the character of the relationship between the baseline and temperature in the region of conformational changes. It was accepted that the baseline is approximated by a straight line connecting the points before and after this transformation. These values were taken into account as the initial conditions for numerical fitting of experimental data of the function consisting of a sum of dependences describing $C_p(T)$ for the individual two-state transformation.

As is shown in Table 1, the sum of the enthalpy values, $\Delta H$, for the individual domains differs negligibly from $\Delta H_{exp}$. The concordance between $\Delta H$ and $\Delta H_{exp}$ is fully satisfactory, taking into account that the determination of $\Delta H_{exp}$ cannot be very precise. As it shown in Figs. 1 and 2 the courses of DSC curves are complicated and it is necessary to choose arbitrarily the initial and final values of the transition temperature. This is the reason for our detailed consideration of the results presented by microcalorimetric recording, i.e. the DSC curves.

The reproducibility and reversibility of the results were also the subjects of detailed analysis. It was stated that the difference in the temperature of peaks of microcalorimetric recordings for the measurements of 5SrRNA from LS and WG in the basic buffer obtained in different time periods was not bigger than 0.1 K. Also only small differences in the shape of DSC curves were observed. The calculated and experimental $\Delta H$ values are different from each other by several percent. The reproducibility of DSC curves was satisfactory also in the cases where the same sample was used in

TABLE 1

Deconvolution of 5SrRNA melting curves into components [a]

| No. | PN | $T_m$ (K) | $\Delta H$ (kJ mol$^{-1}$) |
|---|---|---|---|
| LS + 0.00 mM $MgCl_2$ | | | |
| 1 | 1 | 301 | 113 |
| 2 | 2 | 308 | 421 |
| 3 | 3 | 320 | 220 |
| 4 | 4 | 323 | 616 |
| 5 | 5 | 329 | 293 |
| | | Total | 1663 |
| | | $\Delta H_{exp}$ = | 1630 |
| LS + 2.00 mM $MgCl_2$ | | | |
| 6 | 1 | 312 | 139 |
| 7 | 2 | 330 | 381 |
| 8 | 3 | 330 | 381 |
| 9 | 4 | 342 | 518 |
| 10 | 5 | 352 | 280 |
| | | Total | 1622 |
| | | $\Delta H_{exp}$ = | 1628 |
| LS + 4.00 mM $MgCl_2$ | | | |
| 11 | 1 | 306 | 198 |
| 12 | 2 | 319 | 235 |
| 13 | 3 | 332 | 272 |
| 14 | 4 | 340 | 498 |
| 15 | 5 | 346 | 550 |
| 16 | 6 | 352 | 309 |
| | | Total | 2062 |
| | | $\Delta H_{exp}$ = | 2087 |
| LS + 8.00 mM $MgCl_2$ | | | |
| 17 | 1 | 299 | 220 |
| 18 | 2 | 309 | 289 |
| 19 | 3 | 319 | 296 |
| 20 | 4 | 332 | 292 |
| 21 | 5 | 343 | 542 |
| 22 | 6 | 349 | 508 |
| 23 | 7 | 355 | 305 |
| | | Total | 2452 |
| | | $\Delta H_{exp}$ = | 2535 |
| WG + 0.00 mM $MgCl_2$ | | | |
| 24 | 1 | 308 | 261 |
| 25 | 2 | 315 | 373 |
| 26 | 3 | 320 | 436 |
| 27 | 4 | 325 | 322 |
| | | Total | 1392 |
| | | $\Delta H_{exp}$ = | 1404 |

TABLE 1 (continued)

| No. | PN | $T_m$ (K) | $\Delta H$ (kJ mol$^{-1}$) |
|---|---|---|---|
| WG + 2.00 mM $MgCl_2$ | | | |
| 28 | 1 | 321 | 212 |
| 29 | 2 | 330 | 460 |
| 30 | 3 | 338 | 439 |
| 31 | 4 | 340 | 307 |
| | | Total | 1418 |
| | | $\Delta H_{exp}$ = | 1446 |
| WG + 4.00 mM $MgCl_2$ | | | |
| 32 | 1 | 325 | 144 |
| 33 | 2 | 338 | 209 |
| 34 | 3 | 343 | 460 |
| 35 | 4 | 348 | 448 |
| | | Total | 1261 |
| | | $\Delta H_{exp}$ = | 1262 |
| WG + 8.00 mM $MgCl_2$ | | | |
| 36 | 1 | 319 | 215 |
| 37 | 2 | 332 | 317 |
| 38 | 3 | 341 | 446 |
| 39 | 4 | 346 | 600 |
| 40 | 5 | 350 | 506 |
| | | Total | 2084 |
| | | $\Delta H_{exp}$ = | 2159 |

[a] No., number; PN, peak number; $T_m$, peak temperature; $\Delta H$, transition enthalpy.

more than one determination, with an interval longer than 12–14 h. For the determinations made one after the other in a short time interval (about 3 h), the DSC curves obtained had practically the same shape and peak temperatures, but the $\Delta H_{exp}$ calculated from the second measurement was lower by about 10‰. It was also found that the 5SrRNA samples do not change their properties with time. Reproducible results were obtained, using the same sample, after several months.

## RESULTS

In Fig. 1 the DSC curves for LS in the basic buffer alone and with the addition of 0.27, 0.67, 1.00, 1.33, 2.00, 2.66, 4.00 and 8.00 mM $MgCl_2$ are presented. On the curve without salt there are two intense peaks at temperatures 308.7 and 323.9 K. The addition of $Mg^{2+}$ ions shifts these peaks towards higher temperatures, to an extent depending on the concentration of $MgCl_2$. In the concentration range below 0.6 mM $MgCl_2$, the change in temperature location of the second peak is not large (0.4 K). In the concentration range from about 0.6 to 2.5 mM $MgCl_2$ the changes in temperatures of the peaks are considerable. Any further increase in $MgCl_2$

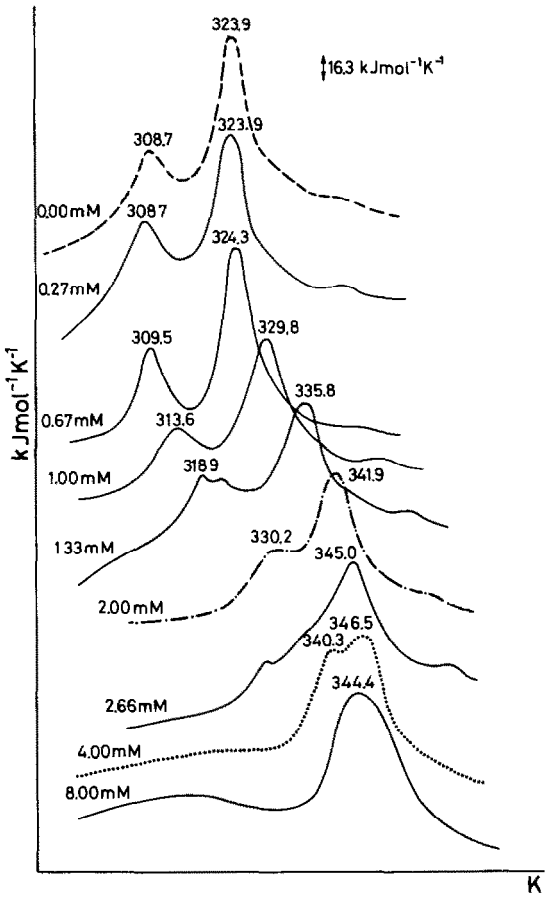


Fig. 1. DSC plots for LS 5SrRNA after addition of $MgCl_2$.

concentration influences the temperatures of the peaks to a smaller degree. Lastly, for the concentrations 4.00 and 8.00 mM $MgCl_2$ the temperature of the second peak remains practically constant.

Similarily in the case of WG, changes in the temperatures of the peaks are also observed. This is illustrated graphically in Fig. 2, where DSC curves for 5SrRNA from WG alone and with the addition of 0.67, 2.00, 4.00 and 8.00 mM $MgCl_2$ are presented. On the DSC curve for WG (Fig. 2) one peak at 320.3 K can be distinguished. Similarily, as in the case of 5SrRNA from LS, the addition of a small amount of $MgCl_2$ causes only a small change in the temperature of the location peak. For example, the addition of 0.67 mM $MgCl_2$ to the solution causes a change in the peak temperature of only 0.5 K. The addition of larger amounts of $MgCl_2$, corresponding to 2.00 mM $MgCl_2$ causes a split of the peak into two, at temperatures 331.0 and 339.0 K. However, for the highest concentrations of $MgCl_2$ (4.00 and 8.00 mM) only one peak is observed on the DSC curve. As in the case of LS the biggest

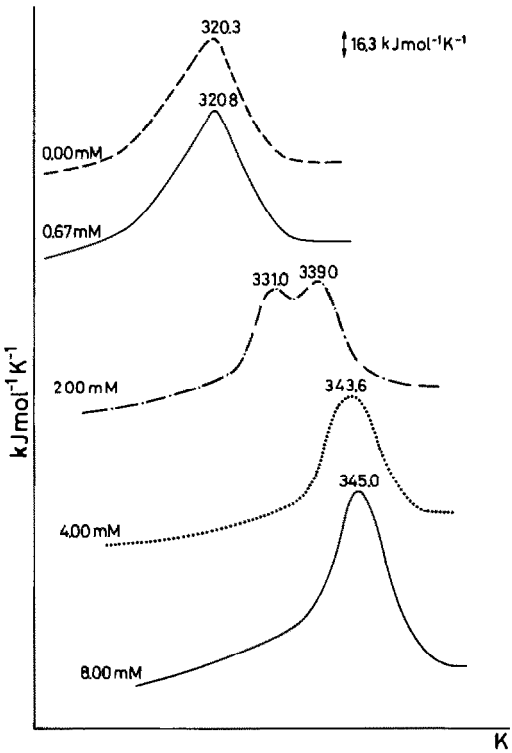


Fig. 2. DSC plots for WG 5SrRNA after addition of $MgCl_2$.

changes in peak temperatures are observed in the concentration range 0.6–2.5 mM $MgCl_2$ (Fig. 3). In Fig. 3 the dependence of peak temperatures (in the case of two peaks, the second peak temperature) on the $MgCl_2$ concentration is presented. It was found that practically the same peak temperature for the highest concentrations were obtained. This indicates $MgCl_2$ "saturation". This is probably due to the existence of a new equilibrium state, which can be assumed when the stabilization role of $Mg^{2+}$ is complete. This fact should be confirmed by the results of deconvolution analysis of DSC curves. The results of this analysis are presented in Figs. 4 and 5 and Table 1. In Table 1 the values of the temperatures of peaks and

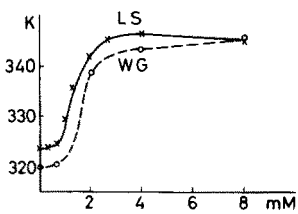


Fig. 3. Peak temperatures vs. $MgCl_2$ concentrations.

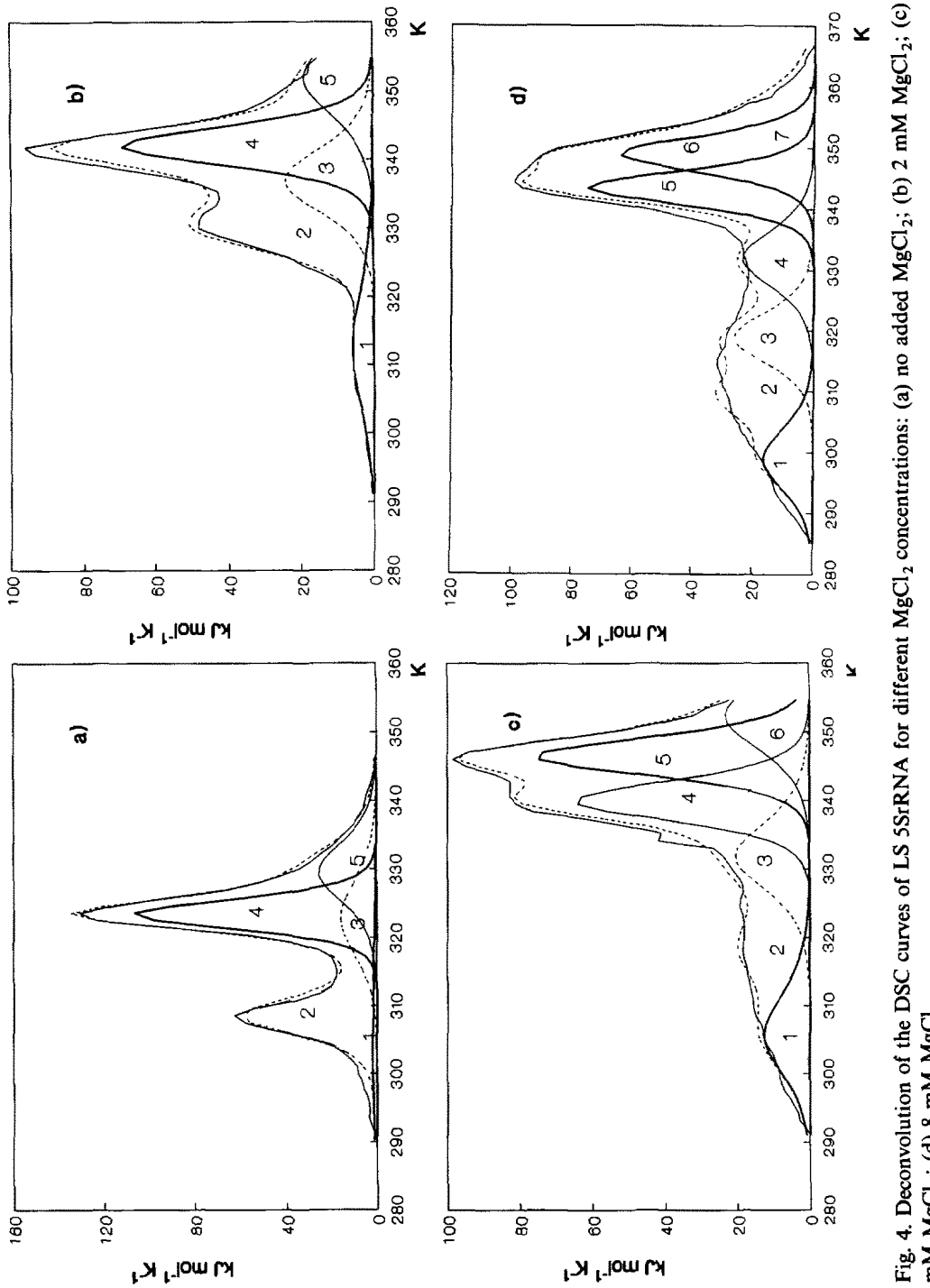


Fig. 4. Deconvolution of the DSC curves of LS 5SrRNA for different $MgCl_2$ concentrations: (a) no added $MgCl_2$; (b) 2 mM $MgCl_2$; (c) 4 mM $MgCl_2$; (d) 8 mM $MgCl_2$.

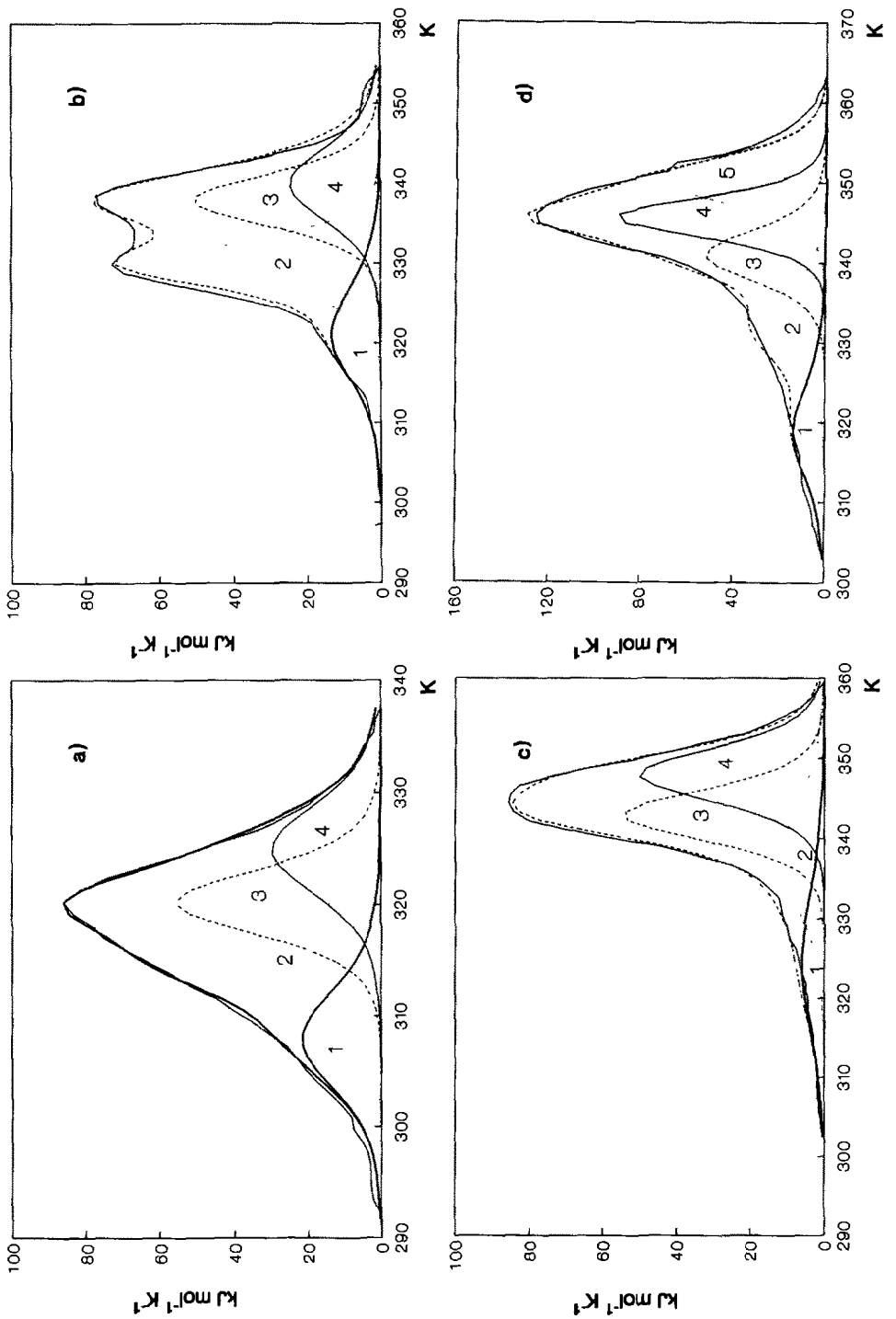


Fig. 5. Deconvolution of the DSC curves of WG 5SrRNA for different $MgCl_2$ concentrations: (a) no added $MgCl_2$; (b) 2 mM $MgCl_2$; (c) 4 mM $MgCl_2$; (d) 8 mM $MgCl_2$.

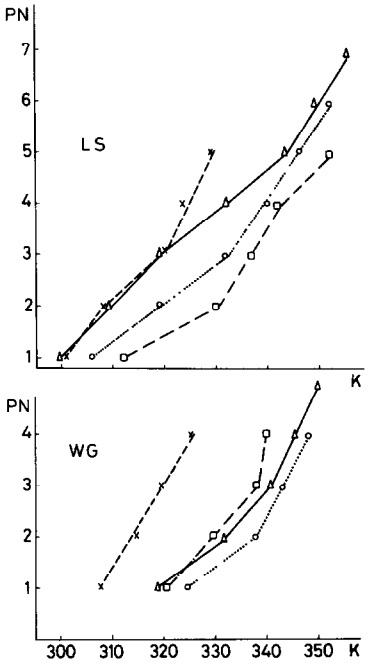


Fig. 6. Peaks number vs. temperature for LS and WG 5SrRNA for different salt concentrations; ×, no added $MgCl_2$; □, 2 mM $MgCl_2$; ○, 4 mM $MgCl_2$; △, 8 mM $MgCl_2$.

the $\Delta H$ values of the domains distinguished are given. The numbers PN correspond to consecutive domains; the bigger the number, the later the appearance of the domain. It was noted that the deconvolution analysis distinguishes seven domains for LS and five for WG in the case of the highest concentration. In the case of solutions without $MgCl_2$ or with only a small concentration, deconvolution of the DSC curve for 5SrRNA of LS enables us to distinguish five domains and four for 5SrRNA of WG.

In Fig. 6 the changes in peak temperatures for the observed domains are presented. Only for WG without $Mg^{2+}$ ions is a linear dependence observed; for the remaining cases it is only possible to observe the linear dependence for three or four domains. On the basis of the $\Delta H$ data presented in Table 1 it was possible to note the existence of five groups of domains having the following mean values of enthalpies: 215 kJ $mol^{-1}$ (Nos. 3, 17, 28, 33, 36); 287 kJ $mol^{-1}$ (Nos. 5, 10, 13, 18, 19, 20); 311 kJ $mol^{-1}$ (Nos. 8, 16, 23, 27, 31, 37); 438 kJ $mol^{-1}$ (Nos. 2, 26, 30, 35, 38) and 507 kJ $mol^{-1}$ (Nos. 9, 14, 22, 40). For 40 domains distinguished, 26 were taken into account. When the concentration of $Mg^{2+}$ ions increases, the domain of lower enthalpy value appears earlier, and those of higher enthalpy values later. From the analysis of the sequence of domain appearance, for 5SrRNA from LS and WG in the presence of the same concentrations of $Mg^{2+}$ ions, the domains of a given enthalpy appear later for LS than for WG.

The results can be summarized as follows.

(1) Heat adsorption on heating the 5SrRNA from LS and WG is a process taking place over a very broad temperature range (Figs. 1 and 2). It starts at about 288 K and ends at 371 K. All the calorimetric curves are complicated, but they are very characteristic of 5SrRNA from LS and WG.

(2) In 5SrRNA from LS and WG an increase in $MgCl_2$ concentration in solution shifts the 5SrRNA melting curve to a higher temperature. In the case of 5SrRNA from LS two peaks are present on the DSC curves, the temperatures of which become closer to each other when the $MgCl_2$ concentration increases, which finally results in one peak at the highest concentration.

(3) There is a dependence of the peak temperature on $MgCl_2$ concentration. For the 5SrRNA studied it can be noted that in the concentration range 0–0.69 mM $MgCl_2$ and at more than 4 mM $MgCl_2$ the changes are small in comparison with the changes in the concentration range 0.69–2.0 mM $MgCl_2$.

(4) The deconvolution analysis of the results shows that in all cases (without and with addition of $MgCl_2$) there exists a certain number of domains characterized by particular values of enthalpy. They can be treated as corresponding to the elementary transformations which always occur. The larger number of domains is probably connected with the appearance of other additional structures, e.g. tertiary structure.

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