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THE CONFORMATIONAL CHANGES OF 5SrRNA FROM LUPIN SEEDS IN PRESENCE OF Na^+, K^+, Cu^{2+}, Pb^{2+} CATIONS BY ADIABATIC SCANNING DIFFERENTIAL CALORIMETRY

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Abstract

The results of calorimetric studies of 5SrRNA solutions isolated from lupin seeds both in the absence and in the presence of different concentration of the cations Na^+, K^+, Cu^{2+}, Pb^{2+} were reported. Using the deconvolution method proposed by Freire and Biltonen the elementary transitions were distinguished and discussed.

Keywords: 5SrRNA comformational changes enthalpy, adiabatic scanning calorimetry

Introduction

5SrRNA as an integral part of the ribosome have been the object of intensive functional as well as structural studies [1]. The numerous nucleotide sequences collected so far, have led to the construction of the general model of the secondary structure [2]. Tertiary interaction which organize the spatial structure of the molecule have also been postulated [3, 4].

The studies of the dynamic conformation of 5SrRNA in presence of different ions, in conditions of temperature changes and at various ionic strengths of the solution may lead to better understanding of the structural propensity encoded in their sequence. As it is well known scanning adiabatic differential calorimetry is a convenient method for this kind of study. So far, it was applied by us for investigations of conformational changes of 5SrRNA from lupin seeds and wheat germ in presence of tetra-protonated spermine, spermidine and magnesium salts [5, 6]. Structural interpretation of the thermal unfolding patterns for lupin seeds and wheat germ was proposed [7].

The investigation presented here are treated as a part of a long term project of calorimetric comparative studies of conformational changes of 5SrRNA solutions with addition of different amount of various salts.

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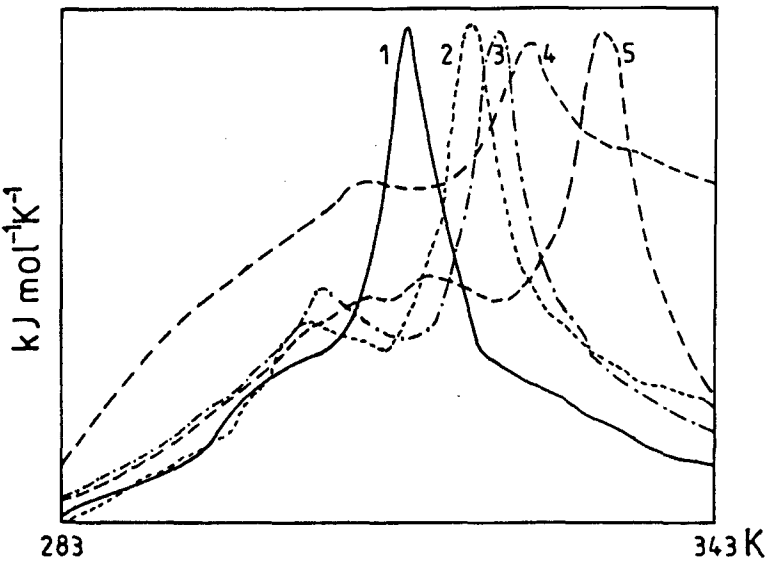

Fig. 1 DSC plots for 5SrRNA after addition of KCl: 1–0 *mM*; 2–10 *mM*; 3-20 *mM*; 4–50 *mM*; 5–100 *mM*

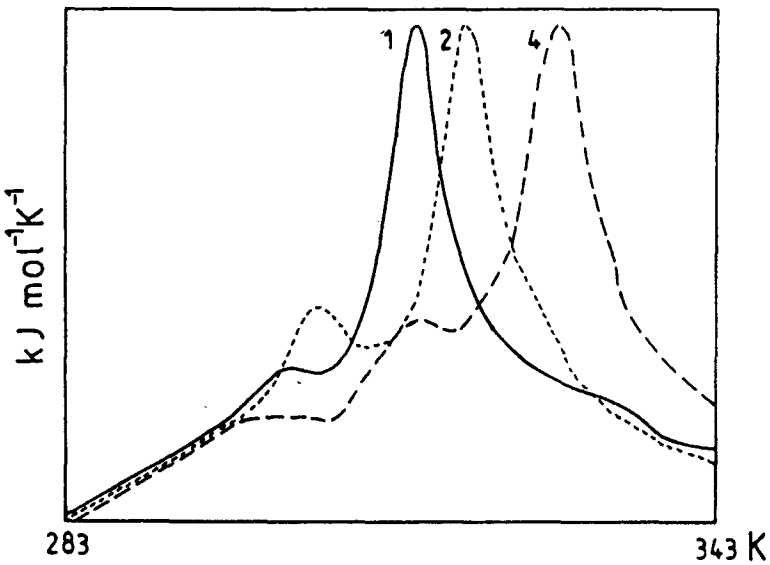

Fig. 2 DSC plots for 5SrRNA after addition of NaCl: 1–0 *mM*; 2–10 *mM*; 4–50 *mM*

In this paper we present the new experimental data obtained by calorimetric measurements for 5SrRNA from lupin seeds in presence of Na^+, K^+, Cu^{2+}, Pb^{2+}

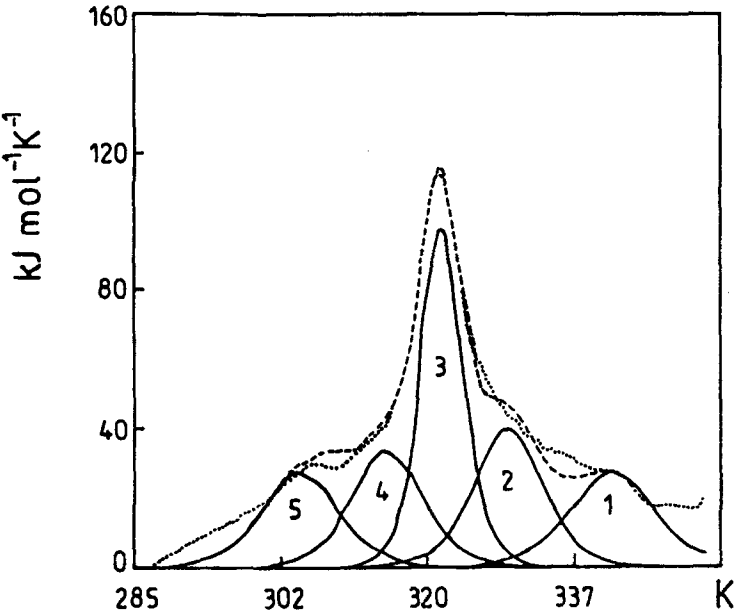

Fig. 3 Decomposition of DSC curve of 5SrRNA without addition the salts (buffer: *pH* 7.2; 10 *mM* sodium cacodylate, 1 *mM* Na_2 EDTA)

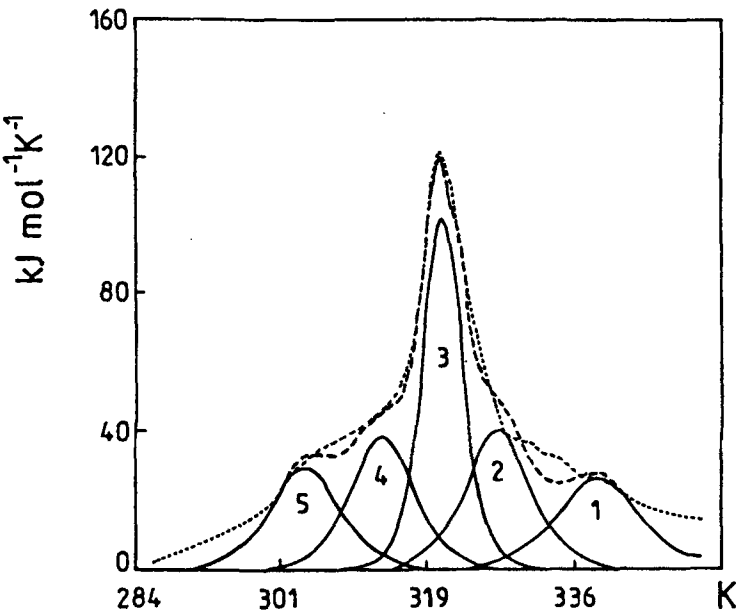

Fig. 4 Decomposition of the DSC curve of 5SrRNA without addition the salts (buffer: *pH* 7.2; 10 *mM* potassium cacodylate, 1 *mM* Na_2 EDTA)

Table 1 Decomposition of 5SrRNA melting curves into components[a]

No.	*PN*	T_m/K	ΔH / kJ·mol^{-1}	ΔG^{298} / kJ·mol^{-1}
5SrRNA				
1	5	301	283	2.82
2	4	310	327	12.66
3	3	316	570	32.47
4	2	323	371	28.72
5	1	334	311	33.52
		Total	1862	110.19
		ΔH_{exp}	1991	
5SrRNA + 10 *mM* NaCl				
6	5	298	254	0
7	4	307	383	11.23
8	3	316	405	23.07
9	2	321	572	40.98
10	1	329	392	36.94
		Total	2006	112.22
		ΔH_{exp}	2253	
5SrRNA + 50 *mM* NaCl				
11	5	301	266	2.65
12	4	314	380	19.36
13	3	324	417	33.46
14	2	329	596	56.16
15	1	337	404	46.75
		Total	2063	158.38
		ΔH_{exp}	2247	
5SrRNA				
16	5	300	298	1.99
17	4	309	350	12.46
18	3	315	586	31.63
19	2	321	374	26.80
20	1	332	311	31.85
		Total	1919	104.73
		ΔH_{exp}	2087	

Table 1 Continued

No.	*PN*	T_m/K	ΔH / kJ·mol^{-1}	ΔG^{298} / kJ·mol^{-1}
5SrRNA + 10 *mM* KCl				
21	6	300	299	1.99
22	5	308	387	12.56
23	4	316	413	23.53
24	3	321	630	45.14
25	2	328	442	40.43
26	1	338	386	45.68
		Total	2557	169.33
		ΔH_{exp}	2703	
5SrRNA + 20 *mM* KCl				
27	6	300	297	1.98
28	5	308	406	13.18
29	4	317	375	22.48
30	3	324	612	49.11
31	2	329	400	37.69
32	1	339	311	37.61
		Total	2401	162.05
		ΔH_{exp}	2526	
5SrRNA + 50 *mM* KCl				
33	4	300	1893	12.62
34	3	315	2386	128.77
35	2	329	2899	273.16
36	1	342	2752	354.06
		Total	9930	768.61
		ΔH_{exp}	9854	
5SrRNA + 100 *mM* KCl				
37	6	300	271	1.81
38	5	309	352	12.53
39	4	318	388	24.40
40	3	326	383	32.90
41	2	334	577	62.19
42	1	339	364	44.02
		Total	2335	177.85
		ΔH_{exp}	2421	

Table 1 Continued

No.	*PN*	T_m/K	ΔH / kJ·mol^{-1}	ΔG^{298} / kJ·mol^{-1}
5SrRNA + 4 *mM* $Pb(ACO)_2$				
43	2	304	1400	27.63
44	1	326	1370	117.67
		Total	2770	121.93
		ΔH_{exp}	2861	
5SrRNA + 4 *mM* $CuCl_2$				
45	7	296	275	− 1.86
46	6	305	373	8.56
47	5	313	417	19.98
48	4	321	426	30.52
49	3	328	431	39.42
50	2	336	420	47.50
51	1	345	431	58.72
		Total	2773	202.84
		ΔH_{exp}	2816	

*No, number; *PN*, peak number; T_m, peak temperature; ΔH, transition enthalpy; $\Delta G^{298} = \Delta H(T_m - 298) / T_m$, free energy of melting [13]

cations. The plot of differential adiabatic scanning calorimeter (DSC) curves, corresponding to the changes of heat power with temperature and existing peaks characterizing the melting temperatures T_m of transitions occurring in the sample are presented. It represents a unique cooperative transition of investigated system and is the superposition of the number of partial components of the two-state transitions of the unfolding subunits of the system.

Materials and methods

The 5SrRNA isolated [8, 9] from lupin seeds was dissolved in the basic buffer of *pH* 7.2 containing: a) NaCl −10 *mM* sodium cacodylate and 1 *mM* Na_2EDTA; b) KCl −10 *mM* potassium cacodylate and 1 *mM* Na_2EDTA; c) $CuCl_2$ and $Pb(ACO)_2$ −10 *mM* sodium cacodylate, 1 *mM* Na_2EDTA and 20 *mM* NaCl. The substances were kindly supported by prof. dr. M. Wiewiórowski from the Institute of Bioorganic Chemistry of the Polish Academy of Sciences.

In all the measurements the concentration of 5SrRNA corresponds to 9.13×10^{-6} *M*. The differential adiabatic scanning calorimeter DASM-4 [10] was

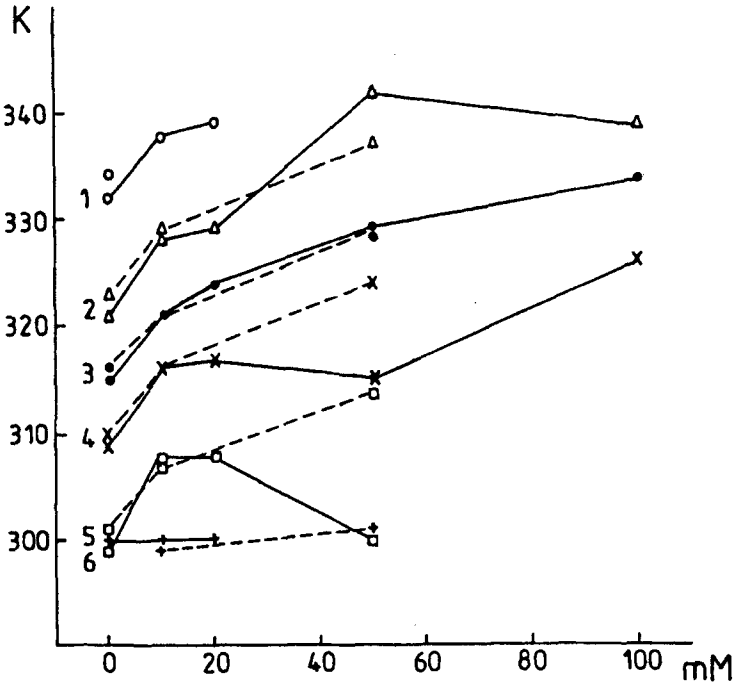

Fig. 5 The temperature of peaks characterizing the domains vs. the concentrations of KCl (—) and NaCl (- - -)

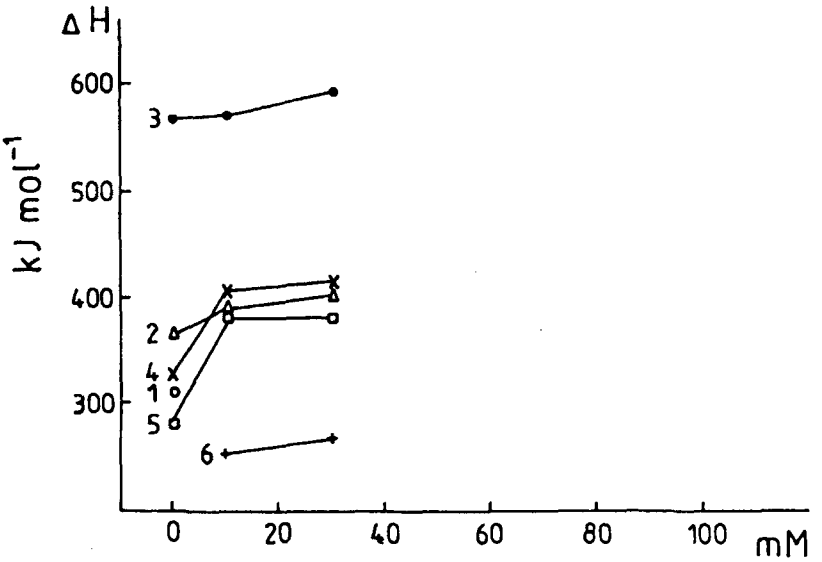

Fig. 6 The enthalpy of peaks characterizing the domains *vs* . the concentrations of NaCl

used for the measurements. The DSC curves were obtained at the scanning rate of 1 deg·min^{-1} in the temperature range 283–345 K.

Experimental data were used for the analysis of the complex unfolding process, according to a method of deconvolution proposed by Freire and Biltonen [11] and Chang [12].

Results

The calorimetric studies were carried out for a range of salts concentrations, in which it was possible to observe the changes on DSC curves. These curves are presented in Figs 1–2. In Fig. 1 the DSC curves for 5SrRNA solutions with addition of 0, 10, 20, 50, 100 *mM* NaCl are presented; in Fig. 2 the curves for 5SrRNA with addition of 0, 10, 20, 50 *mM* KCl are shown. The values of the total enthalpies (Table 1) in the buffer correspond to only 1991–2087 kJ·mol^{-1}, whereas in presence of salts: NaCl and KCl they increase to 2253–2703 kJ·mol^{-1}. For 5SrRNA+50 *mM* KCl the value of the enthalpy is much higher. In the case of addition of KCl to the buffer solution of 5SrRNA the values of the enthalpy are always higher then in the case of addition of corresponding amount of NaCl to the 5SrRNA solution.

The results of the deconvolution analysis for the chosen DSC curves are presented in Figs 3–4 and Table 1. In Table 1 the values of temperatures T_m of peaks, ΔH and ΔG^{298} of distinguished domains are given. The numbers (*PN*) correspond to the consecutively occurring transformations, with the smaller

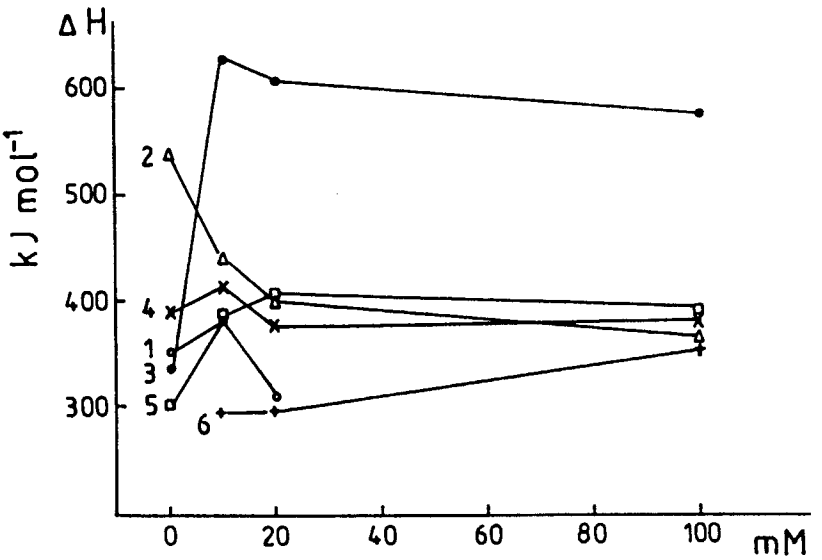

Fig. 7 The enthalpy of peaks characterizing the domains *vs.* the concentrations of KCl

number indicating the transformation of the highest temperature. The dependence of the temperature of peaks versus the concentration of salts NaCl and KCl is presented in Fig. 5. It was found that with the increase of Na^+ ions concentration the location the peak shifts towards higher temperature, whereas addition of K^+ ions causes in domains 2,3,5 firstly the increase of its temperatures with increasing concentration and then decrease; temperature of 3 domain increases monotonically with increasing of the concentration, whereas the temperature of domain 6 remains constant.

On the basis of ΔH data presented in the Table 1, it is possible to note the existence of 5–6 domains, whereas the domain of the biggest value of enthalpy appears as second or third, when the first occurs in the highest temperature.

In Figs 6, 7 the dependences of the enthalpies on the NaCl and KCl concentration are presented. In the case of NaCl (Fig. 6) the values of the enthalpies of the domains 2–6 increase with increasing concentration. The increase of K^+

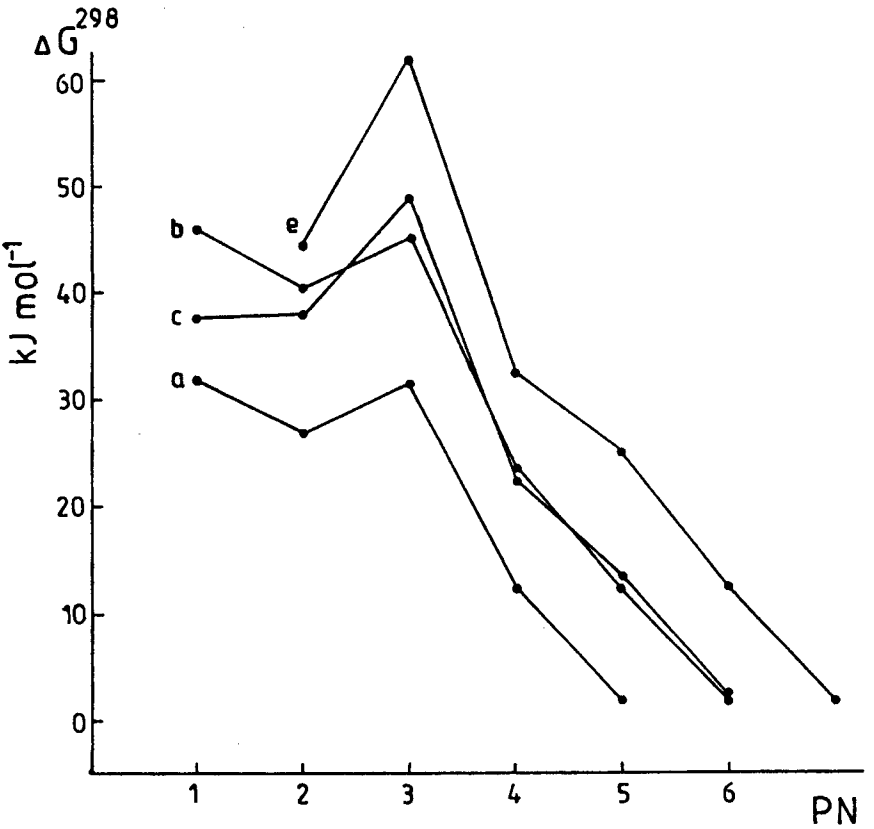

Fig. 8 The free energies of melting of the particular domains for the concentrations of KCl; a - 0 *mM*; b - 10 *mM*; c - 20 *mM*; e - 100 *mM*

ions concentration in solution (Fig. 7) causes initially the increase of the domains 1,3,4 enthalpy, reaching the maximum value at 10 *mM* KCl and then decrease. The changes of enthalpy of the third domain are the biggest. For the domain 2 the value of the enthalpy decreases in the whole range of concentration. Completely different course of DSC curves occurs for 5SrRNA with addition of Pb^{2+} and Cu^{2+} ions. The changes of the plots of DSC curves (Fig. 9) start rapidly just in the lowest temperature (10–15°C). For such changes in the course of curves several times smaller concentration of ions are sufficient. Just in the presence in the 5SrRNA solution of 4 *mM* Pb^{+2} ions the distinctive peak in temperature 304 K, occurs. It is, in comparison with the observed before (Figs 1, 2), higher than the following one in the temperature 326 K. The second one is much less distinctive. The deconvolution analysis of 5SrRNA solution with addition of Pb^{2+} enables to distinguish only two domains of similar enthalpy values (Table 1), corresponding to 1400 and 1370 $kJ \cdot mol^{-1}$, respectively.

In the case of addition of Cu^{2+} ions (Fig. 9) 5SrRNA melts in the large interval of temperatures and it is very difficult to distinguish the peaks characterizing temperatures T_m. Nevertheless the deconvolution of the DSC curve was possible and as a result 7 domains (in which 5 are of the same enthalpy value) were observed. It can be underlined, that independently of the presence of Pb^{2+} or Cu^{2+} ions in the solution the total enthalpy remains constant (about 2800 $kJ \cdot mol^{-1}$).

The experimental data presented in this paper, complied from the investigations of 5SrRNA solutions in presence of the different concentrations of various

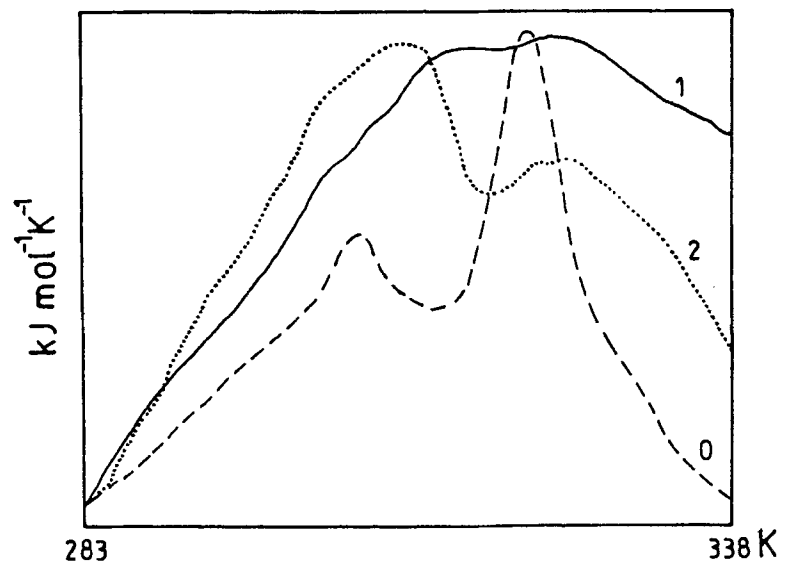

Fig. 9 DSC plots for 5SrRNA after addition: 0 - 0 *mM* Cu^{2+} and Pb^{2+}; 1 - 4 *mM* $CuCl_2$; 2 - 4 *mM* $Pb(ACO)_2$

anions, enriches undoubtly our structural interpretation of thermal folding patterns.

* * *

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Zusammenfassung — Es werden die Ergebnisse einer kalorimetrischen Untersuchung an 5SrRNA-Lösungen, isoliert aus Lupinensamen, sowohl in der Gegenwart als auch in der Abwesenheit verschiedener Konzentrationen von Na^+, K^+, Cu^{2+} und Pb^{2+} dargelegt. Unter Anwèndung der von Freire und Biltonen vorgeschlagenen Dekonvolutionsmethode wurden die grundlegenden Umwandlungen voneinander unterschieden und diskutiert.