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The conformational changes of 5SrRNA of plant origin in presence of anions PO_4^{-3}, NO_3^-, ClO_4^-, Cl^-, of tetra-protonated spermine and magnesium salts by adiabatic scanning differential calorimetry

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Abstract

The results of calorimetric studies of specific transfer, for lupin seed (L.S.) and wheat germ (W.G.) 5SrRNA with addition of different concentration of the anions PO_4^{-3}, NO_3^-, ClO_4^-, Cl^-, of tetra-protonated spermine and magnesium salts were reported. Using the deconvolution technique, the elementary transitions were distinguished and discussed. The dependences between type and concentration of anions of the salts studied were presented.

INTRODUCTION

The results of calorimetric studies of 5SrRNA water solution isolated from lupin seeds (L.S.) and wheat germs (W.G.), both in the absence of and in the presence of the salts $MgCl_2$ and tri-protonated, tetra-protonated or permethylated spermine and spermidine cations have been presented [1–3]. The subject of this study is the analysis of the influence of PO_4^{-3}, NO_3^-, ClO_4^-, Cl^- anions in magnesium and tetra-protonated spermine salts on conformational changes in 5SrRNA.

MATERIAL AND METHODS

The preparation of 5SrRNA from L.S. and W.G. and the method for carrying out calorimetric measurements were described previously [2]. The 5SrRNA was dissolved in basic buffer of pH 7.2, containing 10 mM sodium cacodylate, 1 mM Na_2 EDTA and 20 mM NaCl. The DSC curves were

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obtained at a scanning rate of 1 K min^{-1} in the temperature range 348–373 K.

Experimental data were used for the analysis of the complex unfolding process, according to a method proposed by Freire and Biltonen [4] and Chang [5]. All the compounds used, 5SrRNA from L.S. and W.G. and salts added to the solution, were synthesized at the Institute of Bioorganic Chemistry of PAS.

RESULTS

The salts $Spm^{343} \cdot 4H_3PO_4$; $Spm^{343} \cdot 4HNO_3$; $Spm^{343} \cdot 4HClO_4$; $Mg(NO_3)_2$; $Mg(ClO_4)_2$ were added at various concentrations to the solution of 5SrRNA from L.S. and W.G.

In Fig. 1 the DSC curves for 5SrRNA of L.S. with addition of 0.333 mM of the following salts $Spm^{343} \cdot 4H_3PO_4$; $Spm^{343} \cdot 4HNO_3$; $Spm^{343} \cdot 4HClO_4$ are presented. On this figure the curve for 5SrRNA with addition of $Spm^{343} \cdot 4HCl$ [3] is also presented. Two peaks on the curves can be distinguished. The first of them changes location towards higher temperatures, depending on the ion type, from PO_4^{-3}, NO_3^-, ClO_4^- to Cl^-. The changes of the second peak location are not evident. In Fig. 2 the DSC curves for 5SrRNA from L.S. with addition of the salts $Mg(NO_3)_2$ and $Mg(ClO_4)_2$ are shown. In this figure the DSC curve for 5SrRNA with addition of 2 mM $MgCl_2$ is also presented [2]. From the course of the melting curves the significant changes of location of the first and second peaks depending on the type of anion from NO_3^-, ClO_4^- to Cl^- can be noted. The sequence of changes of the location of peaks, depending on the type of anions added,

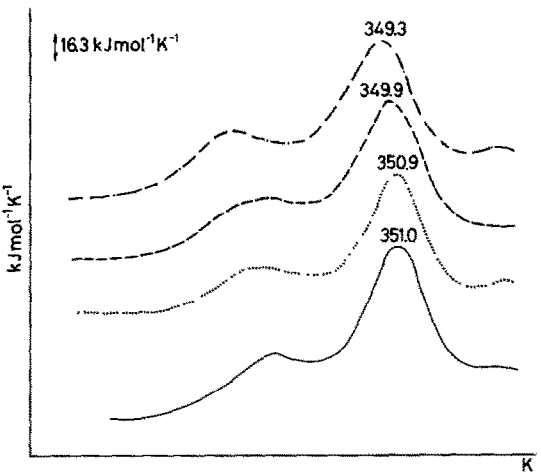

Fig. 1. DSC plots for L.S. 5SrRNA after addition of 0.333 mM of different Spm^{343} salts: ·–·–·, PO_4^{-3}; — — —, NO_3^-; ·····, ClO_4^-; ——, Cl^-.

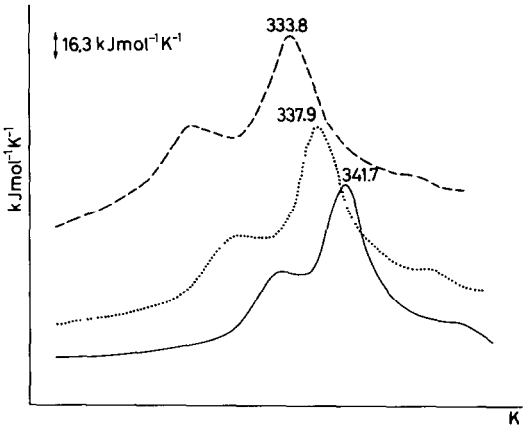

Fig. 2. DSC plots for L.S. 5SrRNA after addition of 2.0 mM of different magnesium salts: — — —, NO_3^-; ·····, ClO_4^-; ——, Cl^-.

occurs in the case of 5SrRNA from L.S. with addition of 1 mM $Mg(ClO_4)_2$ or 1 mM $MgCl_2$ (Fig. 3).

The presence of ClO_4^-, NO_3^- or Cl^- anions in 5SrRNA from W.G. solution gives another picture of the DSC curves (Fig. 4) for the case of the same solution of 5SrRNA from L.S. In this case the biggest change in location of peaks is observed in the solution containing NO_3^- anion (in the case of L.S., Cl^-). In the case studied, the peaks observed are closer to each other (Figs. 2 and 4).

The results of the deconvolution analysis of DSC curves are presented in Figs. 5–7 and Table 1. In Table 1 the temperature values of peaks and ΔH values of the domains distinguished are given. The numbers PN correspond to consecutively occurring transformations with smaller numbers referring to

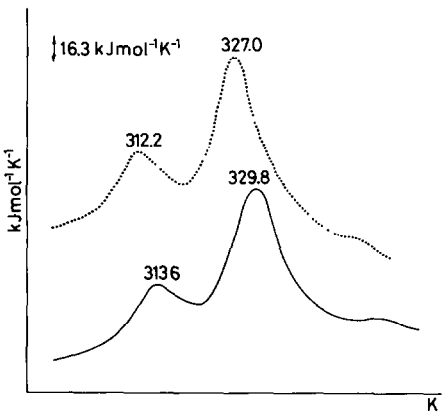

Fig. 3. DSC plots for L.S. 5SrRNA after addition of 1.0 mM of different magnesium salts: ·····, ClO_4^-; ——, Cl^-.

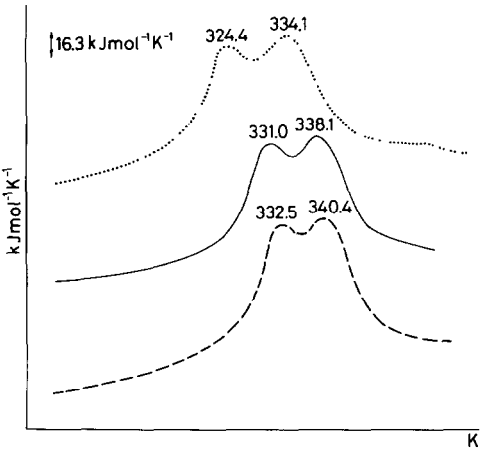

Fig. 4. DSC plots for W.G. 5SrRNA after addition of 2.0 mM of different magnesium salts: ·····, ClO_4^-; ——, Cl^-; — — —, NO_3^-.

earlier appearances of transformation. The dependence of PN vs. peak temperature characteristic for the given domain is presented in Fig. 8.

On the basis of ΔH data presented in Table 1, it is possible to detect the existence of several groups of domains, with the following mean values of enthalpies: 208 kJ mol^{-1} (numbers 10, 14, 37); 248 kJ mol^{-1} (numbers 2, 9, 33); 301 kJ mol^{-1} (numbers 1, 5, 13, 18, 21, 25, 29, 31, 36, 40); 369 kJ mol^{-1} (numbers 11, 17, 22, 23, 28); 410 kJ mol^{-1} (numbers 3, 15, 20); 452 kJ mol^{-1} (numbers 7, 19, 35, 38, 39) and 491 kJ mol^{-1} (numbers 4, 8, 12, 16, 30, 34). For the 40 domains distinguished, 35 were taken into account. As stated previously [2,3], it was noted that the domains of a given enthalpy value are present earlier in W.G. solution than in L.S. solutions.

The results obtained in this work complete the picture of the conformational changes appearing in the nucleic acids studied. A wide spectrum of salts added to the 5SrRNA solutions can be used for the general analysis [1,3]. The results can be summarized as follows.

(1) In the cases studied, heat adsorption on heating 5SrRNA from L.S. and W.G., with addition of different amounts of salts, is a process taking place over a very broad temperature range. It starts at about 288 K and ends at 370 K.

(2) All the curves with salts added are of the same shape, corresponding to the 5SrRNA curves in the basic buffer. The differences in the curves which depend on concentration and type of salts are changes in location, height and width of peaks.

(3) In all cases the increase in salt concentration shifts the peak locations towards higher temperatures.

(4) The shape of the curves is complicated and shows the multistage character of transformations occurring in 5SrRNA solutions, as confirmed

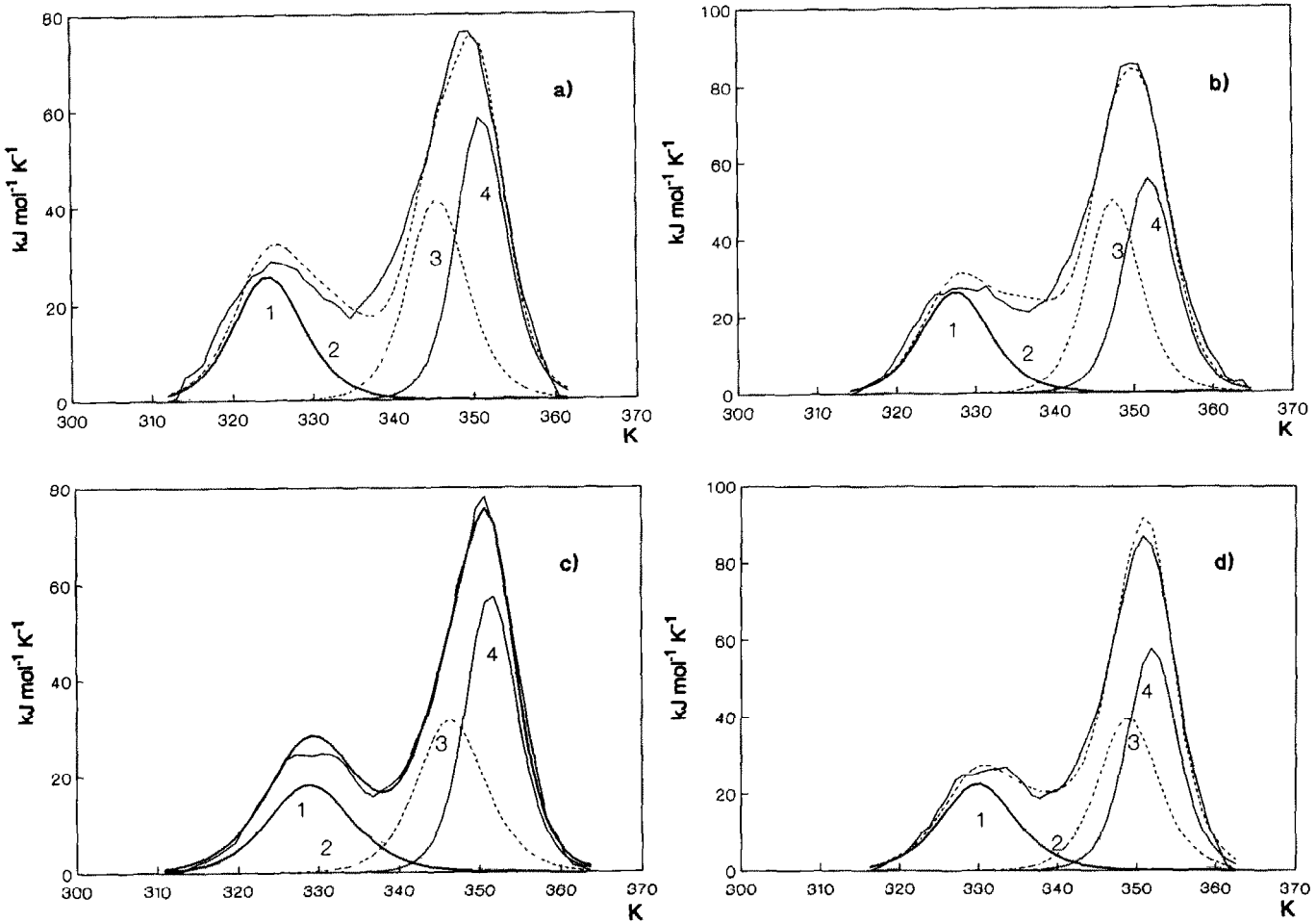

Fig. 5. Decomposition of the DSC curves of L.S. 5SrRNA for 0.333 mM of different salts: (a) $Spm^{343}\cdot 4H_3PO_4$; (b) $Spm^{343}\cdot 4HNO_3$; (c) $Spm^{343}\cdot 4HClO_4$; (d) $Spm^{343}\cdot 4HCl$.

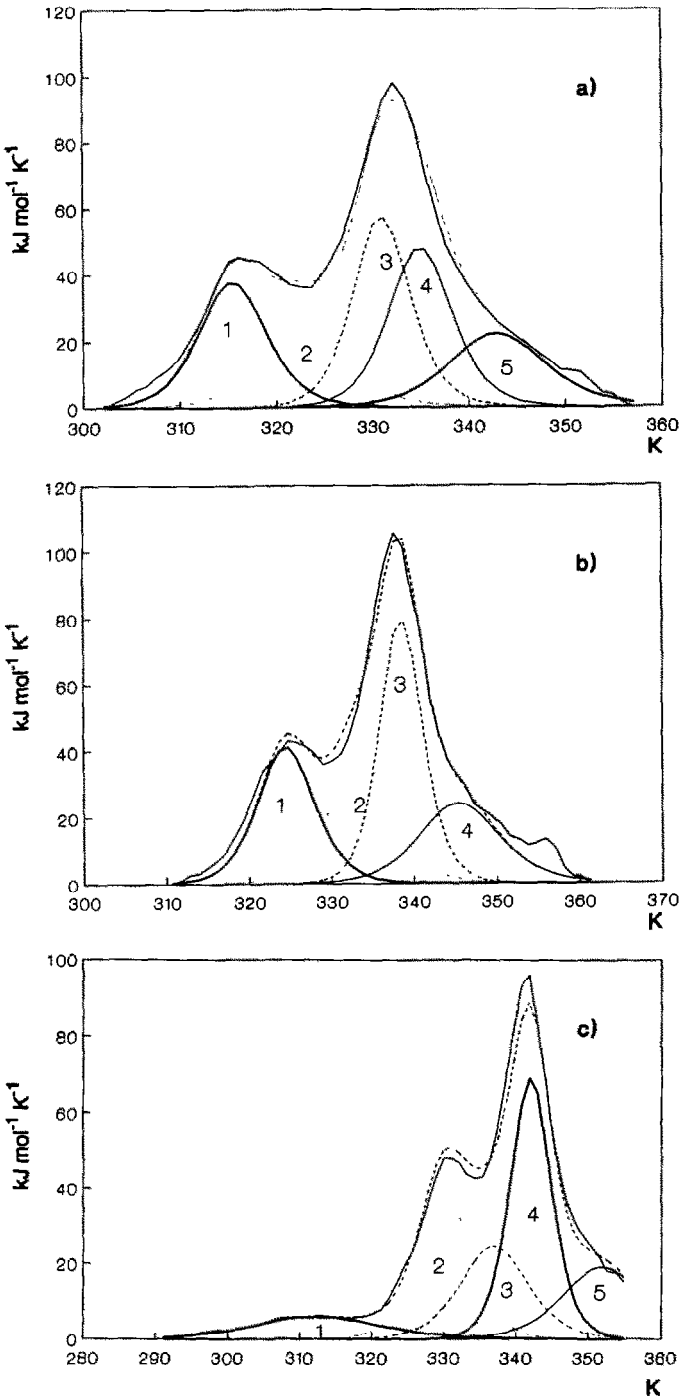

Fig. 6. Decomposition of the DSC curves of L.S. 5SrRNA for 2.0 mM of different salts: (a) $Mg(NO_3)_2$; (b) $Mg(ClO_4)_2$; (c) $MgCl_2$.

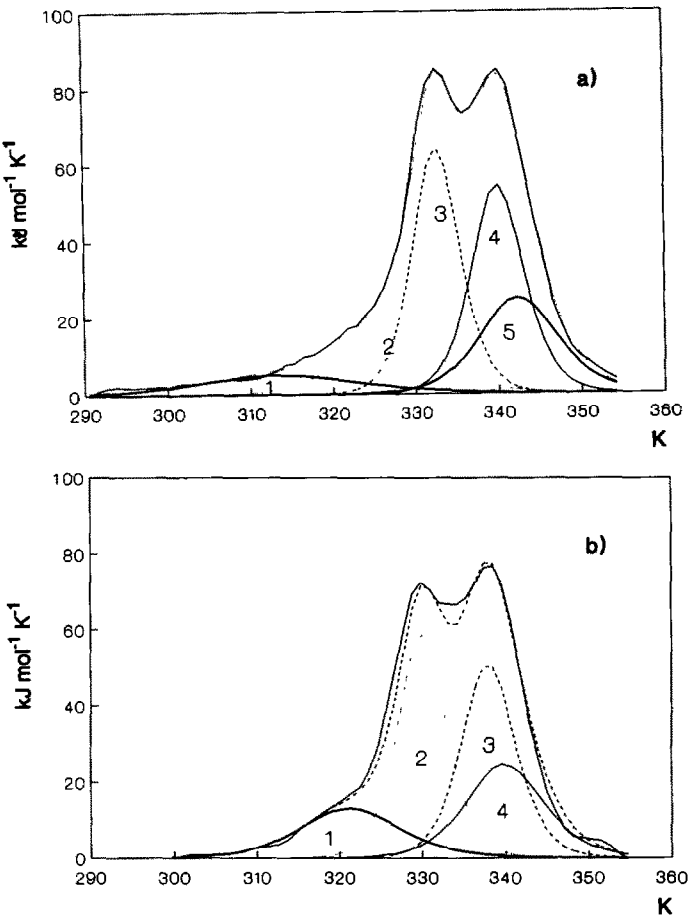

Fig. 7. Decomposition of the DSC curves of W.G. 5SrRNA for 2.0 mM of different salts: (a) $Mg(NO_3)_2$; (b) $MgCl_2$.

by deconvolution analysis, from which several independent domains are distinguished.

(5) The studies carried out in this work show that magnesium cations have the biggest influence on conformational changes. In the case of spermidine ions, this influence is small and appears in the initial stage of transformation. The results obtained will be useful in the structural interpretation of the thermal unfolding patterns for 5SrRNA from L.S. and W.G.

TABLE 1

Decomposition of 5SrRNA melting curves into components

No.	PN	T_m (K)	ΔH (kJ mol^{-1})
L.S. + 0.333 mM $Spm^{343} \cdot 4H_3PO_4$			
1	1	324	301
2	2	332	240
3	3	345	407
4	4	351	490
			Total 1438
			$\Delta H_{exp} = 1458$
L.S. + 0.333 mM $Spm^{343} \cdot 4HNO_3$			
5	1	327	306
6	2	337	267
7	3	347	449
8	4	352	478
			Total 1500
			$\Delta H_{exp} = 1529$
L.S. + 0.333 mM $Spm^{343} \cdot 4HClO_4$			
9	1	329	256
10	2	331	194
11	3	346	356
12	4	351	487
			Total 1293
			$\Delta H_{exp} = 1275$
L.S. + 0.333 mM $Spm^{343} \cdot 4HCl$			
13	1	330	285
14	2	339	218
15	3	349	401
16	4	352	489
			Total 1393
			$\Delta H_{exp} = 1375$
L.S. + 2.000 mM $Mg(NO_3)_2$			
17	1	315	354
18	2	323	309
19	3	331	457
20	4	335	424
21	5	349	294
			Total 1838
			$\Delta H_{exp} = 1873$
L.S. + 2.000 mM $Mg(ClO_4)_2$			
22	1	324	380
23	2	333	374
24	3	338	552
25	4	345	310
26	5	356	65
			Total 1622
			$\Delta H_{exp} = 1628$

No.	PN	T_m (K)	ΔH (kJ mol^{-1})
L.S. + 2.000 mM $MgCl_2$			
27	1	312	139
28	2	330	381
29	3	337	304
30	4	342	518
31	5	352	280
			Total 1622
			$\Delta H_{exp} = 1628$
W.G. + 2.000 mM $Mg(NO_3)_2$			
32	1	314	129
33	2	327	248
34	3	332	487
35	4	340	458
36	5	342	319
			Total 1641
			$\Delta H_{exp} = 1667$
W.G. + 2.000 mM $MgCl_2$			
37	1	321	212
38	2	330	460
39	3	338	439
40	4	340	307
			Total 1418
			$\Delta H_{exp} = 1446$

No., number; PN, peak number; T_m, peak temperature; ΔH, transition enthalpy.

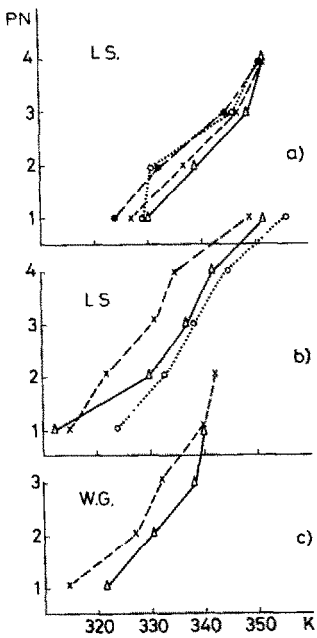

Fig. 8. Peak number versus temperature for L.S. (a, b) and W.G. (c) 5SrRNA for different salt additions: (a) ●, 0.333 mM $Spm^{343}{\cdot}4H_3PO_4$; ×, $\cdot 4HNO_3$; ○, $\cdot 4HClO_4$; △, $\cdot 4HCl$; (b) ×, 2 mM $Mg(NO_3)_2$; ○, $\cdot(ClO_4)_2$; △, $\cdot Cl$; (c) ×, 2 mM $Mg(NO_3)_2$; △, $\cdot Cl_2$.

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