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The conformational changes of 5SrRNA of plant origin in presence of sperminium and spermidinium cations 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 different concentrations of tri-protonated and tetra-protonated spermine and spermidine are reported. Decomposition of the DSC curves are made. The dependences between type and concentration of salts added to the 5SrRNA solution are discussed.

INTRODUCTION

The results of calorimetric studies of 5SrRNA solutions isolated from lupin seeds (L.S.) and wheat germ (W.G.) both in the absence and in the presence of the salts $MgCl_2$ and tetra-protonated and permethylated spermine cations have been presented [1,2]. Encouraged by the results obtained, we decided to enlarge the range of studies by analysing the influence on 5 SrRNA of various salts of biogenic amines: i.e. hydrochlorides of tetra-protonated and tri-protonated spermine Spm^{343} and Spm^{333}, and hydrochlorides of tetra-protonated and tri-protonated spermidine Spd^{34} and Spd^{33}.

MATERIALS AND METHODS

The preparation of 5SrRNA (from L.S. and W.G.) salts of spermine and spermidine has been described previously [3–5]. 5SrRNA was dissolved in 2ml of 10 mM sodium cacodylate buffer (pH 7.2) containing 1 mM Na_2EDTA and 20 mM NaCl [6] and dialysed extensively against the same

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buffer. Samples of 45 A_{260} units of 5SrRNA in 1.5 ml of buffer were usually used for the measurements (final concentration 2.9×10^{-5} M).

RESULTS

The calorimetric studies of 5SrRNA solutions isolated from L.S. and W.G. were carried out for a range of salt concentrations in which it was possible to observe the changes on DSC curves. These curves are presented in Figs. 1–8. In Fig. 1 the DSC curves for 5SrRNA of L.S. in the buffer with addition of 0.067, 0.333, 0.500 and 0.666 mM $Spm^{343} \cdot 4HCl$ are presented; in Fig. 2 the curves for 5SrRNA of W.G. with addition of 0.067 and 0.333 mM $Spm^{343} \cdot 4HCl$ are shown. With the increase of Spm^{343} cation concentration, the change of peak locations to higher temperatures takes place. Comparing the curves presented in Figs. 1 and 2, the significant differences in melting curves for the concentrations 0.067 mM and 0.333 mM can be noted. In Figs. 3 and 4 the DSC curves for 5SrRNA of L.S. with addition of 0.067, 0.134, 0.333 and 0.666 mM $Spd^{34} \cdot 3HCl$ and for 5SrRNA of W.G. with addition of 0.067 and 0.333 mM $Spd^{34} \cdot 3HCl$ are presented, respec-

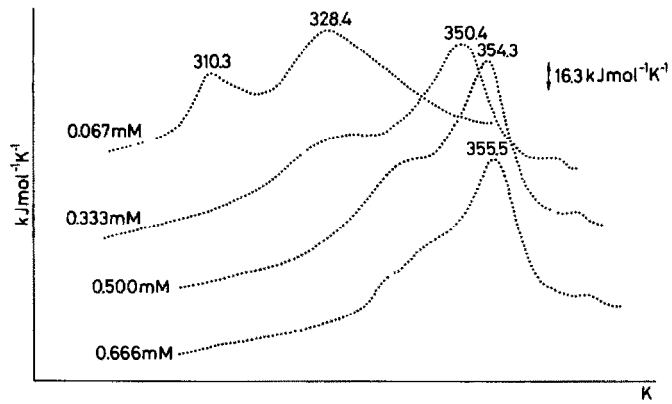

Fig. 1. DSC plots for L.S. 5SrRNA after addition of $Spm^{343} \cdot 4HCl$.

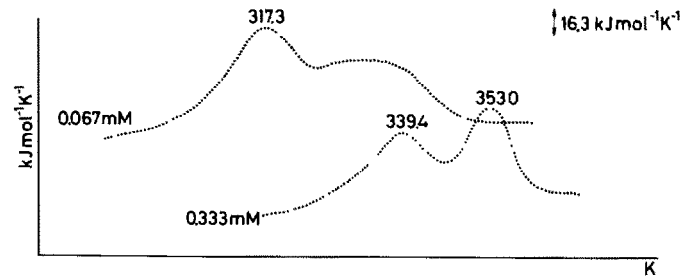

Fig. 2. DSC plots for W.G. 5SrRNA after addition of $Spm^{343} \cdot 4HCl$.

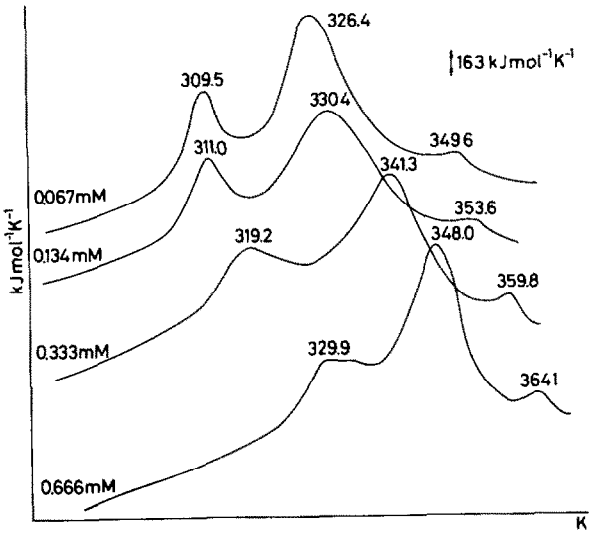

Fig. 3. DSC plots for L.S. 5SrRNA after addition of $Spd^{34}\cdot 3HCl$.

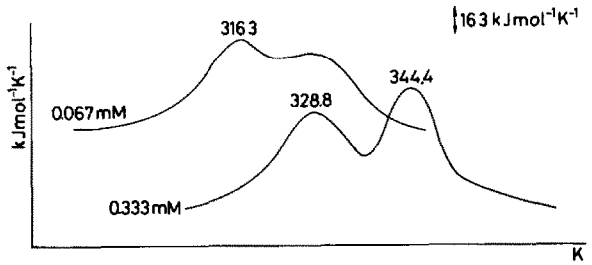

Fig. 4. DSC plots for W.G. 5SrRNA after addition of $Spd^{34}\cdot 3HCl$.

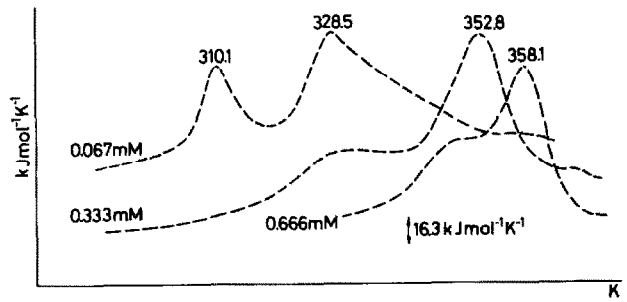

Fig. 5. DSC plots for L.S. 5SrRNA after addition of $Spm^{333}\cdot 4HCl$.

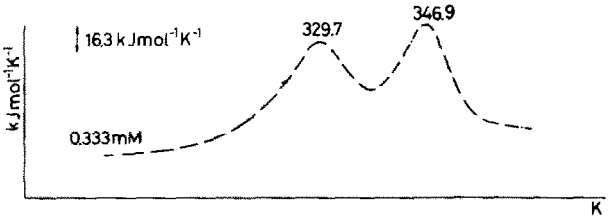

Fig. 6. DSC plots for W.G. 5SrRNA after addition of $Spd^{33}\cdot 3HCl$.

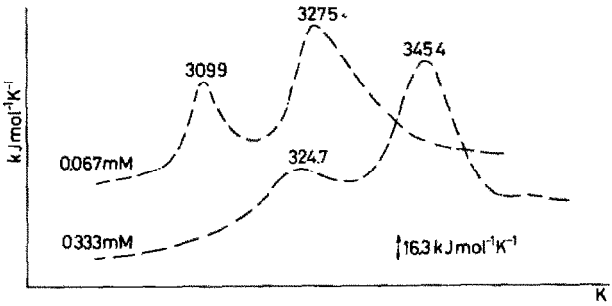

Fig. 7. DSC plots for L.S. 5SrRNA after addition of $Spd^{33}\cdot 3HCl$.

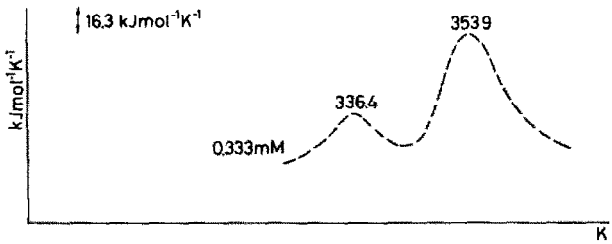

Fig. 8. DSC plots for W.G. 5SrRNA after addition of $Spm^{333}\cdot 4HCl$.

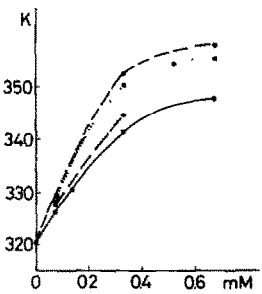

Fig. 9. Highest temperature peaks versus concentration of different salts added to the solution of L.S. 5SrRNA; — — —, Spm^{333}; · · · · · ·, Spm^{343}; ·—·—·, Spd^{33}; ———, Spd^{34}.

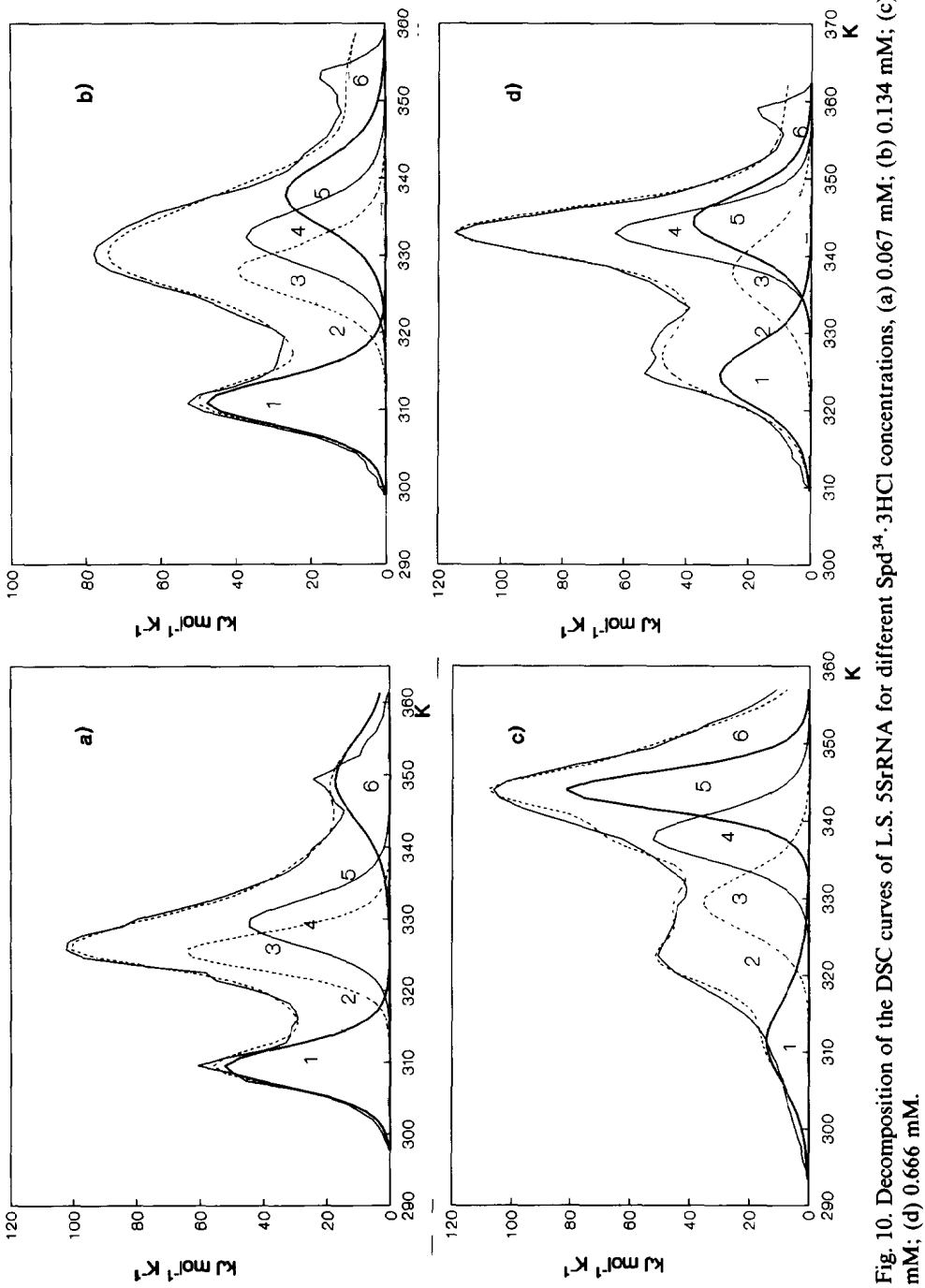

Fig. 10. Decomposition of the DSC curves of L.S. 5SrRNA for different Spd^{34}·3HCl concentrations, (a) 0.067 mM; (b) 0.134 mM; (c) 0.333 mM; (d) 0.666 mM.

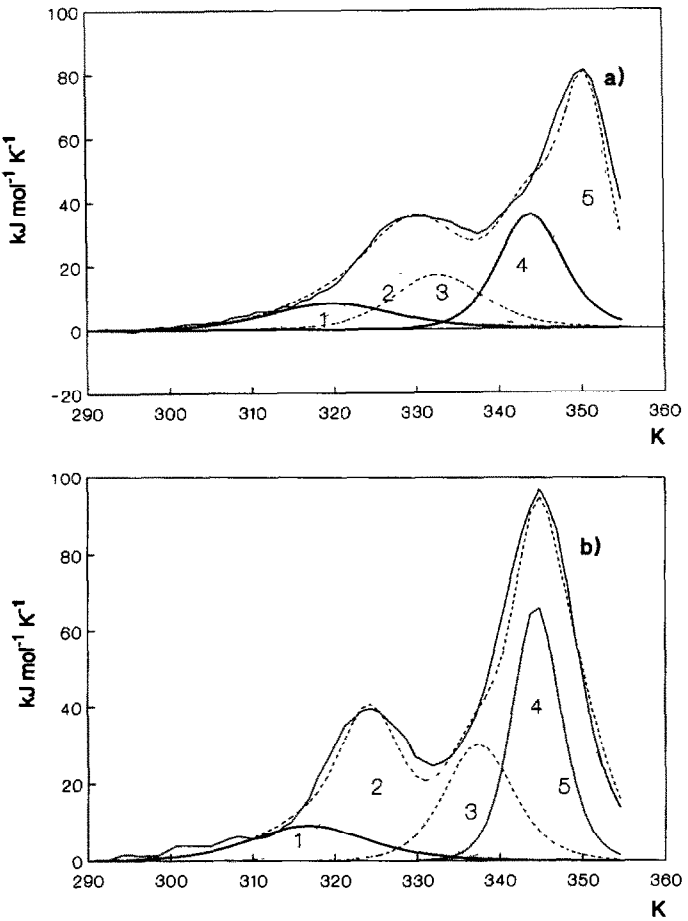

Fig. 11. Decomposition of the DSC curves of L.S. 5SrRNA for 0.333 mM of different salts, (a) $Spm^{343} \cdot 4HCl$; (b) $Spd^{33} \cdot 3HCl$.

tively. In Figs 5–8 curves for 5SrRNA of L.S. and W.G. with addition of 0.067, 0.333 and 0.666 mM $Spm^{333} \cdot 4HCl$ and $Spd^{33} \cdot 3HCl$ are given.

It was found that in all the cases studied the increase of spermine and spermidine cation concentration shifts the location of peaks towards higher temperatures. These changes are dependent on the cation type. This is graphically ilustrated in Fig. 9 which shows the dependence of the second temperature peak versus concentration of the salts added to the L.S. 5SrRNA solution.

The results of the deconvolution analysis [7,8] for the chosen DSC curves are presented in Figs. 10–14 and Table 1. In Table 1 the values of temperatures of peaks and ΔH of the distinguished domains are given. The numbers (PN) correspond to the consecutively occurring transformations, with a smaller number indicating the earlier appearance of transformation. The dependence of PN versus peak temperature characteristic for the given

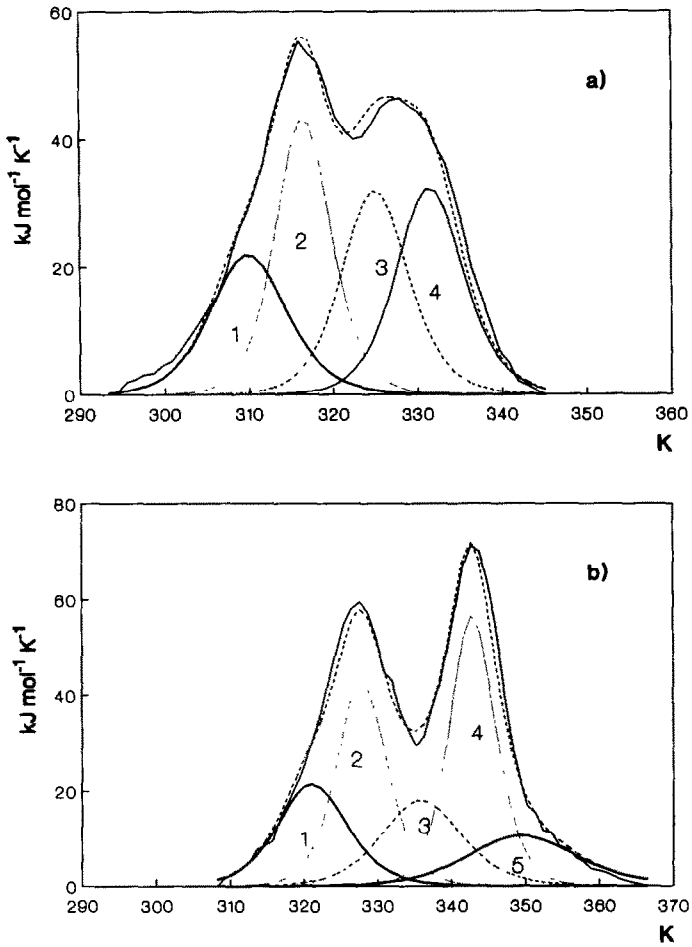

Fig. 12. Decomposition of the DSC curves of W.G. 5SrRNA for different $Spd^{34} \cdot 3HCl$ concentrations, (a) 0.067 mM; (b) 0.333 mM.

domain is presented in Fig. 15. In the case of 5SrRNA from W.G. solution with addition of 0.067 mM $Spd^{34} \cdot 3HCl$, 0.333 mM $Spd^{34} \cdot 3HCl$ and 0.333 mM $Spm^{343} \cdot 4HCl$, the dependence is linear, as in the case of 5SrRNA solution from W.G. without any addition [1]. For the other solutions examined, such linear dependences (Fig. 15) were not observed. These dependences are also not observed in the presence of Mg^{2+} ions.

On the basis of ΔH data presented in Table 1, it is possible to note the existence of five groups of domains having the following mean values of enthalpies: 188 kJ mol^{-1} (numbers 17, 29, 30, 52); 261 kJ mol^{-1} (numbers 2, 3, 11, 13, 43, 47, 49); 326 kJ mol^{-1} (numbers 6, 9, 23, 54, 56) and 483 kJ mol^{-1} (numbers 8, 27, 42, 50). Of the 56 domains distinguished 39 were taken into account. Analysing the appearance of the domains for 5SrRNA from L.S. and W.G. in the presence of the same concentrations of spermine

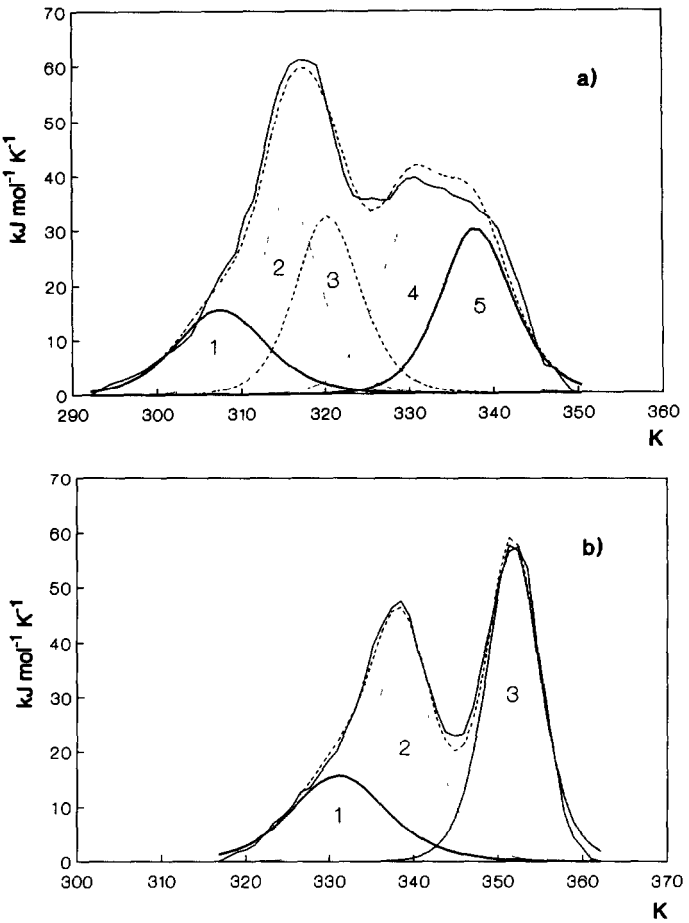

Fig. 13. Decomposition of the DSC curves of W.G. 5SrRNA for different Spm^{343}·4HCl concentrations. (a) 0.067 mM; (b) 0.333 mM.

and spermidine salts, L.S. domains appear later than W.G. domains of the same enthalpy values.

The results can be summarized as follows.

(1) Heat adsorption on heating 5SrRNA from L.S. and W.G. with addition of different amount of spermine and spermidine salts is a process taking place over a very broad temperature range (Figs. 1–8). It starts at about 288 K and ends at 375 K. All calorimetric curves are complicated, with at least two peaks, indicating the multistage nature of the process.

(2) In 5SrRNA from both L.S. and W.G., the increase of spermine and spermidine salt concentration shifts the melting curve to higher temperatures.

(3) It can be stated that there is a dependence between the salt concentration and peak temperatures (Fig. 9). In the case of 5SrRNA, the biggest

TABLE 1

Decomposition of 5SrRNA melting curves into components

No.	PN	T_m (K)	ΔH (kJ mol^{-1})
L.S. + 0.333 mM $Spm^{343} \cdot 4HCl$			
1	1	320	167
2	2	328	251
3	3	333	251
4	4	344	376
5	5	350	535
		Total	1580
		ΔH_{exp} =	1680
L.S. + 0.067 mM $Spd^{34} \cdot 3HCl$			
6	1	309	408
7	2	319	295
8	3	325	478
9	4	329	403
10	5	336	302
11	6	349	265
		Total	2151
		ΔH_{exp} =	2132
L.S. + 0.134 mM $Spd^{34} \cdot 3HCl$			
12	1	311	391
13	2	321	269
14	3	328	375
15	4	332	369
16	5	338	318
17	6	354	199
		Total	1921
		ΔH_{exp} =	1938
L.S. + 0.333 mM $Spd^{34} \cdot 3HCl$			
18	1	311	213
19	2	322	378
20	3	329	358
21	4	338	447
22	5	344	566
23	6	349	410
		Total	2372
		ΔH_{exp} =	2428
L.S. + 0.666 mM $Spd^{34} \cdot 3HCl$			
24	1	324	320
25	2	329	301
26	3	338	308
27	4	343	497
28	5	344	387
29	6	359	187
		Total	2000
		ΔH_{exp} =	1994

TABLE 1 (continued)

No.	PN	T_m (K)	ΔH (kJ mol^{-1})
L.S. + 0.333 mM Spd^{33}·3HCl			
30	1	317	199
31	2	324	346
32	3	337	338
33	4	344	512
34	5	349	385
		Total	1780
		ΔH_{exp} =	1879
W.G. + 0.067 mM Spm^{343}·4HCl			
35	1	308	220
36	2	316	346
37	3	320	333
38	4	330	337
39	5	338	338
		Total	1574
		ΔH_{exp} =	1570
W.G. + 0.333 mM Spm^{343}·4HCl			
40	1	331	239
41	2	338	385
42	3	352	489
		Total	1113
		ΔH_{exp} =	1107
W.G. + 0.067 mM Spd^{34}·3HCl			
43	1	310	264
44	2	316	380
45	3	325	336
46	4	331	345
		Total	1325
		ΔH_{exp} =	1340
W.G. + 0.333 mM Spd^{34}·3HCl			
47	1	321	270
48	2	328	392
49	3	336	260
50	4	342	470
51	5	350	206
		Total	1598
		ΔH_{exp} =	1599
W.G. + 0.333 Spd^{33}·3HCl			
52	1	315	168
53	2	324	328
54	3	330	402
55	4	343	352
56	5	346	399
		Total	1649
		ΔH_{exp} =	1681

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

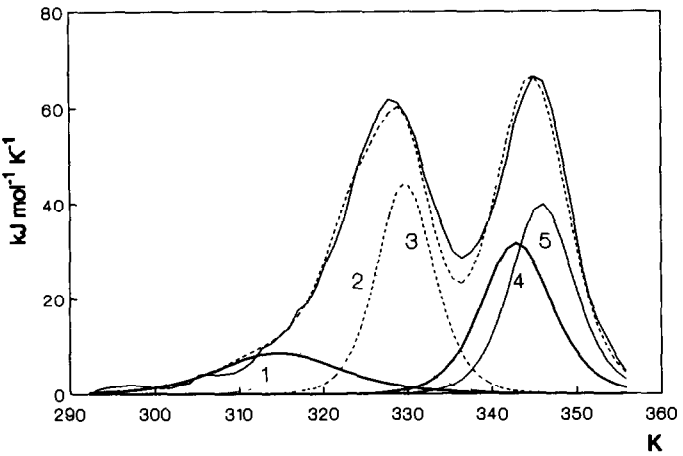

Fig. 14. Decomposition of the DSC curves of W.G. 5SrRNA for 0.333 mM $Spd^{33} \cdot 3HCl$.

change of peak localization occurs for $Spm^{333} \cdot 3HCl$, being smaller for $Spm^{343} \cdot 4HCl$ and for $Spd^{33} \cdot 3HCl$. The smallest influence on the change of peak position occurs with addition of $Spd^{34} \cdot 3HCl$. These changes occur even when very low salt concentrations are added to the solutions. For the concentration 0.067 mM significant changes of melting curves can be

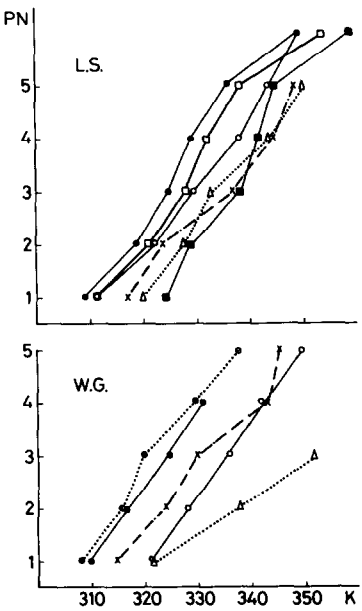

Fig. 15. Peak number versus temperature for L.S. and W.G. 5SrRNA for different salt concentrations, △, 0.333 mM $Spm^{343} \cdot 4HCl$; ●, 0.067 mM $Spd^{34} \cdot 3HCl$; □, 0.134 mM $Spd^{34} \cdot 3HCl$; ○, 0.333 mM $Spd^{34} \cdot 3HCl$; ■, 0.666 mM $Spd^{34} \cdot 3HCl$; ×, 0.333 mM $Spd^{33} \cdot 3HCl$; ⊙, 0.067 mM $Spm^{343} \cdot 4HCl$.

observed relative to the melting curve without salt addition. The curve illustrating the changes of localization of peak temperatures in relation to the salts concentration is exponential. As shown in Fig. 9 the "saturation" of solution by the salts takes place for all salts starting from ≈ 0.6 mM concentration. It has to be noted that this concentration is about ten times lower than for the case of "saturation" of solutions by Mg^{2+} ions [1]. These dramatic changes in the influence of spermine salts on the melting curve can be illustrated by the fact that in the case of 0–0.67 mM concentrations of $MgCl_2$ the changes on the melting curve are negligible.

(4) The deconvolution analysis of results in Table 1 shows that there exists a certain number of domains which can be treated as corresponding to the elementary transformations which always occur. It was noted that the number of domains distinguished in the case of spermine and spermidine salts does not change or changes only slightly.

The results of the studies presented will be useful for structural interpretation of the thermal unfolding patterns for 5SrRNA from lupin seeds and wheat germ.

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