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THE CONFORMATIONAL CHANGES OF 5SrRNA FROM LUPIN SEEDS IN PRESENCE OF ClO_4^-, NO_3^-, Br^-, BF_4^-, SO_4^{2-}, Cl^-, COO^- ANIONS BY ADIABATIC SCANNING CALORIMETRY

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Abstract

The results of calorimetric studies of 5SrRNA solutions isolated from lupin seeds in the presence of the ClO_4^-, NO_3^-, Br^-, BF_4^-, SO_4^{2-}, Cl^-, COO^- (maleic and fumaric acids) anions were reported. The plots of calorimetric curves, enthalpy of conformational changes of two state transitions were presented. Using the deconvolution method proposed by Freire and Biltonen the elementary transitions were distinguished and discussed.

Keywords: conformational changes of 5SrRNA, lupin seeds, scanning adiabatic calorimetry

Introduction

The adiabatic scanning calorimetry is a convenient method for studies of conformational changes occurring in proteins and nucleic acids in the conditions of temperature changes and presence of various ions. So far, it was applied by us for investigations of conformational changes of 5SrRNA solutions from lupin seeds and wheat germs in presence of tetra-protonated spermine, spremidine, magnesium salts [1], Na^+, K^+, Cu^{2+}, Pb^{2+} cations [2]. Structural interpretation of the unfolding patterns for lupin seeds and wheat germ 5SrRNA was proposed [3]. The observed conformational changes in the presence of relatively low concentrations of anions (for example: 2 *mM* $MgCl_2$; 0.33 *mM* spermine salt) were so interesting, that it seemed to be useful to enlarge our investigations by studies of conformational changes of 5SrRNA solutions from lupin seeds in the presence of much higher anion concentrations and by additional investigations of the role of Br^-, BF_4^-, SO_4^{2-}, Cl^-, COO^- anions.

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Materials and methods

The 5SrRNA isolated from lupin seeds was dissolved in the basic buffer of *pH* 7.2 containing 10 *mM* sodium cacodylate and 1 *mM* Na_2EDTA. The solu-

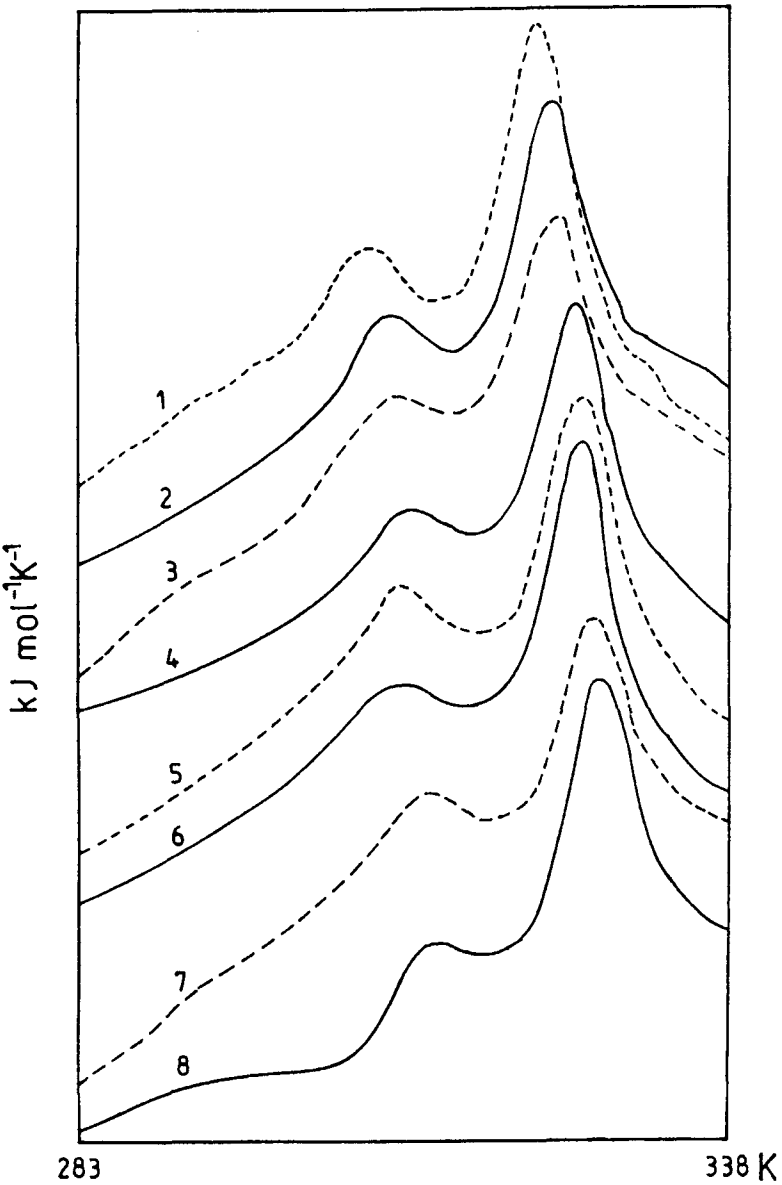

Fig. 1 DSC plots for 5SrRNA after addition of 20 *mM*: 1-NO_3^- ; 2-ClO_4^- ; 3-Br^-; 4-BF_4^- ; 5-SO_4^{2-} ; 6-Cl^-; 7-COO^- (maleic acid); 8-COO^- (fumaric acid)

tions of 5SrRNA in buffer were studied in presence of 20 m*M* of: $NaNO_3$, $NaClO_4$, NaBr, $NaBF_4$, Na_2SO_4, NH_4Cl, the mixtures of maleic and fumaric acids with NaOH (for keeping the *pH* at 7.2). In all the measurements the concentration of 5SrRNA solutions correspond to $9.13\times10^{-6}M$. The substances were kindly supported by Wiewiórowski from the Institute of Bioorganic Chemistry of the Polish Academy of Sciences.

The differential adiabatic scanning calorimeter DASM-4 [4] was used for the measurements. The DSC curves were obtained at the scanning rate of 1 deg·min^{-1}. Experimental data were used for the analysis of the complex unfolding process, according to a method of deconvolution proposed by Freire and Biltonem [5] and Chang [6].

Results

In the Fig. 1 the plot of DSC curves for 5SrRNA solutions with addition of 20 *mM* of salts are presented. The addition of various salts differently shift the localization of peaks towards higher temperatures.

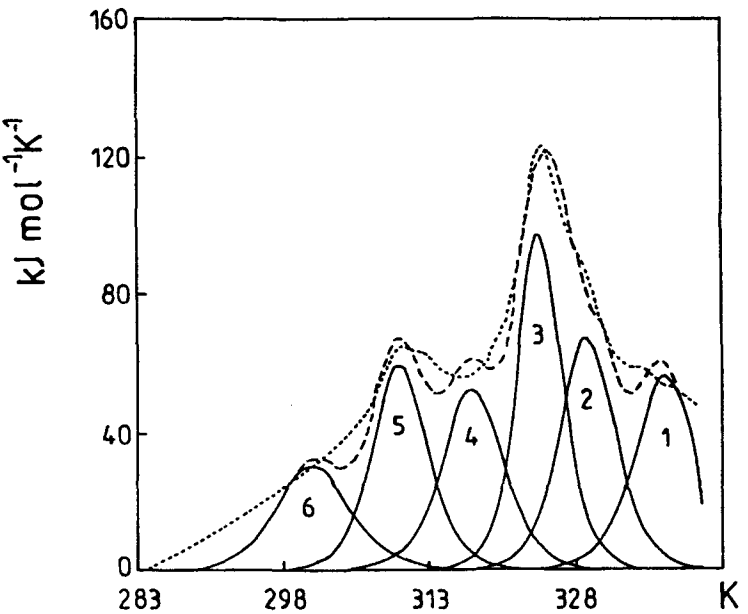

Fig. 2 DSC deconvoluted curves of 5SrRNA after addition of NO_3^-

The results of the deconvolution analysis for the DSC curves are presented in Figs 2–6 and Table 1. In the Table 1 the values of temperature T_m of peaks, ΔH and ΔG^{298} of distinguished domains are given. The numbers (*PN*) corre-

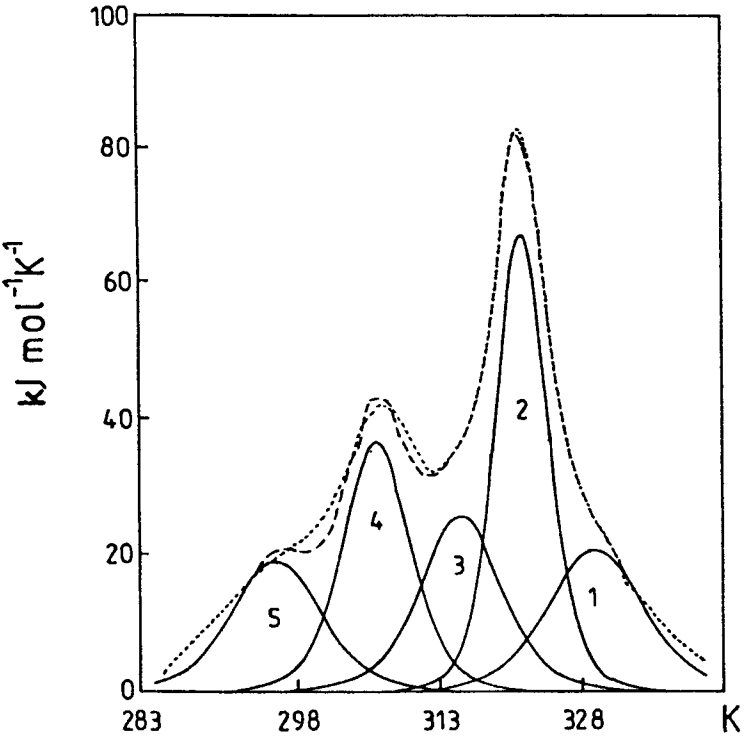

Fig. 3 DSC deconvoluted curves of 5SrRNA after addition of ClO_4^-

spond to the consecutively occurring transformations, with the smallest numbers indicating the transformation at highest temperatures. The number of distinguished domains varies from 2 to 6 (Table 1, Figs 2–9). The addition of 20 *mM* of $NaNO_3$ causes the displacement of the highest peak towards the higher temperatures (peak No 9, Table 1) by only 6 K relative to respective peak on DSC plot of 5SrRNA in the buffer solution. This is the smallest change in peak position in all studied solutions with anion additives. The biggest change, of about 12 K is present after addition of 20 *mM* fumaric acid. Other anions cause the change in peak localization from 6 K to 12 K. It is noteworthy that notable differences, especially in the initial course of DSC plots, occur in solutions with additions of maleic and fumaric acids, whereas the T_m temperatures of the highest peaks (peaks No 39 and 43) are practically the same. The dependence of peaks numbers *vs.* temperature for different anions addition show the similar character of changes except the dependencies observed for maleic acid and NaBr. The same can be observed for the total enthalpy which for solutions with addition of maleic acid and NaBr are 3960 kJ·mol^{-1} and

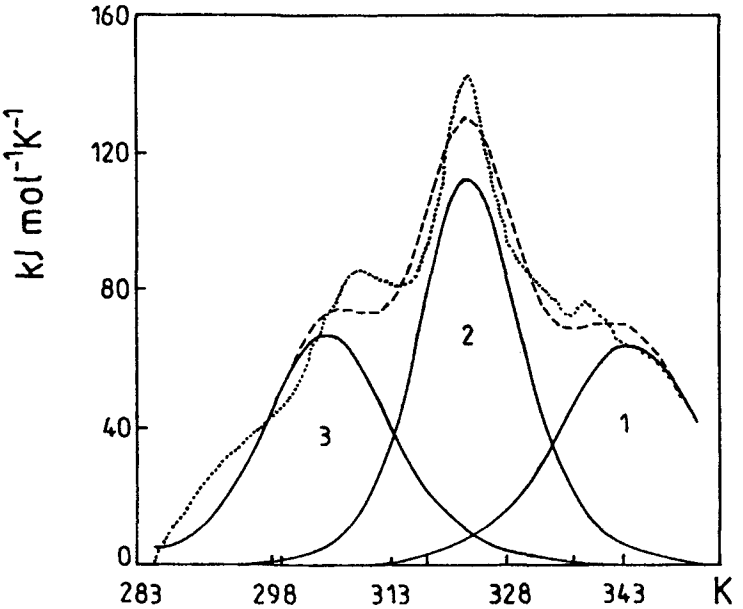

Fig. 4 DSC deconvoluted curves of 5SrRNA after addition of Br^-

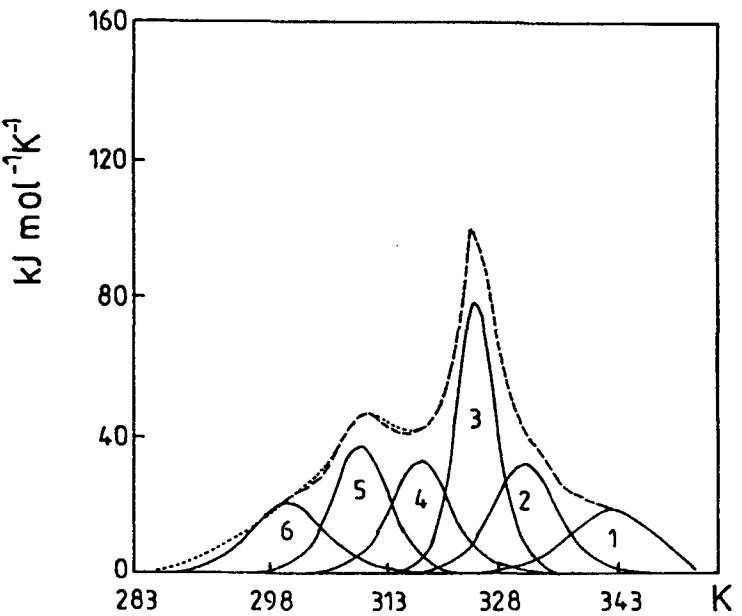

Fig. 5 DSC deconvoluted curves of 5SrRNA after addition of BF_4^-

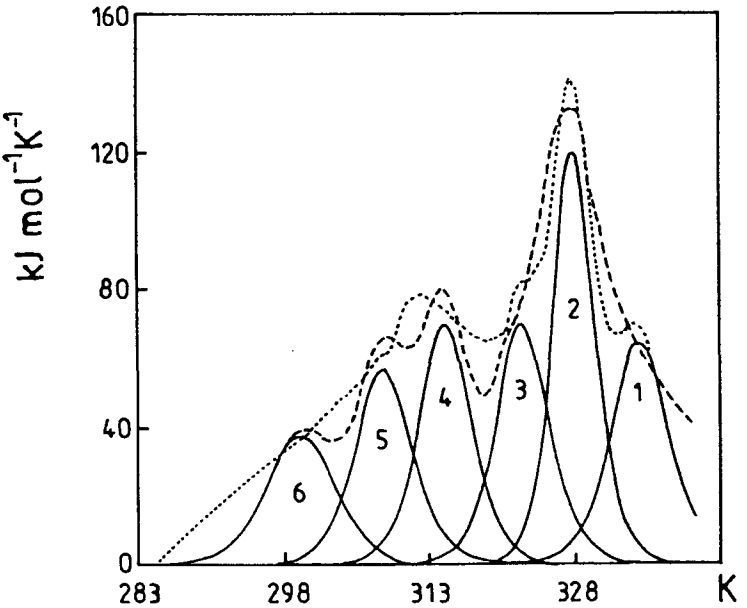

Fig. 6 DSC deconvoluted curves of 5SrRNA after addition of SO_4^{2-}

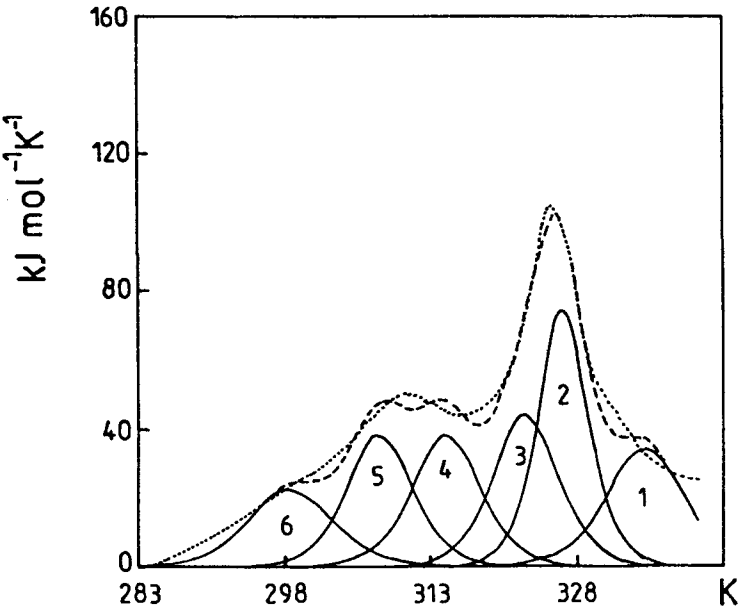

Fig. 7 DSC deconvoluted curves of 5SrRNA after addition of Cl^-

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+NO_3^-				
6	6	299	306	1.02
7	5	308	437	14.19
8	4	316	421	23.98
9	3	322	586	43.68
10	2	327	492	43.63
11	1	335	460	50.81
		Total	2702	177.31
		ΔH_{exp}	2812	
5SrRNA+ClO_4^-				
12	5	296	238	–1.61
13	4	307	340	9.97
14	3	316	294	16.75
15	2	322	483	36.00
16	1	330	277	26.86
		Total	1632	87.97
		ΔH_{exp}	1676	
5SrRNA+Br^-				
17	3	306	1453	37.99
18	2	324	1995	160.09
19	1	345	1598	217.70
		Total	5046	415.78
		ΔH_{exp}	4832	
5SrRNA+BF_4^-				
20	6	301	246	2.45
21	5	310	347	13.43
22	4	318	334	21.01
23	3	325	529	43.95

Table 1 Continued

No	PN	T_m/K	ΔH / kJ·mol^{-1}	ΔG^{298} / kJ·mol^{-1}
24	2	331	341	34.00
25	1	343	270	35.42
		Total	2067	150.26
		ΔH_{exp}	2098	
5SrRNA+SO_4^{2-}				
26	6	298	336	0
27	5	306	424	11.08
28	4	313	481	23.05
29	3	321	494	35.40
30	2	326	658	56.52
31	1	332	492	50.38
		Total	2885	176.43
		ΔH_{exp}	3184	
5SrRNA+Cl^-				
32	6	298	256	0
33	5	307	348	10.20
34	4	314	354	18.04
35	3	323	392	30.34
36	2	327	516	45.76
37	1	335	359	39.65
		Total	2225	143.99
		ΔH_{exp}	2278	
5SrRNA+COO^-	(maleic acid)			
38	2	309	1643	58.49
39	1	328	2225	203.51
		Total	3868	262.00
		ΔH_{exp}	3960	
5SrRNA+COO^-	(fumaric acid)			
40	5	297	206	–0.69
41	4	313	388	18.59
42	3	322	409	30.48
43	2	328	587	53.69
44	1	335	468	51.69
		Total	2058	153.76
		ΔH_{exp}	2119	

[a]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 [7]

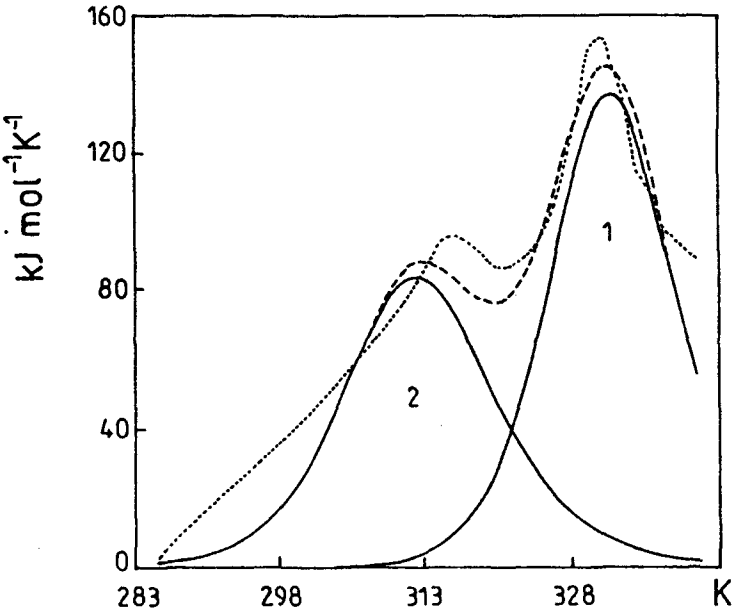

Fig. 8 DSC deconvoluted curves of 5SrRNA after addition of COO^- (maleic acid)

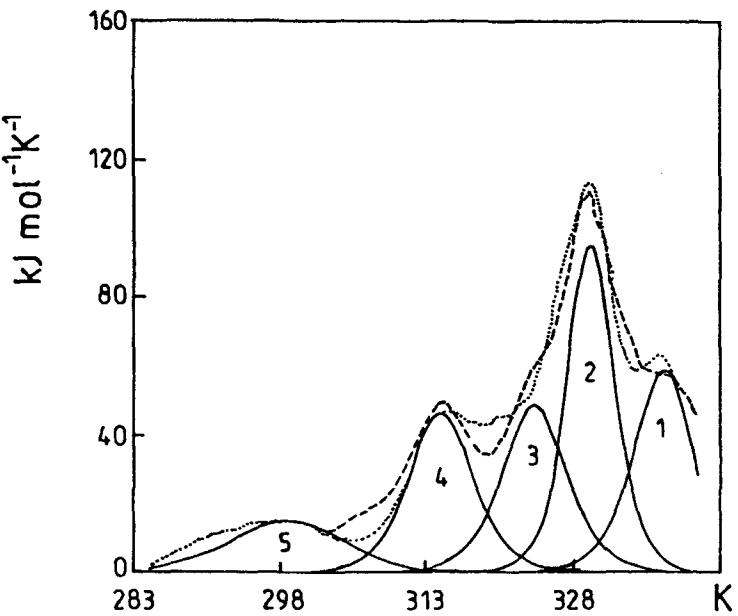

Fig. 9 DSC deconvoluted curves of 5SrRNA after addition of COO^- (fumaric acid)

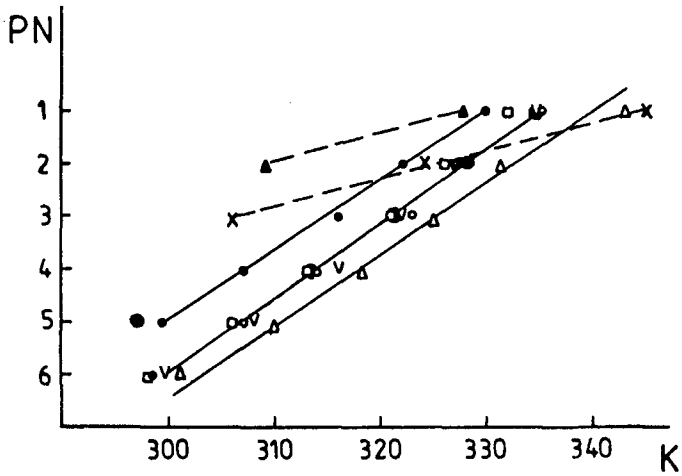

Fig. 10 Peak number *vs.* temperature for different anion additions: • ClO_4^- ; x Br^-; o Cl^-; Δ BF_4^- ; v NO_3^- ; □ SO_4^{2-} ; ▲ COO^- (maleic acid); ● COO^- (fumaric acid)

4832 kJ·mol^{-1} respectively, and are higher than the other values ranging form 1676 kJ·mol^{-1} to 3184 kJ·mol^{-1}.

The conclusions drawn here are only preliminary, they do however enrich our knowledge concerning different effector effects on the conformational changes of 5SrRNA and help to better understand structural unfolding patters.

* * *

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References

1 A. Zielenkiewicz, M. Żółkiewski, W. Zielenkiewicz and M. Wiewiórowski, Thermochim. Acta, 182 (1991) 165.
2 A. Zielenkiewicz, J. Thermal Anal., in press.
3 T. Kuliński, M. D. Bratek-Wiewiórowska, M. Wiewiórowski, A. Zielenkiewicz, M. Żółkiewski and W. Zielenkiewicz, Nucleic Acids Res., 19 (1991) 2449.
4 P. L. Privalov and S. A. Potekhin, in 'Methods in Enzymology', Vol. 131, Enzyme Structure, p. 4–51, Ed. C.H.W. Hirs, S. L. Timashef, Acad Press. Inc. New York, 1986.
5 E. Freire and R. L. Biltonen, Biopolymers, 17 (1978) 463.
6 L. H. Chang, S.-J. Li, T. L. Ricca and A. G. Marshall, Anal. Chem., 56 (1984) 1502.
7 S.-J. Li and A. G. Marshall, Biochemistry, 24 (1985) 4047.

Zusammenfassung — Es werden die Ergebnisse einer kalorimetrischen Untersuchung an 5SrRNA-Lösungen, isoliert aus Lupinensamen, in der Gegenwart von ClO_4^-, NO_3^-, Br^-, BF_4^-, SO_4^{2-}, Cl^- und COO^- (Maleinsäure- und Fumarsäure-) Anionen dargelegt. Gezeigt werden die kalorimetrischen Kurven und die Enthalpie für Konformationsänderungen von zwei Zustandsumwandlungen. Unter Anwendung der von Freire und Biltonen vorgeschlagenen Dekonvolutionsmethode wurden die grundlegenden Umwandlungen voneinander unterschieden und diskutiert.