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THE CONFORMATIONAL CHANGES OF 5*S*rRNA FROM LUPIN SEEDS AND *t*RNA^{Phe} IN PRESENCE OF Ca^{2+}, Mn^{2+} CATIONS BY DSC METHOD

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Abstract

The results of calorimetric studies of 5*S*rRNA isolated from Lupinus luteus and of *t*RNA^{Phe} both in the absence and in the presence of different concentrations of cations Ca^{2+}, Mn^{2+} were reported. The temperature and the enthalpy of melting were determined. Using the deconvolution method the elementary transitions were distinguished and discussed.

Keywords: adiabatic scanning calorimetry, conformational changes, 5*S*rRNA, *t*RNA^{Phe}

Introduction

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

The studies of dynamic conformation of 5*S*rRNA in presence of different ions in conditions of temperature changes and at various ionic strengths of the solution may lead to better understanding of structural propensity encoded in their sequences. As it is well known, scanning adiabatic differential calorimetry DASM is convenient method for this kind of study. So far, adiabatic scanning differential calorimetry was applied by us for experimental investigations of conformational changes of 5*S*rRNA solutions from lupin seeds and wheat germs without and with addition of different amount of various anions: PO_4^{3-}, NO_3^-, ClO_4^-, Cl^-, Br^-, BF_4^-, SO_4^{2-}, COO^- [5, 6] and cations: Spm^{4+} (spermine), Spd^{3+} (spermidine), Mg^{2+}, Na^+, K^+, Cu^{2+}, Pb^{2+} [7–11]. Structural interpretation of thermal unfolding patterns for lupin seeds and wheat germs was proposed [7, 10,12].

Encouraged by the results obtained we decided to enlarge the range of studies by analyzing the influence on 5*S*rRNA and *t*RNA^{Phe} of various concentrations of Ca^{2+}, Mn^{2+} cations.

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Experimental

The samples of 5*S*rRNA from lupin seeds and tRNA^{Phe} were kindly supported by Prof. Dr. M. Wiewiórowski, Institute of Bioorganic Chemistry of the Polish Academy of Sciences. These were dissolved in a basic buffer of pH 7.2 containing 10 mM sodium cacodylate, 1 mM Na_2EDTA and 20 mM NaCl. In all the measurements the concentration of 5*S*rRNA corresponds to $9.13 \cdot 10^{-6}$ M whereas the concentration of tRNA^{Phe} was equal to $1.47 \cdot 10^{-5}$ M. The cations Ca^{2+}, Mn^{2+} from $CaCl_2$ and $MnCl_2$ salts of concentrations 2, 4 and 8 mM were used. Differential adiabatic scanning microcalorimeter DASM-4 [13] of 0.47 ml volume cell was applied for the measurements. Calorimetric recordings were usually started around 283 K and continued at the rate of 1 K min^{-1} up to 348 K or 358 K, depending on the character of the observed phenomena. Experimental data were used for the analysis of the complex unfolding process. The plot of DSC curve presents the dependence of volumetric specific heat capacity on the temperature; existing peaks characterize the melting temperatures T_m occurring in the sample. The region below DSC curve corresponds to the total enthalpy $\Delta_m H$ of melting of the structure. It represents a unique cooperative transition of investigated system and its superposition of the number of components of two-state transitions of the unfolding subunits of the system. The DSC curves were decomposed into particular phase transitions characterized by appropriate contribution of the enthalpy and transition temperatures according to a method of deconvolution proposed by Freire and Biltonen [14] and Chang [15].

Results

In Figs 1, 2, 6, 7 the experimentally determined DSC plots of 5*S*rRNA and tRNA^{Phe} solutions were presented. In Tables 1, 2 the values of temperatures T_m of peaks, enthalpy ΔH, and free energy of melting ΔG^{298} of the distinguished domains are reported. The numbers (*PN*) correspond to the consecutively occurring

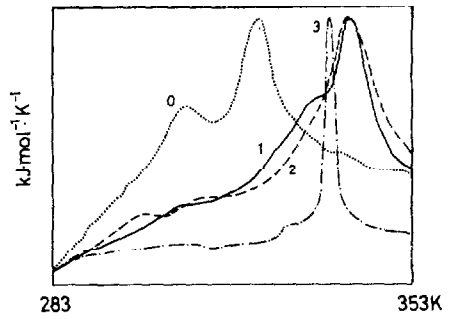

Fig. 1 DSC plots for 5*S*rRNA after addition of $CaCl_2$; 0 – 0 mM; 1 – 2 mM, 2 – 4 mM; 3 – 8 mM

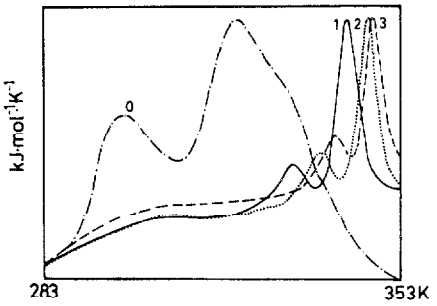

Fig. 2 DSC plots for tRNA^{Phe} after addition of $CaCl_2$; 0 – 0 mM; 1 – 2 mM, 2 – 4 mM; 3 – 8 mM

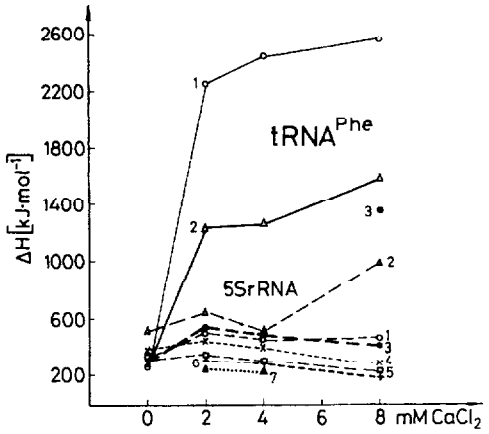

Fig. 3 The enthalpy of peaks characterizing the domains: o – 1; Δ – 2; • – 3; × – 4; □ – 5; + – 6; ▲ – 7 *vs.* the concentration of $CaCl_2$ for 5*Sr*RNA (- - -) and tRNA^{Phe} (—)

transformations, with a smaller number indicating the earlier appearance of transformation. The dependence of *PN vs.* peak characteristic for a given domain is presented in Figs 4, 5.

In Figs 1, 2 the DSC curves for 5*Sr*RNA and tRNA^{Phe} solutions in the basic buffer alone and with the addition of 2, 4, 8 mM $CaCl_2$ are presented. The addition of various concentration of Ca^{2+} differently shifts the localisation of peaks towards the temperature. On the curves without the salts two peaks are present, in the case of 5*Sr*RNA they are more intensive and appear at higher temperatures than in the case of tRNA^{Phe}. The addition of 2 mM Ca^{2+} ions shifts these peaks towards higher temperatures of about 18 K (Fig. 1, curve 1) in 5*Sr*RNA case and of about 22 K (Fig. 2, curve 1) in the case of tRNA^{Phe}. The increase of concentration of Ca^{2+} cations up to 4 and 8 mM, results in shifting the temperature co-ordinates of the peaks towards lower temperature values for 5*Sr*RNA and higher tempera-

ture values for tRNA^{Phe}. In the transition of 5SrRNA without the addition of Ca^{2+} cations, five domains can be distinguished (Table 1). In the transitions obtained at higher concentrations of Ca^{2+} the greater number of domains can be distinguished: 7 domains in the case of 2 and 4 mM $CaCl_2$ solutions; 6 domains in the case of 8 mM $CaCl_2$. In the case of tRNA^{Phe} transition without Ca^{2+} cations we obtain 5 domains, whereas when the cations are present, we obtain less domains (2 or 3) – see Table 1. The addition of Ca^{2+} cations to 5SrRNA as well as to tRNA^{Phe} solutions induces the increase of a total value of enthalpy of transition ΔH_{exp} in regards to the enthalpy of transition, when the cations are absent. The dependence of enthalpy of particular domains on concentration of Ca^{2+} cations is shown on Fig. 3. When the Ca^{2+} cations are present the values of enthalpy of domains are much higher in the case of tRNA^{Phe} than in the case of 5SrRNA. It is a consequence of the fact, that in the whole melting process of tRNA^{Phe} less domains have been distinguished (2 or 3).

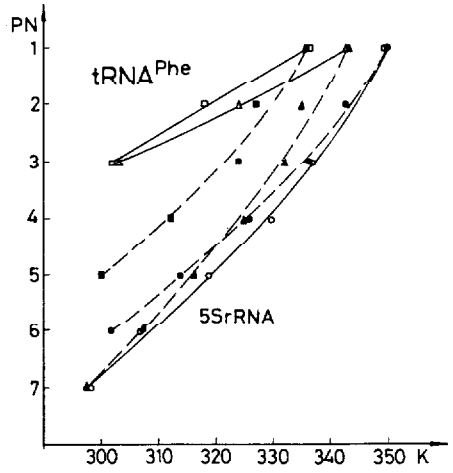

Fig. 4 Peak number *vs.* temperature for 5SrRNA (- - -) and tRNA^{Phe} (—) for different $MnCl_2$ concentration: •o – 2 mM; ▲Δ – 4 mM; ■□ – 8 mM

Apart from the differences between the enthalpy of domain values, some similarities for tRNA^{Phe} and 5SrRNA may be noticed. For example domains 1 and 2 are characterized by a violent increase of the enthalpy value, while the concentration of $CaCl_2$ is changed from 0 to 2 mM. These domains appear in the final stage of the transition at the highest temperatures (Fig. 5). At these temperatures the melting of the most stable structures of 5SrRNA and tRNA^{Phe} occurs. The change of free energy of melting ($\Delta G^{298} = \Delta H(T_m - 298)/T_m$) is also, for these domains, most visible (Table 1).

In Figs 6 and 7 the DSC plots for 5SrRNA and tRNA^{Phe} in the presence of 0, 2, 4 and 8 mM $MnCl_2$ are shown. The addition of 2 mM $MnCl_2$ induces the melting of 5SrRNA in the wide range of temperature (Fig. 6, curve 1), while the addi-

Table 1 Decomposition of 5*Sr*RNA and *t*RNA^{Phe} melting curves in the presence of Ca^{2+} [a]

No.	*PN*	T_m/K		ΔH/kJ mol^{-1}		ΔG^{298}/kJ mol^{-1}	
		5*Sr*RNA	*t*RNA^{Phe}	5*Sr*RNA	*t*RNA^{Phe}	5*Sr*RNA	*t*RNA^{Phe}
0 mM $CaCl_2$							
1	5	297	297	301	200	–1.01	–0.67
2	4	306	314	389	306	10.17	15.59
3	3	313	322	340	298	16.29	22.21
4	2	321	332	541	265	38.76	27.14
5	1	327	344	352	262	31.22	35.02
			Total	1923	1331	95.43	99.30
			ΔH_{exp}	2016	1265[b]		
2 mM $CaCl_2$							
6	7	301		260		2.59	
7	6	311		324		13.54	
8	5	321		380		27.23	
9	4	329		469		44.19	
10	3	336		538		60.85	
11	2	342	317	662	1243	85.17	74.50
12	1	349	343	510	2260	74.53	296.50
			Total	3143	3503	308.10	331.00
			ΔH_{exp}	3345	3651		
4 mM $CaCl_2$							
13	7	299		258		0.86	
14	6	310		300		11.61	
15	5	321		332		23.79	
16	4	331		409		40.78	
17	3	338		496		58.70	
18	2	343	317	545	1295	71.50	77.62
19	1	350	346	475	2442	70.57	338.77
			Total	2815	3737	277.81	416.39
			ΔH_{exp}	2863	4116		

Table 1 Continued

No.	*PN*	T_m/K		ΔH/kJ mol^{-1}		ΔG^{298}/kJ mol^{-1}	
		5*Sr*RNA	*t*RNA^{Phe}	5*Sr*RNA	*t*RNA^{Phe}	5*Sr*RNA	*t*RNA^{Phe}
8 mM $CaCl_2$							
20	6	296		223		-1.51	
21	5	307		255		7.48	
22	4	320		284		19.53	
23	3	330	306	421	1360	40.82	35.56
24	2	338	331	1006	1589	119.05	158.42
25	1	345	349	487	2584	66.34	377.60
			Total	2676	5533	251.71	571.58
			ΔH_{exp}	2571	5928		

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 [17]
b) 1213 kJ mol^{-1} Privalov and Filimonov [16]

tion of the same amount of $MnCl_2$ to the *t*RNA^{Phe} solution results in obtaining sharp featured melting curve (Fig. 7, curve 1). In both cases, the increase of the concentration of Mn^{2+} cations to 4 and 8 mM results in shifting of the melting curve peaks towards lower temperatures. The phenomenon of the peak shifting towards lower temperature values, was observed only in few cases: in the presence of 4 and 8 mM $CaCl_2$ (5*Sr*RNA) – Fig. 1; in the presence of 4 and 8 mM $MnCl_2$ (5*Sr*RNA) and in the presence of 4 and 8 mM $MnCl_2$ (*t*RNA^{Phe}). In all

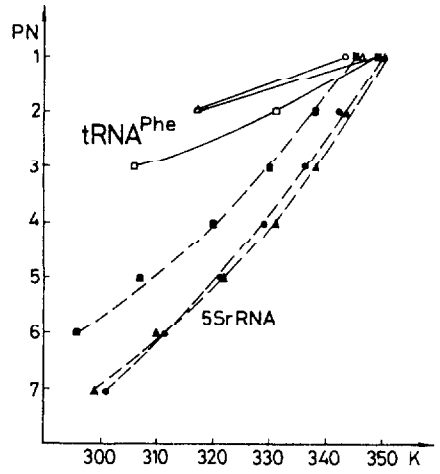

Fig. 5 Peak number *vs.* temperature for 5*Sr*RNA (- - - -) and *t*RNA^{Phe} (—) for different $CaCl_2$ concentration: •○ – 2 mM; ▲△ – 4 mM; ■□ – 8 mM

other cases, including investigations of 5*S*rRNA in the presence of magnesium cations [15, 16], sperminium and spermidium cations [16, 17], sodium and potasium cations [18], increasing of the salt concentration results in shifting the position of peaks towards higher temperature values.

In Table 2 the results of decomposition of 5*S*rRNA and tRNA^{Phe} melting curves in the presence of 2, 4 and 8 mM $MnCl_2$ are contained. In the case of 5*S*rRNA 5÷7 domains are distinguished, whereas in the case of tRNA^{Phe} – 3÷7 domains. The increase of $MnCl_2$ concentration in the case of 5*S*rRNA does not induce considerable changes of the enthalpy values of particular domains (Fig. 8), with one exception of domain 2. Its enthalpy value at concentration 4 mM $MnCl_2$ is quite high. On the other hand, in the case of tRNA^{Phe} the large increase of enthalpy of domains is observed, particularly when the concentration of $MnCl_2$ is changed from 2 to 4 mM. Although the changes of values of the domain enthalpy are similar in the case of presence of Mn^{2+} cations (Fig. 8) and Ca^{2+} cations (Fig. 3), there is no simple dependence found on their temperature values (Fig. 5 and Fig. 4). For example, the temperatures of domain 2 in the case of tRNA^{Phe} have a tendency to decrease with the growing $MnCl_2$ concentration

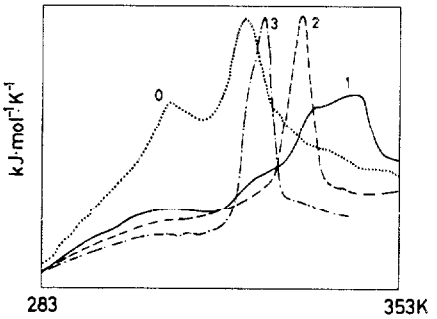

Fig. 6 DSC plots for 5*S*rRNA after addition of $MnCl_2$: 0 – 0 mM; 1 – 2 mM, 2 – 4 mM; 3 – 8 mM

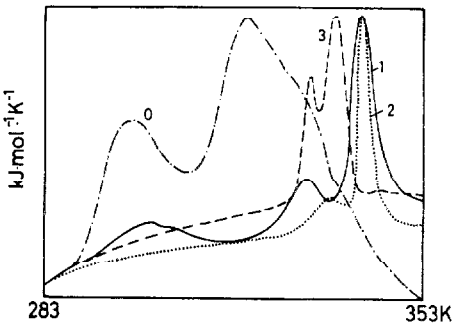

Fig. 7 DSC plots for tRNA^{Phe} after addition of $MnCl_2$: 0 – 0 mM; 1 – 2 mM, 2 – 4 mM; 3 – 8 mM

Table 2 Decomposition of 5*Sr*RNA and *t*RNA^{Phe} melting curves in the presence of Mn^{2+} a)

No.	*PN*	T_m/K		ΔH/kJ mol^{-1}		ΔG^{298}/kJ mol^{-1}	
		5*Sr*RNA	*t*RNA^{Phe}	5*Sr*RNA	*t*RNA^{Phe}	5*Sr*RNA	*t*RNA^{Phe}
2 mM $MnCl_2$							
1	7		298		290		0
2	6	302	307	269	310	3.56	9.09
3	5	314	319	289	295	14.73	19.42
4	4	326	330	386	403	33.15	39.08
5	3	336	337	491	356	55.53	41.20
6	2	343	343	522	725	68.48	95.12
7	1	350	350	448	471	66.56	69.98
			Total	2405	2850	242.01	273.89
			ΔH_{exp}	2559	2912		
4 mM $MnCl_2$							
8	7	297		284		−0.96	
9	6	307		344		10.08	
10	5	316		372		21.19	
11	4	325		440		36.55	
12	3	332	303	571	961	58.48	15.86
13	2	335	324	760	1232	83.94	98.86
14	1	343	343	528	2539	69.27	333.10
			Total	3299	4732	278.55	447.82
			ΔH_{exp}	3447	4425		
8 mM $MnCl_2$							
15	5	300		246		1.64	
16	4	312		280		12.56	
17	3	324	302	479	933	38.44	12.36
18	2	327	318	677	1240	60.04	77.90
19	1	336	337	423	2728	47.84	315.70
			Total	2105	4901	160.52	406.05
			ΔH_{exp}	2146	4629		

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 [17]

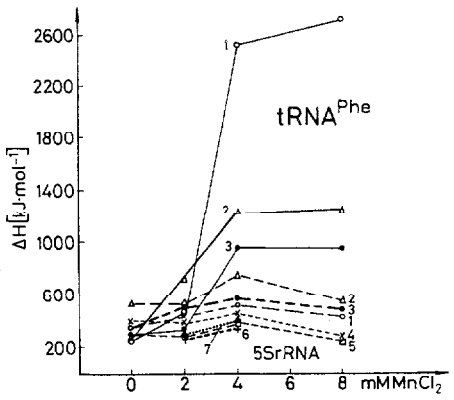

Fig. 8 The enthalpy of peaks characterizing the domains: o – 1; Δ – 2; • – 3; × – 4; □– 5 ; ı – 6; ▲ – 7 *vs.* the concentration of $MnCl_2$ for 5*S*rRNA (- - -) and $tRNA^{Phe}$ (—)

and increase with the concentration of $CaCl_2$. In the case of 5*S*rRNA the temperatures of distinguished domains are decreasing or increasing with the increasing concentration of $MnCl_2$ and $CaCl_2$, depending on *PN* number of domain (obtained curves of *PN vs.* temperature *T* have a crossing point).

Conclusions

Summarizing, we can confirm the following: DSC plots are diverse, depending on the melting process of individual elements of molecules of 5*S*rRNA and $tRNA^{Phe}$. The cations of Ca^{2+} and Mn^{2+} added in a small amount (2 mM) usually stabilise a structure, increasing temperatures of melting. The comparison of obtained DSC plots leads us to a statement, that in the presence of studied cations the transitions in 5*S*rRNA solutions occur at temperature range 292÷355 K, while in the case of $tRNA^{Phe}$ – usually, at temperatures few degrees lower, confirming that the melting process of $tRNA^{Phe}$ is running faster. The applied method of decomposing whole melting process (in the range of temperatures studied) to the individual elements usually enables to distinguish 5÷7 domains in the case of 5*S*rRNA. In the case of $tRNA^{Phe}$ the number of distinguished domains is smaller (2÷3). The choice of decomposing to more or less domains has an influence on the calculated values of enthalpy domain enthalpy, usually much higher in the case of $tRNA^{Phe}$ than for 5*S*rRNA. It also confirms, that in $tRNA^{Phe}$ the individual transitions occur in a wider range of temperatures. The enthalpies of individual domains have similar values. However, the collected experimental results can be used for the interpretation of stability of 5*S*rRNA and $tRNA^{Phe}$ in the presence of double charged cations, in particular, if there would be possible to attach individual distinguished domains to adequate molecule fragments, melting as a result of

increasing temperature. Such attempts have been undertaken by: Privalov and Filimonov [16], who studied the influence of Mg^{2+} ions by means of calorimetric method; Barciszewski *et al.* [7]; Kuliński *et al.* [12] and Li *et al.* [17], who investigated melting of 5*S*rRNA in the presence of sodium or magnesium cations and spermine.

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