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PROVIDING EXTRA-SENSITIVE TO TEMPERATURE TRANSITIONS IN BIOLOGICAL MACROMOLECULES IN THE CONTEXT OF ENTROPY-DRIVEN UNFOLDING PROCESSES

The two-configuration approximation based on the thermodynamic approach to describing conformational transitions in physiologically significant biological macromolecules, e.g. some specific RNA molecules known as RNA-thermometers, is used to derive the consistent equation for determining the thermodynamic probability of the entropy-driven unfolded process which is extra-sensitive to ambient temperature. We show that with the given accuracy of the involved parameters this equation coincides with the equation obtained using the microscopic approach. We then compare the derived equation with experimental data and finally discuss and conclude the results obtained.

K e y w o r d s: temperature sensitivity, entropy-driven processes, biological macromolecules.

1. Introduction

It is well known that a closed dynamical (mechanical) system tends toward its equilibrium due to the spontaneous processes taking place within it. As a result, the potential energy of a system must decrease reaching its minimum and the system's equilibration process is enthalpy-driven. On the other hand, if a system exchanges matter or energy with its surrounding medium, then the system is open (e.g. a biological system). In this case, the system's entropy comes into play in such a way that it increases up to reaching its maximum. Therefore, in this case the system's equilibration process is entropy-driven. Moreover, from the thermodynamic point of view, the enthalpy-driven process provides a system with an equilibrium state whose population does not increase with temperature, whereas the entropy-driven process provides a system with an equilibrium state population of which increases with temperature. Sometimes the latter phenomenon is very pronounced so that one can even speak in this case of an entropy-driven temperature extra-sensitivity of a system.

A schematic example illustrating the entropy-driven extra-sensitive to temperature transitions between the unfolded configuration and the folded configuration of mRNA molecule is shown in Fig. 1. Notably, it is precisely these mRNA molecules of specific bacterial microorganisms that have a unique property: they are able to extraordinarily sharply regulate their translation initiation rate with a change in ambient temperature. Such a property of those mRNAs – therefore called RNA thermometers, – was identified as being completely due to their specific structural and sequence based features. It has been established that the most important stage in the thermosensing process is the melting of the mRNA's ribosome-

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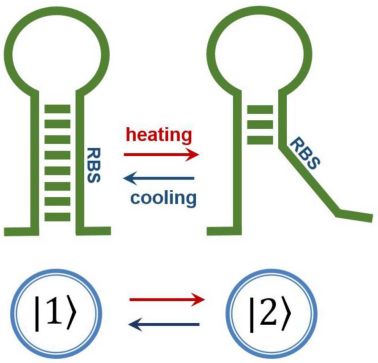

Fig. 1. A schematic representation of RNA structural elements undergoing folding-unfolding transitions that mask or unmask the ribosome binding site (RBS) in response to temperature change, shown as a blue (cold) or red (hot) arrow (thermometer), that can be associated with states $|1\rangle$ or $|2\rangle$ in Eq. (1), respectively.

binding site [1–3] by initiating the unfolding process as depicted in Fig. 1. However, there is a lack of understanding of the background physical mechanism that underlies this melting phenomenon, as well as of the extent to which various approaches developed to date for the transitions in mRNA molecules can be regarded as common or uncommon from a thermodynamic viewpoint concerning even the simplest two-configuration approximation of Fig. 1.

In this work we use the two-configuration approximation to derive the consistent equation for determining the thermodynamic probability of an entropy-driven unfolded process. Then we coincide this equation with that obtained in the microscopic two-state approach, compare these equations with the experimental data and finally discuss and conclude by summarizing the results obtained.

2. Thermodynamic Probability of an Entropy-Driven Unfolded Configuration

In order to analyze different possibilities for the description of experimental data on the unfolding of RNA molecules, let us start with a simple but general two-state model for the probabilities of RNA being found in distinct structural states $|j = 1\rangle$ and $|j = 2\rangle$ defined with respect to the bases involved in base pairing. Let us associate state $|1\rangle$ with a stem-like structure, in which all bases are paired, so adopting for the thermosensing region a structure that sequesters the ribosome binding site (RBS) of a gene. On the contrary, state $|2\rangle$ is associated with the open RNA structure, in which some specific remaining acid-base residues are unpaired (for instance, a guanine residue of the repressor of heat-shock gene expression at position 83 paired opposite the Shine–Dalgarno sequence in the hairpin structure is shown to play a pivotal role in the ability of RNA to change its expression with temperature [1, 3–5]). The resulting kinetic scheme for transitions between such generalized states can be represented as follows

$$|1\rangle \rightleftarrows |2\rangle. \tag{1}$$

Let each state $|j = 1, 2\rangle$ in (1) be characterized by the macromolecular free energy

$$G_{j=1,2} = (j-1)(\varepsilon - \mu) \equiv (j-1)(\Delta H - T\Delta S), \tag{2}$$

which is an additive sum of all respective contributions from the intrinsic energy ε (enthalpy ΔH) and the chemical potential μ (partial entropy ΔS) at a given temperature T. As a rule, the latter quantities increase with each single act of base unpairing by an approximate value of $\varepsilon - \mu$ that remains the same for all base pairs. Such an assumption is supported by the finding that enthalpy and entropy contributions of the base pairs to the heat capacity increment of the DNA duplex (analogous to the stem-like structure of RNA) are additive and equal for the AT and CG base pairs [6]. Then, denoting by $P_j(T) = \exp(-G_j/RT)/\sum_j \exp(-G_j/RT)$ the Gibbs probability of state $|j\rangle$ as a function of temperature, the cumulative probability $P_2(T) = 1 - P_1(T)$ for the melted (i.e. unfolded) base pair structure present in RNA reads

$$P_2(T) = \left\{1 + \exp\left[\frac{(\Delta H/T - \Delta S)}{R}\right]\right\}^{-1}, \tag{3}$$

where R is the gas constant, approximately estimated as $8.314\,\mathrm{J}\cdot\mathrm{mol}^{-1}\cdot\mathrm{K}^{-1}$.

Let us compare the afore-derived distribution with its analogs known from the literature for the RNA thermometers. Here, however, we should note that there exist two scenarios for describing the data. One scenario, commonly used in most papers, is entropy-enthalpy compensation mechanism. It argues that a strict correlation between contributions of the entropic and enthalpic increments is of primary importance in fitting the data across temperatures. Really, for large as well as correlated entropy and enthalpy

changes $\Delta S \sim \Delta H/T \gg R$, equation (3) describes the experimental data well. Indeed, compensating the enthalpy and entropy at the melting temperature T_m as $\Delta S = \Delta H/T_m$ equates (3) to

$$P_2(T) = \frac{1}{2}\left\{1 + \tanh\left[\frac{(T_m^{-1} - T^{-1})\Delta H}{2R}\right]\right\}. \qquad (4)$$

Remarkably, this very expression was used in fitting experimental curves in [7] for the process of unfolding (melting) of the cyanobacterial hsp17 ribonucleic acid thermometer with the following thermodynamic parameters $\Delta H = 431\,\mathrm{kcal}\cdot\mathrm{mol}^{-1}$; $\Delta S = 1278\,\mathrm{J}\cdot\mathrm{mol}^{-1}\cdot\mathrm{K}^{-1}$; $T_m = 64\,^\circ\mathrm{C}$. In that case, both the criterion for the entropy-enthalpy correlation and the condition for compensation of the increments of entropy and enthalpy at the melting temperature, $\Delta S/R = \Delta H/RT_m \approx 154 \gg 1$, are satisfied a fortiori, which directly indicates the reliability of study [7]. At the same time, this raises a principle question that, if the number of experimental data points are well described by the relation (4) inferring an entropy-enthalpy correlation, why did the authors of this study not used the physical model to explain those well compensated changes of the enthalpy ΔH and the entropy ΔS? Furthermore, both these quantities appear far too large to obey the fundamental assumptions of any DNA/RNA unfolding model. On the other hand, however, they hardly satisfy experimental data for the values of their increments as well. For example, as was established in [6], both these values were found to be about twenty times lower than those that might be assumed to describe the unusually steep melting curves in the RNA thermometers (cf. [6, 7]).

Another scenario in reproducing the steep unfolding curves of the RNA thermometers is entropy driven scenario. It points to the plausibly increasing role of entropy due to its linear growth with temperature observed in many organic materials such as glasses or polymers [8]. In this case, one can scale a linear dependence of the entropy increment on the temperature difference $\Theta = T - T_0$ in degrees centigrade as follows $\Delta S(\Theta) = \Delta S^\circ + \sigma_T\Theta$, with $T_0 \geq 0\,^\circ\mathrm{C}$ and $\sigma_T = \partial\Delta S/\partial T$ denote a reference temperature and the temperature derivative, respectively, and $\Delta S^\circ \equiv \Delta S(0)$. In this case, one becomes free to choose the form necessary for successful incorporation of the low enthalpy increments when fitting the steep unfolding curves because of the possibility of omitting the enthalpy contribution in dependence (3), by substituting the entropy contribution for it. The only thing that will be required in this case is to employ the entropy-driven criterion in the form $|\Delta S| \gg \Delta H/T$. As a result, in the centigrade temperature scale, one can obtain the following logistic equation with two effective parameters $s \equiv \Delta S^\circ/R < 0$ and $q \equiv \sigma_T/R > 0$

$$P_2(\Theta) = \{1 + \exp[-(s + q\Theta)]\}^{-1}. \qquad (5)$$

Such a phenomenological form of Eq. (5) with not well defined parameters was used in [9] for studying the thermosensing property of 100 randomly selected mRNAs as well as three known thermometers, that is, rpoH, ibpA and agsA sequences from E. *coli*. It was shown that these mRNAs have an inherent tendency to melt with an increase in temperature according to the logistic law in the cases examined, but they exhibit a noticeable negative entropy increment $\Delta S^\circ < 0$; $|\Delta S^\circ| \gg R$. This finding, unfortunately remained unexplained in [9], nevertheless, it makes it possible to describe RNA thermometers as secondary structures that realize in themselves just the entropy-driven scenario of configurational transitions for implementing their thermosensing feature. However, this realization occurs under the condition of a negative entropy increment for these transitions which can happen, for instance, due to a particular structuring, in an unknown way, of water molecules around the non-polar residues upon going from higher to lower temperatures.

To give more consistency and clarity to the entropy-driven scenario, it is noteworthy that from a purely thermodynamic point of view [10], there is always a temperature interval, say from T_0 to T, in which the excess heat capacity ΔC_p at constant pressure p is nearly constant. In this interval, which should be distanced from that one where an unfolding transition in the vicinity of the melting temperature T_m is accounted for, the corresponding increments of enthalpy and entropy are the following

$$\Delta H(T) = \int_{T_0}^{T} \Delta C_p\, dT + \Delta H(T_0) \qquad (6)$$

and

$$\Delta S(T) = \int_{T_0}^{T} (\Delta C_p/T)\, dT + \Delta S(T_0) \qquad (7)$$

respectively, with $\Delta H(T_0)$ and $\Delta S(T_0) = \Delta S^\circ$ being their corresponding changes at temperature T_0. With the constant ΔC_p equations (6) and (7) can be written in the scaled form as follows

$$\begin{aligned} &\Delta H(T) = \Delta H(T_0) + \Delta C_p \cdot (T - T_0); \\ &\Delta S(T) = \Delta S^\circ + \Delta C_p \ln(T/T_0) \cdot \theta(T - T_0), \end{aligned} \tag{8}$$

where $\theta(x)$ is the Heaviside step function, needed to preserve a finite entropy at 0 K, taking the value 0 for $x < 0$ and 1 for $x \geq 0$. Remarkably, the fact of independence of temperature of the heat capacity increment at the level of $\Delta C_p \approx \approx 0.1\ \mathrm{kJ \cdot mol^{-1} \cdot K^{-1}}$ and the linear temperature dependence of enthalpy change in (8) with $\Delta H(T_0) - \Delta C_p T_0 \approx 25\ \mathrm{kJ \cdot mol^{-1}}$ have both been observed recently in [6] for cooperative association/dissociation of the DNA duplex. Consequently, once more setting the entropy-driven criterion $|\Delta S(T)| \gg \Delta H(T)/T$ reduces Eq. (3) in this interval to the specific temperature extra-sensitive equation of the hyperbolic form

$$P_2(T) = \frac{T^h}{T_m^h + T^h}. \tag{9}$$

This equation resembles the classical Hill equation, but is reformulated in terms of temperature instead of ligand concentration, to describe cooperative binding of a soluble ligand to a macromolecule, with parameters $h = \Delta C_p/R$ and $T_m = T_0 \exp(-\Delta S^\circ/\Delta C_p)$ being the so-called "Hill coefficient" and melting temperature, respectively.

Here we should note that Eq. (9), which was in fact postulated in Ref. [11] (see also [4]) in an ad hoc manner as the "Hill equation" and stated without its derivation, was used to describe the admittedly not well-defined notion of RNA cooperativity for the case of fourU RNA thermometer as means to deal with its sensitivity to temperature. However, in spite of the correspondence of fitting this ad hoc equation to data with "Hill coefficients" of $h = 10.6$ and $h = 18.6$, as well as the similar $h \approx 12$ fitted to the model (5), estimating a measure of the cooperativity number as to be say from ten to eighteen or more seems too large.

Another drawback of using both the ad hoc "Hill equation" in [11] and the logistic equation (5) in [9] is the loose use of the centigrade temperature instead of the kelvin one. To overcome that insufficiency means in fact, rejecting the "Hill equation" from consideration. Therefore, in the following we use the thermodynamically correct equation (9) to quantitatively reconcile it with the description of existing data.

Thus, in terms of the thermodynamic framework a simple probabilistic analysis of the behavior of two-state thermosensing structures makes it possible to plausibly conclude that the RNA thermometer operates according to the entropy-driven scenario, in which the change of enthalpy is considered insignificant, whereas that of entropy is regarded as dominant and increasing with temperature, irrespective of being augmented logarithmically or linearly. Therefore, it is of interest to propose a concrete molecular model of two-state thermodynamic system for which incremental contributions to both the linear domain and the logarithmic domain of entropy additions to free energy (2) can separately be determined and then compared with one another. As a result, one could conclude about which contribution really prevails and which thermodynamic parameters of the system are commonly responsible for that prevailing. However, there is the practical problem in calculating the contribution of the lattice normal vibrations akin to that performed for some ceramic crystals such as MgO [12] and TcC [13] with the use of the density functional perturbation theory in the quasiharmonic approximation. In this case, one can indeed obtain the linear temperature growth of entropy in the working temperature interval, but only by calibrating the entropy value at a low level of $30\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$, which appears as nearly forty times lower than that justified in the experiment $1.3\ \mathrm{kJ \cdot mol^{-1} \cdot K^{-1}}$ [7]. At the same time, the one order of magnitude smaller value $\sim 1\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$ is estimated for the entropy increment of graphite when logarithmic growth of its entropy with temperature is taken into account [14]. Nonetheless, it is precisely this value is that which is required to be small enough to allow one to adequately fit the obtained formula (9) to existing experimental data while providing a necessary melting temperature.

3. A Simplified Two-State Microscopic Model

To combine the different approaches discussed above in a unified physical model, let us consider as before only a simplistic two-state case (1) of the RNA unfolding process with the two main configurations, as shown in Fig. 1. There are two ways to construct a physical model for the structural states of the mRNA

molecule in solution. One way is to do this macroscopically, that is, to operate with quantities such as the total reactive volume, number of moles, energy, entropy and temperature, to describe the thermodynamic behavior of the system as a whole. Another way is microscopic, which is intended to operate with the quantities which do not describe the whole system, but specify the states of separate atoms. Knowing in this way the atom's initial positions and momenta allows one to follow the atomic dynamics according to the equations of motion, given the total energy of the system including the kinetic energy as well as the energy of the most relevant interatomic interactions represented as a potential of the mean force. Further, this approach makes it possible to obtain the equilibrium probabilities of a system's structural states, by averaging over all possible atomic configurations, i.e., microscopic states. But if the final molecular configurations of a total system are in equilibrium, i.e. if macroscopic states are taken into account, then we must consider various structural states of the mRNA molecule as separate systems whose instances form a statistical ensemble. For the RNA unfolding process with two structural configurations, we will consequently arrive at two different systems coexisting in solution (Fig. 1), namely, the mRNA molecule with a stem-like structure (system $|1\rangle$) and the mRNA molecule with a particular number of unpaired base pairs in the unfolded region (system $|2\rangle$). Therefore, the objective is to represent for each of such systems its probability coupled to the entropy that in turn is logarithmically proportional to the number of all accessible molecular configurations, while the other macroscopic parameters have certain values. As such, due to the basic postulate of statistical mechanics we have to assume that, while both systems are in mutual thermodynamic equilibrium, all macroscopic configurations of them will occur with the same probability, i.e. they will be specified by a single contour of equal free energy levels intrinsic to the systems. However, both the number of those energetically isoergic levels in the corresponding set, forming an entropic contribution to a free energy of every system, and the enthalpic contribution to it from the energy gap between respective sets of levels, are in general altered because of their dependance on the environment due to the interaction with which cumulative probabilities of these two systems are equilibrated.

The arguments above allow us to represent two mRNA systems under study as systems constantly interconverting in aqueous solution from one to another with the transition probabilities W_{12} and W_{21} that, in the thermodynamic equilibrium, obey the principle of macroscopic reversibility (the condition of full balance), namely $W_{12}/W_{21} = P_1/P_2$. In fact, this means that now we can speak of a single two-state system, each state of which designated as before by $|1\rangle$ and $|2\rangle$ is characterized by a single, but degenerate, level of energy $E_{i=1,2} = E_{i=1,2}(T)$ and degeneracy $g_{i=1,2} = g_{i=1,2}(T)$, both being functions of temperature at constant volume and pressure. Furthermore, without any loss of generality, the temperature dependence of the latter can be scaled as follows

$$g_i(T) = z_i^{\circ}(T) \cdot m_i(T),$$

where $m_i(T)$ is the number of moles of the mRNA molecule as a function of temperature in state $|i = 1, 2\rangle$ and $z_i^{\circ}(T) = \exp[\mu_i^{\circ}(T)/RT]$ is the standard fugacity related to the standard chemical potential μ_i° defined as the chemical potential μ of (2) at the reference level of $m_i(T) = 1\,\text{mol}$.

In this context, considering different scenarios for describing temperature-induced behaviors of RNA molecules is straightforward. Indeed, since the mRNA molecule represents one and the same object of interest in both states $|1\rangle$ and $|2\rangle$, a state's equilibrium probability $P_i(T)$ is defined as

$$P_i(T) = m_i(T)[m_1(T) + m_2(T)]^{-1}. \tag{10}$$

Therefore, the mutual equilibrium between states $|1\rangle$ and $|2\rangle$ is attained at $P_1(T_m) = P_2(T_m)$ or at $m_1(T_m) = m_2(T_m)$, where T_m is the melting temperature. Any shift of temperature T from T_m perturbs the mole numbers $m_1(T)$ and $m_2(T)$, which causes the displacement of these quantities from their equilibrium values $m_2(T_m)$ and $m_1(T_m)$. This occurs at the cost of an enhanced increment of the chemical potential $\Delta\mu(T) \equiv \mu_2(T) - \mu_1(T) = \ln[m_2(T)/m_1(T)]$ providing a net driving force for the melting/folding process of the mRNA molecule. However, to retain the thermodynamic equilibrium in the total reaction volume, such a "local" mutual disequilibrium between the states in (10), induced by the corresponding change of ambient temperature T, should be compensated by a respective change in the standard entropy term $\Delta S^{\circ}(T) =$

$= [\mu_2^\circ(T) - \mu_1^\circ(T)]/T$ or in the base-pair dissociation enthalpy term $\Delta H(T)/RT \equiv \varepsilon(T)/RT = [E_2(T) - E_1(T)]/RT$ or in the both terms, respectively. Depending on which of these terms prevails, we will obtain different scenarios for the RNA molecules. Certainly, for temperature-independent (or constant) standard entropy $\Delta S^\circ(T) \equiv \Delta S^\circ = \text{const}$ and dissociation enthalpy $\Delta H(T) \equiv \varepsilon = \text{const}$, we arrive at the scenario of entropy-enthalpy compensation, discussed above in (3) but its occurrence in RNA molecules would require unrealistically large values of both quantities, related as $\Delta S^\circ = \varepsilon/T_m$. Instead, if the dissociation enthalpy is negligibly small as compared to the standard entropy the scenario is entropy-driven, but only when the corresponding entropy increment becomes linearly (5) or logarithmically (8) temperature dependent. Finally, for very large dissociation energy $\varepsilon(T) \gg \Delta S(T)$ with high and positive temperature dependence such, at least, as $\varepsilon(T) \sim T \ln T$ or $\varepsilon(T) \sim T^{3/2}$ and higher, an enthalpy-driven scenario comes into play. This scenario, however, becomes almost infeasible with respect to the enthalpy constraints for RNA molecules [6].

Among various variants discussed above, the entropy-driven behavior of the mRNA unfolding is reproduced by another realization as well. This is a situation in which an intrinsic disequilibrium occurs in the total thermodynamic system, which is not in the full thermodynamic equilibrium, but only in the partial thermodynamic equilibrium with respect to two solutes dissolved in aqueous solution. As applied to the RNA thermometers, this leads to the difference in solubility levels for the two forms of the mRNA structures, that is, the stem-like form of mRNA molecule and its form of it with a number of unpaired base pairs. As generally known [15], change of temperature T is the factor affecting equilibrium of any solute in solution that, with increasing the T, either enhances its saturation molar concentration, if dissolution is endothermic, or diminishes this quantity, if dissolution is exothermic. Frequently, nearly a linear temperature dependence of solubility is the case [16]. In more general cases, however, that dependence is non-monotone exhibiting saturation or a temperature maximum [17]. This effect is accounted for by expression (10) if the current mole numbers $m_i(T)$ of the mRNA molecule are replaced by those of mRNA solubility at saturation $m_i^{\text{sat}}(T)$, allowing them to have their own temperature dependence, and thus considering the emergent chemical potential difference $\Delta\mu^{\text{sat}}(T) = \ln[m_2^{\text{sat}}(T)/m_1^{\text{sat}}(T)]$ as a new driving force for the mRNA unfolding. By doing so according to the modified Apelblat equation [18] for an aqueous solution at room temperature $T > T_0$, we scale $m_{i=1,2}^{\text{sat}}(T)$ as

$$m_i^{\text{sat}}(T) = A_i(T/T_0)^{n_i} \exp(B_i/RT), \tag{11}$$

where A_i, B_i and n_i are the empirical constants, and $T_0 > 0\,^\circ\text{C}$ is the calibration temperature. At $n_2 - n_1 \equiv n > 0$ and $T_m(T) = T_0(A_2/A_1)^{1/n} \exp[(B_2 - B_1)/nRT]$ expression (10) again reduces to the temperature sensitivity equation analogous to Eq. (9)

$$P_2(T) = \frac{T^n}{T_m^n(T) + T^n} \tag{12}$$

However, this form now contains both a not well-defined temperature exponent n and the exponentially temperature dependent melting temperature $T_m(T)$. However, at sufficiently large $n \gg 1$, say $n > 10$ or more as in (9), and at $B_1 \approx B_2$, temperature dependence of $T_m(T)$ disappears thus approximating the melting temperature simply as $T_m(T) \approx T_0$. Furthermore on the other hand, if $n_2 - n_1 \equiv n < 0$ one obtains an opposite temperature sensitivity equation

$$P_2(T) = \frac{T_m^{-n}(T)}{T^{-n} + T_m^{-n}(T)} \tag{13}$$

providing an inverse temperature dependence as compared to that of Eq. (12).

4. Comparison with Experiment

Given the derived equations (9) and (12) for the temperature extra-sensitive probability of the unfolded configuration of RNA thermometers, let us provide these equations with the respective experimental data. We constrain ourselves here to the comparison with only a few examples of temperature extra-sensitive data obtained by different experimental methods. We use a self-developed Python code to identify the model parameters h and T_m in Eq. (9) by minimizing the root mean square error (RMSE) of the model compared to the experimental data. The obtained best fitting curves are plotted in Fig. 2 and Fig. 3, and corresponding estimated parameters are given in Table 1 (see Sec. 5).

Fig. 2 shows the data on the 4U RNA studied by fast relaxation imaging. As we see the normalized

D/A FRET signal as a function of temperature for both 4U RNA and its low-melting variant (4U and lm-4U, respectively) are well described by Eq. (9) with the respective parameters of h and T_m (given in the inset).

Fig. 3 reproduces the temperature-induced unfolding curves monitored by CD-spectroscopy of hsp17 RNA, as well as its two stabilized mutants hsp17$^{\rm rep}$ and hsp17$^{\rm stab}$ RNA, respectively, with estimated model parameters of Eq. (9) given in the insets. Similar to that in Fig. 2, Fig. 3 also provides a good description of the respective set of CD spectroscopic data by Eq. (9) given the appropriate model parameters. Thus, the comparatively high quality fit demonstrated in both Figs. 2 and 3 can serve as direct evidence for the correctness and usefulness of the derived in Sec. 2 thermodynamic model.

5. Discussion and Conclusion

In this paper the problem of adequately describing thermodynamic properties of temperature extra-sensitive processes observed in particular bacterial RNA molecules is considered in Sec. 2. To simplify the problem, we constrain ourselves to only the two main RNA configurations, that is folded and unfolded, which spontaneously transition to one another according to scheme (1) as depicted in Fig. 1. In this two-state model, defining the thermodynamic probability of an unfolded state in the general form (3) for entropy-driven processes reduces it to the hyperbolic master equation (9) that exhibits a power law dependence on the absolute temperature with non-integer exponent. Although this equation resembles the classical Hill equation if speculatively reformulated with respect to centigrade temperature instead of ligand volume concentration, it cannot, in principle, be regarded as this type of equation in any reasonable meaning. The main incorrectness of the latter is the loose interpretation of temperature exponent as a parameter of cooperativity in a "Hill equation"

Table 1. **Estimated parameters h and T_m in Eq. (9) and the excess heat capacity ΔC_p obtained by minimizing the RMSE of the model with respect to the experimental data**

No.	RNA type	h	T_m, °C	ΔC_p, $\frac{\rm J}{\rm mol\cdot K}$	RMSE	T_m, °C (exper.)
1	4U	80.34	53.2	668	6.58×10^{-3}	53 [19]
2	lm-4U	61.74	47.4	513	4.82×10^{-3}	47 [19]
3	hsp17	38.80	45.7	323	4.45×10^{-3}	46 [7]
4	hsp17$^{\rm rep}$	121.12	63.9	1007	1.11×10^{-2}	64 [7]
5	hsp17$^{\rm stab}$	105.09	50.8	874	2.69×10^{-3}	51 [7]

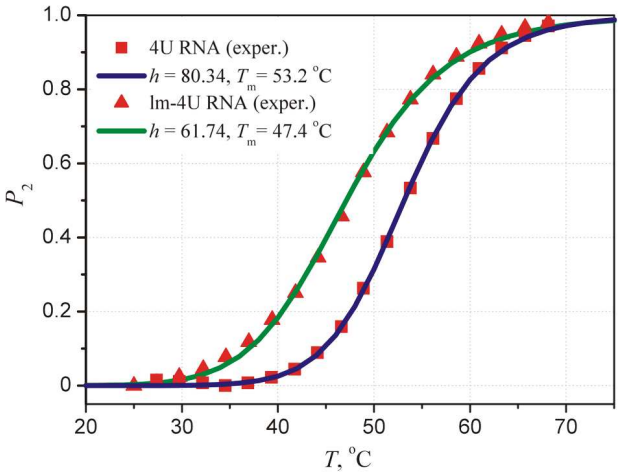

Fig. 2. Theoretical cumulative probability distributions for the unfolded base pair structures as a function of temperature (curves calculated from (9) with the parameters shown in the insets), which correspond to experimental data (adapted from [19] and separately normalized to 1) for 4U RNA and its low-melting derivative lm-4U RNA studied in [19] by the fast relaxation imaging (FReI) technique

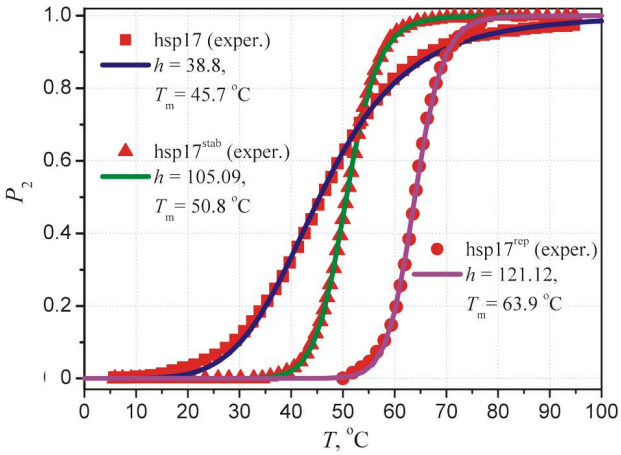

Fig. 3. Theoretical cumulative probability distributions for the unfolded base-pair structures as a function of temperature (curves calculated from (9) using the parameters shown in the insets), which correspond to the temperature-induced unfolding experimental data (adapted from [7] and separately normalized to 1) for hsp17 RNA and its two stabilized mutants hsp17$^{\rm rep}$ and hsp17$^{\rm stab}$ RNA monitored by circular dichroism (CD) spectroscopy [7]

in fact postulated ad hoc [11]. On the contrary, in the consistently derived above equation (9), the corresponding temperature exponent is defined with appropriate accuracy as the reduced molar excess heat capacity at constant pressure measured in the gas constant units. Consequently, strictly speaking equation (9) should be called the temperature sensitivity equation for entropy-driven processes in biological macromolecules with a sensitivity power (temperature exponent) proportional to a molecular heat capacity.

The comparison of Eq. (9) with experimental data is provided in Sec. 4 in Figs. 2, 3. We see that the unity-normalized curves for the temperature dependence of the probability of the unfolded state in different RNAs can be well described by Eq. (9) with two estimated model parameters, that is, the melting temperature T_m and the temperature exponent h. The first parameter is easily calculated as the temperature at which folded and unfolded states of a functional conformational unit of RNA's structural elements, schematized in Fig. 1, are equally populated. The second parameter, characterizing temperature sensitivity power of this unit, is its reduced molar excess heat capacity $\Delta C_p = Rh$. The latter, however, is very difficult to determine in the case of polyatomic molecule even in the gas phase (see e.g. [20]). The estimated values of these parameters are given in Table 1. It is worthy of noting that the first parameter is independent of the particular model description, whereas the second appears to be critical for the equation used in modeling the data. Thus, the comparison of the postulated ad hoc "Hill equation" with experimental data gives the following range for variation of $h = 10.6 \div 18.6$ [11], while the thermodynamically consistent equation (9) provides the same data with the greatly broader interval of $h = 38.80 \div 121.12$ (see Sec. 4 and Figs. 2, 3). Since correlated processes of say from forty to one hundred twenty cooperative simultaneous ligand transitions seem to be almost infeasible, avoiding the concept of cooperativity in the interpretation of temperature extra-sensitive properties of RNAs might be taken for granted. Instead, the concept of excess heat capacity occurring in RNA functional conformational units should be accounted for as playing a pivotal role in the explanation of variable extra-sensitivity to temperature in different RNAs. In this context it indeed occurs that the temperature sensitivity power of Eq. (9) coincides with the temperature exponent which in turn is directly proportional to the excess heat capacity of a functional conformational unit.

Another aspect of RNA calorimetry deals with the common assumption that heat capacity changes in RNA folding/unfolding are almost negligible. For instance, an approximately constant value of the heat capacity $\Delta C_p \approx 100\,\mathrm{J \cdot mol^{-1} \cdot K^{-1}}$ is observed in cooperative association/dissociation of the DNA duplex irrespective of its composition with respect to complementary nucleotides [6]. At the same time, this value being characteristic of a small RNA unit appears as much lower compared to that observed for the hydrophobic hydration of nonpolar functional units and polyatomic ion structures, whose heat capacity values are usually much greater ranging within hundreds of $\mathrm{J \cdot mol^{-1} \cdot K^{-1}}$ (see e.g. [21–23]). Similarly such an extended interval between possible values of $\Delta C_p = 323 \div 1007\,\mathrm{J \cdot mol^{-1} \cdot K^{-1}}$ is characteristic of experiments for different RNA types in Figs. 2, 3 and in Table 1. Because, in general, the exact determination of ΔC_p for polyatomic molecules in polar solutions is impossible since it requires accurately determining the contributions to the value of ΔC_p of various additional components of movement within a functional conformational unit (e.g. flexural or rotational components), the problem of directly calculating the heat capacity for different RNAs remains unsolvable hitherto. Therefore, it is important to try to monitor heat capacity values with a help of indirect methods presented above in Sections 2-4 and in Table 1. Nevertheless, in the recent paper of [24] it has been observed that longer alkyl chains were associated with an increased heat capacity of ionic liquids. By analogy with this observation, the solution of RNA molecules possessing longer free polynucleotide chains in the unfolded configuration can be hypothesized as having a larger excess heat capacity compared to that of RNA molecules possessing shorter polynucleotide chains. Unfortunately, this hypothesis, though plausible yet currently unverifiable, cannot be confirmed by any of the currently employed means.

Also, in the framework of the microscopic approach, the problem of extra-sensitive to temperature transitions between two degenerate states is considered in Sec. 3. We find that using the modified Apelblat model for the unfolding of macromolecules in an aqueous solution at room temperature the form of

the resulting equation for the probability of unfolded state surprisingly coincides, either directly (12) or invertedly (13), with the form of temperature sensitivity equation (9) for entropy-driven transitions in biological macromolecules derived in Sec. 2. The only difference is a poor interpretation of the temperature exponent and melting temperature in (12) or (13) as compared with (9) where this interpretation is physically straightforward. Moreover, polyatomic molecules like RNAs generally possess additional degrees of freedom corresponding to various rotations about and flexions of the chain axis. This requires an accurate equipartition of the heat energy among different microscopic translational, rotational and vibrational degrees of freedom characteristic of functional conformational states as well as taking into account microscopic flexures of apparently motionless conformational chains. Obviously, these problems cannot be resolved with traditional approaches. Nevertheless, both equations (9) and (12) can serve as a form of the temperature sensitivity master equation for entropy-driven processes in different biological macromolecules.

In conclusion, the phenomenon of temperature extra-sensitivity observed in some biological macromolecules, e.g. RNA thermometers [7, 11], can be understood as an entropy-driven process accompanying the unfolding of these macromolecules with temperature due to the increase of their heat capacity increment because of the increasing number of degrees of freedom contributing to them and leading to an increased degeneracy in the unfolded configuration.

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НАДАННЯ НАДЧУТЛИВИМ ДО ТЕМПЕРАТУРИ ПЕРЕХОДАМ У БІОЛОГІЧНИХ МАКРОМОЛЕКУЛАХ ЗМІСТУ ЕНТРОПІЙНО-КЕРОВАНИХ ПРОЦЕСІВ РОЗГОРТАННЯ

Для узгодженого виводу рівняння, яке характеризує термодинамічну ймовірність надчутливих до температури ентропійно керованих процесів розгортання використано двоконфігураційне наближення, яке ґрунтується на термодинамічному підході до опису конформаційних переходів у фізіологічно значущих біологічних макромолекулах. Показано, що з точністю до введених параметрів це рівняння збігається з рівнянням, яке отримано з використанням мікроскопічного підходу. Результати розрахунків порівнюються з експериментальними даними.

Ключові слова: термочутливість, ентропійно керовані процеси, біологічні макромолекули.