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LETTERS TO THE EDITOR

Dear Editor,

In the last issue of *Croatica Chemica Acta* appeared a paper by S. Jakša. L. Kralj and J. Kobe (**The Synthesis and Hybridization Studies of Oligodeoxyribonucleotides Containing the 2'-Deoxyguanosine Modification, 8-Aza-3-deaza-2'-deoxyguanosine**, *Croatica Chemica Acta*, **75** (1) (2002) 175–187) which in the section dealing with the thermodynamics of duplex to single strands transition contains a number of unacceptable errors. To justify this rather strong statement and to show how thermodynamics should be used when dealing with the problem of duplex denaturation let me point out the following:

1. The quantities $\Delta H°$ and $\Delta S°$ presented in Table III are defined in lines 8–11 of the text on the page 182 with the following sentence: Transition enthalpies $\Delta H°$ and transition entropies $\Delta S°$ were determined from the dependence of T_m on the DNA concentration (ranging from 11.5 to 3.5 mmol dm^{-3}). All $\Delta H°$ values and $\Delta S°$ values reported in Table III are negative which means that the denaturation of each duplex is an exothermic process accompanied by an increased order in the solution. This simply cannot be true. Obviously, the authors misunderstood the theoretical treatment developed by Marky and Breslauer they are referring to in ref. 18. In this reference Marky and Breslauer discuss the single strands $\rightleftharpoons$ association complex equilibria as association equlibria and therefore the $\Delta H°$ and $\Delta S°$ values calculated from their theoretical expressions refer to the association and not to the denaturation processes. Thus, the correct $\Delta H°$ and $\Delta S°$ values describing denaturation of the measured duplexes should be of the same magnitude as those presented in Table III, but of the opposite sign.

2. From the discussion on duplex stability given on p. 182 (based on the measured melting temperatures and incorrect $\Delta H°$ values) it is clear that authors do not understand the basic principles of thermodynamic stability. Namely, when comparing stabilities of various duplexes one has to compare their free energies of denaturation, $\Delta G°_{den(T)}$, at the same T that is usually chosen to be 25 °C (in this way one is actually comparing their equilibrium denaturation constants, $K_{den(T)}$, at given T). These $\Delta G°_{den(T)}$ values are determined from the general relation $\Delta G°_{den(T)} = \Delta H°_{den} - T\Delta S°_{den}$ assuming that the $\Delta H°_{den}$ and $\Delta S°_{den}$ values do not depend on the temperature (the same assumption was used by Marky and Breslauer in deriving their $1/T_m = [(n–1)R / \Delta H°] \ln c_{DNA} + [\Delta S° – (n–1)R \ln 2n] / \Delta H°$ relation from which the $\Delta H°_{den}$ and $\Delta S°_{den}$ values are determined as $\Delta H°_{den} = -\Delta H°$ and $\Delta S°_{den} = -\Delta S°$; ref. 18). Finally, from the relation $K_{den(T)} = \exp(-\Delta G°_{den(T)} / RT)$ one can easily see that an increase in duplex stability (lower $K_{den(T)}$) must be reflected in more positive $\Delta G°_{den(T)}$ value.

3. The method of determining $\Delta H°$ and $\Delta S°$ (of association) from the $1/T_m$ *vs*. $\ln c_{DNA}$ plots was applied in a much too narrow concentration range for a safe determination of $\Delta H°$ (from the slope) and $\Delta S°$ (from the intercept on the *y*-axis). Namely, due to the error in T_m values determined from the experimental melting curves, which amounts to at least ± 0.5 °C, the slope of the $1/T_m$ *vs*. $\ln c_{DNA}$ line constructed over so narrow c_{DNA} range is unsafe within more than 100 %. Consequently $\Delta H°$ and $\Delta S°$ values obtained from these plots cannot be used in any serious discussion on the stability of the measured duplexes.

I believe that this discussion clearly shows that the thermodynamic part of the above mentioned paper is incorrect and thus cannot lead to any meaningful conclusions on the stability of the measured duplexes.

Sincerely yours,

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Dear Editor:

We are much obliged to receive comments appealing our work (**The Synthesis and Hybridization Studies of Oligodeoxyribonucleotides Containing the Guanosine Modification, 8-Aza-3-deaza-2'-deoxyguanosine**) concerning the interpretation of the subtitled part of the article. It is obvious that the objectives of the article have not been focused on in depths studies of thermodynamic interpretations of the preliminary calculations. However it seemed to us, that the disclosure of the data can be easily compared to the most reliable and comparable sources, like by Seela *et al*.[1] and/or Turner *et al*.,[2] where the authors have used identical approach and interpreta-

tions. Still, we appreciate dr. Vesnaver's concern, and agree partly with his comments, though overlooked by referees and ourselves. Having this in mind, we would like to add some additional corrections in details.

1. We have subtitled a part of the article, which is dealing with the stability of the modified duplexes as »Thermal Denaturation Studies«. It would be logical that the thermodynamical results (the quantities $\Delta H°$, $\Delta S°$) which follow would be presented as $\Delta H°_{den}$, $\Delta S°_{den}$ (the same magnitude but the opposite sign). As we wanted to compare our data with the results of similar work on aza-deaza modified oligonucleotides published by Seela *et al*.,[1] the results in Table III on page 182 were presented as $\Delta H°$ and $\Delta S°$ of formation (We appoligize for uncorrect subtitles.). We may now correct the title of the Table III to »*T_m values and thermodynamic data of 16 mer DNA duplexes formation*« and also replace the text in lines 8 – from »Transition enthalpies $\Delta H°$ and transition entropy ΔS°« to »*Transition enthalpies $\Delta H°$ and entropy $\Delta S°$ of duplex formation*«.

2. We certainly do understand the theoretical proceedings of Marky and Breslauer (refered in article) and the principles of thermodynamic stability as do the referees and leading authors in the field, and we do point out that unsufficient exactitude on the measurements as preliminary data still gives important message to further work in the field. It is clear, when comparing stabilities of various duplexes, one has to compare their free energies $\Delta G°$ at the same temperature that is usually chosen to be 25 °C or 37 °C. From our results it is obvious that entropy changes do not prevail over enthalpy of formation. Unfortunately, the sentence in lines 18–21 on page 182 is not written correctly. It should be as follows: »*When comparing the thermodynamic data of duplexes II : V, III : V, IV : V with the unmodified control duplex I : V, we may conclude that the less favorable free enthalpy terms lead to a minor duplex destabilization and that entropy changes do not prevail over enthalpy of formation*«. The calculated $\Delta G°$ values at 25 °C are as follows –100.2 kJ mol^{-1}, –97.2 kJ mol^{-1}, –88.0 kJ mol^{-1} and –83.0 kJ mol^{-1} for I : V, II : V, III : V and IV : V. As we have realized the uncertainty of our results, we have not compared any stability of our anticipated duplexes, especially not on the basis of ΔH of formation. **We have only found out that every *G** contributes to the lowering of T_m and that there is an almost linear dependance of the T_m values and the number of modified base residues**.

3. As mentioned above, we have realized our problems first of all with the instrumentation, the inability of melting measurements within the concentration range of 200 μmol dm^{-3} to 1 μmol dm^{-3}, the deficiency of modified oligodeoxynucleotides, *etc.* Therefore we want to emphasize that these results are only preliminary thermodynamic calculations.

In conclusion we may add that these preliminary results by no means presented a wrong picture and give erroneous results. We would be extremely enthusiastic that someone would initiate a thorough in depth calorimetric studies on all available 3–deaza-guanosine modified oligonucleotides.

Sincerely,

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References:

1. F. Seela and A. Jawalekar, *Helv. Chim. Acta* **85** (2002) 1857–1868.
2. J. A. McDowell and D. H. Turner, *Biochemistry* **35** (1996) 14077–14089.