A COMPUTER PROGRAM FOR DIFFERENTIAL SCANNING MICRO- AND NANO-CALORIMETERS. THE USE FOR DNA COMPLEXES WITH ANTITUMOR PLATINUM COMPOUNDS

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Cisplatin is an effective antitumor drug that exerts its biological effect by DNA chemical modification. Complexes of cisplatin and other platinum compounds with short oligonucleotide duplexes (short DNAs) are carefully studied with differential scanning calorimetry (DSC). So far the method was not used for long DNAs, therefore we have carried out investigation DNA chemically modified with cisplatin and its ineffective stereoisomer transplatin using micro and nano differential scanning calorimeters (DASM-4 and CSC 6300).

At the start of this work, we found: (i) a primary DSC curves registered with the nano-calorimeter were not reproducible, (ii) the parts of the primary DSC curves before and after the temperature range of DNA melting were far from horizontality and linearity, (iii) those parts were often not parallel to the instrumental base line; (iv) subtraction from the primary DSC curve makes the shape of final part of the curve unsuitable for calculation of the second (chemical) base line. Because of these reasons, the programs developed by the producer of calorimeters give unrepeatable shape of the final curves of the excess heat capacity and values of thermodynamic parameters. The same is true in part for microcalorimeter, but for this device the distorting effects are much weaker. However, the noise for it is much higher relative to nano-calorimeter (compare Figures 1A and 1B).

In this work, we developed a processing procedure and corresponding computer program for calculation of excess heat capacity caused by the helix-coil transition. The method is suitable for cases of bad reproducibility of DSC curves when their shape is not similar to the instrumental base line. There are two ways of the use of the method. If the instrumental base line is close in shape to the regions preceding and succeeding the helix-coil transition, then the line is subtracted from the raw data before further calculations. If there is no shape similarity, then the calculated baseline is used instead of instrumen-

tal. Usually, subtraction of instrumental base line did not give good results for nano-calorimeter in contrast to microcalorimeter. However, application of the calculated baseline after subtraction of the instrumental base line strongly increases reproducibility of the final procedure of calculation of the curve of excess heat capacity for microcalorimeters.

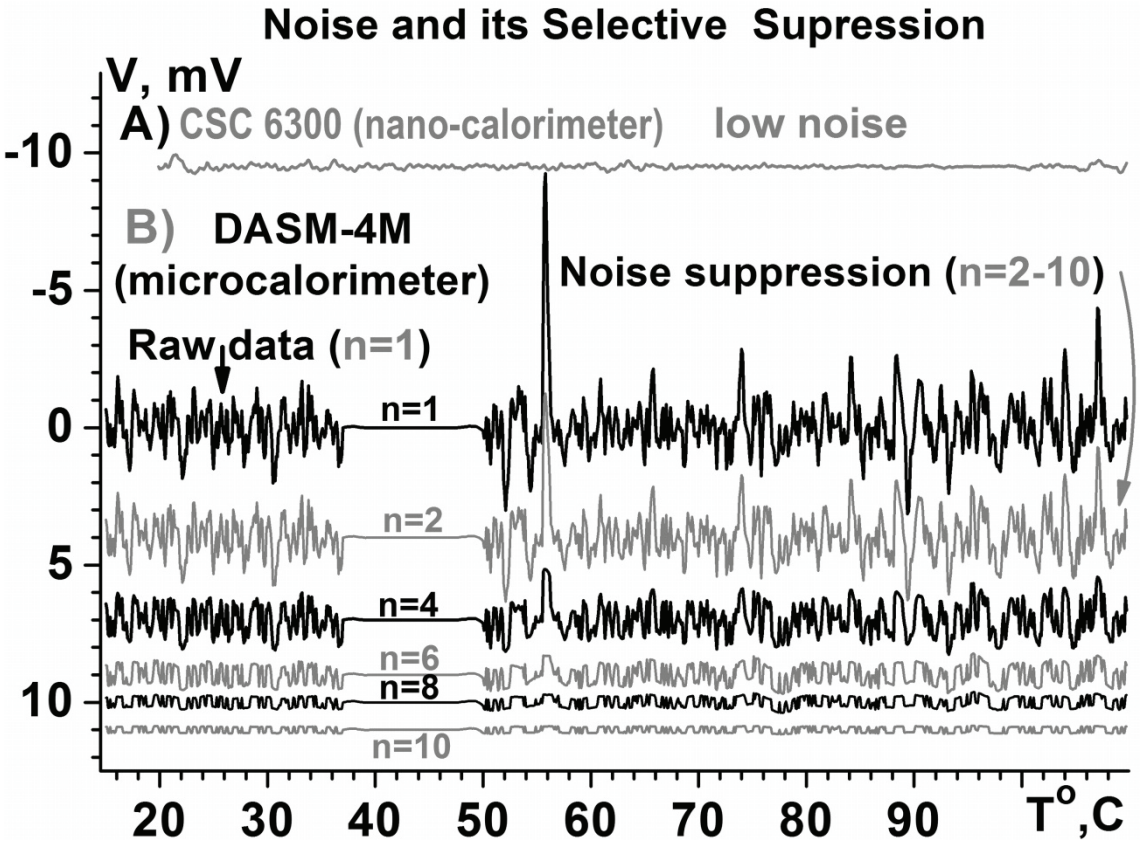

Figure 1 – A) A noise of nanocalorimeter CSC 6300 registered and calculated for the solution used for DNA melting (0.03 M NaCl, 0.1 mM Na_2CO_3, $5 \cdot 10^{-5}$ M EDTA) (Er_{av}=0.071 mV, Er_{max}=0.51 mV, T_{Er}=0.53°C). B) Noise of microcalorimeter DASM-4M (*n*=1) and its selective gradual suppression with the elaborated program (Er_{av}=0.67 mV, Er_{max}=8.2 mV, T_{Er}=0.82°C). The definition of the noise characteristics is given in the text. The smooth region without noise (37-50°C) is the site of the deleted calibration pulse

We have also developed a special method for noise smoothing (Figure 1B). Instead of direct smoothing, smoothed curves are used for preliminary "noise correction". That allows more accurate calculation of the smoothed curve at every consequent step.

To characterize the noise and its decrease under processing, we calculate the noise average value (Er_{av}), maximal value (Er_{max}) and period (T_{er}) using Eqs (1)-(3):

$$Er_{av} = \Sigma|P(i)-P_{sm}(i)| \ / \ N \quad (1)$$

$$Er_{max} = \max \{|P(i)-P_{sm}(i)|\} \quad (2)$$

$$T_{Er} = (T_2-T_1) / N_{prd} \quad (3)$$

where T_1, T_2 are the first and last temperature points of the primary ("raw") DSC curve, and N_{prd} is the number of pairs of neighboring points characterized with negative and then positive values of the noise (Figure 2A and 2B (n=1)).

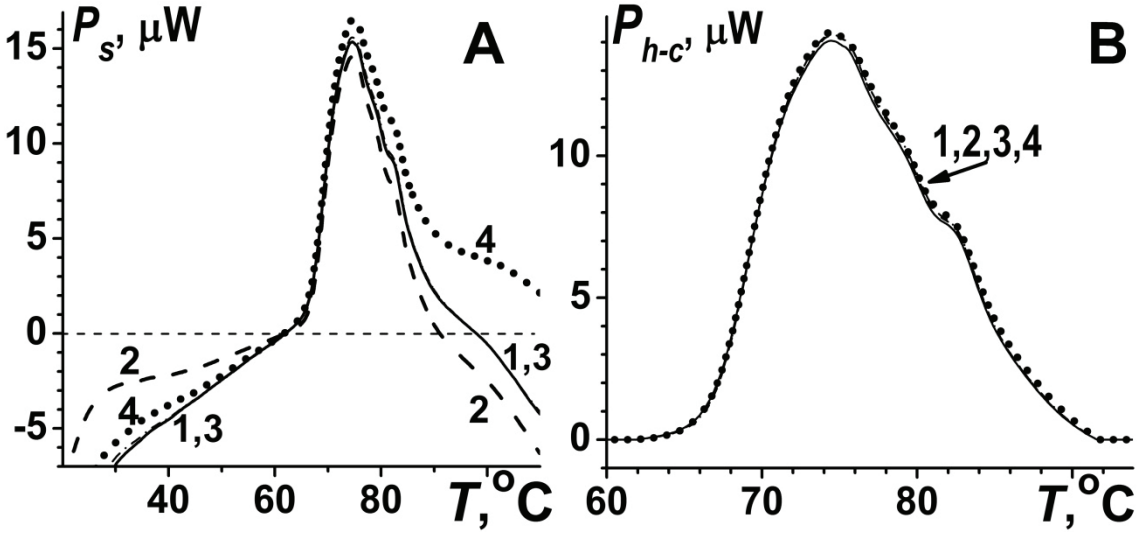

Figure 2 – A) Four primary DSC curves (raw data) measured for DNA incubated in 0.01 M $NaClO_4$ at 37°C for 48 hours with antitumor drug cisplatin at relative per nucleotide concentration r=0.025. The registration was carried out in 0.03M NaCl, 0.1mM Na_2CO_3, $5 \cdot 10^{-5}$M EDTA, pH ~7. B) The curves of excess capacity caused by the helix-coil transition obtained by processing of "raw data" from Figure 1A

In this study, a change in enthalpy and entropy of the helix-coil transition under DNA chemical modification with cisplatin (23 kcal/mole and 65 cal/(deg mole of modifications) and very similar but inactive transplatin (38 kcal/mole and 78 cal / (deg mole of modifications)) was determined.

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