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Biophysics

Chromatin Thermostability in Breast Carcinoma Tissue Composition

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ABSTRACT. Thermostability and energetics of melting process of chromatin in composition of normal and ductal breast carcinoma tissues at various stages of disease have been studied. It has been shown that the melting process of normal tissues proceeds in five transition stages at 55, 66, 78, 97 and 103°C. The first, second, and third transition stages are connected with melting of proteins and the fourth and fifth transitions correspond to chromatin denaturation. In the given paper we consider only the melting of chromatin. It was found that stability of both domains of carcinoma chromatin decreased by 5.0 and 3.5°C compared to norm, and, which was more important, nearly 20% of ΔH_d from transition V was lost and added to transition IV without change of total chromatin denaturation enthalpy. We explain this change by strong rearrangement of structural organization of both domains of chromatin – hetero and active chromatin. In particular, unfolding about 20% of 30nm fiber structure and its pass to 10 nm fiber and partial unfolding of 10nm fiber due to loss of H1 histone and core histones H2A/H2B, that coincide with published data. © *2011 Bull. Georg. Natl. Acad. Sci.*

Keywords: *breast ductal carcinoma, calorimetry, chromatin.*

Over the last twenty years with the help of highly sensitive differential scanning calorimeters (DSC) designed for study of complex biological systems the thermodynamic characteristic of some proteins and chromatin in composition of tissues, cells and nuclei have been investigated. It has been shown that membranes, nuclear proteins, cytoplasmatic complexes and extra cellular proteins melt at 40-85°C and chromatin melts in the range of 85- 110°C [1-5].

At present some different mechanisms of mammary cancer development are considered. According to data [6,7], a leading role in ductal breast cancer induction should be ascribed to epigenetically inactivated two tumor suppressor genes, the fragile histidine triad (FHIT) gene the WW domain containing oxidoreductase (WWOX). Other data suggest that shortened telomeres are the main cause of ductal breast cancer growing [8, 9]. In [10-12] it is shown that extracellular matrix (ECM) takes active part in mammary gland development and breast cancer through integrin receptor signals sending into the cell, which modifies gene expression. But in spite of significant results reached in the study of the mammary cancer development mechanism [6-12], there are no data as tumor cell transformation influences on stability of chromatin assembly. We tried to evaluate these fine changes of chromatin structure stability which are observed in the case of tumor cell transformation. Differential scanning calorimetry (DSC) was chosen for our investigation as it is a reliable and direct method of chromatin study *in situ* [1-5].

Materials and Methods. The biopsy tissues were diagnosed at the Oncological Center and Medical State University, and the stage and grade of breast ductal carcinoma were determined using macroscopic description and morphological analysis.

The measurements were performed on 11 samples of carcinoma biopsy tissues at II and III stages at the Institute of Physics, Tbilisi State University, using a differential scanning microcalorimeter (DSC) with sensitivity of 0.1 μW [2, 13]. The volumes of measuring vessels varied from 0.03 to 0.1 cm^3; the chosen heating rate was 0.75°C/min and the temperature range of measurements was from 25 to 140°C. The precision of the temperature measurements was not less than 0.05°C. The error in determination of melting enthalpy (ΔH_d), heat capacity dQ/dT (ΔC_d) was not more than 10%. The microcalorimeter (DSC) processor was equipped with all software needed for determination of the denaturation thermodynamic parameters of the investigated biopolymer solutions and tissues, as well as deconvolution of calorimetric curves [13]

The weight content of biomass in both normal and carcinoma was determined by dry weight at 105°C, DNA content in tissues was determined by method [2, 13].

Results. Fig.1(a,b) presents the heat absorption curves of norm and ductal carcinoma tissues. It is seen that the denaturation process norm begins at 39°C and completes at 115°C. Five different transition temperatures were detected: transition I – a weak diffusion heat absorption in the temperature range from 39°C to 58°C; transition II – a dominant clear peak with a maximum at 66±1°C and ΔT_d=3.2±0.2°C, and ΔC_d^{max} = 8.9 J/gK; transition III – the heat absorption at 78°C is weaker than the heat absorption at transition II, ΔC_d^{max} = 1.8J/gK and weakly expressed transitions IV and V with ΔC_p^{max} equal 0.1 and 0.2J/gK at 97°C and 103°C, respectively. The total denaturation heat calculated from the area under transitions I-V equals 1.68 J. This value per gram of dry biomass gives a value of ΔH_d equal to 69.5±7 J/g biomass for the denaturation of norm. This value is obtained on the basis of five measurements. The heat absorption at transitions IV and V corresponds to the melting of chromatin and equals 10.1±1.0 mJ which, recalculated per gram DNA, gives transitions IV and V with values 32±3 and 56±6 J/g DNA, respectively. As is seen in Fig.1(a,b), the curve profile of norm significantly differs from profiles in the case of carcinoma. We can see a strong decrease of ΔH_d, ΔC_d^{max} and T_d of carcinoma compared to norm, and a shift of endotherms IV and V of carcinoma compared to healthy tissue to lowly temperatures by 5.0 and 3.5°, respectively, and transition of heat (ΔH_d) from stage V to stage IV is about 12 J/g. Here we consider only the denaturation process of chromatin, because transitions I, II, III in the range of temperatures 39-85°C correspond to both subcellular proteins and RNP complexes, as well as to melting of extracellular components that demand further investigation.

Weakly expressed transitions V and IV compared to dominant transitions around 66 and 78°C in the case of norm and around 60 and 85°C in the case of carcinoma are connected with the fact that duct tissues have small quantity of DNA about 0.6 % and large quantity of structural proteins including collagen round 40% which give powerful heat absorption peaks in the temperature range 55-85°C.

Fig.2(a,b) shows the high-temperature region of microcalorimetric records, and Fig.3(a,b) demonstrates excess heat capacity deconvoluted curves of healthy and carcinoma tissues for a more distinct image of the chromatin denaturation process. The deconvolution was made by Gaussian components.

Discussion. Chromatin of cells in metaphase has complex hierarchical organization, involving at least four main levels: the first level – nucleosomal – 10nm fibers, its nucleosomes are separated from each other by linker DNA;

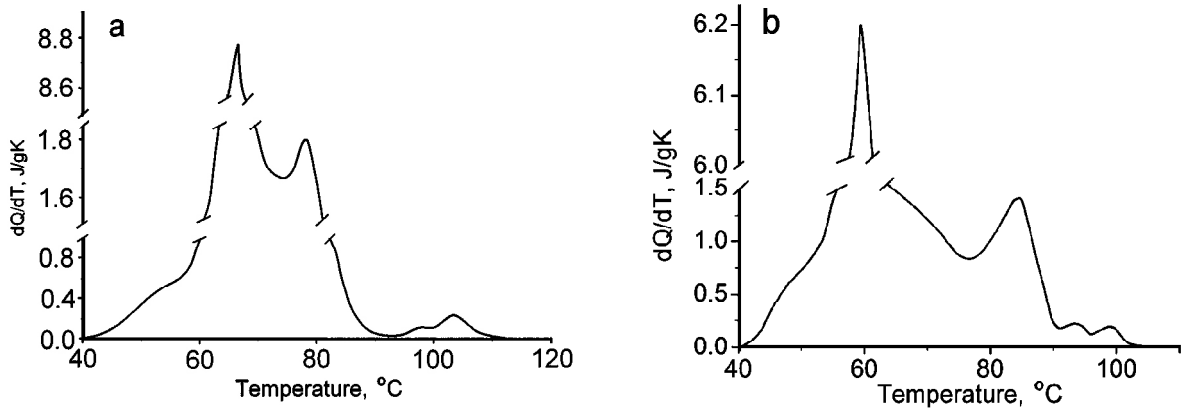

Fig.1. (a,b) The excess of heat capacity, dQ/dT, at various temperatures; recalculated per gram of dry biomass; healthy mammary duct (norm – a) and breast ductal carcinoma (carcinoma – b). The amount of protein in the measuring cell was about 20 mg dry biomass, DNA was ~0.125 mg. The curve has been built on the basis of 9 independent measurements of biopsy norm and carcinoma tissues of patients, from 40 to 60 years old.

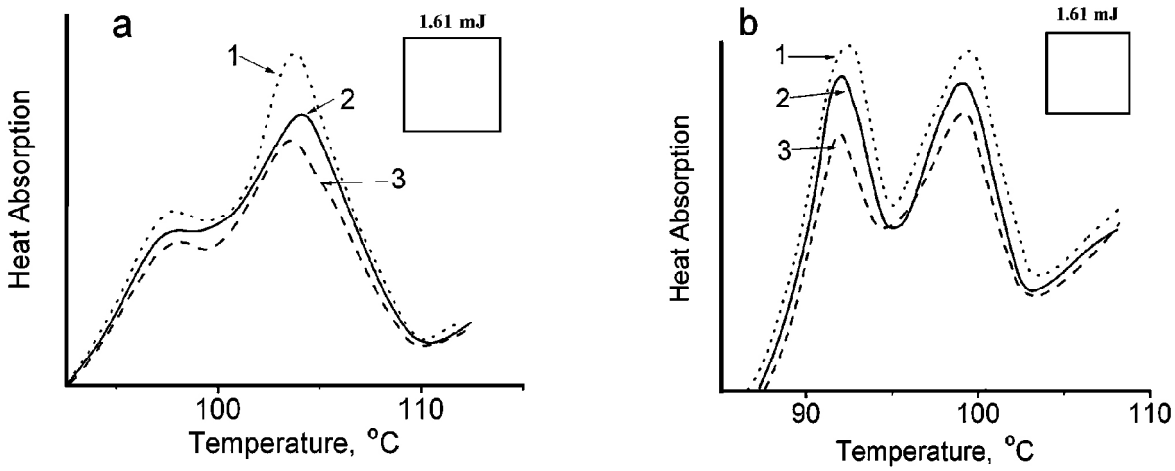

Fig. 2. (a,b). Microcalorimetric records of healthy (a) and carcinoma (stage III) breast duct tissues isolated from different patients. In both cases transition stages IV and V correspond to denaturation of chromatin. The deconvolution of the curves a2 and b1 with an arrow in Fig. 2(a, b) is presented in Fig.3 (a, b). The differences in the area under the thermal profiles depend on various sizes of samples which contain: a) the first curve – DNA–0.140mg; the second curve - DNA – 0.125 mg; the third curve - DNA – 0.120 mg; b) the first curve – DNA – 0.150 mg; the second curve – DNA- 0.130; the third curve – DNA – 0.11 mg.

the second one packing of 10nm fibers due to its superspiralization into 30nm fibers; the third one - looping organization - in this case 30nm fiber forms loops, which may be both open and twisted, and bound to protein structures of chromatin; the fourth level, when looped structure is packed in metaphase chromosome. In interphase cellular nuclei, chromatin ready for transcription is in state of 30nm fiber-untranscriptable "silent chromatin". The structure of 30nm fiber is dynamic, the degree of fiber decondensation is determined by transcription activity of corresponding genes. At relatively low transcription level, chromatin regions located between transcribed ferments have diameter of 10nm and depleted H1 histone (active chromatin). In the case of tumor cell transcription when effectiveness of transcription is increased, certain parts of 30nm fiber turn into 10 5nm fiber. In this case simultaneously with whole nucleosomes, there are also unfolding ones - hexasomes that have lost not only H1 histone but also core histones H2A/H2B [14].

Proceeding from numerous experimental and theoretical data on the denaturation of proteins, nuclear acids and their complexes, a direct connection between melting enthalpy and degree of their native structure exists [15]. Consequently, about 20% of ΔH_d decrease of transition stage V and increase of ΔH_d of transition stage IV by the same value without changing their total value (Fig.1,2,3) may be considered as a result of considerable unfolding of 30nm fiber into 10nm fiber. We suggest that this unfolding is not connected directly with an increase of the activity of "silent chromatin" by 20% but it is connected with readiness to start transcription.

The decrease of T_d of stage IV without decrease of this stage ΔH_d is evidence of the fact that active chromatin, presumably in 10-5nm fiber, may be additionally activated compared to norm. This supposition is also supported by data, according to which the loss of H1 histone from active 10nm chromatin leads to a decrease of its T_d by several degrees [16].

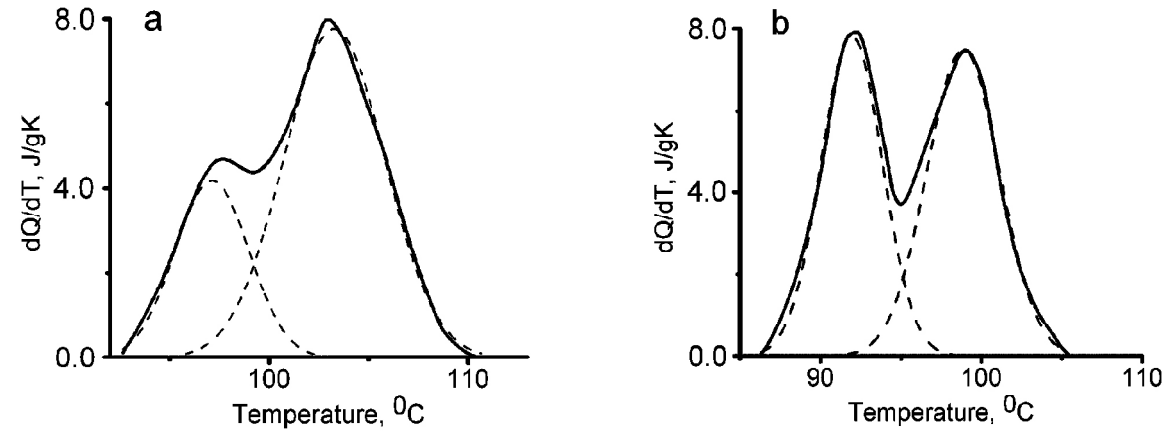

Fig.3. (a,b) Deconvoluted excess heat capacity curves of chromatin in the composition of healthy (a) and carcinoma tissues in the temperature range 90-110°C.

ბიოფიზიკა

ძუძუს კარცინომის ქსოვილის ქრომატინის თერმოსტაბილურობა

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შესწავლილ იქნა ჯანმრთელი და სხვადასხვა სტადიის ძუძუს კარცინომის ქსოვილებში ქრომატინის დენატურაციის თერმოსტაბილურობა და სითბური მახასიათებლები. ნაჩვენებია, რომ ნორმალურ ქსოვილში დენატურაციის პროცესი მოიცავს ზუთ სტადიას გადასვლის ტემპერატურებით: 55, 66, 78, 97 და 103°C. გადასვლის პირველი, მეორე და მესამე სტადია დაკავშირებულია ცილების დენატურაციასთან, ხოლო მეოთხე და მეხუთე გადასვლა შეესაბამება ქრომატინის დენატურაციას. აღმოჩნდა, რომ ქრომატინის ორივე დომენის სტაბილურობა შემცირდა 5 და 3.5°C გრადუსით ნორმასთან შედარებით და, რაც უფრო მნიშვნელოვანია, V გადასვლისას შთანთქმული სითბოს (ΔH_d) 20% გადადის IV სტადიაზე შთანთქმულ სითბოში ისე, რომ ქრომატინის დენატურაციის ჯამური ენთალპია უცვლელი რჩება. ჩვენ ამ ცვლილებას განვიხილავთ, როგორც ქრომატინის ორივე სტრუქტურის (ჰეტერო და აქტიური ქრომატინის) რემოდელირებას, კერძოდ, 30 ნმ-იანი ფიბრილის სტრუქტურის დაახლოებით 20%-ის გაშლას 10 ნმ-იან ფიბრილამდე და 10 ნმ-იანი ფიბრილის ნაწილობრივ გაშლას, H1 და ბირთვის H2A/H2B ჰისტონების დაკარგვის გამო, რაც ეთანხმება გამოქვეყნებულ მონაცემებს.

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