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THE DECONDENSATION PROCESS OF NUCLEAR CHROMATIN AS INVESTIGATED BY DIFFERENTIAL SCANNING CALORIMETRY

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Owing to its orderly subdivision into structural domains, the nucleus from resting cells has a remarkably simple denaturation profile, made up of five gaussian heat-absorption peaks in the temperature range 330-380 K (1). They are marked by roman numerals in Fig. 1A. Transitions I and II remain after nuclease digestion and high-salt extraction, and reflect the co-operative melting of the nuclear scaffold. The linker DNA undergoes a conformational change in transition III. Importantly, the endotherms at 365 and 380 K (IV and V) split up the unfolding of the core particle, and we succeeded in establishing their structural origin. The core particle gives rise to IV or to V depending on its location in the unordered polynucleosomal chain or in the helical 30 nm fibre. Thus, the corresponding transition enthalpies ΔH_m^T can be related to the extent of chromatin condensation.

When an intercalative agent, ethidium bromide (EB) binds to nuclear chromatin, sharp changes are induced in the higher-order structure. A transition from the ordered fibre to the expanded loop has been detected in our laboratory by electron microscopy and we took advantage of this circumstance to inquire into the dependence of the level of structuration on the intercalation process, which essentially entails a reduction of the DNA helix winding number β. The value of r, the ratio of bound EB to DNA phosphate, have been determined on suspensions of nuclei from rat liver by a modification of the spectrofluorimetric method by Le Pecq and Paoletti (2). The results are shown in Fig. 1B. Between r=0 and r=0.04 a strong decrease of ΔH_m^{380} (4.4 Kcal·(mol bp)$^{-1}$), comparable with the increase in ΔH_m^{365} (3 Kcal·(mol bp)$^{-1}$) is observed. This effect reveals an extensive loosening of the internucleosomal interactions. The high affinity sites of chromatin are within the DNA linker segments and become saturated over the same range of r;

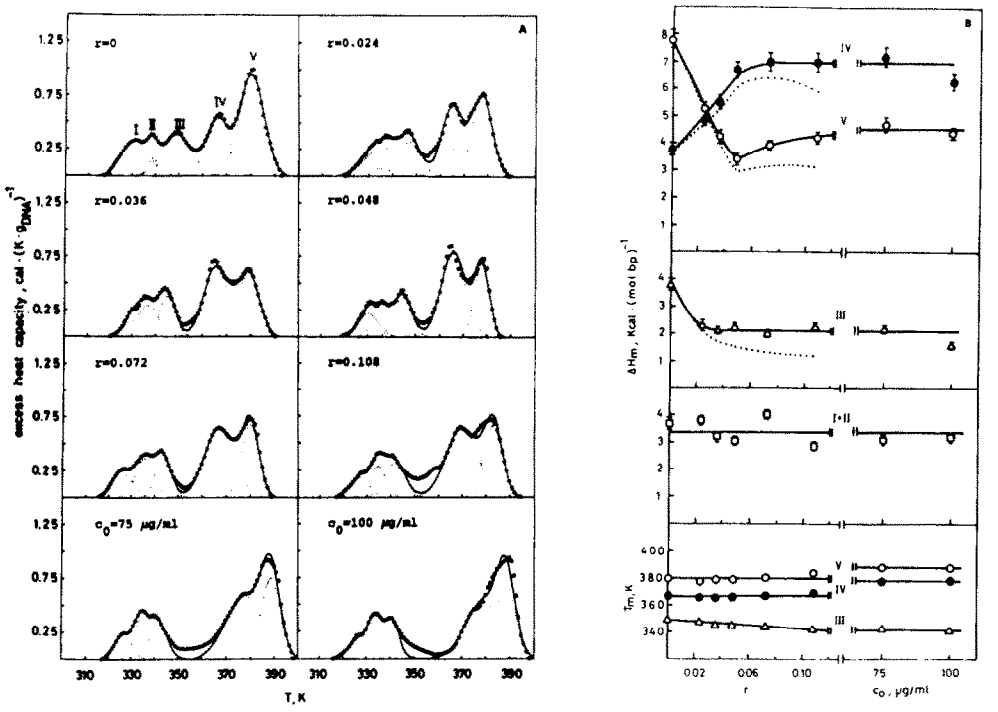

Fig. 1.A. The melting profiles of rat liver nuclei in the presence of different amounts of EB. The values of r or of the total dye concentration c_0 are indicated. Experimental data (•); gaussian component transitions (···);resolved envelope, corresponding to the sum of the transitions (——). B. The values of ΔH_m and T_m plotted against r or c_0. The dotted lines indicate the value of ΔH_m corrected for the intercalation heat.

this process brings about chromatin decondensation. Further binding of EB to the core-particle DNA, while inducing a slight supercoiling in the chromatin loops, does not involve large enthalpy changes.

The interpretation of these results rests on the following consideration: 1) for closed circular DNA $\Delta\beta = -\Delta\tau$, where τ is the superhelix winding number; furthermore in a relaxed chromatin loop unwrapping of the DNA from the histone core is converted into superhelical turns. These are simple outcomes of the topological theory. 2) The forces responsible for the stability of the higher-order structure, depending on the internucleosomal interactions, will oppose unwrapping. Hence, a decrease in β can result in both chromatin decondensation and supercoiling through an increase in length (from 50 to 100 bp) of the linker segment. The decondensation process of chromatin induced by intercalation mimics, in several aspects, the structural changes underlying gene activation.

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