The Influence of Formamide on Thermal Denaturation Profiles of DNA and Metaphase Chromosomes in Suspension

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Systematic photometric studies are presented to analyze the thermal denaturation behaviour with and without formamide of metaphase chromosome suspensions in comparison to DNA solutions. Temperature dependent hyperchromicity measurements at 256 nm and 313 nm were performed using an appropriately designed computer-controlled photometer device. Due to an upright optical axis, this allowed absorbance measurements with negligible sedimentation effects not only for solutions of pure DNA, but also for particle suspensions of isolated metaphase chromosomes. This device has a temperature resolution of ± 0.5 °C and an optical sensitivity of 10^{-3} to 10^{-4} optical density. For calf thymus DNA the reduction of the melting point with the increase of formamide in the solution was measured at pH 7.0 and pH 3.2. The good correlation of the theoretical approximation to experimental data indicated the suitability of the apparatus to quantitatively describe DNA conformation changes induced by thermal denaturation. For metaphase chromosome preparations of Chinese hamster culture cells, absorbance changes were measured between 20 °C and 95 °C with a temperature gradient of 1 °C/min. These measurements were performed at pH 7.0 and at pH 3.2. The denaturation profiles (= first derivative of the absorbance curve) resulted in a highly variable peak pattern at 256 nm and 313 nm indicating complex conformation changes. A statistical evaluation of the temperature values of the peak maxima resulted in temperature ranges typical for chromosomal conformation changes during thermal treatment. Especially the range of highest temperature values was independent from pH modifications. For pH 3.2 the influence of formamide on the denaturation behaviour of metaphase chromosome preparations was analyzed. In contrast to pure DNA solutions, a reduction of the "melting point" (i.e. the maximum temperature at which a conformation change takes place) was not found. However, the denaturation behaviour depended on the duration of formamide treatment before the measurement.