# THE EFFECT OF TEMPERATURE ON HISTONE GRK AGGREGATION

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## SUMMARY

The aggregation of histone GRK, induced by the addition of salt, is found to be highly dependent on temperature. The rate of this aggregation decreases with decreasing temperature and can be essentially stopped at low temperatures.

## INTRODUCTION

The aggregation of histones has long been known (1-4) and this phenomenon has presented severe problems in studying their physical properties. Our laboratory has studied conformational changes in histone GRK and has shown that a critical salt concentration exists for the onset of aggregation (5,6). Recently, it was noted that histone GRK aggregation is affected by temperature (7). Since the ability to stop histone aggregation is so important for physical studies, we expanded these observations and report our results here.

## MATERIALS AND METHODS

Histone GRK, prepared by the method of Ogawa et al. (8), was electrophoretically pure and its amino acid composition agreed with the published sequence (8,9). Its concentration was determined spectrophotometrically using a molar extinction coefficient of $5.4 \times 10^3$ $cm^{-1}$/mole histone/liter at 275 nm (10).

Stock solutions of phosphate were used as their own buffers and adjusted to pH 7.4 by the addition of NaOH. Both phosphate solutions and histone GRK in water were brought to the temperature at which they were to be studied. At this temperature aliquots of each were mixed; the time of mixing is called time zero.

The fluorescence anisotropy $[r = (I_{\parallel} - I_{\perp})/I_{\parallel} + 2I_{\perp})]$ and light scattering were measured on a computer controlled polarization spectrometer constructed in our laboratory (11).

For the fluorescence polarization measurements, the samples were excited at 279 nm and the emissions were measured at 325 nm. The light scattering measurements were made at 90° by setting the excitation and emission monochrometers at 365 nm and the incident and emission polarizers in a vertical position. We call this intensity $I_E$. To correct for fluctuations in the lamp, a partially reflecting quartz window was placed in line with the incident beam and the reflected incident light was measured with a separate photomultiplier. $I_E$ and the lamp intensity, L, were measured at the same time; the fluctuations in $I_E$ were corrected by measuring $I_E/L$.

Temperatures of the samples were monitored throughout the experiments with a thermistor probe imbedded in the sample jacket and a YSI telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Control measurements showed less than 0.25°C between the sample and jacket temperatures.

## RESULTS AND DISCUSSION

The florescence anisotropy of histone GRK, measured after the addition of salt, undergoes an initial rapid

increase which is followed by a further increase over a much longer period of time (5,6). The initial rapid increase, or fast step, is associated with a conformational change in the histone GRK monomer and dimerization. The subsequent slow increase, or slow step, is associated with aggregation. The total anisotropy change for the slow step is a function of both the concentration and the type of salt used, and, for phosphate, is a maximum at around 0.007M.

As can be seen from figure 1, the slow step is highly

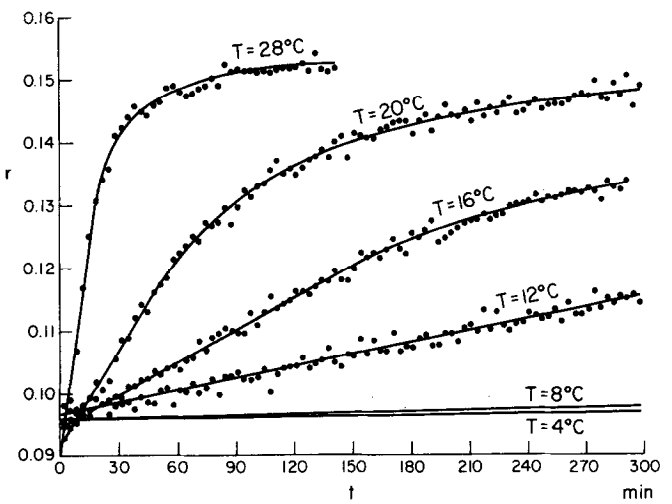


Fig. 1. Fluorescence anisotropy of 1.23 x $10^{-5}$ M histone GRK at various temperatures in 0.007 M phosphate, pH 7.4, as a function of time. Original data for 4°C and 8°C are omitted to show the difference between the two temperatures. The lines shown for these two sets of data were made by a least squares fit of the data.

dependent on temperature. At 28°C the slow step is almost completed after only 90 min. However, at 4°C the slow step is essentially stopped for at least a 5 hour period. To see if the aggregation could be stopped for a longer period of time, a sample of 8 x $10^{-6}$ M histone GRK in 0.007M

phosphate was kept at 0°C for 72 hours. Fluorescence anisotropy measurements were made at regular intervals and, as shown in figure 2, there was no slow step over that time period.

Figure 3 shows that the light scattering intensity

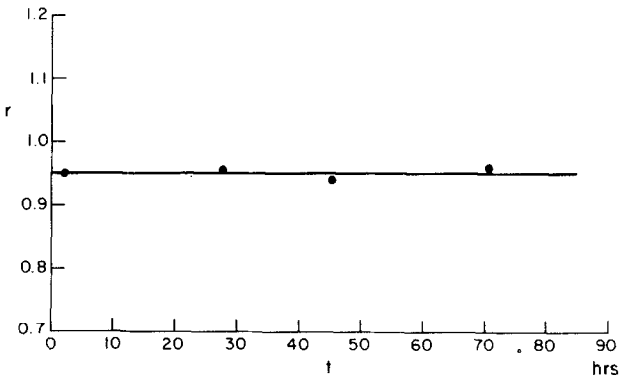


Fig. 2. Fluorescence anisotropy of 8 x $10^{-6}$ M histone GRK at 0°C in 0.007 M phosphate, pH 7.4. The anisotropy is constant over the time tested.

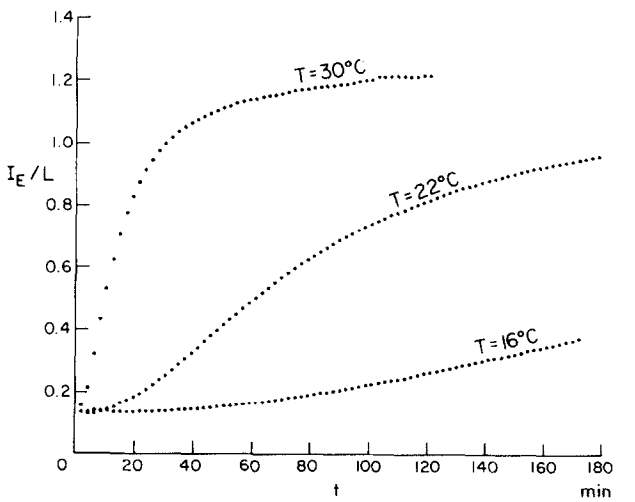


Fig. 3. Light scattering intensity at 365 nm and 90° of 1.23 x $10^{-5}$ M histone GRK in 0.007 M phosphate, pH 7.4, as a function of time.

during the slow step also decreases with decreasing temperature. The sigmoid nature of the curves is in accordance with theory, in that the scattered light intensity is proportional to $M^n$ where M is the molecular weight and n is greater than unity.

Both fluorescence anisotropy and light scattering measurements show that histone GRK aggregation can be controlled by changing the temperature. It is also possible to completely avoid this aggregation by lowering the temperature sufficiently. Physical studies may then be made on conformational changes in histone GRK molecules without the complications of aggregation.

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