

A CALORIMETRIC STUDY OF POLYGUANYLIC ACID AT NEUTRAL pH

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The structure of polyguanylic acid (poly G) at neutral pH has been studied by optical and calorimetric methods. It can be shown that diverging from earlier findings Poly G reversibly undergoes a cooperative thermal transition. Thermal denaturation curves are recorded at 253 nm as a function of the sodium ion concentration. The denaturation enthalpy of poly G in dilute aqueous solution is determined to 2.2 kcal/mole g. It is concluded, that the part of the ordered poly G structure, which gives rise to a temperature dependent cooperative transition, arises from stacking interactions of adjacent bases in the single strands.

1. Introduction

Our aim in these studies is to provide a ground work for subsequent study of naturally occurring nucleic acids. In order to interpret the thermodynamic parameters which characterize the helix formation of two polynucleotides correctly it has been necessary to study first the individual polymers. Little definitive work has appeared on the behaviour of polyguanylic acid in dilute aqueous solutions. It has been claimed that such studies are very difficult to carry out with poly G because of the occurrence of stable self-aggregated structures of the synthetic homopolymer [1–3]. Another difficulty of the investigation of poly G arose from the fact that earlier preparations of poly G contained only very short polynucleotide chains so that these investigations actually dealt with oligo G [4,5].

In this paper we will describe a thermally induced cooperative dissociation of single stranded poly G. The stacking enthalpy will be calculated from the calorimetric measurements.

2. Material and methods

Highly polymerized poly G (potassium salt, lot 7265204) was purchased from Boehringer (Mannheim,

West Germany). S_{20w} was determined with the help of an analytical ultracentrifuge in 10 mM salt solution to 25 at 25 deg. centigrade. The average degree of polymerization was calculated from the sedimentation coefficient to about 1000 giving a mean molweight of about 360 000. Three 25 ml portions of poly G solution, each containing 4 mg poly G/ml were dissolved in quartz distilled water and then dialyzed for 72 h against several changes (2 l each) of unbuffered NaCl solution with a sodium ion concentration of 10 mM · pH measurements were performed at room temperature with the aid of a Radiometer Type 22 pH meter. All chemicals were of reagent grade. The concentration of the polymer solution was determined via a calorimetric phosphorous analysis [6]. All solutions were once heated to 85 deg. centigrade for 10 min to force all poly G into a denaturated conformation and were slowly cooled to room temperature to allow poly G to renature. Heat denaturation experiments were carried out at the maximum absorbance at 253 nm with a Hitachi–Perkin Elmer Mod. 124 spectrophotometer. Changes of the temperature were registered with a Hewlett–Packard 2 FAM x-y recorder. The heating rate was 0.2 deg/min. 10 mM sodiumchloride solution was used as an optical blank. Solutions for spectrophotometric measurements were prepared by diluting 50 μ l of the solution used for the calorimetric measurements with 2 ml of the appropriate salt sol-

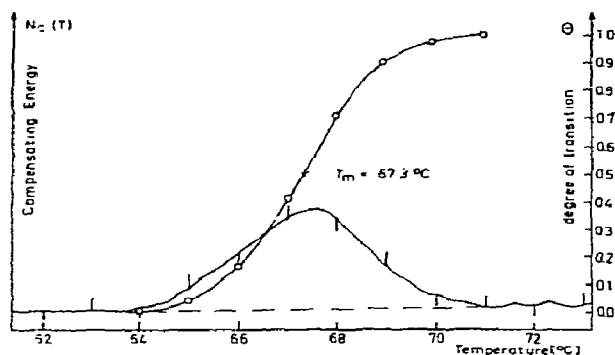


Fig. 1. Heat-induced helix-coil transition curves of poly G in 10 mM NaCl solution, pH 7.0. Left-hand ordinate: compensating energy (\times). Right-hand ordinate: degree of transition θ vs temperature (\circ).

vent. Calorimetric measurements were performed with the aid of a newly developed adiabatic twin calorimeter [7] as described in detail earlier [8].

3. Results

When heating highly polymerized poly G at pH 7 in NaCl solution the result shown in fig. 1 is obtained. The curve in the lower part of fig. 1 shows the temperature course of the electrical compensating energy needed to maintain adiabatic conditions in the calorimeter. The sigmoid curve with open circles (upper curve in fig. 1) represents the degree of transition calculated from the experimental calorimetric recorder tracing as a function of the temperature.

The area under the heat absorption peak is proportional to the enthalpy change accompanying the thermal transition of poly G. The temperature, at the maximum of the heat absorption peak is defined as the melting temperature T_m of poly G. One point of considerable interest is the finding that the compensating energy-temperature curve is rather flat at the first measurement (not shown in fig. 1). A second heating after cooling the solution slowly down to room temperature (40 deg. centigrade in 24 h) leads to the transition curve shown in fig. 1. A third reiteration of the cooling and heating process results in the entirely identical transition curve as shown in this figure. In fig. 2 the heat denaturation profile, obtained by plot-

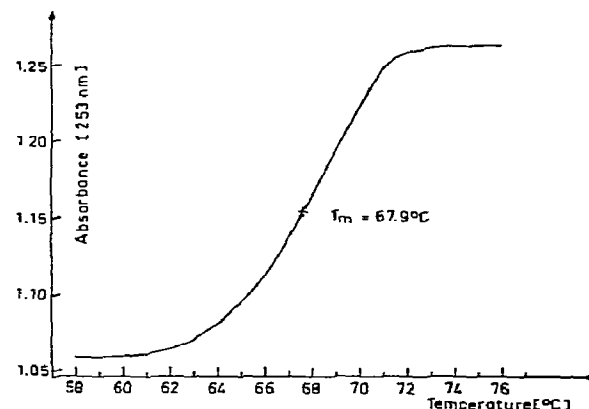


Fig. 2. Heat-induced helix-coil transition curve of poly G UV absorbance recorded at 253 nm as a function of temperature. Sodium ion concentration 10 mM, pH 7.0.

ting the absorbance of poly G at 253 nm against temperature is shown.

The ultraviolet absorption increases a few degrees lower compared to the temperature at which the heat content temperature function starts to increase, and the temperature interval attached to the transition in the optically measured curve is a little broader. This may be due to the lower polymer concentration in the solution used throughout the optical measurements. It is evident, however, that the temperature course of the two parameters (additional heat capacity and UV absorption) signify the same transition of poly G from an ordered single stranded structure to a randomly coiled state. The temperature, at which the absorbance increase reaches half of its final value, is identical with T_m calculated from the calorimetric transition curve within the limits of experimental error.

4. Discussion

The course of the experimental transition curves (cf. figs. 1 and 2) supports strongly the idea that highly polymerized poly G forms ordered single stranded structures which can undergo a temperature induced cooperative order-disorder transition under certain experimental conditions. A basic presupposition for this cooperative melting of poly G is the development of a

method to prepare of a pure product with a high degree of polymerization ($S_{20,w} = 23 - 25$). This product is now available. It is prepared by Boehringer, Mannheim, by the enzymatic polymerization of GDP by the action of *E. coli* polynucleotide phosphorylase [9]. The transition enthalpy for the single-strand helix-coil transition is measured at low ionic strength (0.01 M) with the help of an adiabatic scanning calorimeter. ΔH yields 2.2 ± 0.3 kcal per mole monomer. ΔS yields about 6.5 eu. Because the poly G single-strand helix is stabilized solely by stacking interactions [10] the transition enthalpy is equivalent to the stacking enthalpy of poly G. The value of 2.2 kcal per mole monomer obtained for poly G is of the same order of magnitude as the extrapolated value of the stacking enthalpy of 5'-GMP at pH 7 obtained by calorimetric methods [11].

The cooperative length can be calculated from the ratio of ΔH (van't Hoff) and ΔH (cal). ΔH (van't Hoff) may be calculated according to the following equation

$$\Delta H_{v.H.} = 4RT_m^2 \frac{d\theta}{dT}.$$

$d\theta/dT$ is the slope at the midpoint of the transition and it is taken from the curves in fig. 1 and fig. 2 respectively. $\Delta H_{v.H.}$ yields 136 kcal calculated from the UV melting curve and 260 kcal calculated from the calorimetric transition curve. The cooperative length calculated from the UV melting curve is 62 and 118 calculated from the degree of transition vs temperature in fig. 1. The difference of the two values arises from the great uncertainties in determining the slope at T_m when the transition is highly cooperative. The value of the stacking enthalpy is small compared to the stacking enthalpies so far published. But since the value of 2.2 kcal/mole G is the first directly measured value we assume that this value is correct and the values calculated from optical measurements are too high.

Conclusion: Poly G can undergo a heat induced single-strand helix-coil transition at low ionic strength in dilute aqueous solution. The transition is cooperative when poly G used in the measurements has a high degree of polymerization. The transition enthalpy is 2.2 ± 0.3 kcal/mole G. The cooperative length is about 120.

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