THE VISCOSITY CHANGE DURING THE POLYMERIZATION OF NUCLEOSIDE DIPHOSPHATES

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1. Introduction

During the polymerization of nucleoside diphosphates to polynucleotides – catalysed by polynucleotide phosphorylase – the viscosity of the reaction mixture increases, reaches a maximum and then decreases. Although this effect has already been observed by Beers in 1957 [1], it is still not clearly understood. Recently Harvey et al. [2] suggested that the maximum in the viscosity could be explained by the existence of a slowly dissociating complex between polynucleotide phosphorylase and growing polynucleotide chains. In this paper it will be shown that the decrease in viscosity can also be interpreted as a thermal degradation of the newly synthesized polynucleotides.

2. Materials and methods

ADP and UDP were products of P-L Biochemicals Inc., Milwaukee, Wisconsin. Polynucleotide phosphorylase obtained from *Micrococcus lysodeikticus* cells purchased from Mile Chemical Company, Elkhart, Indiana. The enzyme was extracted according to Brenninkmeyer and Veldstra [3]. The enzyme preparation so obtained is rather impure. Bentonite was obtained from Serva, Heidelberg and purified as described elsewhere [4].

Viscosities were determined with Ubbelohde capillary viscometers placed in a thermostat with a temperature constancy better than 0.02°. Absorbance was measured with a Zeiss PMQ II spectrophotometer, optical rotation with a Bendix automatic spectropolarimeter type Polarmatic 62.

Inorganic phosphate was determined by the method of Fiske and Subbarow [5].

3. Results and discussion

The reaction mixture for the polymerization of ADP consisted of 9 mM ADP, 0.8 mM $MgCl_2$ and 7.5 mM tris and was brought to pH 8.5 with KOH. After addition of the enzyme, the mixture was placed in a thermostat at 37° and the synthesis of poly A started immediately. The progress of the reaction was followed by measuring as a function of time: the specific viscosity, the absorbance at 260 nm, the change in optical rotation at 291 nm and the liberated inorganic phosphate (see fig. 1). The most remarkable features of this figure are that the viscosity decreases after having reached a maximum and that this maximum is reached about half an hour before the other measured quantities become constant. This can be explained by assuming that initially very large molecules are formed which for some reason are broken into fragments that are still polymeric. Of the four methods of measurements, only the viscosity is sensitive to the degree of polymerization. The decrease in viscosity is not coupled with a decrease in the amount of polymer synthesized.

To investigate whether the degradation was caused by ribonuclease, we carried out the synthesis in the presence and absence of bentonite, a clay which is known to adsorb ribonuclease and many other

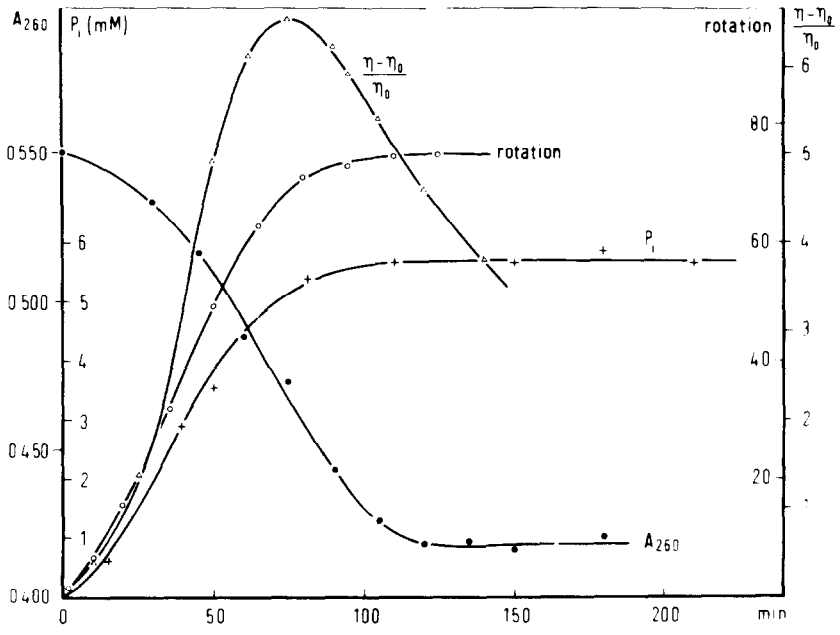

Fig. 1. The course of the synthesis of poly A determined by: change in specific viscosity, △ – △; decrease in absorbance at 260 nm, ● – ●; increase in optical rotation at 291 nm in arbitrary units, ○ – ○; release of inorganic phosphate, + – +.

enzymes [6]. The results are given in fig. 2. Although the viscosity becomes higher and the poly A chains accordingly larger in the presence of bentonite, there is still a maximum in the viscosity and so there must be another cause of degradation besides enzymic adsorbtion by bentonite.

To obtain more information about the change in molecular weight during the polymerization, we measured the concentration dependence of the reduced viscosity of the reaction mixture at the times A, B, C and D indicated in fig. 2. The relation between reduced viscosity and concentration c is given by [7]:

$$\frac{\eta - \eta_\circ}{\eta_\circ c} = BM^{\alpha} + B'M^{2\alpha}c \qquad (1)$$

in which η and $\eta_\circ$ are the viscosity of the solution and the solvent respectively, M is the viscosity average molecular weight and B, B' and α are constants dependent on polymer, solvent and temperature. When the left side of this equation is plotted versus c, straight lines are obtained with both intercept and slope increasing with increasing molecular weight

The results of these measurements are given in fig. 3. It appears that the highest molecular weight occurs at the beginning of the reaction. This means that the enzyme builds up a polynucleotide molecule completely before initiating the synthesis of another. The same mechanism was found by Klee and Singer [8] for the reverse reaction, the hydrolysis of a polynucleotide chain by polynucleotide phosphorylase. Of course, in our experiments also this enzyme degrades the polymer and it was shown by Peller and Barnett [9] that the action of polynucleotide phosphorylase alone could explain the observed maximum in viscosity. To eliminate this as the sole cause of the anomalous viscosity change, we added EDTA to the reaction mixture, since it is known [10] that polynucleotide phosphorylase does not work without Mg^{2+} ions. The synthesis – shown by a rise in viscosity – stopped immediately at any stage of the reaction when EDTA was added, but the degradation – as shown by a fall in viscosity – proceeded. We thus conclude that thermal degradation of the synthesized, initially very large poly A molecules, plays an important role in bringing about the viscosity maximum. This is in agreement with

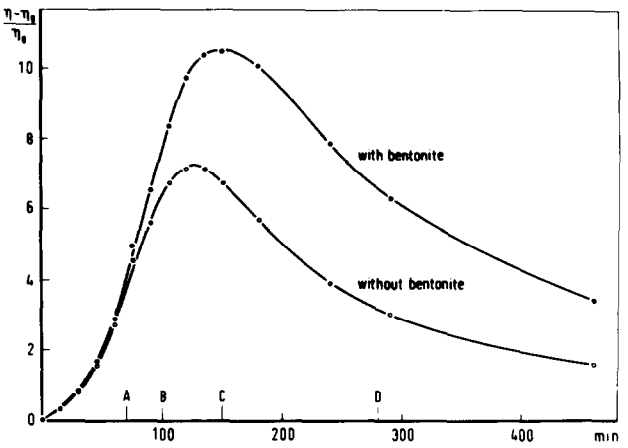

Fig. 2. Effect of bentonite on the viscosity observed during the polymerization of ADP.

experiments of Eigner et al. [11] and Fitt and Wille [12]. However, we can not exclude degradation by enzymes that may be present in our crude polynucleotide phosphorylase preparation and which are neither adsorbed by bentonite nor inhibited by EDTA. Experiments with UDP gave similar effects as described above for ADP.

The possibility of degradation of initially very large molecules is considered as less obvious by Harvey et al. [2] because they could not isolate polynucleotides of very high molecular weight. This, however, is quite understandable because they isolated the polynucleotide by gel filtration on Sephadex before determining the molecular weight. Evidently this method takes so much time that the degradation of the polynucleotide has taken place during isolation. However, it must be said that our experiments do not completely exclude the possibility they suggest viz. that the viscosity maximum is caused by the formation of a slowly dissociating complex between the enzyme and growing polynucleotide chains.

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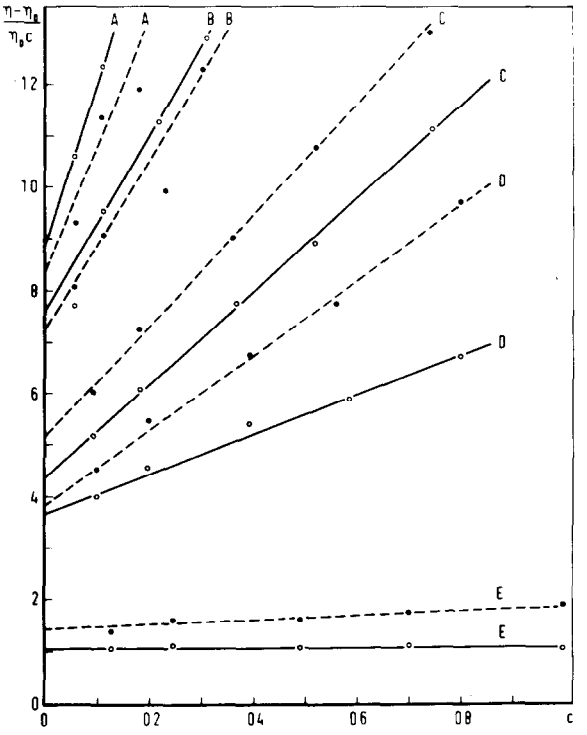

Fig. 3. Reduced viscosity at 25° of poly A at different times during the synthesis with (• – •) and without (○ – ○) bentonite. The concentration of poly A is expressed as fraction of the final concentration. The times at which samples were taken from the reaction mixture (A, B, C and D) are given in fig. 2. Sample E is taken after the reaction mixture stood 16.5 hr at 25°. The measurements of samples A and B are not very accurate because synthesis is going on and the viscosity is changing rapidly during these measurements.

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