UNPRIMED SYNTHESIS OF POLYMERS WITH RNA-POLYMERASE

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The induction of interferon can be stimulated by synthetic polyribonucleotides. A particularly active polymer is poly rI/poly rC [1]. In this context the activity of the alternating polymer poly r(I–C) is of interest.

Although the synthesis of poly r(I–C) by an unprimed polymerisation of ITP and CTP by DNA-dependent RNA-polymerase [E.C.2.7.7.6] purified according to Berg has been reported [2], the T_m-value of this polymer is not published. While repeating the reported synthesis, we obtained polymers with different T_m-values using different enzyme

Table 1
Synthesis of polymers[a].

Enzyme	Concentration of each ITP and ^{32}P-CTP[b] (mM)	Amount of enzyme (U/ml incubation sol.)	Isolated Polymer				Polymer
			Amount (A_{260}-units)	T_m (°C) (0.1 M Na^+)	Distribution of radioactivity[f] (cpm)		
					IPM	CPM	
Zillig	1.0[c]	220	1.6	47	4128	213	r(I–C)
Zillig	4.5	280	7.4	47	31177	1271	r(I–C)
Zillig	0.6	190	2.4	47	–	–	r(I–C)
GG	0.6	190	3.9	57	192	6735	rI/rC
GG	4.5	360	13.1	47 + 57	13372	29718	r(I–C) and rI/rC
GG	1.0[c]	200	1.9	47	–	–	r(I–C)
GG	4.5[d]	540	1.5	–[e]	–	–	poly rC
PC	0.6	190	3.3	57	326	6163	rI/rC
PC	4.5	360	13.5	47/57	–	–	r(I–C) and rI/rC

[a] Polymers were worked up as described [7] by removal of protein with isoamylalcohol/chloroform.
[b] Mn^{2+}-concentration: 3 mM.
[c] in presence of poly d(I–C); Mg^{2+}-concentration: 0.03 M.
[d] CTP only.
[e] After annealing with poly rI $T_m = 57°$
[f] Nearest neighbour analyses of the isolated polymers were carried out as described [8], except that aliquots of elution solutions of electrophoretograms were counted directly.

preparations. We therefore undertook an investigation to establish the nature of these polymers as well as the conditions for the synthesis of poly r(I–C).

To synthesize poly r(I–C) in an unambigious way we polymerized ITP and α-^{32}P-CTP on poly d(I–C) as template with DNA-dependent RNA-polymerase from *E. coli* purified according to Zillig [3]. The polymer isolated has a T_m = 47° (table 1). In the nearest neighbour analysis, all the radioactivity was associated with IMP. This polymer, therefore, was poly r(I–C). As can be seen from table 1, we could obtain this polymer in a *de novo* synthesis only with Zillig enzyme. This enzyme preparation contains only part of the σ-factor [4]. The Burgess enzyme containing σ-factor (GG-enzyme) and the enzyme without σ-factor (PC enzyme) [5], yielded a polymer with T_m = 57°. The nearest neighbour analysis showed this to be poly rI/poly rC. For an authentic sample of poly rI/poly rC we also found T_m = 57° under our conditions. The use of larger concentrations of substrates than recommended by Krakow yielded polymeric material with two T_m-values, 47° and 57°, respectively, which suggested the presence of both poly r(I–C) as well as poly rI/poly rC. This was confirmed by the nearest neighbour analysis, where only 1/3 of the radioactivity was transferred to IMP.

In an attempt to understand the differences observed between the various enzyme preparations, we observed that ^{14}C-CTP alone could be polymerized to poly rC by the GG-enzyme as well as the PC-enzyme. The isolated polymer was characterized by its sensitivity to KOH and pancreatic RNAase, and its ability to form a double-stranded polymer with poly rI having T_m = 57°. ^{14}C-ITP alone was polymerized more slowly with a considerable lag phase (fig. 1). The Zillig enzyme cannot use either CTP or ITP alone as substrate. [6].

Since the GG- as well as the PC-enzyme catalyze the synthesis of poly rI/poly rC, the σ-factor apparently is not involved in this reaction. It is therefore unlikely that the amount of σ-factor contained in the Zillig enzyme is responsible for the synthesis of poly r(I–C). There must be other differences between these enzyme preparations which direct the synthesis of either the alternating copolymer or the homopolymer pair. It is interesting that at higher substrate concentrations, even the GG- and the PC-enzyme can synthesize some of the alternating polymer.

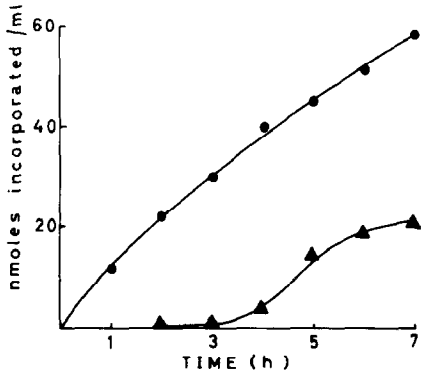

Fig. 1. Incorporation of ^{14}C-CTP (●——●) and ^{14}C-ITP (▲——▲) into acid precipitable material by Burgess GG- and PC-enzyme. Conditions as described in table 1.

Further work is in progress to determine the differences between the Zillig and the Burgess enzyme in their ability to catalyze the synthesis of different polymers using ITP and CTP.

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