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Synthesis and Properties of Poly(9-vinylpurine-6-thiol), a Polyvinyl Thiopurine Analog of Polyriboinosinic Acid

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The chemical synthesis and some of the properties of poly(9-vinylpurine-6-thiol), a polyvinyl derivative of 6-mercaptopurine, are described. Analogous to some other polyvinyl compounds, this polymer interacts with polyribonucleotides by formation of double-stranded complexes. Its antiviral and cytostatic properties are currently under investigation.

INTRODUCTION

Amongst a variety of compounds known to interfere with the metabolism in proliferating cells, antagonists of nucleic acids and their constituents have gained special significance since some of them exhibit considerable cytostatic and antiviral activities (*1, 2*). In particular, sulfur-containing analogs of purine bases, such as 6-mercaptopurine[2] and its derivatives, have been widely used as drugs in cancer chemotherapy (*3–8*). However, an acquired resistance (*5, 9, 10*) against MP and related compounds, induced by long-term application of the drugs, has often limited the success of treatment; and novel cytostatic agents continue to be developed in the hope that these drawbacks will be overcome.

Since polyvinyl analogs of nucleic acids exhibit a variety of interesting properties (*11*), e.g., selective activity against RNA tumor viruses, we were interested in investigating sulfur-containing analogs, particularly the polyvinyl derivative of MP for activity against transformed cells. As indicated in the scheme, polymerization of the monomer (**II**) does not lead to the compound in question (*12, 13*) but yields a cross-linked polymer of inadequate solubility. Sulphydrolysis of **III**, however, produces a polymer (**IV**) which is sufficiently soluble in water and interacts with nucleic acids by complex formation analogous to some of the polyvinyl compounds described earlier (*14–16*). Preliminary results suggest that **IV** is active against proliferating cells by a mechanism which is not yet well understood.

[1] Many fruitful discussions with George W. Kenner resulted in my interest in nucleotides, particularly as ligands in affinity chromatography. Our research on this group of compounds eventually led to the development of new chemotherapeutic agents.

[2] Abbreviations: MP, 6-mercaptopurine; polyVMP, poly(9-vinylpurine-6-thiol); polyU, polyribouridylic acid; polyC, polyribocytidylic acid.

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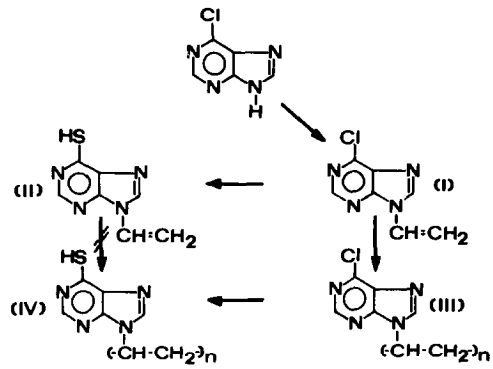

scheme of reactions

EXPERIMENTAL

PolyU and polyC were products obtained from Sigma Ltd., London. Poly(6-chloro-9-vinylpurine) was synthesized according to published procedures (*14*). All other chemicals were purchased from BDH and were of the highest purity available. Optical measurements were carried out on a Gilford 240 spectrophotometer.

Synthesis of poly(9-vinylpurine-6-thiol). Poly(6-chloro-9-vinylpurine) (300 mg) was dissolved in dimethylacetamide (1 ml) and cooled to 4°C. Potasssium hydrogen sulfide, 1.5 *M* (60 ml), freshly prepared by saturating a solution of KOH with hydrogen sulfide at 0°C, was added and the suspension was stirred at 4°C for 3 days. After neutralization with 1 *M* acetic acid, the reaction mixture was concentrated to dryness under reduced pressure (1 Torr, 40°C), redissolved in 1 *M* K_2CO_3 containing 1 m*M* dithiothreitol (DTT), and desalted by extensive dialysis against deionized water. PolyVMP was obtained by lyophilization of the aqueous solution. Yield 280 mg (91%);

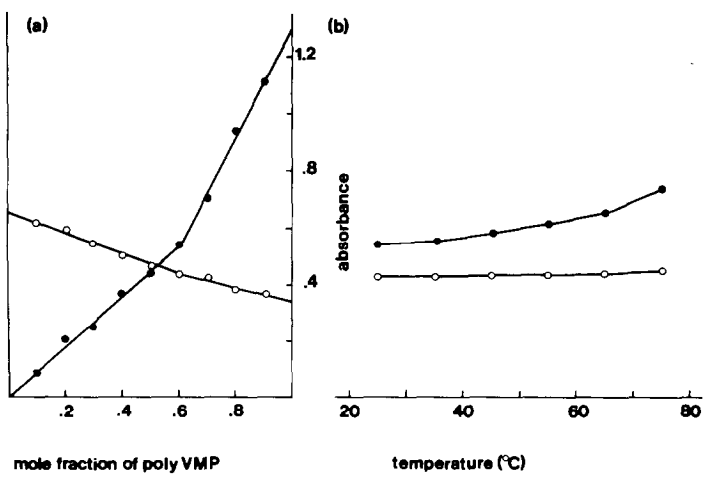

FIG. 1. Continuous variation diagram (a), and thermal dissociation profile (b) of the polyVMP polyC complex. (●——●) absorbance at 318 nm; (○——○) absorbance at 271 nm.

λ_{max} 318 nm, λ_{min} 258 nm (pH 8.1); ν: 1120 cm^{-1}; $(C_7H_6N_4S)_n$ calc. S/N, 0.572 found S/N, 0.563.

Complex formation of polyVMP with pyrimidine polyribonucleotides. PolyVMP and the polynucleotide (molar ratio 3 : 2) were dissolved in 10 m*M* sodium cacodylate buffer (pH 8.1) at a concentration of 0.5 mg of polyVMP per ml and incubated at 40°C for 60 min. The solution was then slowly cooled to 4°C (0.2°C/min) and extensively dialyzed against deionized water. The complex was isolated by lyophilization of the aqueous solution.

Continuous variation studies of complex formation. Mixtures of different molar ratios of the respective polymer (as indicated in Fig. 1) were prepared by diluting 10 m*M* standard solutions into 5 m*M* sodium cacodylate buffer (pH 8.1) containing 10 m*M* potassium chloride. After equilibration at room temperature for 48 hr, the absorbance at the λ_{max} of either polymer was measured in quartz cuvettes of 1 cm path length. Melting profiles of polymer complexes (temperature increase 0.5°C/min) were taken from mixtures of maximal hyperchromicity.

DISCUSSION

Some of the vinyl polymers introduced by Pitha and Ts'o (*17*) have recently shown interesting properties in both *in vivo* and *in vitro* systems. Not only do they interact with nucleic acids of complementary base composition by formation of rather stable complexes (*1*), but they have also selective antiviral activities on account of their ability to induce interferon (*18*) and to inhibit RNA-directed DNA polymerases of certain tumour viruses (*19–21*). Moreover, some derivatives markedly inhibit DNA-dependent DNA synthesis (*22*), transcription (*23*), and translation (*24–26*).

In this paper we describe the synthesis of polyVMP, the polyvinyl derivative of 6-mercaptopurine. Starting from 6-chloro-9-vinylpurine, which is easily obtained in a one-step vinylation reaction from 6-chloropurine (*17*), the polymer in question is prepared by sulphydrolysis after polymerization of the monomer in dimethylacetamide (*14*). Its water solubility is quite adequate, particularly in slightly alkaline solutions (pH not below 8.0). At lower pH values, an increase of solubility can be achieved by addition of an anionic detergent. On the other hand, strongly alkaline solutions may result in slow decomposition of the polymer. Upon exposure to oxygen, the compound loses some of its solubility, which is due to a certain amount of cross-linking via disulfide bonds. This oxidation is reversed by dithiothreitol.

As with some of the polyvinyl compounds described by Pitha (*11*), polyVMP forms complexes with pyrimidine ribonucleotide polymers, the absorbance of which is decreased when compared to their uncomplexed components. As indicated by continuous variation experiments (Fig. 1), this polymer shows less tendency for complex formation than some of the other vinyl polymers, thus resembling the behaviour of poly(9-vinyl-hypoxanthine) rather than poly(9-vinyladenine). In case of complex formation between polyC and polyVMP, a mixture of a molar fraction of 0.6 for polyVMP gives a maximum hyperchromic effect upon thermal denaturation (Fig. 1). Some of the properties of the latter complex, particularly its potential ability to induce interferon, are currently being investigated.

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Note added in proof: Recent results from our laboratory suggest that polyVMP inhibits the replication of avian myeloblastosis virus by a reversible interaction with its RNA-directed DNA polymerase.

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