The Synthesis and Enzymatic Polymerization of Nucleotides Containing Mercury: Potential Tools for Nucleic Acid Sequencing and Structural Analysis

(acetoxymercuration/mercaptans/*Escherichia coli*/polymerases)

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ABSTRACT A simple acetoxymercuration reaction for introducing covalently bound mercury atoms into nucleotides is described. The 5-mercuriacetate derivatives of UTP, CTP, dUTP, and dCTP, as well as the 7-mercuriacetate derivative of 7-deazaATP, have been prepared by this procedure and tested as substrates for nucleic acid polymerases. These nucleotides, in the absence of added mercaptan, are not polymerized and in most instances are potent enzyme inhibitors. However, conversion of these mercuriacetates to mercurithio compounds *in situ* by the addition of one of various mercaptans, yields nucleoside triphosphates that are excellent substrates for all polymerases tested: *Escherichia coli* and T7 RNA polymerases, DNA polymerase I of *E. coli*, DNA polymerase of avian myeloblastosis virus, and calf-thymus terminal deoxynucleotidyl transferase. By varying the mercaptan used to promote syntheses it is possible to access certain structural limitations in the enzyme's nucleoside triphosphate binding site. These mercurinucleotides appear to have a diversity of potential applications: (1) as heavy-atom reagents for crystallographic and microscopic studies; (2) as affinity probes for enzymes sensitive to sulfhydryl modification; (3) as steric probes of substrate-binding sites on enzymes; and (4) as reagents for forming covalent protein-polynucleotide complexes.

Numerous nucleoside analogues have been synthesized during the past decade, mainly for examination of their chemotherapeutic activity. One group of compounds that appears to have escaped attention is the metallonucleosides. Although likely to be of limited potential as chemotherapeutic agents, nucleotide, polynucleotide, and coenzyme derivatives of such compounds could provide a set of useful tools for various biochemical studies, a few of which are outlined below.

Recent electron microscope technology has progressed to the state where single heavy-atoms have been visualized (1-4). Although further refinements are needed (e.g., reduction of the background noise in support grids), microscopic sequencing of polynucleotides is potentially feasible. Metallonucleoside triphosphates that are able to replace their natural triphosphate counterparts quantitatively as *in vitro* substrates for nucleic acid polymerases would yield polymeric products suitable for such sequence analysis. The enzymatic polymerization of chemically stable metallonucleotides would

also circumvent most of the present problems of preparing heavy-atom polynucleotide derivatives. The current methods of attaching electron-dense atoms onto polynucleotides, involving chemical modification of preexisting DNA or RNA polymers (5, 6), often give unstable products, incomplete substitution, or fragmentation of the polynucleotide chain (7). The availability of a simple method of preparing polymers with specific heavy-atom base labels should make sequence analysis limited only by the resolving power of the electron microscope.

Metallonucleoside derivatives can also be readily used for analysis of protein or polynucleotide structures, as well as investigation of specific protein-polynucleotide complexes. In addition to the obvious utility of heavy-atom analogues as isomorphous replacements in crystallographic studies, appropriately designed compounds should provide electron spin resonance or affinity probes for investigation of macromolecular structure in solution.

With objectives like those outlined above in mind, we have undertaken the preparation of various metallonucleotides. In this report, we describe the synthesis and some properties of nucleotides containing covalently bound mercury atoms.

METHODS AND MATERIALS

The standard ribo- and deoxyribonucleoside 5'-triphosphates were purchased from P. L. Laboratories, while deoxyuridine and deoxyuridine 5'-triphosphate were obtained from Sigma. Radioactive nucleotides were products of New England Nuclear Corp. Tubercidin (7-deazaadenosine), the generous gift of Dr. G. Fonken, The Upjohn Co., Kalamazoo, Mich., was converted to the 5'-triphosphate as described (8).

The pyrimidine nucleoside triphosphates (in both ribo- and deoxyribo-series) and the 5'-triphosphate of 7-deazaadenosine were converted to the corresponding mercuriacetate compounds by the following general procedure. The nucleoside triphosphate (1 mmol) was dissolved in 5.0 ml of 0.5 M sodium acetate buffer (pH 5.0), and mercuric acetate (5.0 mmol) was added. The reaction mixture was then heated at 50° for 3 hr. The mixture, after cooling, was diluted 10- to 20-fold with water and applied to a column (110-ml) of DEAE-cellulose (bicarbonate form). The column was washed exhaustively with water (about 4 liters) to remove excess mercuric salts before the products were eluted with a linear gradient (2 liters) of triethylammonium bicarbonate (0-0.5 M), pH 7.5. Fractions containing the mercurinucleoside triphosphate were pooled and desalted by rotary evaporation. After several washes with methanol the nucleotide was dissolved in water, adjusted to pH 7.0, and stored at -20°. Conversion to the mercurinucleotide was in all cases quantitative, al-

Abbreviations: dUMP-HgX, dUTP-HgX, and CTP-HgX, the 5-mercuriacetate derivatives of dUMP, dUTP, and CTP. 7-DeazaATP-HgX the 7-mercuriacetate derivative of 7-deazaATP (tubercidin 5'-triphosphate). dUTP-HgSR, UTP-HgSR, etc. the 5-mercurithio derivatives of dUTP and UTP prepared by reacting the appropriate mercuriacetate compound with an excess of a mercaptan.

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though heating at 50° did cause a small degree of hydrolysis to the corresponding mono- and diphosphates (which were readily removed chromatographically). Spectral, chromatographic, and electrophoretic analyses were used to demonstrate that the mercuri-products are free from unmodified starting material.

Radiolabeled mercurinucleoside triphosphates were prepared as above with [²⁰³Hg]mercuric acetate (New England Nuclear Corp.). The standard conditions were: 5 μmol of nucleoside triphosphate in 0.05 ml of acetate buffer and 25 μmol of [²⁰³Hg]mercuric acetate (20 Ci/mol).

Mid-log-phase *Escherichia coli* B cells were obtained from Grain Processing Corp. *E. coli* DNA polymerase I (Fraction VII) was prepared as described by Jovin *et al.* (9) with the exception that the final Sephadex G-100 gel-filtration column was eluted with phosphate buffer (pH 7.0) containing no 2-mercaptoethanol. *E. coli* B RNA polymerase, purified by the procedure of Burgess (10), was the gift of R. Ludwig and R. Condit. Commercial preparations of this enzyme (Grand Island Biological Co.) gave similar results. The T7 RNA polymerase and T7 DNA were the generous gifts of Drs. E. Niles and W. Summers. Calf-thymus terminal deoxynucleotidyl transferase (11) was kindly supplied by Dr. R. Ratliff, Los Alamos, Calif.

The RNA-dependent DNA polymerase of avian myeloblastosis virus was prepared from a viral preparation supplied by Dr. J. Beard, Duke University, as described by Green *et al.* (12). For enzyme assays the virus solution was preincubated in 0.5% NP-40 detergent (Shell) at 4° for 30 min before addition to the polymerization mixture.

Calf-thymus DNA was obtained from Sigma (Grade V), while poly(rA)·oligo(dT) and oligo(dT)₆ were procured from Collaborative Research Corp. Poly[d(A-T)] was prepared according to the method of Richardson *et al.* (13). All other reagents were obtained from regular commercial sources.

RESULTS

The utility of metallonucleotides as biological probes would appear to be dependent on their ability to interact with, or be used by, the various nucleotide-binding proteins. Since one of our major objectives was to prepare metallocompounds that could be enzymatically polymerized, several structural criteria, based on the known substrate requirements of nucleic acid polymerases, were applied in designing the synthetic approach. First, it was felt that substituents should not be located on ring positions that would sterically, or otherwise, interfere with the normal Watson-Crick hydrogen-bonding potential of the bases. Compounds with blocked H-bonding potential, for example *N*₆-dimethyl dATP (14), are inactive as polymerase substrates. Secondly, no substituent would be introduced onto ring positions that would alter the normal "anti" nucleoside conformation found in natural polynucleotides (15, 16). Previous studies have shown that functional groups on the C-6 of a pyrimidine ring or on the C-8 of a purine ring lead to conformational changes that render such nucleotide derivatives unacceptable as substrates for Qβ replicase, *E. coli* DNA polymerase I, and polynucleotide phosphorylase (17, 18). These considerations effectively limit potential substituents to the 5-position of the pyrimidine base and the 7-position of the purine ring. Various nucleoside triphosphates with small substituents on either of these ring positions have been shown to be effective polymerase sub-

FIG. 1. The structure of 5-mercuriuridine (deoxyuridine), (I); 5-mercuricytidine (deoxycytidine), (II); and 7-mercuri-7-deazaadenosine, (III). The counterion, X, varies according to the ionic environment but is normally acetate, carbonate, or chloride. In the presence of mercaptans the compounds are converted to the corresponding mercurithio compounds (nucleoside-HgSR). The spectral properties of the carbonate salts at pH 7.5 are: I, $\lambda_{\max}$, 267 nm ($\epsilon = 10,100$), $\lambda_{\min}$, 242 ($\epsilon = 3600$); II, $\lambda_{\max}$, 275 ($\epsilon = 9200$), $\lambda_{\min}$, 255 ($\epsilon = 5600$); III, $\lambda_{\max}$, 278 ($\epsilon = 12,000$), $\lambda_{\min}$, 244 ($\epsilon = 2900$). Although the absorption maximum is unaffected by the nature of the counter ion present, the wavelength and extinction of the absorption minimum does vary somewhat.

strates (17, 19), provided that the substituent is not charged at the pH of the enzyme incubation.

In another communication† we describe properties of 5-methylmercurithio- and 5-trimethyllead pyrimidine nucleoside compounds. Since the synthetic routes to these compounds were rather extensive, simpler approaches were considered. Methods of preparing mercury-carbon organomercurials by hydrogen substitution have been known since the mid-1800s. These reactions ($RH + HgX_2 \rightarrow RHgX + HX$) proceed by way of electrophilic substitution and occur at the carbon of greatest electron density. For nucleoside compounds, the C-5 of pyrimidines, the C-8 of purines, and the C-7 of 7-deazapurines possess the highest electron density (20, 21). We therefore attempted direct mercuration of nucleoside derivatives in buffered (pH 5.0) aqueous solution using mercuric acetate, the most potent mercurating agent (22). Although no appreciable reaction occurred within 8 hr at room temperature (24°), 3 hr of heating at 50° resulted in quantitative yields of stable mercurated compounds. Mercurated nucleosides, unlike their phosphorylated derivatives, are extremely insoluble and precipitate from the reaction mixture. Mercuration of dCTP, CTP, dUTP, UTP, and 7-deazaATP yielded the 5'-triphosphates of the nucleosides shown in Fig. 1. The relatively mild reaction conditions should permit direct mercuration of polynucleotides and nucleotide coenzymes as well. Preliminary experiments with poly(uridylic acid) suggest that this is indeed the case.

Several investigators had shown previously that mercuric chloride and mercuric perchlorate form complexes in solution with the bases of nucleosides and polynucleotides (23, 24) resulting in spectral shifts similar to those of the nucleotides containing covalently bound mercury (see legend to Fig. 1). However, in contrast to the compounds described here, these complexes, involving ring nitrogen and amino groups (25), are completely reversed by the addition of agents that complex Hg^{++} , such as Cl^- and CN^- . Several factors could account for the absence of detectable covalent products in their experiments: (1) mercuric chloride and mercuric perchlorate

† Livingston, D. C., Dale, R. M. K. & Ward, D. C., submitted to *Biochemistry*.

TABLE 1. Polymerization of [^{203}Hg]dUTP-HgX by *E. coli* DNA polymerase I with poly[d(A-T)] as a template: Effect of mercaptans

Mercaptan added	[^3H]dATP + TTP		dATP + [^{203}Hg]dUTP-HgX		
	[^3H]dAMP polymerized (nmols)		[^{203}Hg]dUMP-HgSR polymerized		
			(cpm)	nmol	% Total
None	10.2	617	—	—	0
2-Mercaptoethanol	12.2	225,001	13.5	26.9	
Thioglycerol	11.9	221,000	12.9	26.6	
1-Thioglucoase	11.6	87,100	5.25	10.5	
Dithiothreitol	11.6	52,000	3.12	6.2	
Thioethylamine	12.2	38,800	2.34	4.7	
Thioacetic acid	12.5	34,500	2.07	4.2	
3-Thiopropionic acid	11.7	31,800	1.91	3.8	
Cysteine	12.3	22,100	1.34	2.7	
N-Ethylcysteine	12.1	5,690	0.34	0.7	
Thiourea	11.5	7,920	0.48	0.9	
Thiosuccinic acid	11.8	762	—	0	
No enzyme	0.004	823	—	0	

The standard reaction (0.1 ml) contained: Tris·HCl buffer, pH 7.4, 0.05 M; MgCl_2 , 0.01 M; poly[d(A-T)], 0.2 $A_{260\text{nm}}$ (0.03 mM); NTPs, 0.5 mM; [^3H]dATP and [^{203}Hg]dUTP-HgX (specific activities, 30,000 and 16,600 cpm/nmol, respectively); 0.01 M the appropriate mercaptan (all stock solutions were made at 0.1 M in 1 M Tris·HCl buffer, pH 7.4); and 2 units of *E. coli* DNA polymerase, Fraction VII. Reactions were incubated at 37° for 15 min, then terminated by the addition of 10% Cl_3CCOOH solution (100% Cl_3CCOOH -saturated sodium pyrophosphate-saturated trisodium phosphate-water; 1:1:1:7). The acid-precipitable material in each sample was collected on glass-fiber filters (Whatman GF/A) and washed with 30 ml of Cl_3CCOOH solution followed by 50 ml of cold 1.2 M 2-mercaptoethanol solution and 10 ml of ethylether. The filters were dried and counted in a liquid scintillation counter. The mercaptoethanol is essential to reduce a high, nonspecific adsorption of ^{203}Hg label to the filter. —, Indicates a value of zero.

are known to be less efficient mercurating agents than mercuric acetate (22) and (2) the reactions were usually done at room temperature over short time intervals. Since our attempts to mercurate nucleotides with mercuric chloride by prolonged heating at 50° were without success, it is not surprising that the earlier studies gave similar results.

The mercury-carbon bond of the mercurinucleotides is stable to both acidic and alkaline conditions. For example, the spectral and chromatographic properties of the 5-mercuriacetate derivative of UMP (UMP-HgX) showed no significant change on standing for several days at room temperature in solutions of 0.01 N HCl or 0.01 N NaOH. The bond is also stable to cold 10% trichloroacetic acid, and to excess sulfhydryl compounds.

Having determined that the mercurinucleotide compounds possess sufficient chemical stability to withstand the normal conditions of polynucleotide isolation, we turned our attention to the evaluation of their potential as polymerase substrates. Preliminary experiments tested the substrate properties of *E. coli* DNA polymerase I. With poly[d(A-T)] as a template and by monitoring the incorporation of [^3H]dAMP,

it was shown that dUTP-HgX was not polymerized (Fig. 2). However, upon the addition of excess 2-mercaptoethanol, substantial incorporation was obtained. Since up to 40% of the total dATP label could be polymerized in such reactions, it was apparent that the observed polymerization could not be due to contamination by unreacted dUTP. Although unlikely in view of the observations described above, it was possible that under the enzyme reaction conditions mercaptoethanol promoted the regeneration of nonmercurated dUTP, which was then being incorporated. To eliminate such a possibility, further experiments were done with [^{203}Hg]dUTP-HgX. The data in Table 1 demonstrate several significant points. First, the extensive polymerization of [^{203}Hg]dUMP-HgX in the presence of mercaptoethanol conclusively demonstrates that the mercurinucleotide is indeed a substrate. Furthermore, the data show that extremely bulky substituents (e.g., 1-thioglucoase) are capable of being added to the 5-position and yet give efficient synthesis. The fact that the mercaptan is also incorporated has been demonstrated by use of [^{14}C]cysteine to follow the reaction. Although nucleoside triphosphates with small ionizable functional groups (e.g., OH and SH) are not polymerized in their ionic form† (26), certain charged polymers can be utilized. These charged groups would, however, be expected to have little effect on the electronic character of the pyrimidine ring itself. All mercaptans, even those unable to promote significant incorporation of dUTP-HgX (e.g., thiourea and thiosuccinic acid), are fully capable of supporting maximal rates of DNA synthesis when dATP and TTP are used as substrates. The differential effect of the mercaptans is not due to differences in the rate at which the mercuritriphosphate is converted to the mercurithio-compound; the same profile of activity is obtained even when the triphosphate and mercaptan are mixed together 1 hr before addition to the enzyme reaction. The efficiency of the various mercaptans to effect polymerization must reflect steric or ionic properties of the enzyme in the region of the nucleotide-binding site. One can

TABLE 2. Addition of [^3H]TTP and [^{203}Hg]dUTP-HgX to oligo(dT)₆ by calf-thymus terminal transferase: Effect of mercaptans

Mercaptan added	[^3H]TMP incorporated (nmol)	[^{203}Hg]dUMP-HgSR incorporated		
		(cpm)	(nmol)	% Total
None	3.2	236	0.01	0.02
Methylmercaptan	8.9	164,000	7.3	14.6
Ethylmercaptan	9.0	155,000	6.9	13.8
2-Mercaptoethanol	9.2	531	0.02	0.04
Dithiothreitol	9.2	218	0.01	0.02
No enzyme	0.03	235	0.01	0.02

The standard reaction (0.1 ml) contained: Tris·HCl buffer, 7.4, 0.05 M; MgCl_2 , 0.01 M; oligo(dT)₆, 0.27 A_{260} (27 μM); [^3H]TTP or [^{203}Hg]dUTP-HgX (specific activities, 11,200 and 22,500 cpm/nmol, respectively), 0.5 mM; 0.01 M of the appropriate mercaptan [all stock solutions (0.1 M) made in 0.1 M Tris·HCl buffer, pH 7.4]; enzyme, 10 μg . Reaction mixtures were incubated at 37° for 1 hr. Time courses of polymerization (not shown) indicate that the reactions proceed in a linear manner for up to 2 hr. The acid precipitation and washing procedure was as described in Table 1.

also conclude from the relative incorporation of TTP and unlabeled dUTP-HgX in the presence of thiosuccinic acid that the maximum contamination of dUTP-HgX with dUTP is less than 0.20%.

In contrast to *E. coli* DNA polymerase I, calf-thymus terminal deoxynucleotidyl transferase has a much more restrictive substrate requirement. Of the mercaptans tested, only methylmercaptan and ethylmercaptan were capable of supporting the utilization of dUTP-HgX (Table 2).

In the presence of 2-mercaptoethanol, *E. coli* RNA polymerase utilizes all three ribo-mercurinucleoside triphosphates, either singly or in combination (Table 3). Similar results (not shown) were obtained with T7 RNA polymerase with T7 DNA as a template. As with DNA polymerase I, *E. coli* RNA polymerase activity with natural substrates is stimulated maximally by various mercaptans (Table 4). The polymerization of mercurinucleotides is, however, dependent on the mercaptan used. The inability of RNA polymerase to use the thioglucose derivative of UTP-HgX is in striking contrast to the facile polymerization of the corresponding deoxy compound by *E. coli* DNA polymerase I. This suggests that the steric properties of the nucleoside triphosphate-binding region are different in these two polymerases. A detailed study of the ability of DNA and RNA polymerases to use mercurinucleotides as a function of added mercaptan should provide insight into the substrate specificity of these enzymes.

TABLE 3. Utilization of mercurinucleoside triphosphates by *E. coli* RNA polymerase with calf-thymus DNA as a template

Substrates	2-Mercapto-ethanol	[³ H]GMP incorporated		
		(cpm)	(nmol)	% Total
[³ H]GTP, ATP, CTP, UTP	—	13,630	1.10	2.2
	+	35,600	2.87	5.8
[³ H]GTP, ATP, CTP	—	496	0.04	0.08
	+	1,240	0.10	0.2
[³ H]GTP, ATP, CTP, UTP-HgX	—	47	0.004	0.0
	+	29,200	2.36	4.7
[³ H]GTP, ATP, UTP	—	372	0.03	0.06
	+	992	0.08	0.16
[³ H]GTP, ATP, UTP, CTP-HgX	—	38	0.003	0
	+	27,900	2.25	4.5
[³ H]GTP, ATP, UTP-HgX, CTP-HgX	+	18,500	1.49	3.0
	—	248	0.02	0.04
[³ H]GTP, UTP, CTP	—	248	0.02	0.04
	+	868	0.07	0.14
[³ H]GTP, UTP, CTP, 7-deazaATP-HgX	—	25	0.002	0
	+	21,200	1.71	3.4
No enzyme	+	45	0.004	0

The standard reaction (0.1 ml) contained: Tris·HCl buffer, pH 8.0, 0.05 M; 0.01 M MgCl₂; 2 mM MnCl₂; calf-thymus DNA, 150 μg/ml; nucleoside triphosphates, 0.5 mM; RNA polymerase, 20 μg; and 2-mercaptoethanol (where indicated), 0.01 M. Specific activity of [³H]GTP, 12,400 cpm/nmol. The reactions, after incubation for 15 min at 37°, were terminated and washed with the 10% Cl₃CCOOH solution described in Table 1.

TABLE 4. Effect of mercaptans on the utilization of [³⁰³Hg]-UTP-HgX by *E. coli* RNA polymerase

Mercaptan added	[³ H]ATP + UTP	ATP + [³⁰³ Hg]UTP-HgX		
		[³⁰³ Hg]UMP-HgSR polymerized		
	[³ H]AMP polymerized (nmol)	(cpm)	(nmol)	% total
None	6.0	1,210	—	0
2-Mercaptoethanol	15.6	540,000	14.9	29.6
Thioglycerol	15.1	276,000	7.6	15.2
Dithiothreitol	14.8	45,400	1.20	2.5
Thiosuccinic acid	15.3	4,670	0.10	0.20
1-Thioglucose	15.5	4,000	0.075	0.15
No enzyme	0.003	1,200	—	0

The standard reaction (0.1 ml) contained: Tris·HCl buffer, pH 8.0, 0.05 M; MgCl₂, 0.01 M; poly[d(A-T)], 0.2 A₂₆₀ (0.03 mM); NTPs (as indicated), 0.5 mM (specific activities: [³H]dATP, 15,700 cpm/nmol; [³⁰³Hg]UTP-HgX, 26,400 cpm/nmol); 0.02 M mercaptan (all stock solutions were made at 0.2 M in 0.01 M Tris-HCl buffer, pH 8.0); and 20 μg of purified *E. coli* RNA polymerase. The reactions, after incubation at 37° for 15 min, were terminated and processed as described in Table 1. —, Indicates a value of zero.

Mercurinucleoside triphosphates are also efficient substrates for the RNA-dependent DNA polymerase of avian myeloblastosis virus (Table 5), for *E. coli* polynucleotide phosphorylase, and for calf-thymus DNA polymerase (data not shown). These results further demonstrate the diversity of polymerases that can utilize these compounds.

DISCUSSION

The acetoxymercuration reaction described in this report can be applied to the synthesis of numerous nucleoside compounds including 8-mercuri-derivatives of adenosine and guanosine. The simplicity of the method and the chemical stability of the resultant products should facilitate their utility as structural probes. Use of such compounds in conjunction with a spectrum of mercaptans provides a simple method for mapping steric properties of an enzyme's nucleo-

TABLE 5. Polymerization of [³⁰³Hg]dUTP-HgX by avian myeloblastosis virus DNA-polymerase

Substrate	2-Mercapto-ethanol	pmol of radioactive dNMP polymerized		
		(cpm)	(nmol)	% total
[³ H]TTP	—	17,400	112	0.22
[³ H]TTP	+	35,800	320	0.64
[³⁰³ Hg]dUTP-HgX	—	560	16.0	0.03
[³⁰³ Hg]dUTP-HgX	+	7,350	245	0.49
No enzyme	+	730	18.0	0.04

The reactions (0.1 ml) contained: Tris·HCl buffer, pH 8.3 0.04 M; MgCl₂, 0.01 M; poly(rA)·oligo(dT), 5 μg; [³H]TTP or [³⁰³Hg]dUTP-HgX, 0.4 mM (specific activities, 155 and 35 cpm/pmol, respectively); 0.01 M 2-mercaptoethanol (as indicated), and 40 μg of avian myeloblastosis virus protein. Incubation: 20 min at 37°. The reactions were terminated and processed as described in Table 1.

FIG. 2. Time-course of the DNA polymerase I reaction with poly[d(A-T)] template: Effect of 2-mercaptoethanol (0.01 M) on the polymerization of dUTP-HgX. [^3H]dATP, used in all reactions, had a specific activity of 5700 cpm/nmol. dATP + TTP: + mercaptan, $\circ$ — $\circ$; — mercaptan, $\bullet$ — $\bullet$. dATP + dUTP-HgX: + mercaptan, Δ — Δ ; — mercaptan, $\square$ — $\square$.

tide-binding site. In addition, perfusing protein crystals with these compounds in the presence of an appropriate mercaptan should provide a method of preparing isomorphous crystal derivatives, particularly if the mercurated compound is an effective enzyme inhibitor. Mercuration of commercially available nucleoside di- and triphosphates that contain a methylene group between the β or γ phosphates should readily provide one group of such inhibitors.

The facile polymerization of mercurated nucleotides by various polymerases provides a method for preparing specifically-mercurated polynucleotides for electron microscopic examination. Synthetic polymers that contain mercury atoms in defined and repeating sequences are needed to test (1) the fidelity of mercurated nucleotides in the replication or transcription process, (2) the chemical stability of the mercury bonds in an electron beam, and (3) whether, with the aid of image superimposition, one can deduce the basic nucleotide repeat frequency. Our observations on the utilization of pyrimidine deoxynucleotides containing large substituents on the 5-position of the base suggests that nucleotides possessing two heavy atoms per base can be polymerized, thus further enhancing the possible microscopic detection of individual nucleotide residues.

Mercurated polynucleotides incubated with proteins containing free sulfhydryl groups should be capable of forming covalent polynucleotide-protein complexes by sulfhydryl exchange. The possibility of using covalent ferritin-polynucleotide complexes for electron microscopic localization of specific regions in polymer duplexes is currently under investigation.

In vivo studies with the mercurinucleosides described here may be somewhat limited because of potential problems with

sulfhydryl exchange. We are, however, preparing nucleosides that contain small, nonexchangeable, mercury substituents (e.g., base-HgCH₃) that will not have this drawback. Should these compounds, like 5-ethyldeoxyuridine (27), be utilized by bacterial and mammalian cells, numerous other applications (e.g., isolation of early viral replicative intermediates and localization of regions of genetic recombination) become apparent.

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