CHARACTERISTIC OPTICAL ROTATORY DISPERSION SPECTRA OF THIRTEEN DINUCLEOSIDE PHOSPHATES WITH MERCURIALS

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Ultraviolet absorption spectra have been widely used to characterize nucleic acids and their constituent bases, nucleosides and nucleotides because the method is simple and rapid, and requires only a small sample. However, two sequence isomers of dinucleoside(3'→5')-phosphate (DNP) have very similar ultraviolet absorption spectra. Ultraviolet rotatory dispersion (ORD) is a method with advantages similar to those of ultraviolet absorption and the ultraviolet rotatory dispersion spectra of the sequence isomers usually differ more than do their ultraviolet absorption spectra. The difference is still insufficient, however, to be useful for distinguishing the isomers, except in the case of ApG[1] and GpA (Warshaw and Tinoco, 1965; 1966). In view of the drastic and differential changes produced by mercuric ion in the ORD spectra of deoxyribonucleic acid (Cheng, 1965), ribonucleic acid and homopolyribonucleotides (Cheng and Honbo, 1965), it seemed worthwhile to determine whether the ORD spectrum of a DNP with a mercurial can be used for identifying the dinucleoside. The present communication reports such an investigation for thirteen DNP with three mercurials. The results indicate that the ORD spectrum in the presence of mercuric chloride (and to a lesser extent propyl mercuric chloride) is useful for identifying a dinucleoside phosphate.

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[1] The DNP adenylyl-(3'→5')-guanosine is abbreviated ApG, etc.

Materials and Methods

ApG, GpA, ApU, UpA, GpU, UpG, GpC, CpG, and CpU were obtained from California Biochemical Corporation, and ApC, CpA, ApA, and CpC from Gallard-Schlessinger Chemical Manufacturing Company. Methyl mercuric acetate and propyl mercuric chloride were obtained from Merck and Company. The ORD of a DNP was measured at 0.5×10^{-4} M using a solution of 0.1 M sodium perchlorate/0.01 M sodium acetate, pH 5.70, as medium. To obtain the ORD spectrum of the DPN in the presence of a mercurial, a sample was prepared by a 100-fold dilution of a stock solution of the mercurial (2.0×10^{-2} M) with an aliquot of the DNP solution; the sample was allowed to stand at 27° for about 30 minutes before the measurement. All ORD spectra were obtained at 26° with an automatic scanning Cary Model 60 recording spectropolarimeter, using cell paths of 0.1 and 1 cm for the wavelength ranges below 200 mμ and of 220-400 mμ, respectively. Data below 220 mμ were less accurate because of higher noise of the instrument.

Results

Our results are summarized in Fig. 1. Mercuric chloride, methyl mercuric acetate, and propyl mercuric chloride each produced distinct changes in the ORD of DNP. These changes are clearly attributable to the specific action of mercurials,[2] since the molarity of the mercurial compound was only 1/2000 of that of sodium perchlorate. The alterations produced by mercuric chloride differed qualitatively from those by propyl mercuric chloride and methyl mercuric acetate and were usually more severe. Propyl mercuric chloride in every case caused modifications similar to but of a greater degree than those produced by methyl mercuric acetate.

In the presence of mercuric chloride, CpG, ApG, GpC, GpA and CpA exhibit negative Cotton effects, while CpU, CpC, UpG, UpA, GpU, ApU, ApA

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[2] In the absence of knowledge of their identity, the active forms of the mercurials for producing these changes are regarded in this paper as the forms added to DNP solutions.

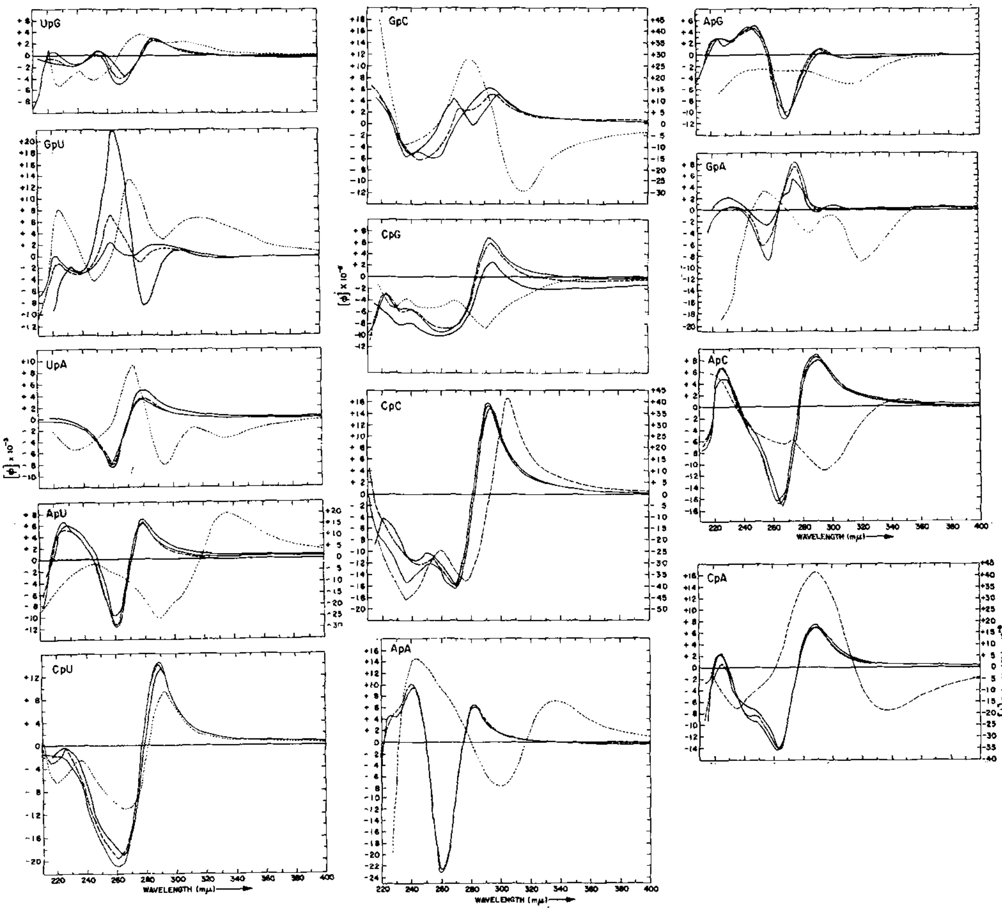

Fig. 1. Optical rotatory dispersion spectra of dinucleoside phosphates with mercurials. Dotted line, with $HgCl_2$; dashed line, with methyl mercuric acetate CH_3HgAc; heavy solid line, with propyl mercuric chloride light solid line, without mercurial.

and ApC show positive Cotton effects. The two wavelengths of peak and trough of the first Cotton effect are sufficient to distinguish any one curve from the others. The large differences in amplitude further facilitate the differentiation. Thus, any one of these thirteen DNP can be readily distinguished from the other twelve by the ORD spectrum of the DNP with $HgCl_2$.

TABLE I

Optical Rotatory Dispersion of 13 Dinucleoside Phosphates With and Without Mercurial[a]

	λ_{p1} (mμ)	λ_{t1} (mμ)	$\phi_{p1} - \phi_{t1}$[b]	λ_{p2} (mμ)	$\phi_{p2} - \phi_{t1}$[b]	λ_{t2} (mμ)
UpG	292	269	1.0	≃256	0.67	–
+ $HgCl_2$	≃ 310	≃ 298	<0.05	283	0.18	250
+ C_3H_7HgCl	291	273	0.80	254	0.51	–
GpU	290	272	1.0	258	1.2	240
+ $HgCl_2$	320	295	1.5	273	4.4	248
+ C_3H_7HgCl	304	282	4.0	262	13.0	237
CpG	292	261	1.0	237	0.25	232
+ $HgCl_2$	–	292	–	270	0.30	253
+ C_3H_7HgCl	296	261	0.77	238	0.13	232
GpC	293	≃260	1.0	–	–	–
+ $HgCl_2$	–	316	–	278	4.6	235
+ C_3H_7HgCl	299	282	0.44	269	0.38	240
ApG	293	269	1.0	245	1.3	235
+ HgCl	–	316	–	248-270	0.22	–
+ C_3H_7HgCl	294	270	0.92	249	1.3	230
GpA	277	258	1.0	233	0.55	–
+ $HgCl_2$[c]	–	321	–	300	0.48	285
+ C_3H_7HgCl	301	292	0.07	275	0.33	257
UpA	279	259	1.0	–	–	–
+ $HgCl_2$[d]	314	295	0.60	274	1.44	235
+ C_3H_7HgCl	280	260	1.2	–	–	–
ApU	279	262	1.0	227	1.0	–
+ $HgCl_2$	335	290	2.5	247	1.35	–
+ C_3H_7HgCl	280	260	0.90	229	0.88	–
CpA	288	263	1.0	225	0.76	–
+ HgCl	–	335	–	288	2.74	235
+ C_3H_7HgCl	290	264	0.97	225	0.75	–
ApC	289	262	1.0	224	0.92	–
+ $HgCl_2$	348	294	0.49	272	0.20	–
+ C_3H_7HgCl	292	266	1.0	226	0.87	–
CpU	289	261	1.0	–	–	–
+ $HgCl_2$	293	267	0.58	234	0.24	220
+ C_3H_7HgCl	291	265	0.90	225	0.53	–
CpC	291	269	1.0	252	0.13	236
+ $HgCl_2$	305	276	2.5	260	0.41	236
+ C_3H_7HgCl	294	270	0.94	250	0.16	242
ApA	282	261	1.0	241	1.11	229
+ $HgCl_2$	335	300	0.51	243	0.77	–
+ C_3H_7HgCl	282	261	1.0	241	1.0	–

[a] Experimental conditions detailed in Materials and Methods section.

[b] All amplitudes of an ORD spectrum of a dinucleoside phosphate with or without mercurial are expressed on the basis that the amplitude of first Cotton effect of the dinucleoside phosphate without mercurial is set equal to 1.0.

[c] Also has a taller peak at 255 mμ with the amplitude $\phi - \phi_{t2} = 0.43$.

[d] Also has a shallower trough at 334 mμ with $\phi_{p1} - \phi = 0.17$.

The wavelengths and amplitudes of three extrema (peak and trough of first Cotton effect and the following extremum) are sufficient to differentiate the ORD spectra of these DNP with propyl mercuric chloride, except that the three curves of CpA, CpU and ApC show only minor differences. The contrast produced by propyl mercuric chloride for the isomer pair of UpG and GpU was remarkable.

Any two sequence isomers of DNP show comparatively low contrast either in their ORD spectra in the presence of methyl mercuric acetate or in their ultraviolet absorption curves in the presence of any of these three mercurials.

Discussion

On the basis of these results, we propose that the ORD of a DNP with $HgCl_2$ obtained by the aforementioned procedure can be used to identify the dinucleoside. The procedure requires 1.8×10^{-4} millimoles of DNP and can be completed within an hour. Since the samples used for the measurements of ultraviolet absorption and ORD of the DNP can be reused to obtain the ORD spectrum of the DNP with $HgCl_2$, information on the kind of bases contained in the sample can also be obtained without increasing the sample size. Should an additional amount of 1.8×10^{-4} millimoles of the DNP be available, it is advisable to obtain the ORD curve for the sample in the presence of propyl mercuric chloride as well. Multiple confirmation is recommended because an optical property can in principle be exhibited by more than one structure. Besides, each of these additional measurements can be completed within 0.5 hr of additional time; the ORD baseline for the perchlorate buffer was found unchanged by the presence in the perchlorate buffer of 2×10^{-4} $\underline{M}$ $HgCl_2$ or propyl mercuric chloride.

To facilitate the identification of DNP, data on the wavelengths and amplitudes of the peak and trough of first Cotton effect, and also of the following two extrema in most cases, are given in Table I for DNP, DNP with $HgCl_2$, and DNP with propyl mercuric chloride. To eliminate possible errors from such factors as DNP concentration and the rotation scale of the spectropolarimeter, all amplitudes for either a DNP or the DNP plus a mercurial, were expressed in terms of the amplitude of the first Cotton effect of the DNP without a mercurial.

That sharp contrast between two ORD spectra of sequence isomers was observed for all five pairs of isomers studied with $HgCl_2$ demonstrates the power of the method to distinguish base sequence. It would seem that the ORD spectra with $HgCl_2$ and with propyl mercuric chloride will be useful for identifying trinucleoside diphosphates as well as three DNP not yet studied (UpC, GpG and UpU).

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