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Di- and Oligonucleoside Phosphates of Adenine and Cytosine. Electrochemical Study of Reduction, Adsorption, and Association

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Abstract: Similarities and differences in the mechanisms for electrochemical reduction of a series of interrelated adenine- and cytosine-based dinucleoside monophosphates and adenine oligonucleotides have been considered in terms of their structure, including kinetics of intervening chemical reactions, associations in solution, and adsorption and association at the charge-transfer interface. Every adenine and cytosine moiety in a dinucleoside monophosphate is reduced. A maximum of three rings is reduced in the adenine oligomer series; the ease of reducibility decreases and levels off with increasing chain length. The reduction products are similar to those of the free bases except as affected by the rates of intervening chemical reactions. At pH 3.5, the overall protonation rates for the carbanions derived from adenine and ApA are 2×10^5 and 5.5×10^5 sec^{-1}, respectively. The deamination rate of the adenine ring is less than 1 sec^{-1}. The dinucleoside phosphates are very strongly adsorbed, suggesting the presence of more adsorption sites and possible ring interactions in the stacked configuration. They seem to be adsorbed with rings planar to the electrode surface; in the case of the adenine oligomers, a maximum of four rings can be thus oriented. Most of the compounds exhibit pH-dependent and concentration-dependent self-association in the adsorbed state, even from bulk solution concentration as low as 0.05 m*M*; the principal mode of association is probably that of vertical overlapping or stacking of bases.

As a part of a systematic investigation of nucleic acid components by electrochemical methods,[1-11] a study of di- and oligonucleoside phosphates of adenine and cytosine has been initiated for the following reasons. (a) Adenine (6-aminopurine) and cytosine (4-amino-2-hydroxypurine) belong to the most important biologically occurring purine and pyrimidine derivatives.[12] (b) Dinucleoside phosphates, which are the lowest chain-length derivatives capable of base–base intramolecular interactions, *e.g.*, stacking, similar to those operative in the corresponding polymers (polynucleotides and nucleic acids), are the most suitable models for initiating study of the effects of incorporation of a base in a polynucleotide chain on its electrochemical behavior. (c) There is a need to determine which electrochemical techniques can provide reliable data for such relatively complex compounds. (d) The gap should be filled between the extensive electrochemical investigations of purines, pyrimidines, and their nucleosides and nucleotides[15] on the one hand and those of polynucleotides and nucleic acids[16] on the other.

A variety of electrochemical technics are being utilized to obtain information concerning the behavior of

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(2) B. Janik and P. J. Elving, *J. Amer. Chem. Soc.*, **92**, 235 (1970).
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(12) Only adenine[2] and cytosine[2,6,9,13,14] of the major nucleic acid bases and their nucleosides and nucleotides[2,3,6,13,14] are reducible under normal polarographic conditions at the dropping mercury electrode (dme); consequently, oligomers containing these bases are expected to be reducible.
(13) B. Janik and E. Paleček, *Arch. Biochem. Biophys.*, **105**, 225 (1964).
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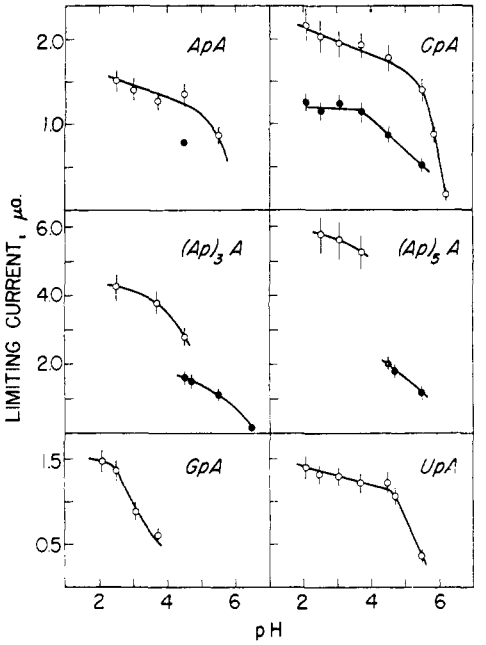

Figure 1. Variation with pH of limiting current of polarographic waves of di- and oligonucleotides in chloride and McIlvaine buffers at 25°. (○) Normal wave or sum of normal wave and prewave; (●) prewave. Concentration, m*M*: ApA, 0.051; CpA, 0.061; $(Ap)_3A$, 0.047; $(Ap)_5A$, 0.043; GpA, 0.052; UpA, 0.057. Estimated maximum experimental error in current measurement is indicated.

nucleic acid components in solution, *e.g.*, structure, conformation, and association including stacking, and their behavior at the electron-transfer interface, *e.g.*, adsorption, association in the adsorbed state, and chemical reactions accompanying electron transfer. The study of adsorption phenomena is particularly relevant since many biological reactions in the living cell involve adsorption of nucleic acids at charged boundaries such as membrane or ribosome surfaces.

Electrode Mechanisms and Processes[17]

Nature of the Electroactive Species. Presence of the protonated form of di- and oligonucleotides containing the adenine and/or cytosine moieties as the reducible species is supported by the facts that (a) the polarographic wave in the pH region where it begins to disappear has a kinetically controlled current component, suggesting combination of the nonprotonated form with protons, and (b) pH plots of the dc polarographic wave limiting current (i_l) (Figure 1), of the ac polarographic peak current (Δi_s), and of the main cyclic voltammetric peak current (i_p) exhibit sigmoidal current decrease, centering 1 to 3 pH units higher than pK_a for the protonated oligomer.[18] Such current decrease is characteristic of the polarographic reduction of the acid form of a conjugated acid–base system; it has been observed for adenine, cytosine, and their nucleosides and nucleotides.[1,2,6,13]

N(1) in adenine and N(3) in cytosine are the initial reduction sites, as well as the most likely protonation sites in the simple nucleotides.[2,3,13] Protonation may be expected to occur at the same sites in di- and oligonucleotides; however, equilibria may exist involving nonprotonated, monoprotonated, and polyprotonated forms.[19]

Current Control. Drop-time dependencies of i_l, temperature coefficients, and *i–t* curves recorded at limiting currents indicate essentially diffusion control with possible kinetic control at higher pH.

Based on log *i*–log *t* and $i_p/v^{1/2}$–*v* plots, and ac polarographic data, the current controlling factors for ApG and GpA differs, at least quantitatively, from those of the other compounds. Catalytic hydrogen reduction (presence supported by i_p–*v* relation[20a]) is a complicating factor, *e.g.*, close proximity of the polarographic wave and background discharge due to marked positive shift in potential of the latter. Adsorption, though not clearly indicated by cyclic voltammetry, is likely involved, since ac polarograms of ApG and GpA indicate strong adsorption nearly up to background discharge.

Reduction Processes. Adenine itself is initially reduced electrolytically at the 1,6 N=C bond at a potential sufficient also to reduce the 3,2 N=C bond; reduction of each double bond is a two-electron process involving hydrogen addition. Hydrogenation of the 1,6 N=C bond produces a *gem*-diamine centered on the C(6), which results in elimination of NH_3 and regeneration of the 1,6 N=C bond producing 3,4-dihydropurine, which is reduced as formed. Consequently, electrolytic reduction of adenine is a six-electron process under conditions where the compound is completely reduced. At the dme, however, the intervening deamination does not occur to an appreciable extent and the reduction is a four-electron process.[1,4,7] The electrode processes in the reduction of adenine nucleosides and nucleotides are essentially the same, being centered in the pyrimidine ring but modified by association of the compound in solution, its orientation and adsorption at the interface, and the changes in electron density and other characteristics of the two pyrimidine ring N=C bonds.[2,3,8]

Cytosine both at the dme and under conditions of exhaustive electrolysis undergoes a three-electron reduction, involving two-electron hydrogenation of the 3,4 N=C bond (corresponds to 1,6 N=C bond in adenine), deamination to form 2-hydroxypyrimidine, and one-electron reduction of the latter to a free radical which dimerizes.[6,7,9] Cytosine nucleosides and nucleotides are similary reduced with modifications of the type indicated in the previous paragraph.[3,6,13,14]

(17) (a) Experimental results are summarized in the subsequent Experimental Section. Some preliminary results obtained in the present investigation were previously reported;[17b] this included presentation of Figures 4, 9, and 10 of the present paper. Additional data on CpC, CpU, and CpG are given in ref 6; in order to facilitate comparison, some of the latter data have been included in the present figures and tables. Tables and summaries of additional dc and ac polarographic and cyclic voltammetric data for the various compounds indicating the effects of concentration, temperature, and scan rate are available from the authors. To save space, nucleotides will be occasionally referred to as monomers, dinucleoside monophosphates, *e.g.*, ApA, as dimers (or dinucleotides), and oligonucleotides, *e.g.*, $(Ap)_2A$, as oligomers. (b) P. J. Elving and J. W. Webb, "The Purines: Theory and Experiment," E. D. Bergmann and B. Pullman, Ed., Israel Academy of Sciences and Humanities, Jerusalem, 1972, pp 371–391.

(18) (a) Values of pK_a are available only for several oligonucleotides;[18b,c] however, they are expected to approximate pK_a for the constituting nucleosides;[18d] (b) B. Janik, "Physicochemical Characteristics of Oligonucleotides and Polynucleotides," Plenum Press, New York, N. Y., 1971; (c) "Handbook of Biochemistry," 2nd ed, H. A. Sober, Ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1970; (d) J. C. Maurizot, J. Blicharski, and J. Brahms, *Biopolymers*, **10**, 1429 (1971).

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Table I. Variation with pH of Total Ac Polarographic Data for Di- and Oligonucleotides[a]

Compd	Depression				Peak I		Peak II	
	pH	i_{min},[b] μA	pH	ΔE,[c] V	pH	$-E_s$,[d] V	pH	$-E_s$,[d] V
ApA	3.7	0.15			2.5–5.5	1.08 + 0.030 pH	2.5–5.5	1.16 + 0.060 pH
$(Ap)_3A$	3.5	0.20	4.5	0.72	4.5–6.5	1.33	2.5–4.5	1.15 + 0.060 pH
	5.5	0.30						
$(Ap)_5A$			4.5	0.96	4.5–5.5	1.34	2.5–4.0	1.19 + 0.052 pH
ApG	3.1	0.26	3.7	0.89	2.0–4.5[e]	1.15 + 0.047 pH		
GpA	4.5	0.27	4.5	0.87	2.0–4.0[e]	1.06 + 0.068 pH		
					4.0–5.5	1.57 + 0.067 pH		
ApU	5.3	0.26	5.5	0.60	2.0–6.5	1.16	2.0–5.5	1.15 + 0.061 pH
UpA					2.0–5.0	1.14	2.0–5.0	1.13 + 0.066 pH
ApC	4.5	0.27	~3	0.64	2.5–5.5[f]	1.08 + 0.022 pH	2.0–5.8	1.14 + 0.060 pH
			5.5	0.56				
CpA	3.7	0.22	3.7	0.55	2.5–4.5[g]	0.99 + 0.040 pH	2.0–4.5	1.14 + 0.061 pH
	6.2	0.18	5.5	0.63	6.0–8.0	1.70 − 0.089 pH		
CpC	4.5	0.21	3.7	0.80	2.0–4.5	1.08 + 0.019 pH	2.0–4.2	1.21 + 0.041 pH
			5.5	0.73	4.5–5.7	1.18	4.2–8.0	1.02 + 0.108 pH
					5.7–8.0	1.72 − 0.094 pH		
					9.0–10.0[h]	1.11 − 0.019 5H		
CpG	5.5	0.31	3.5	0.80	2.0–3.7	1.11 + 0.015 pH		
			7.7	0.82	3.7–9.6[i]	1.24 − 0.018 pH		
CpU	4.5	0.25	4.5	0.60	2.5–5.5	1.13 − 0.007 pH		
					5.5–9.2[i]	1.30 − 0.038 pH		

[a] Data for *ca.* 0.05 m*M* solutions in chloride and McIlvaine buffers of 0.5 *M* ionic strength at 25°. [b] Data are the maximum values of i_{min} taken from i_{min} *vs.* pH plots, where the difference in current from the background electrolyte base current was measured at the potential of the lowest part of the depression. Blank spaces indicate that the maximum i_{min} value could not be estimated from the plot. A second value in the case of $(Ap)_3A$, ApC, and CpC is for the second maximum observed on the i_{min}–pH plot. The maximum errors in estimating i_{min} are ±0.3 pH and ±0.01 μA. [c] The width of the depression, ΔE, is the difference between potentials at the 0.05-μA level above the minimum of the depression or above that of a pit or step if the latter occur. Data are the maximum widths obtained from ΔE–pH plots; blank spaces indicate that ΔE could not be estimated from such plots. The maximum errors in estimating ΔE are ±0.3 pH and ±50 mV. [d] Maximum deviations from the equations are 25 mV for peak I and 15 mV for peak II. [e] Data for a shoulder (*cf.* Figure 2). [f] E_s at pH 6.2 is 50 mV more positive. [g] E_s–pH plot is curved between pH 4.5 and 6.0 with a maximum E_s of −1.19 V at pH 5.1 to 5.4. [h] Ammonia and carbonate buffers. [i] McIlvaine and carbonate buffers.

Reduction of the base moiety (adenine and/or cytosine) of the di- and oligonucleotides studied occurs by mechanisms which are essentially those for the constituent base or its nucleosides and nucleotides; *e.g.*, current–pH and potential–pH variations obtained by different electrochemical techniques are of the same type for each homologous series.[2,6,13] The shift in $E_{1/2}$ with pH is due to the presence of the protonated form as the reducible species.

The dimer and trimer *n* values (ApA, 6.0; $(Ap)_2A$, 12.0; ApU, 3.6; UpA, 4.2; ApC, 6.2; CpA, 6.2), although not exact due to the approximations made and other influences on the *I* values (*e.g.*, adsorption, association, and experimental error), are in good agreement with the four-electron and two-electron reduction of each adenine[4] and cytosine[6] moiety, respectively; similarly, each cytosine in CpC undergoes a two-electron reduction.[6] Low *n* values for ApA may be at least partially due to uncertainty in extrapolating the *I–C* plot to zero concentration;[21] an *n* of 6.7 was obtained at the 0.1 m*M* level.

In addition, *I* for $(Ap)_2A$ is about three times that of ApU, indicating reduction of all three adenine rings. The similar *n* values of 12.0, 12.7, and 13.0 for $(Ap)_2A$, $(Ap)_3A$, and $(Ap)_5A$, respectively, may indicate that a maximum of three adenine rings are reduced per oligomer. The degree to which intramolecular association and adsorption affect *I* is, at present, uncertain; however, $E_{1/2}$ and the extent of adsorption also show a leveling effect with increasing chain length (*cf.* subsequent discussion).

(21) Since the values of *I* are strongly concentration dependent, the dependence being different for each derivative, the *n* values were calculated from the *I* values extrapolated to zero concentration.

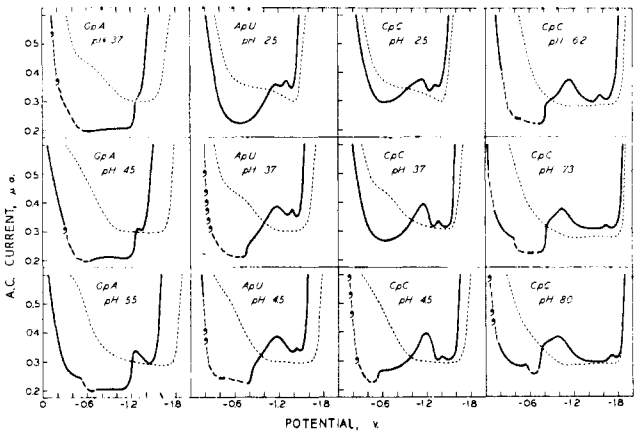

Figure 2. Total ac polarograms for *ca.* 0.05 m*M* GpA, ApU, and CpC at various pH values in McIlvaine buffer at 25°: dashed lines denote distorted current oscillations; dotted lines denote background electrolyte base current.

The dc wave and cyclic voltammetric peak of ApG and GpA seem to have no counterpart to the ac polarographic shoulder (Figure 2), which is slightly below background current in 0.05 m*M* solutions. However, variation with pH of the shoulder potential (Table I) suggests that a faradaic component might be present below *ca.* pH 4; above pH 4, the shoulder gradually changes into a desorption peak. The failure of analogous cytosine dimers (CpG and CpU) to undergo ac polarographic reduction is consistent with the much lower reversibility of cytosine derivatives compared to adenine ones.[10] Similar to the monomer, dimers containing cytosine as the only reducible moiety are polarographically reducible[6] up to about pH 9.

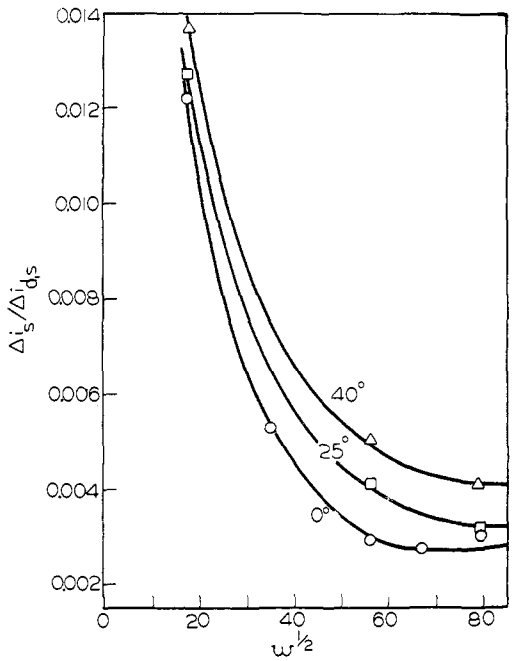

Figure 3. Variation of the ratio of $\Delta i_s \Delta i_{d,s}$ with frequency and temperature for ApA, 0.0936 m*M*, in pH 3.5 McIlvaine buffer (ionic strength, 0.1 *M*).

Kinetics of Accompanying Chemical Reactions. Cyclic voltammetry and ac polarography indicate reduction of dimers and oligomers based on adenine and cytosine to be an overall irreversible process. Based on earlier studies, the observed irreversibility is due to protonation of the carbanion produced by a two-electron reduction of the equivalent 1,6 purine or 3,4 pyrimidine N=C bond and, in the case of adenine and cytosine, also to subsequent deamination.[5,8,22] The only anodic peak, produced by guanine-containing dimers at potentials *ca.* 1.2 V more positive than that of the main cathodic peak, is due to oxidation of the guanine reduction product (*cf.* Experimental Section).

The rates of protonation, k_p, for purine, pyrimidine, and cytosine were calculated from the limiting value at high frequencies of the $\Delta i_s/\Delta i_{d,s}$ *vs.* $\omega^{1/2}$ plot (*e.g.*, Figure 3), where Δi_s is the observed ac in-phase current, $\Delta i_{d,s}$ is that expected for a reversible, diffusion-controlled process, ω is $2\pi f$ (f = frequency of applied alternating voltage), and t is the drop-time in seconds.[23]

$$k_p^{1/2} = \frac{1}{1.387 t^{1/2}}\left[\left(4.78\frac{\Delta i_{d,s}}{\Delta i_s}\right)^{1/2} - 1\right]^2 \qquad (1)$$

Since adenine and ApA produce only one ac in-phase peak, calculations of k_p were based on the total four-electron process per ring (simultaneous reduction of 1,6 and 3,2 N=C bonds).[24] Since two reductions and, therefore, two protonations occur, the resulting k_p values may be equivalent to an overall rate; k_p for adenine at pH 4.5 is between those calculated for the two individual two-electron reduction steps in purine. The values, however, provide a suitable basis for comparison.

As expected, the rate for adenine increases as pH decreases. However, k_p for ApA exhibits an unexpected negative temperature coefficient.[24] Since the purine k_p temperature coefficients are positive, the negative value may be related to the lower degree of association of ApA at higher temperature (*cf.* subsequent discussion).

The rates of deamination for the adenine series could not be determined by cyclic voltammetry as they were for the cytosine series;[6] $i_p/ACv^{1/2}$ ratios increase exponentially with increasing v for adenine, ApA, and $(Ap)_3A$, indicating that adsorption at the interface is a predominant factor[20] (*cf.* discussion of adsorption). The rate of deamination of CMP, which occurs slowly but to a measurable extent, was calculated[6] to be about 3 sec^{-1}. Since deamination of adenine does not occur to an appreciable extent at the dme, the rate is estimated to be less than 1 sec^{-1}.

Effect of Primary Structure on Reduction. 1. Effect of Heterocyclic Base. One effect of changing a heterocyclic base in a dinucleoside is manifested in $E_{1/2}$; *e.g.*, difficulty of reduction at pH 3.4 and 25° (Table II) increases in the order ApA < GpA ≤ ApG < UpA ≤ ApU < ApC < CpA. Since (a) the heterocyclic bases in these dimers can be in stacked conformation,[25] (b) the electronic interaction of both bases cannot be effectively mediated through the ribose–phosphate–ribose backbone, and (c) association of dimers would be minimized at 25° (moreover, tendency of the individual bases to associate decreases: adenine > guanine > cytosine > uracil[26]), the order of decreasing reducibility reflects at least partially the stacking interactions between the two bases.

At pH 2.5, 3.4, and 4.0, ApA $E_{1/2}$ values are significantly more positive and those for ApC, CpA, and CpC more negative than the mean $E_{1/2}$ for the dinucleoside series; ApG and GpA are more positive than the mean at pH 3.4 and 4.0. On generalizing the observed dme behavior, CpC is less easily reducible than ApA, similar to the corresponding base–nucleoside–nucleotide series,[3] and incorporation of cytosine decreases the ease of reducibility of the dimer even if the other moiety is adenine. At the hmde, an opposite effect is observed; *e.g.*, dimers containing cytosine or cytosine and adenine are more easily reducible than those containing adenine as the only reducible base; this is probably due to relatively strong adsorption of the reduction product. Because of strong adsorption of depolarizer, guanine-containing dimers are reduced at more negative potentials.

Effectiveness of purine and pyrimidine moieties in shifting the background discharge to more positive potential decreases in the order: G > A > C > U.

2. Effect of Chain Length. In the adenine oligomer series, ease of reducibility decreases in the order: adenine > ApA > $(Ap)_2A$ > $(Ap)_3A$, adenosine > $(Ap)_5A$ > AMP (Table III). In the cytosine series,

(22) S. J. Pace and P. J. Elving, private communication.

(23) G. H. Aylward and J. W. Hayes, *Anal. Chem.*, **37**, 195, 197 (1965).

(24) The calculated protonation rate constants, $k_p \times 10^{-5}$ sec^{-1}, are as follows (pH and temperatures in parentheses): adenine, 2 (3.5, 0°), 0.1 (4.5, 0°); ApA, 5.5 (3.5, 0°), 3 (3.5, 25°), 2 (3.5, 40°). For purine (pH 4.2–5.2; 25°), k_p following reduction at the 1,6 and 3,2 N=C bonds is 4.9×10^3 and 1.5×10^4 sec^{-1}, respectively;[8] for cytosine and pyrimidine (pH 4; 25°), k_p, corresponding to the first purine reduction stage, is approximately 5×10^4 sec^{-1} for both compounds.[6,8]

(25) (a) S. I. Chan and J. H. Nelson, *J. Amer. Chem. Soc.*, **91**, 168 (1969); (b) J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, **25**, 465 (1967); (c) *ibid.*, **25**, 481 (1967); (d) B. W. Bangerter and S. I. Chan, *J. Amer. Chem. Soc.*, **91**, 3910 (1969).

(26) P. O. P. Ts'o, M. P. Schweizer, and D. P. Hollis, *Ann. N. Y. Acad. Sci.*, **158**, 256 (1969).

Table II. Variation with pH of $E_{1/2}$ for Polarographic Reduction of Di- and Oligonucleotides[a]

Compd	Prewave pH	Prewave $-E_{1/2}$, V	Normal wave pH	Normal wave $-E_{1/2}$, V
ApA	4.0–4.5	0.935 + 0.076	1.0–2.0	0.920 + 0.127 pH
			2.5–5.5[b]	0.980 + 0.070 pH
$(Ap)_2A$	4.5–6.4	1.035 + 0.055 pH	2.5–4.5	1.060 + 0.053 pH
$(Ap)_5A$	4.0–5.5	1.115 + 0.039 pH	2.5–4.0	1.120 + 0.043 pH
ApG			1.0–2.5	0.945 + 0.100 5H
			2.5–4.5[c]	1.050 + 0.057 pH
GpA			2.0–4.0[c,d]	1.035 + 0.059 pH
ApU			2.0–5.5	0.985 + 0.080 pH
UpA			2.0–5.5	0.980 + 0.080 pH
ApC	2.0–5.5	1.010 + 0.059 pH	2.0–5.5	1.055 + 0.064 pH
CpA	2.0–5.5	1.015 + 0.058 pH	2.0–5.5	1.075 + 0.061 pH
CpC	2.5–5.5	1.040 + 0.041 pH	2.5–6.0	1.085 + 0.056 pH
			4.6–8.1	0.800 + 0.104 pH
			8.1–10.6[e]	1.225 + 0.051 pH

[a] Data for *ca.* 0.05 m*M* solutions in chloride and McIlvaine buffers of 0.5 *M* ionic strength at 25°. The maximum deviation from the equations given is 10 mV. [b] $E_{1/2}$ in pH 4.0 acetate buffer is 25 mV more positive but fits the equation for pH 4.9 and 5.9 acetate solutions. [c] $E_{1/2}$ in pH 4.0 acetate buffer fits the equation. [d] $E_{1/2}$ in pH 1.0 chloride buffer is 25 mV more positive. [e] Carbonate buffer. $E_{1/2}$ in pH 9.1 ammonia buffer is 65 mV more positive.

Table III. Dc and Ac In-Phase Polarography of the Adenine Nucleoside Series[a]

Compd	pH	Concn, m*M*		Dc $-E_{1/2}$, V	Dc Slope, mV	Dc i_d, μA	Dc I	Dc $D^c \times 10^6$, cm²/sec	Ac[b] $-E_s$, V	Ac i_s, μA	Ac i_s/concn
Adenine	3.4	0.1		1.231	50	1.2	8.7	12.8	1.30	0.135	1.53
		0.5		1.274	76	6.4	9.5	15.3	1.32	0.56	1.12
	4.5	0.5		1.344	84	6.8	10.2	17.7	1.38	0.62	1.24
	5.0	0.1		1.372	69	1.3	9.9	16.6	1.43	0.13	1.30
Adenosine	3.4	0.1		1.272	48	1.1	7.8	10.3	1.33	0.14	1.40
	5.0	0.1		1.375	47	0.89	6.5	7.2	1.43	0.08	0.80
AMP	3.4	0.1		1.289	43	1.1	8.2	11.4	1.35	0.12	1.20
		0.26		1.293	50	2.6	7.2	8.8	1.33	0.28	1.08
	5.0	0.1		1.409	45	0.9	6.5	7.2	1.44	0.07	0.70
ApA	3.4	0.084		1.256	29	1.4	12.0	6.1	1.31	0.20	2.4
	4.5	0.094		1.307	46	1.4	11.1	5.3			
$(Ap)_2A$	3.4	0.091		1.267	18	2.17	17.3				
$(Ap)_3A$	3.4	0.087		1.271	14	2.09	17.3				
$(Ap)_5A$	3.4	0.078		1.277	19	1.95	18.1				
ApU	3.4	0.094		1.273	56	0.74	5.6	5.3	1.33	0.07	0.71
	5.0	0.070		1.366	52	0.48	5.0	4.2	1.40	0.05	0.72
UpA	5.0	0.078		1.362	44	0.64	6.0	6.1	1.40	0.06	0.82
ApC	3.4	0.10	Wave I	1.238	27	0.60	4.3	7.4[d]	1.27	0.01	0.15
			Wave II	1.297	31	0.78	5.6		1.32	0.135	1.4
	5.0	0.078	Wave I	1.320	36	0.56	5.1	7.0[d]	1.35	0.035	0.45
			Wave II	1.394	39	0.47	4.4		1.42	0.10	1.3
CpA	5.0	0.084	Wave I	1.320	41	0.53	4.5	7.2[d]	1.36	0.011	0.13
			Wave II	1.409	49	0.60	5.2		1.44	0.092	1.1

[a] All data taken at 0° in McIlvaine buffers at comparable depolarizer concentration (0.08 to 0.10 m*M*). [b] Ac signal: 50 Hz; 3.5-mV rms amplitude. [c] $D^{1/2} = I/607n$. [d] Based on the sum of I values for waves I and II.

cytidine and CMP are more easily reducible than cytosine, reflecting the consequence of adding a ribose and ribosophosphate to a heterocyclic ring which is the reduction site.[6,13]

Di- and oligonucleotides are expected to approach the interface with base planes essentially perpendicular to the surface. The right hand screw conformation of dinucleotides[27] allows the reduction sites in all adenine and/or cytosine moieties to approach the surface at the same time with no previous substantial rotation or reorientation of bases; rotation may be, however, required in long-chain oligo- or polynucleotides. Consequently, adenine di- and short oligonucleotides are generally more easily reducible than adenosine or AMP because of the extra energy needed for orientation of alternate stacks[2,26] in the case of the latter two compounds; *i.e.*, extra energy would be needed for reduction of those adenine moieties which approach the interface with their pyrimidine rings oriented away from the surface.

The increase in $E_{1/2}$ with the oligomer chain length probably reflects the increase in intra- and intermolecular association and/or adsorption at the interface, which cause reduction to be more difficult. The potential increment per nucleoside moiety added decreases with increasing chain length, indicating that these effects level off at higher chain lengths; moreover, only three adenine moieties seem to be reduced in $(Ap)_3A$ and $(Ap)_5A$. The concept of a leveling off effect is supported by the fact that the ease of reduction of poly(adenylic acid), poly(A), ($E_{1/2}$ = −1.286 V)[28] is about the same as that of $(Ap)_5A$ (−1.292).

(27) P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, *Biochemistry*, **8**, 997 (1969).

(28) B. Janik, R. G. Sommer, and A. M. Bobst, *Biochim. Biophys. Acta*, **281**, 152 (1972).

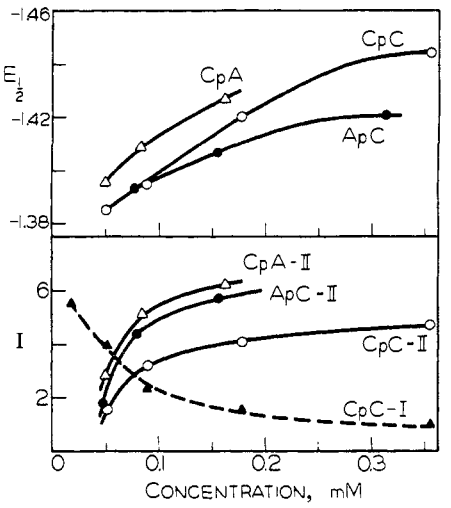

Figure 4. Variation of $E_{1/2}$ and I with concentration of dinucleoside monophosphates in pH 5.0 McIlvaine buffer (ionic strength, 0.13 M) at 0.5°. Roman numerals refer to the waves of each compound.

Another effect which makes reduction more difficult as the chain length increases is film formation at the electrode surface (*cf.* subsequent discussion).

3. Effect of Sequence. ApC is slightly more easily reduced than CpA; although the difference in $E_{1/2}$ is only 20 mV, it is consistent over the concentration range examined (Figure 4). ApU and UpA have similar ease of reducibility, but UpA tends to associate more strongly in the adsorbed state (based on greater potential range covered by its quadrature current depression). The stronger desorption maxima for CpA and ApU in the quadrature current component at −1.2 V indicate that more of these molecules are adsorbed than their respective conformers.

Adsorption and Association at the Interface

Although the polarographic limiting current is essentially diffusion controlled, involvement of adsorption phenomena at the solution interface in the case of each dimer and oligomer is strongly indicated. What is adsorbed and how strongly depend on the base composition, oligomer chain length, electrode potential, and solution pH.

Adsorption of the individual bases, nucleosides, and nucleotides has been discussed.[1,2,6,15,29,30] Adsorption of the dimers and oligomers is generally similar to that of the monomers except that it is greater due to the increased number of adsorption sites: π bonding involving aromatic rings, specific bonds to individual oxygen and nitrogen atoms, and chemical binding involving, for example, negatively charged phosphate groups and metal complex formation.[15]

The lack of an adequate theoretical model allows only qualitative discussion of the extent of and relative differences in adsorption of the dimers and oligomers. There are, thus, some unavoidable but nonetheless real uncertainties in some of the interpretations, particularly in respect to conclusions drawn from ac polarography about the adsorption of faradaically active substances. In dealing with moderately complex molecules involved in complex and interdependent phenomena, it is virtually impossible to say anything about these species without making some assumptions. One purpose of the present investigation is to provide initial but systematic electrochemical data on an interrelated group of di- and oligonucleotides, which will provide a foundation for future studies. The conclusions drawn are, admittedly, tentative but are based on all of the available electrochemical and other pertinent data.

Adsorption of Depolarizer. Ac polarograms indicate adsorption of all electroactive species and also of compounds nonreducible under the experimental conditions used, *e.g.*, depression of base current below that of background at potentials more positive than the reduction peak.[31]

Location of the steep ac depression and distorted current oscillations (when the latter appear) near the potential of zero charge (ecm) of the dme, and the increase in magnitude of the depression with increasing pH (up to pH 3.4 to 5.5), *i.e.*, with increasing solution concentration of uncharged species, suggest that this strong adsorption involves an uncharged portion of the molecule.[2]

As the potential becomes more negative, gradual desorption occurs (*cf.* the broad desorption peak between −1.0 and 1.3 V); some compounds are readsorbed at still more negative potential, at least above pH 6 to 7, as indicated by a second depression. Actually, the adsorbed molecules probably reorient themselves; different orientations at the interface can be expected for positively and negatively charged or uncharged species at potentials around −0.6 and −1.6 V.

The magnitude of the depression reaches a maximum at about 0.08 mM bulk solution concentration for the dimers, 0.05 mM for the oligomers, and 0.5 mM for adenine. These concentrations represent the effective maximum surface concentrations of the adsorbing molecules and are inversely proportional to the effective area occupied. Even if adsorption equilibrium is not completely attained, the trend in the data indicates that a dimer occupies a larger adsorption surface area than the sum of constituent monomers, which suggests that both heterocyclic rings in a dimer are involved in adsorption. At the ecm, the bases themselves are adsorbed planar to the surface.[30,32] If a generally similar orientation of bases in a dimer were assumed, these data would further suggest dimer adsorption with rings planar to the electrode surface, which would depend on the relative energies of adsorption and of base orientation in the solution species. The similar maximum surface concentrations for each oligomer also suggests that there is a maximum number of rings which can be thus oriented.

The depression for adenine-containing dimers and oligomers varies less with pH than that for cytosine-containing dimers (Figures 2 and 5; *cf.* Figures 7 and 8 of ref 6), which would indicate less involvement of species with differing electric charge in the adenine case.

(29) V. Vetterl, *J. Electroanal. Chem.*, 19, 169 (1968); *Biophysik*, 5, 255 (1968).
(30) V. Vetterl, *Collection Czech. Chem. Commun.*, 31, 2105 (1966).
(31) B. Breyer, T. Biegler, and H. H. Bauer, "Modern Aspects of Polarography," T. Kambara, Ed., Plenum Press, New York, N. Y., 1963, pp 50–57; B. Breyer and H. H. Bauer, "Alternating Current Polarography and Tensammetry," Interscience, New York, N. Y., 1963.
(32) B. E. Conway and R. G. Barradas, *Electrochim. Acta*, 5, 319 (1961); R. G. Barradas and B. E. Conway, *Electrochim. Acta*, 5, 349 (1961).

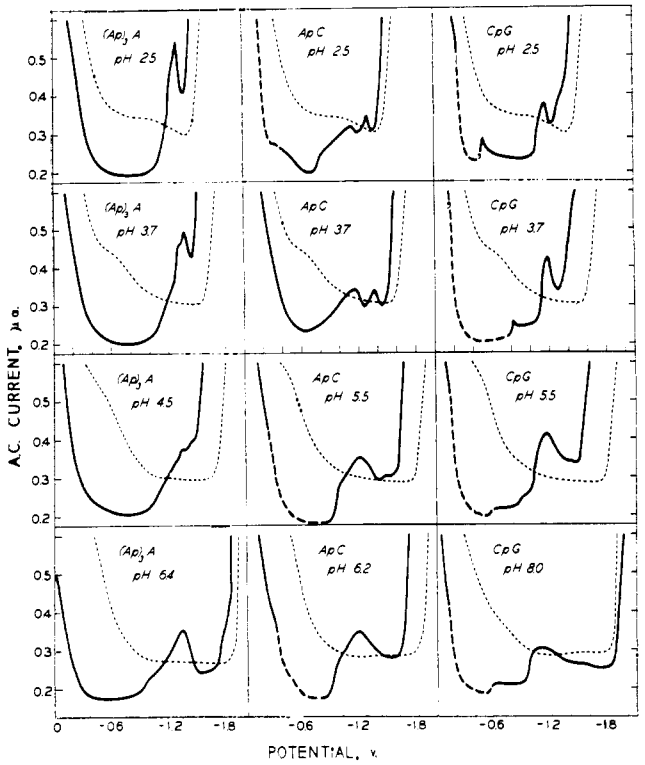

Figure 5. Total ac polarograms for *ca.* 9.05 m*M* $(Ap)_3A$, ApC, and CpG at various pH values in McIlvaine buffer at 25°: dashed lines denote distorted current oscillations; dotted lines denote background electrolyte base current.

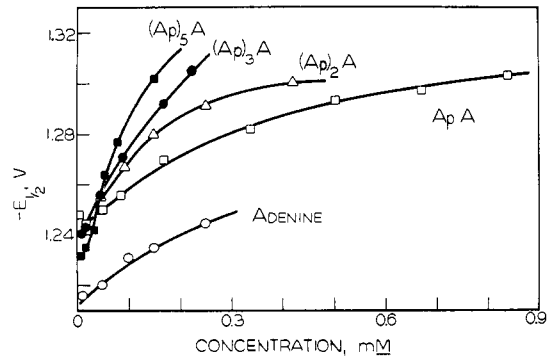

Figure 6. Variation of $E_{1/2}$ with concentration of adenine and oligonucleotides in pH 3.5 McIlvaine buffer (ionic strength, 0.1 *M*) at 0.5°: adenine (○); ApA (□); $(Ap)_2A$ (△); $(Ap)_3A$ (●); $(Ap)_5A$ (■).

Adsorption of Reduction Product. The reduced form of all adenine dimers and oligomers studied, except ApU and UpA, is also more or less strongly adsorbed. Assignment of the dme prewave to reduction to an adsorbed product and of the normal wave to reduction to a dissolved product is based on variation in the two wave heights with *h*, temperature, and concentration (Figures 6 and 7). A more detailed consideration of the prewave assignment is given in ref 6.

The hindrance to reduction of solution species by adsorbed product is evident from the decrease following the initial increase of the instantaneous current (Figure 8, h–j and p–r). At more negative potential, reduction through the adsorbed layer is possible (*cf.* current increase at end of drop-life in Figure 8, c–f and k–n).

Reduction products of ApC, CpA, and CpC are adsorbed over the whole pH range where reduction

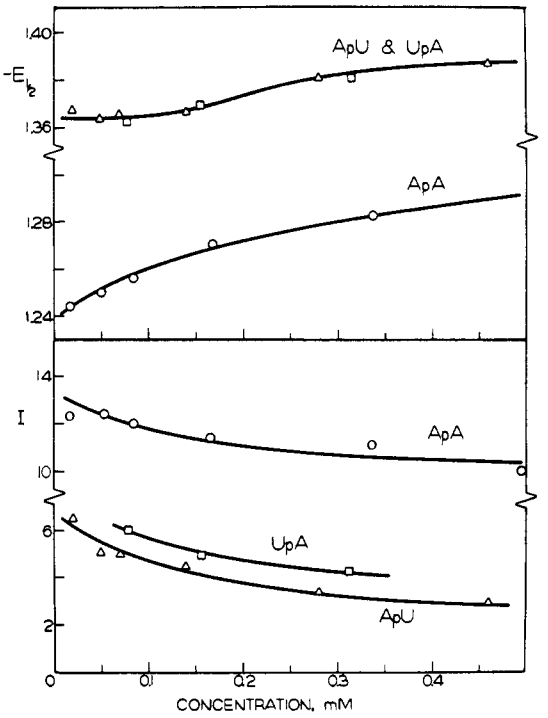

Figure 7. Variation of $E_{1/2}$ and *I* with concentration of dinucleoside monophosphates at 0.5°; ApU (△) and UpA (□) in pH 5.0 McIlvaine buffer (ionic strength, 0.13 *M*); ApA (○) in pH 3.5 McIlvaine buffer (ionic strength, 0.1 *M*).

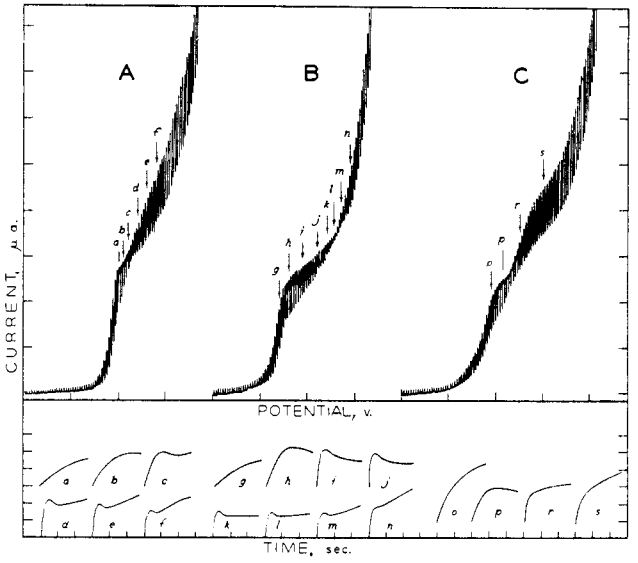

Figure 8. Variation in shape of *i–t* curves at 25° recorded at various potentials on the polarographic curves of $(Ap)_3A$ at pH 4.5 (A) and pH 5.5 (B) and CpA at pH 4.5 (C). Depolarizer concentration *ca.* 0.05 m*M*. Polarographic curves start at −1.1 V (A and C) and −1.2 V (B); potential scale is 100 mV/division. Arrows mark potentials, V, at which the *i–t* curves were recorded: a, −1.300; b, −1.310; c, −1.320; d, −1.340 e, −1.360; f, −1.380; g, −1.340; h, −1.360; i, −1.390; j −1.420; k, −1.440; l, −1.455; m, −1.470; n, −1.490; o, −1.290; p, −1.315; r, −1.350; s, −1.400. Time scale is 1 sec/division. Current scale, μA/division: 0.75 (A), 0.50 (B and C), 1.0 (a–f), 0.5 (g–n and p–s), 0.20 (o).

occurs (Table II). The absence of a prewave for ApU and UpA between pH 2 and 5.5, and for ApA, $(Ap)_3A$, and $(Ap)_5A$ below pH 4, indicates that under these conditions, either the reduction product is not adsorbed or both reduction product and depolarizer are equally

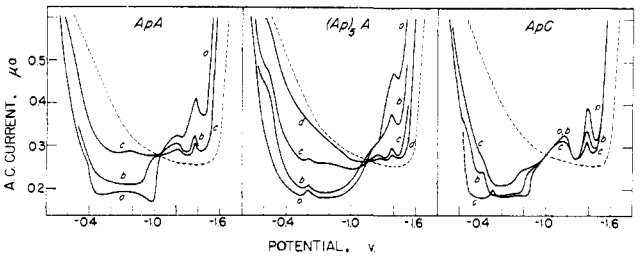

Figure 9. Effect of concentration on total ac polarograms of ApA, $(Ap)_5A$, and ApC in pH 3.5 McIlvaine buffer at 25°. Concentration, m*M*: ApA, (a) 0.16, (b) 0.08, (c) 0.04; $(Ap)_5A$, (a) 0.07, (b) 0.03, (c) 0.01, (d) 0.003; ApC, (a) 0.17. (b) 0.08, (c) 0.04. Dashed lines denote distorted current oscillations; dotted lines denote background electrolyte base current.

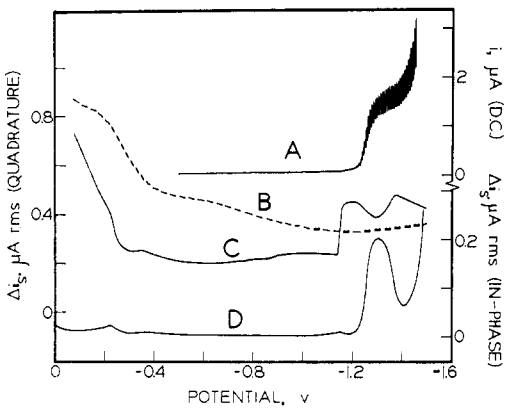

Figure 10. Dc and ac polarograms of 0.084 m*M* ApA in pH 3.5 McIlvaine buffer (ionic strength, 0.1 *M*) at 0.5°: (A) dc polarogram; (B) ac quadrature current component for background electrolyte; (C) ac quadrature current component; (D) ac in-phase current component.

adsorbed. The situation with ApG and GpA is more complicated (*cf.* Experimental Section).

Association of Adsorbed Molecules. Most of the compounds examined exhibit in certain pH ranges depressions, which, even at concentrations as low as 0.05 m*M*, contain more or less well developed pits or wells (*cf.* Figures 2, 5, and 9; *cf.* Figure 7 of ref 17b), which are characteristic of the association of adsorbed molecules[33] and which have been observed for some nucleosides.[29] Adenine associates at 1 m*M* bulk solution concentration at pH 3.4 and 0°; adenosine[29] must be at least 4.4 m*M* to associate at pH 7.0 at 25°, while ApA associates at 0.08 m*M* at pH 3 at 0° (Figure 10) and $(Ap)_3A$ at 0.05 m*M* at pH 6.4 at 25° (Figure 5).

The potential range covered by the pit can be used to evaluate the extent of association in the adsorbed state. For dimers containing adenine, this range increases in an order ApU < CpA, ApC < ApA, which parallels the tendency of nucleosides and nucleotides to associate in solution, *i.e.*, pyrimidine–pyrimidine < purine–pyrimidine < purine–purine,[34] and, consequently, may indicate similar modes of association at the interface and in solution. The extent of this potential range for ApA decreases with increasing temperature as would be expected if association and/or adsorption phenomena were involved; the decrease is approximately linear with the logarithm of the absolute temperature.

The presence of an adsorbed film often hinders the reduction process, since diffusing molecules cannot approach the electrode surface through the film at the potential where reduction would normally begin. Normal reduction occurs only after rupture of the film and is marked by a sudden rise in current. The quadrature current polarograms for ApA indicate that the film breaks at about −1.15 V; this potential is sufficiently positive as not to hinder reduction (Figure 10). However, for $(Ap)_3A$ and $(Ap)_5A$, the film breaks at about −1.27 V (*cf.* Figure 7 of ref 17b), which is sufficiently negative to block reduction; thus, a faradaic process occurs at the potential of film breakage, as indicated by the sudden increase in in-phase current. This sudden rise explains the steep slopes observed for the dc wave.

Effect of Base Composition and Chain Length on Adsorbability. The surface activity of the nucleic acid bases increases in the order cytosine < uracil < adenine < guanine.[30,35] This sequence is followed in the dinucleoside monophosphates (CpC < ApC, ApU < ApA < GpA) and also holds for the degree of association at the interface. At pH 3, ApU is adsorbed to a greater extent than ApC, but the order is reversed with increasing pH, reflecting the pH-dependent adsorption of the cytosine ring.

The extents of adsorption and association increase with increasing oligomer chain length up to $(Ap)_3A$; the ac polarograms of $(Ap)_3A$ and $(Ap)_5A$ are almost identical. As the chain length increases, the transition from the association pit in the quadrature current to the following peak becomes more rounded and occurs over a larger potential range, reflecting the longer time necessary for the film to break and the bulkier molecule to reorient at the electrode surface; unexpectedly, this time is the longest for $(Ap)_2A$. These comparisons suggest that associability and adsorbability, although sensitive to the nature of the molecule, are not simply proportional to molecular size. There appear to be two opposing forces. As the chain length increases, the extents of association and adsorption increase due to the greater number of adenine rings. However, an increase in chain length results in a bulkier molecule, limiting the number of adenine rings which can be oriented and reduced at the electrode surface and requiring a longer time for the molecule to reorient itself. There appears to be a balance of these effects at $(Ap)_2A$ and $(Ap)_3A$. The effect in $(Ap)_2A$ involves the number of rings oriented and reduced at the electrode; the effect in $(Ap)_3A$ involves the maximum degree of association and adsorption. A similar limit for associability in aqueous solution with oligomer chain length has also been postulated[21] (*cf.* next section).

Association in Solution

Nmr measurements (generally in buffered or unbuffered neutral aqueous solutions above 25° at 4 to 20 m*M*) indicate that ApA, ApG, and CpA undergo both inter- and intramolecular associations.[25a,d,27] The extent of the two types of association is less for ApC

(33) W. Lorenz, *Z. Elektrochem.*, **68**, 192 (1958).
(34) P. O. P. Ts'o, J. S. Melvin, and A. C. Olson, *J. Amer. Chem. Soc.*, **85**, 1289 (1963).
(35) In the text, uracil is substituted for thymine; the latter compound appears in ref 30. The substitution is valid, however, since both compounds are structurally similar except for a methyl group which has only minimal effect.

and CpA than for ApA due to the lower degree of base overlapping of the cytosine ring. The mode of intermolecular association is similar to that of the individual bases which involves alternate stacking of the rings. The result of intramolecular interactions is a folded or stacked structure with a conformation in which each nucleoside unit has an anti conformation and the (3′ → 5′) screw axis has a right-handed turn.

Based on optical rotatory dispersion studies of the adenine oligomers,[36] Ts'o and coworkers[27] suggest that ApA may associate more in solution than $(Ap)_3A$, resulting in the possibility that, with increasing chain length, association by stacking of the oligomer increases to a certain size and then decreases.

Electrochemically, association in solution may be related to the change in I and $E_{1/2}$ with concentration. The decreasing reversibility of the electrode process with increasing concentration is in agreement with additional energy being involved; *i.e.*, association increases the activation energy for charge transfer, making reduction more difficult. Correspondingly, I decreases with increasing concentration due to the smaller D for the associated molecules. Since the standard free energy for such association is of the order of the thermal energy, the stacks must break and re-form rapidly and higher stability can be expected at lower temperatures; thus, the decrease of I for the adenine nucleoside–nucleotide sequence with increasing concentration[2] is about two to three times greater at 1.5° than at 25°.

Unfortunately, adsorption and subsequent film formation at the electrode cause similar changes in I and $E_{1/2}$ with increasing concentration, making it difficult to interpret the data solely in terms of association in solution; however, with reasonable assumptions, comparisons are possible. Maximum coverage of the interface by the adsorbed reduced layer is presumably reached above 0.1 mM solution concentration (*cf.* variation of I with C in Figures 4 and 7; a similar effect is seen on plotting depth of the ac depression *vs.* concentration, *e.g.*, Figure 9). Above this concentration, however, $E_{1/2}$ continues to become more negative with increasing concentration for all dimers and oligomers containing an adenine ring (Figures 4, 6, and 8). In addition, extrapolation of the $E_{1/2}$–C plots for the adenine oligomers (Figure 6) to zero concentration (and, thus, to zero degree of association) results in a more or less common $E_{1/2}$ at −1.23 V. Similar extrapolation of the adenine curve results in an $E_{1/2}$ of about −1.21 V. The increasing differences in $E_{1/2}$ between adenine oligomers with increasing concentration can then be explained in terms of increasing differences in the degree of interaction, *e.g.*, association and adsorption, with the latter being greatest for $(Ap)_5A$. The $E_{1/2}$ increment becomes less for each adenine moiety added, indicating that these effects may level off at some higher chain length. The differences in extrapolated $E_{1/2}$ for adenine and its oligomers may be explained in terms of a stacked configuration for dimer and oligomers, indicating the effect one ring has upon the other when the molecule is reduced.

Experimental Section

Chemicals. The reported analytical data for the dinucleoside monophosphates (ApA, ApG, GpA, ApU, UpA, CpA, and ApC) (Calbiochem; Nutritional Biochemical) and adenine oligonucleoside phosphates ($(Ap)_2A$, $(Ap)_3A$, and $(Ap)_5A$) (Miles Laboratories) indicated sufficient purity for polarographic study. The adenine oligomers were received as the lyophilized lithium salts; samples contained 50–60% $LiClO_4$ and LiOAc. Concentrations of stock solutions, prepared in distilled water, were determined from absorbances measured at λ_{max}, using reported molar absorptivities.[25b,c,37]

Buffer solutions were prepared and nitrogen used for deoxygenating was purified as previously described.[6]

Apparatus. Except where noted, apparatus was as previously described.[6,38] Reported current magnitudes for total ac polarograms (Figures 2, 5, and 9) are attenuated by a factor of 1.93. Current–time curves on a single successive drop were photographed on a Tektronix Type 502 oscilloscope with a C-12 Camera (3000 speed Type 47 Polaroid film). The applied dc potential was obtained from a potentiometer employing a 2.7-V mercury battery and a Hewlett-Packard Model 7128B power supply; to eliminate high frequency noise, the cell signal was filtered through a Hewlett-Packard Model 17106 input filter.

Polarographic and voltammetric measurements were made in a jacketed three-compartment cell,[6,39] kept, unless otherwise noted, at 25.0 ± 0.1 or 0.5° (temperature of a circulating ice–water bath).

For pH studies and total ac polarography, dme capillaries (marine barometer tubing) had drop-times, t (measured at potentials of interest), of 3 to 4 sec and m values (open circuit) of 1.7 to 2.2 mg/sec; for all other studies, these values were between 6 and 7 sec and 1.0 mg/sec, respectively. The phase-selective polarograms employed a controlled 3-sec drop-time. All ac measurements used a 50-Hz, unless otherwise noted, frequency of 3.54-mV rms amplitude.

For the cyclic voltammetric pH studies, a hanging mercury drop electrode (hmde) was prepared by collecting one drop from the dme into a platinum disk sealed into glass or by using a Metrohm E-410 microburet assembly, which gave an hmde of 0.022 cm^2 area.

The long times required to reach a constant current level for dimers containing adenine necessitate modification of the coulometric procedures;[6] electrolysis was stopped after an hour, and the concentration was immediately determined spectrophotometrically and subtracted from the initial concentration to determine the amount of compound electrolyzed. This procedure was satisfactorily tested with adenine.

All potentials are *vs.* sce at the experimental temperature.

Electrochemical Behavior. For convenience of comparison, data are discussed by the technique used. Since adenine, adenosine, and AMP have been previously described,[1-5,15,22] only some immediately pertinent results are included. $(Ap)_3A$ and $(Ap)_5A$ behave essentially the same; generally similar behavior patterns are shown by complementary dinucleotide pairs: ApU, UpA; ApC, CpA; ApG, GpA.

1. Dc Polarography. At pH 3.4 (McIlvaine buffer) and 0°, adenine exhibits a well-defined wave whose $E_{1/2}$ becomes more negative and I remains approximately constant with increasing concentration (Figure 6); some wave abnormality appears above 0.5 mM.[40] The pH dependencies[41] of $E_{1/2}$ and i_l for the dimers and oligomers are shown in Tables II and III and Figure 1. Currents are essentially diffusion controlled; *e.g.*, i_l–$h^{1/2}$ plots are linear and pass through the origin in most cases at sufficiently low pH for adenine dimers and oligomers and dimers containing adenine and uracil or cytosine. Examples of other data are given in Figures 4, 6, and 7.

At 25°, ApA shows one moderately well-defined cathodic wave (the change in $E_{1/2}$–pH relation below pH 2.5 is probably due to the change from McIlvaine to chloride buffer; similar behavior is observed with the adenine nucleosides and nucleotides[2]). $E_{1/2}$ and I do not change significantly between 0.25 and 0.75 M ionic strength. The wave height (total height if prewave appears) decreases slightly with increasing pH up to about pH 5 and then suddenly decreases to disappear at about pH 6. At pH 3.4, $E_{1/2}$ is

(36) S. R. Jaskunas, C. R. Cantor, and I. Tinoco, Jr., *Biochemistry*, 7, 3164 (1968).

(37) M. M. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, 13, 54 (1965).

(38) C. O. Schmakel, Ph.D. Thesis, University of Michigan, 1971.

(39) J. E. Hickey, M. S. Spritzer, and P. J. Elving, *Anal. Chim. Acta*, 35, 277 (1966).

(40) B. Janik and P. J. Elving, *J. Electrochem. Soc.*, 117, 457 (1970).

(41) The theoretical value for a reduction involving the same number of electrons and protons in the potential-determining step of the electrode process is 0.06 V/pH.

essentially independent of temperature from 0 to 40°, while the wave slope and I increase. Between pH 4 and 5, the initial portion of the wave of 0.05 mM ApA solutions appears to constitute a more positive wave, whose i_1 is characterized by abnormal oscillations. Above 0.1–0.2 mM, the wave splitting is hardly noticeable; with decreasing concentration, it becomes more evident and the normal wave limiting portion merges with background discharge and/or vanishes. Only one wave can be seen below 0.05 mM: below 0.01 mM, this wave has a normal shape. Plots of prewave i_1 *vs.* C increase linearly from 0.04 to 0.2 mM and extrapolate to finite i_1 values at $C = 0$, indicating a marked increase of i_1 with C below 0.04 mM. The prewave height is directly proportional to h: its slope is about 18 mV compared with 35 mV for the normal wave.

At 0° and pH 3.4, 0.005 to 0.84 mM ApA shows one cathodic wave (Figures 6, 7, and 10); with increasing concentration, $E_{1/2}$ becomes more negative (the $E_{1/2}$–C plot has an inflection at about 0.2 mM), I decreases, and the wave slope remains constant at about 28 mV. For the one wave shown by $(Ap)_2A$, $(Ap)_3A$, and $(Ap)_5A$, $E_{1/2}$ becomes more negative, I decreases, and the slope becomes smaller with increasing concentration (Figure 6); unlike ApA, $E_{1/2}$ becomes slightly more positive with increasing temperature.

At 25°, $(Ap)_3A$ and $(Ap)_5A$ exhibit a split wave similar to ApA. As pH increases, the normal wave gradually merges with background discharge so that only the initial sudden decrease can be observed above pH 4. The normal and prewave slopes are 27 and 18 mV, respectively. Other properties of the waves are similar to those of ApA.

At 25°, ApG and GpA produce only one wave in the concentration and pH ranges studied (Table II). Between 0.05 and 0.2 mM at pH 3.8, wave heights are directly proportional to concentration, and $E_{1/2}$ shift negatively by 30 to 40 mV. The slow initial increase in current of the dc wave with potential (accompanied by distorted current oscillations and characteristic i–t curves), followed by a sharp current increase on the upper rising portion of the wave, may indicate that the initial reduction to an adsorbed product is partially inhibited by a simultaneously adsorbed depolarizer; the film formed by the latter breaks at more negative potential.

ApU and UpA show only one wave in the concentration and pH ranges studied, whose pH characteristics are similar to those of the normal ApA wave. At 25°, 0.05 to 0.2 mM, and pH 3.8, their wave shape is unaltered, the slope is about 50 mV, the height is directly proportional to concentration, and $E_{1/2}$ shifts negatively by 30 to 40 mV. At 0° and pH 5, $E_{1/2}$ for the two compounds are similar but I for UpA is about 20% larger than that of ApU (Figure 7). $E_{1/2}$ is independent of concentration up to 0.14 mM, then becomes 30 mV more negative, and, above 0.46 mM, is again independent. I decreases with increasing concentration.

At 0 and 25°, ApC and CpA exhibit normal waves and prewaves. In pH 5 McIlvaine buffer at 0°, prewave $E_{1/2}$ is relatively constant (−1.32 V) with increasing concentration but I decreases exponentially similar to CpC (Figure 4); normal wave $E_{1/2}$ becomes more negative and I increases exponentially (the wave blends with background above 0.2 mM). Below 0.2 mM, the sum of I for both waves is constant. The normal wave $E_{1/2}$–C plot shows the same general curvature as for CpC[6] except that the CpA plot is shifted about 10 mV more negative than that for CpC and the ApC plot about 10 mV more positive. The temperature coefficient of i_1 for the CpA prewave at pH 3.7 is 0.5% between 8 and 40°; $E_{1/2}$ becomes 10 mV more positive for each 10° increment.

2. Current–Time Curves. The i–t curves recorded at i_1 of the normal wave usually produced log i–log t plots,[42a] which are linear from *ca.* 0.3 sec to the end of the drop-life (about 3 sec) with slopes of *ca.* 0.3, regardless of whether the rising part of the wave is distorted or not; plots for ApG and GpA are either curved or reasonably linear (slope of 0.4–0.5). The shape of i–t curves recorded at other potentials depends on the wave pattern; the following situations can be distinguished.

(a) When the normal waves of ApA, ApU, UpA, and CpC do not show irregularities, i–t curves recorded at potentials on the rising wave have a smooth parabolic shape and give more or less linear log plots.

(b) For ApA, ApC, CpA, and CpC, i–t curves recorded on the prewave limiting portion (Figure 8C) exhibit a small decrease in current at the end of the drop-life, *e.g.*, CpC and CpA at pH 4.5, or resemble a parabola; in the latter case, the log plots depart markedly from linearity.

(c) When the normal wave has disappeared, *e.g.*, $(Ap)_3A$ and $(Ap)_5A$ above pH 4.5, i–t curves recorded along the prewave (Figures 8A and 8B) are characterized by a decrease in current following the initial increase; with increasing negative potential, a current increase occurs at the end of the drop-life.

(d) When abnormal current oscillations appear on the lower part of the normal wave, *e.g.*, ApG and GpA, the i–t curve shapes depend on pH and potential and are generally similar to those described in section (b).

3. Cyclic Voltammetry. Adenine and the dimers and oligomers produced a single cathodic peak, whose pH characteristics are similar to those of the normal dc wave and ac peak (Table IV).

Table IV. Variation with pH of E_p on Cyclic Voltammetry of Di- and Oligonucleotides[a]

Compd	pH	$-E_p$, V
ApA	2.5–5.5[b]	1.21 + 0.056 pH
$(Ap)_3A$	2.5–6.5	1.17 + 0.049 pH
$(Ap)_5A$	2.5–4.7[c]	1.20 + 0.042 pH
ApG	2.5–4.0[d]	1.15 + 0.065 pH
GpA	2.0–3.7	1.13 + 0.062 pH
ApU	2.5–4.7	1.06 + 0.075 pH
UpA	2.0–4.7	1.05 + 0.080 pH
ApC	2.0–6.0	1.09 + 0.061 pH
CpA	2.0–5.5	1.12 + 0.054 pH
CpC	2.0–5.3	1.14 + 0.042 pH
	5.3–6.0	0.23 + 0.210 pH
	6.0–8.0	0.90 + 0.101 pH
	9.0–10.0[e]	1.08 + 0.076 pH

[a] Data for the main cyclic voltammetric peak obtained in *ca.* 0.05 mM solutions in chloride and McIlvaine buffers on the first scan at a scan rate of 0.3 V/sec. The maximum deviation from the equations is 15 mV. [b] E_p in chloride and acetate buffers are 50 to 250 mV more negative. [c] E_p on the first scan at pH 2.5 and 3.0 is 70 and 35 mV more negative, respectively; E_p on the second scan fits the equation. E_p at pH 5.5 is 25 mV more positive. [d] E_p on the first scan in pH 2.0 chloride buffer is 30 mV more negative; E_p on the second scan fits the equation. E_p at pH 1.1 and 4.5 are 65 and 30 mV more positive, respectively. E_p in pH 4.0 acetate fits the equation. [e] Carbonate buffer.

At pH 3.4 and 0°, E_p becomes more negative and $i_p/ACv^{1/2}$ increases sharply with increasing v (0.02 to 20 or 24 V/sec) for adenine, ApA, and $(Ap)_3A$; no anodic peaks are observed on the reverse scan. The $(Ap)_3A$ peak is very narrow at low v and widens at higher v.

With increasing pH, i_p for the compounds containing adenine but not cytosine decreases and disappears above pH 5–6. The peak height decreases with an increasing number of scans at the same drop (the decrease is larger at higher v); stirring normally restores the first scan height.

Between $v = 0.09$ and 0.3 V/sec, the $i_p/v^{1/2}$ ratio for ApU and UpA is constant, but E_p becomes linearly about 10 mV more negative.

Between 0.07 and 0.35 V/sec, ApC and CpA produce a single cathodic peak at the foot of the background discharge; $i_p/v^{1/2}$ is constant and the numerical E_p value increases linearly.

At 25°, ApG and GpA produce a single cathodic peak (v = 0.07 to 3.2 V/sec); $i_p/v^{1/2}$ decreases by 20–50% with increasing v; the decrease is more pronounced at lower pH and often tends to level off at higher v. No anodic peak is formed at v below 2 V/sec or when the reverse scan starts at a potential just after the top of the cathodic peak. However, if the hmde has been polarized to potentials of background discharge, an anodic peak ($E_p = -0.06$ V at pH 2.5) appears on the reverse scan at v above 2 V/sec. The location and nature of this peak, *e.g.*, need for high v and electrode polarization to background discharge potential, is similar to those of the anodic peak due to oxidation of the unstable reduction product produced by guanine, its nucleosides and nucleotides, and

(42) (a) Due to depletion of the solution surrounding the drop,[42b] i–t curves recorded for sequential drops from a vertical capillary produce log i *vs.* log t plots which are curves convex to the time axis, particularly at the beginning of the drop-life; the slope, whose choice is ambiguous, is always greater than the *ca.* 0.19 expected for diffusion-controlled currents, even though the h dependence indicates diffusion control (a log slope of 0.66 is expected for kinetic control). (b) J. Kuta and I. Smoler, *Progr. Polarogr.*, 1, 43 (1962).

some other purine derivatives[43] and reflects the fact that guanine is reduced at potentials slightly more negative than background discharge.

4. Ac Polarography. Total ac polarographic patterns and data are shown in Figures 2, 5, and 9 and Table I and phase-selective patterns and data in Figure 10 and Table III.

At pH 3.4 and 0°, adenine shows one in-phase peak; E_s becomes more negative and $\Delta i_s/C$ decreases with increasing concentration. Above 0.5 mM, there appears to be a small shoulder on the positive potential side of the peak. At low C (0.05 mM), the quadrature current component shows depressions from background centered around -0.5 and -1.2 V and maxima at about -0.85, -1.33, and -1.55 V. All depressions and maxima become more intense with increasing concentration, and, except for the third maximum, shift to more negative potential. At pH 4.5 and 0°, peaks, maxima, and depressions shift to somewhat more negative potential.

The total ac polarograms for the nucleotides at 25° in McIlvaine buffer below pH 5 are typified by that for 0.05 M ApA: a depression centered at -0.6 V, a broad peak (I) at -1.2 V, and a narrow peak (II) at -1.3 V. Peak II is essentially constant in height up to pH 4.0–4.5 and then decreases sharply to disappear at pH 5.0–5.5.

At 0° and pH 3.4, the ApA quadrature current consists of the depression and peaks I and II at about the same potentials as in the total ac polarogram. For peak II, which results from the faradaic process, the in-phase peak is more intense and occurs at more positive potential than the quadrature peak (Figure 10); no other in-phase peaks are observed. At 0.084 mM and above, the depression flattens, extending from about -0.4 to -1.15 V. The transition from the end of the depression to the base of the peak I occurs over a narrow potential range. At 0.5 mM, peak I begins to separate into two (peaks Ia and Ib), which are 100 mV apart at 0.84 mM.

With increasing concentration, E_q of quadrature peak II becomes more negative; its height increases up to about 0.5 mM and then remains constant. E_s for the in-phase peak (the only peak seen at and below 50 Hz) is constant up to about 0.4 mM but then becomes slightly more negative; the difference, $E_{1/2} - E_s$, decreases up to 0.2 mM and then remains constant at about 28 mV. The $\Delta i_s/C$ ratio also decreases gradually.

For 0.1 mM ApA solutions, E_s is constant in the frequency range studied (50–1000 Hz); the peak height is constant up to 700 Hz and then increases slightly. Above 50 Hz, a second in-phase peak appears at about 100 mV more negative than the first and becomes somewhat more negative with increasing frequency. This pattern has also been observed[22] with adenine at concentrations above 0.5 mM at 25°.

With increasing temperature the depression width and peak I height decrease; the transition from depression to peak becomes less sharp.

The quadrature current for $(Ap)_2A$ is similar to that of ApA except that the depression covers a larger potential range, the transition from the depression to peak I is less sharp, and peaks I and II occur at more negative potential. With increasing concentration, the depression width, the transition sharpness, and the peak II intensity decrease; at high C, peak I occurs as a shoulder on peak II. At low C, the in-phase component apparently consists of two closely adjacent peaks; at higher concentration, the second peak occurs as a shoulder on the first, whose leading edge is very sharp. This edge, the transition from depression to peak in the quadrature current, and the rising portion of the dc wave all occur at about the same potential, similar to $(Ap)_3A$ (*cf.* Figure 7 of ref 17b).

The total and phase-selective ac polarograms for $(Ap)_3A$ and $(Ap)_5A$ show the same general pattern except that the quadrature depression occurs over a larger potential region (Figures 5 and 9). The in-phase component shows two apparent peaks whose characteristics and concentration dependence are similar to those of $(Ap)_2A$.

The total ac polarographic patterns of ApU and UpA are similar to those of ApA. At 25° and 0.05 mM, ApU shows pit formation from pH 3.7 on (Figure 2). Distorted current oscillations appear at potentials more positive than -0.5 V and, generally, on appearance of pits.

At 0° and pH 5, E_s and $\Delta i_s/C$ for ApU and UpA change with C similar to $E_{1/2}$ and I. The quadrature component, however, shows some differences. The two UpA peaks are at about the same potentials as those of ApU but of lower intensity. The negative potential end of the large depression is about 50 mV more negative for UpA; consequently, the transition from repression to peak is sharper and relatively constant for all UpA concentrations studied. The base of the depression is more irregular for UPA; in fact, there appears to be within the depression a second minor depression, whose range and depth increase with decreasing C. For both compounds, pits begin to appear at about 0.07 mM.

For ApU at pH 3.4, a smaller pit occurs inside of the larger one toward the more negative side. The potential width of the large pit decreases from 0.85 V at 0° to 0.65 V at 25°. The smaller pit is still present at 25°, but its width has decreased.

At 0° and pH 5, the quadrature component for ApC and CpA shows a broad, flat depression between -0.5 and -1.0 V, which becomes more rounded below 0.5 mM. At 0.05 mM, both show a rounded depression centered around -0.6 V, on which (for CpA alone) is superimposed a flat depression from -0.6 to -1.0 V; at -1.0 V, the current increases very sharply. A rounded peak occurs at -1.20 to -1.25 V, whose height for CpA increases with C to 0.17 mM and then remains constant; the ApC peak is much less intense, more distorted, less concentration dependent, and, above the concentration at which the flat depression occurs (0.08 mM), shifts 20 to 30 mV more negative. A second broad, weak peak appears at -1.5 to -1.6 V. The in-phase component shows two peaks corresponding to the dc waves. Peak I E_s (35 mV more negative than wave I $E_{1/2}$) is constant at -1.35 V which increasing C; i_s/C decreases. Peak II, of ApC, like wave II, occurs at more positive potential than those of CpA.

The total ac polarograms for ApG and GpA exhibit a large flat depression (-0.3 to -1.2 V) and an inflection or shoulder on background discharge, which changes to a step with increasing pH and, finally, to a peak above pH 4.5 (Figure 2). In the depression, current oscillations are distorted at potentials more positive than -0.5 V. At pH 3.7, plots of the shoulder heights against concentration are linear, indicating zero height at *ca.* 0.05 mM; E_s does not change significantly.

5. Controlled Electrode Potential Electrolysis. On electrolysis at pH 3.4 and 0°, 0.25 mM adenine gave a faradaic n of 6.3, in agreement with that of Smith and Elving,[4] and 0.26 mM AMP gave n values of 4.5 and 4.8; since the latter electrolyses took about the same time (one hour) as that of adenine itself, it is apparent that the rate of deamination in AMP is far slower than that in adenine. Electrolyses of 0.11 mM ApA solutions gave high n values of 15 and 16, which are probably due to catalytic hydrogen reduction caused by accumulation of reduced product in solution and on the mercury surface. Electrolysis of 0.1 mM ApU gave an n of about 7, which is only an approximation due to uncertain contributions of background current and catalytic hydrogen reduction. Electrolysis of 0.1 mM ApC in pH 5.0 McIlvaine buffer at 0° yielded an n of about 11. However, the electrolysis curves for ApA, ApU, and ApC are atypical in showing not the characteristic exponential decrease of current with time but a linear decrease, which indicates that the electrolysis is zero order and is probably controlled by the rate of dissolution of adsorbed product.

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