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Circular Dichroism Studies of the Conformational Stability of Dinucleoside Phosphates and Related Compounds in Aqueous Neutral Salt Solutions†

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ABSTRACT: Circular dichroism spectra were recorded for adenylyl-(3′–5′)-adenosine, adenylyl-(3′–5′)-uridine, uridylyl-(3′–5′)-adenosine, adenosine 5′-mononicotinate, and uridylyl-(3′–5′)-adenosine phosphonate in a variety of neutral salt solutions and several organic solvents as a function of temperature and pH. The additives altered the circular dichroism spectra of dinucleoside phosphates and adenosine 5′-mononicotinate as a consequence of induced conformational changes. Uridylyl-(3′–5′)-adenosine phosphonate, however, displayed no optical activity attributable to base–base interactions, even at low temperature and in the presence of stabilizing salts. Perturbation of electronic transitions by solvent additives was ruled out as a major contribution to spectral changes. Most neutral salts, dioxane, and methanol unstacked the dinucleoside phosphates and the observed sensitivities to additives resembled those recorded for DNA. In contrast to the naturally occurring dinucleotides, the degree of stacking in adenosine 5′-mononicotinate was enhanced by inorganic electrolytes while nonpolar additives (such as long-chained tetraalkylammonium salts) disrupted the stacked state; these results suggest that polar and nonpolar additives which alter dinucleotide conformation according to the Hofmeister series act by different mechanisms. The absence of detectable stacking in uridylyl-(3′–5′)-adenosine phosphonate demonstrates the importance of phosphodiester backbone stereochemistry to the maintenance of the stacked state. Both ΔH and ΔS (for unstacked → stacked) were negative and decreased in absolute magnitude in the presence of denaturing agents; stabilizing salts had the opposite effect. Lowering the pH unstacked adenylyl-(3′–5′)-adenosine, adenosine 5′-mononicotinate, and adenylyl-(3′–5′)-uridine. The conformation of uridylyl-(3′–5′)-adenosine, however, did not change over the observed pH range. The results of this study illustrate the contributions of both solvophobic bonding interactions and phosphodiester backbone stereochemistry to the conformational form and stability of dinucleoside phosphates in solution.

Base-stacking interactions are generally thought to provide a major contribution to the overall energetics defining polynucleotide conformation in solution (for a recent review, see Cantor and Katz, 1971). These interactions are characterized as solvophobic (enthalpy driven–entropy opposed) (Lowe and Schellman, 1972) in contradistinction to the Kauzmann hydrophobic bond (entropy driven–enthalpy opposed) which is considered responsible for the association of hydrocarbon moieties in aqueous solution. Recently, conformational energy calculations have also shown the importance of phosphodiester backbone stereochemistry in defining the conformational form of polynucleotides (Olson and Flory, 1972a–c; Govil, 1973; Perahia *et al.*, 1973; Sasisekharan, 1973).

Physical biochemists interested in questions of nucleic acid conformational stability have studied the constituents of polynucleotides in order to separate the contributions of solvophobic bonding from the effects of electrostatic backbone interactions, hydrogen bonding, and cooperativity. Typical examples include studies of monomer base association (for a review, see Ts'o, 1970, Scruggs *et al.*, 1972, and Herskovits and Harrington, 1972), studies of nucleoside and nucleotide conformation by nmr[1] and CD (Prestegard and Chan, 1969; Formoso, 1972; Schleich *et al.*, 1972) and studies of dinucleoside phosphate conformation utilizing nmr and optical spectroscopy (Leng and Felsenfeld, 1966; Brahms *et al.*, 1967; Davis, 1967; Davis and Tinoco, 1968; Chan and Nelson, 1969; Kondo *et al.*, 1970; Lowe and Schellman, 1972; Ts'o *et al.*, 1969; Smith *et al.*, 1973).

We undertook the present circular dichroism experiments to investigate the conformational form and stability of dinucleoside phosphates in solution. We were also interested in obtaining additional information about the mechanism(s) of the Hofmeister effect. Neutral salts, organic solvents, low pH, and temperature were used as conformational perturbants. Model compounds were chosen which might be expected to reveal the contributions of solvophobic interactions and backbone stereochemistry to dinucleotide conformation.

ApA[1] was chosen because it is the most widely studied di-

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[1] Abbreviations used are: ApA, adenylyl-(3′–5′)-adenosine; ApU, adenylyl-(3′–5′)-uridine; UpA, uridylyl-(3′–5′)-adenosine; AMN, adenosine 5′-mononicotinate; UpcA, uridylyl-(3′–5′)-adenosine phosphonate; AMP, adenosine 5′-monophosphate; A, adenosine; U, uridine; TMACl, tetramethylammonium chloride; TEACl, tetraethylammonium chloride; TPACl, tetrapropylammonium chloride; GdmCl, guanidinium hydrochloride; $T_{1/2}$, temperature at the midpoint of a thermally induced transition; CD, circular dichroism; nmr, nuclear magnetic resonance.

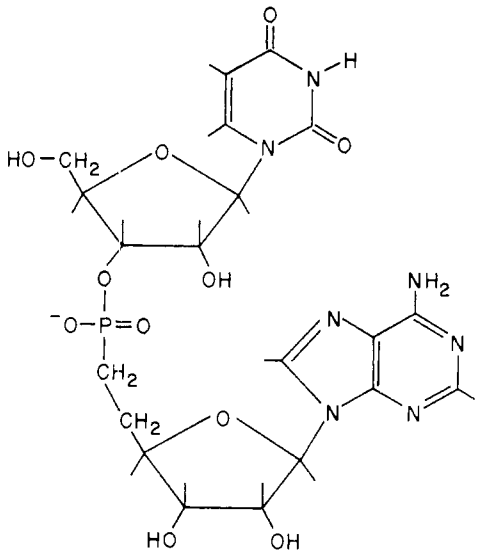

FIGURE 1: Structural formula of UpcA.

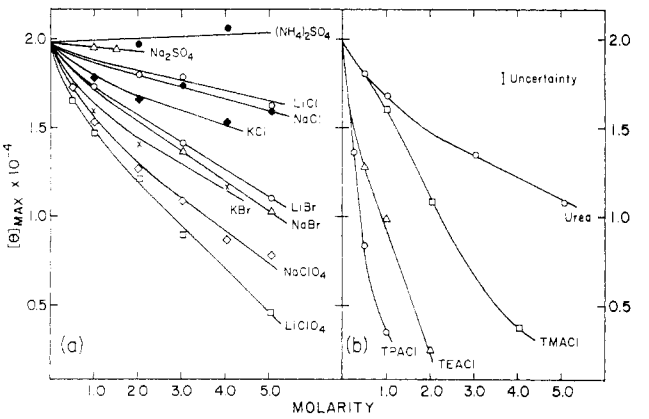

FIGURE 2: The ellipticity (per mole of residue) of ApA at 272 nm (pH 7.0, 24°) in the presence of neutral salt additives as a function of additive concentration: (a) inorganic electrolytes; (b) tetraalkylammonium salts and urea.

nucleotide; ApU and UpA would reveal the effect of sequence; AMN possesses no phosphodiester backbone, has fewer "backbone" bonds and hydrogen-bonding sites than the dinucleotides, but still exhibits a solvophobic interaction between adenine and the pyridine heterocyclic ring (Miles and Urry, 1967); and UpcA (structural formula in Figure 1), with the 5′-adenosine oxygen atom replaced by a methylene group, would give information regarding the role of the phosphodiester backbone in the definition of dinucleotide conformation.

Experimental Section

ApA, ApU, UpA, and AMN were the best grades commercially available and were used as received. UpcA (Jones *et al.*, 1970) was provided through the courtesy of Drs. G. H. Jones, J. Cohen, and J. Griffin. All salts and solvents were reagent grade except GdmCl, dioxane, and methanol which were spectroquality grade. Doubly distilled water was used for all solutions.

Stock solutions of chromophore were prepared by weight in 0.01 M pH 7.0 sodium dimethyl arsenate buffer (sodium cacodylate). An aliquot of stock solution was mixed with a weighed portion of salt or a particular volume of organic solvent and the solution brought to a predetermined final volume with buffer. The pH was then readjusted (if necessary) to pH 7.0 using concentrated acid or base. Volume changes due to pH adjustment were less than 2%. Concentrations of stock solutions were verified by circular dichroism at 25° using published ellipticity values (Warshaw and Cantor, 1970; Miles and Urry, 1967) except for UpcA whose concentration was determined by weight.

A Beckman Acta V spectrophotometer was used to obtain absorption spectra at different temperatures. CD spectra were recorded on a Jasco J-20 spectrometer calibrated against a 1-mg/ml solution of *d*-10-camphorsulfonic acid (twice recrystallized from acetic acid) according to the procedure of DeTar (1969). In some experiments, time-averaged CD spectra were obtained using a Varian 620/i computer interfaced to the Jasco spectrometer. Temperature was measured to ±0.5° by a Yellow Springs telethermometer Model 42 SC connected to a thermistor mounted in the sample cell.

CD spectra monitored at a single wavelength while the temperature was changed at a rate of 1.5°/min yielded thermodynamic data which agreed with the results obtained from full spectra recorded at several temperatures where the solution was allowed to equilibrate for 5 min prior to measurement. Furthermore, previously reported studies have demonstrated that thermodynamic results derived from temperature-dependent dinucleotide CD spectra are independent of the observed wavelength (Lowe and Schellman, 1972; Powell *et al.*, 1972). Therefore temperature-dependent CD was recorded by continuously monitoring a single wavelength at the low-energy extremum while temperature increased. All optical changes were reversible.

Corrections for refractive index and thermal expansion were estimated to be less than 10%, and hence, were not made.

Results

CD spectra were taken of ApA, ApU, UpA, and AMN in a variety of salt solutions and organic solvents and as a function of temperature and pH. For all compounds studied except UpA, the magnitude of the low-energy extremum reflected the relative CD intensity at all wavelengths observed. For UpA hyperchromicity measurements revealed that salt additives such as $NaClO_4$ disrupted the stacked state in agreement with the interpretation based on the amplitude of the low-energy CD maximum. Consequently, for all compounds the CD of the low-energy extremum was taken as a monitor of spectral changes. This procedure is insensitive to additive or temperature-induced wavelength shifts or to changes in spectral line shapes.

Representative plots for the salt induced optical activity changes of ApA and AMN as a function of salt concentration are shown in Figures 2 and 3, and in Figure 4 we have summarized the relative isothermal response of the optical activity for all the dinucleotides and AMN to the salt perturbants employed.

The CD spectrum of UpcA at 14° (Figure 5) resembled the CD spectrum of UpA at 72° and, with the exception of small wavelength shifts, was insensitive to solvent and temperature perturbations. No increases in the optical activity of UpcA could be induced by lowering the temperature to 5° in either the presence or absence of 4 M $(NH_4)_2SO_4$.

The response of the optical activity of ApA, ApU, and UpA follows the pattern, more or less, which has been established for the conformational stability of a variety of biopolymers in aqueous salt solutions (von Hippel and Schleich, 1969a,b). $(NH_4)_2SO_4$ and Na_2SO_4 either increased the optical

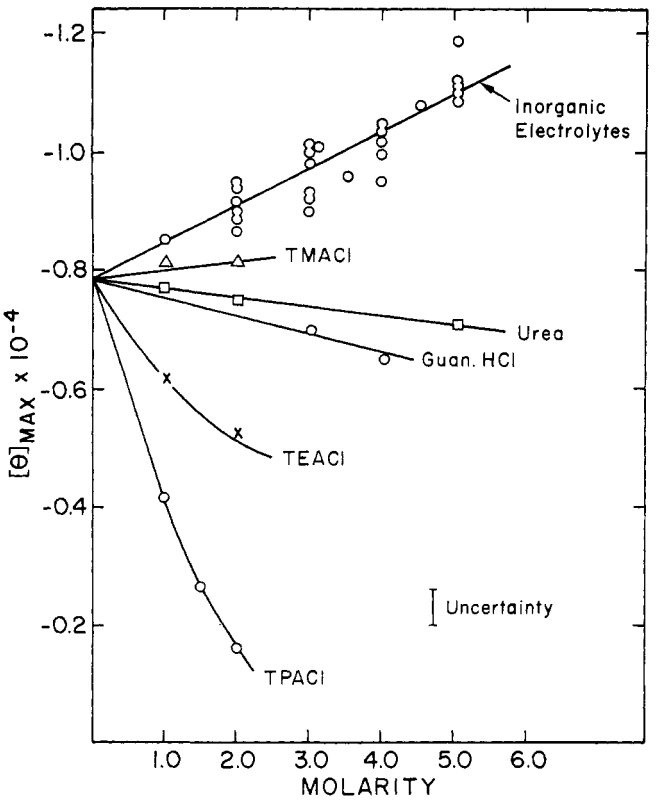

FIGURE 3: The ellipticity (per mole of residue) of AMN at 275 nm (pH 7.0, 24°) in the presence of neutral salt additives as a function of additive concentration.

activity relative to the salt-free case or had little effect. Monovalent anion salts decreased the optical activity with relative effectiveness: $ClO_4^- > Br^- > Cl^-$. Methanol, urea, and GdmCl all decreased the ellipticity and the potency of the tetraalkylammonium salts increased with the length of the alkyl chain. However, the effectiveness of monovalent cation salts depended on the particular dinucleotide. For example, Na^+ salts decreased the low-energy CD of UpA more than Li^+ salts,

TABLE I: Apparent Thermodynamic Parameters[a] for ApA Stacking.

Sample	ΔH (kcal/mol)	ΔS (eu)	$T_{1/2}$ (°K)
No salt	−8.5 ± 0.3	−29.8	286
No salt	−8.6 ± 0.8	−30.1	285
No salt	−8.5 ± 0.4	−29.7	287
1 M $NaClO_4$	−8.7 ± 0.3	−30.7	283
5 M LiCl	−6.3 ± 0.2	−23.0	276
5 M NaCl	−8.1 ± 0.4	−29.6	273
2 M KBr	−4.5 ± 0.3	−16.9	265
2 M TMACl	−5.4 ± 0.3	−20.6	263
4 M KBr	−4.5 ± 0.2	−17.3	262
3 M $NaClO_4$	−5.9 ± 0.6	−22.6	262
5 M LiBr	−4.8 ± 0.3	−18.9	254
5 M NaBr	−4.1 ± 0.3	−16.5	248
5 M $NaClO_4$	−4.9 ± 0.5	−19.9	248
5 M $LiClO_4$	−3.9 ± 0.2	−16.7	231
pH 3	−3.4 ± 0.7	−15.5	221
40% dioxane	−2.8 ± 0.6	−14.1	199

[a] Only relative values have physical significance (see Discussion).

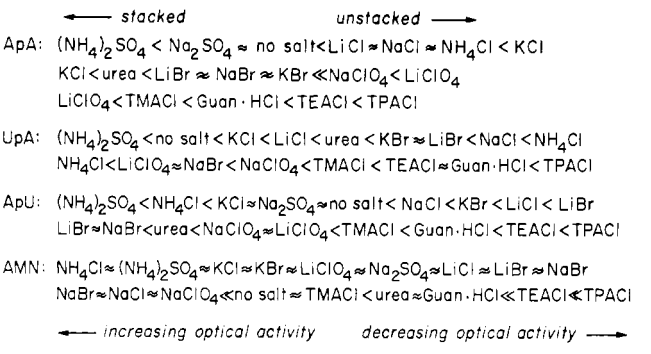

FIGURE 4: Relative effectiveness of various salts in stabilizing or destabilizing stacked conformations in dinucleoside phosphates and AMN (pH 7.0, 24°).

but for ApU (a sequence isomer of UpA) and ApA, Li^+ salts were as effective or more potent than Na^+ salts. Finally, the additive NH_4Cl decreased the optical activity of UpA and ApA, but caused an increase for the ApU dimer (relative to the salt-free case).

In contrast to the naturally occurring 3′–5′-linked dinucleoside phosphates, the optical activity of AMN increased in the presence of inorganic salts. TEACl, TPACl, dioxane, and methanol altered the optical activity of both AMN and the dinucleotides with the same relative effectiveness. Urea and GdmCl were less effective in decreasing the CD of AMN than for dinucleotides while the additive TMACl had virtually no effect on the optical activity of AMN.

We also measured the optical activity of the various dinucleoside phosphates and AMN in aqueous neutral salt solutions as a function of temperature to obtain apparent thermodynamic parameters characterizing the optical activity change. Melting curves were subjected to a van't Hoff analysis (see Discussion). The results of the van't Hoff analysis are listed in Tables I–IV ranked in order of decreasing $T_{1/2}$. Negative ΔH and ΔS values were obtained (for unstacked → stacked) which generally decreased in absolute magnitude in the presence of denaturing salts and organic solvents in proportion to their effectiveness in reducing the optical activity of the dimers at 25°. Salts which increased the optical activity likewise increased the absolute magnitude of the enthalpy and entropy changes.

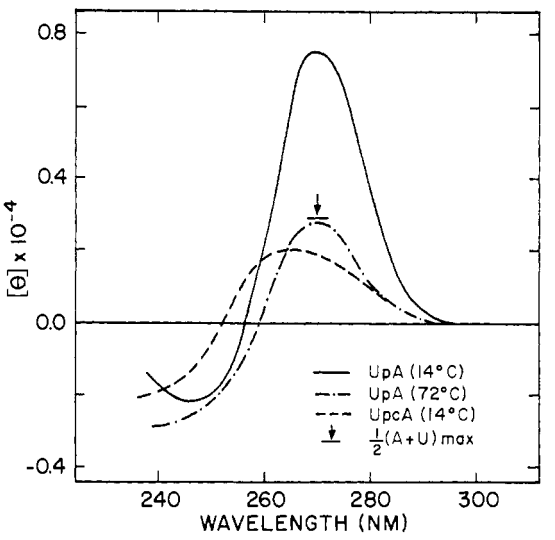

FIGURE 5: The CD of UpA and UpcA at the indicated temperatures (pH 7.0).

TABLE II: Apparent Thermodynamic Parameters[a] for UpA Stacking.

Sample	ΔH (kcal/mol)	ΔS (eu)	$T_{1/2}$ (°K)
No salt	−6.4 ± 0.4	−23.8	267
5 M NaCl	−5.3 ± 1.3	−22.7	237
5 M NaBr	−3.1 ± 0.7	−13.8	224
5 M GdmCl	−1.1 ± 2.2	−10.7	101

[a] Only relative values have physical significance (see Discussion).

The titration curves of the 3′–5′-linked dinucleoside phosphates and AMN are presented in Figure 6. Titration above pH 8 was not performed due to the possibility of base catalyzed hydrolysis of the phosphodiester linkage. The CD spectra of ApA and AMN moved toward higher energy, whereas the spectra of ApU and AMN moved toward lower energy with increasing acidity. Concomitant with these spectral shifts, the optical activity of ApA, ApU, and AMN decreased. The apparent pK values deduced from the spectral shifts and intensity changes were in the range of 3.0 ± 0.5. No changes in the magnitude of the optical activity were observed for UpA with decreasing pH.

Discussion

Interpretation of both nmr (Chan and Nelson, 1969; Ts'o *et al.*, 1969; Smith *et al.*, 1973) and CD spectral data (Leng and Felsenfeld, 1966; Brahms, *et al.*, 1967; Bush, 1967; Bush and Tinoco, 1967; Davis, 1967; Davis and Tinoco, 1968; Johnson and Tinoco, 1969, 1972; Lowe and Schellman, 1972) support the view that increasing temperature unstacks the dinucleoside phosphates by separating the bases. It is reasonable to assume salt-induced optical activity changes are also produced by similar conformational rearrangements. In principle, however, ion binding to a base chromophore could lead to a change in the direction of the transition moment and thus alter the CD without a corresponding change in the relative position of the dinucleotide bases. While some sort of interaction undoubtedly occurs (Lowe and Schellman, 1972; Powell *et al.*, 1972; B. Cross, N. Johnson, and T. Schleich, manuscript in preparation), we suggest it produces minor changes in the optical activity of the dinucleotides and hence, that the magnitude of the CD reflects the average position of the bases relative to each other. This suggestion is based on the following arguments. (a) Dinucleotide melting curves (θ_{max} *vs.* T; see Figure 7) taken in the presence and absence of perturbants can be superimposed on each other by translating the curves along the abscissa (Figure 8) by an increment ΔT. This observation suggests that temperature, neutral salts, organic solvents, and low pH all evoke a predominantly single optical response from dinucleotides which has as its basis a conformational change. Thus the offset required for superimposition is a measure of the extent to which the dimer is unstacked relative to the additive free case. The magnitude of the offset (ΔT) correlates with the intensity of the low-energy extremum at 24° for all of the dinucleoside phosphates and AMN (*e.g.*, see Figures 2 and 8). Hence, changes in the magnitude of the CD at 24° reflect conformational alterations in the dimer. (b) The wavelengths of the extrema and crossover points of the CD spectra of all the dinucleotides and AMN moved toward lower energy in the presence of neutral salt additives. In contrast, where direct binding between chromophore and perturbant is known, the wavelength shifts depend on the chromophore; *e.g.*, for protonation, the maxima shifted toward lower energy in the case of ApU and UpA and moved toward higher energy for ApA and AMN and the observed shifts were larger than those observed for neutral salts. (c) If ion binding decreased the CD, the ellipticity would be expected to increase at higher temperatures as the ion–base complex dissociates; such an effect would introduce additive dependent nonlinearity into the van't Hoff plot. The uncertainty in the enthalpies (Tables I–IV) is a measure of the dispersion in the linear van't Hoff plot and is not dependent on either the nature or concentration of the additive. These results do not rule out the possibility of ion–base complex formation. However, they suggest that such binding does not significantly alter the CD spectrum by electronically perturbing the base; rather the relatively large variations of the optical activity induced by salt additives arise from conformational changes.

CD spectra of ApA taken in the presence of 5 M LiCl at different temperatures (10 scan time-averaged spectra were recorded) displayed isosbestic points at 236 and 260 nm. This

TABLE III: Apparent Thermodynamic Parameters[a] for ApU Stacking.

Sample	ΔH (kcal/mol)	ΔS (eu)	$T_{1/2}$ (°K)
4 M $(NH_4)_2SO_4$	−7.0 ± 0.2	−24.2	288
No salt	−5.9 ± 0.4	−22.0	260
5 M NaCl	−5.8 ± 0.1	−21.8	267
5 M LiCl	−5.8 ± 0.2	−21.8	266
5 M $NaClO_4$	−5.7 ± 0.3	−23.2	247
pH 3	−5.6 ± 0.2	−22.7	246

[a] Only relative values have physical significance (see Discussion).

TABLE IV: Apparent Thermodynamic Parameters[a] for AMN Stacking.

Sample	ΔH (kcal/mol)	ΔS (eu)	$T_{1/2}$ (°K)
5 M NaBr	−2.4 ± 0.1	−9.4	256
4.5 M $NaClO_4$	−2.6 ± 0.3	−10.5	251
5 M NaCl	−2.2 ± 0.2	−9.0	249
2.5 M $NaClO_4$	−3.0 ± 0.1	−12.2	248
3 M NaCl	−2.3 ± 0.2	−9.5	245
1 M NaCl	−2.3 ± 0.2	−9.5	245
4 M $(NH_4)SO_4$	−2.1 ± 0.1	−8.8	243
4 M $NaClO_4$	−2.1 ± 0.1	−8.9	240
2 M $NaClO_4$	−2.5 ± 0.2	−10.4	238
3.2 M KBr	−2.2 ± 0.1	−9.3	236
3.5 M $NaClO_4$	−2.0 ± 0.1	−8.6	236
4 M KCl	−2.0 ± 0.1	−8.6	229
1 M $NaClO_4$	−2.4 ± 0.2	−10.5	229
0.5 M NaCl	−2.0 ± 0.4	−9.0	226
2 M NaCl	−1.8 ± 0.2	−8.0	221
No salt	−2.2 ± 0.2	−10.0	221
No salt	−1.9 ± 0.1	−8.7	215
2 M TEACl	−1.9 ± 0.1	−9.5	198
40% dioxane	−1.4 ± 0.4	−9.3	151

[a] Only relative values have physical significance (see Discussion).

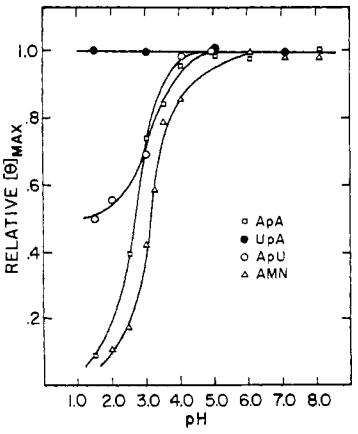

FIGURE 6: Titration of ApA, ApU, UpA, and AMN at 24°. Relative $[\theta]_{max}$ = ellipticity at the observed pH/ellipticity at pH 7.00. Monomer optical activities have not been subtracted.

result is consistent with the assumption of two optical states and indicates that the van't Hoff procedure for the extraction of thermodynamic information is valid for the analysis of the temperature-dependent CD spectra of dinucleotides in salt solutions.

Hence, CD data taken as a function of temperature (θ_{max} *vs.* T) were subjected to a van't Hoff analysis using the methodology outlined by Lowe and Schellman (1972) except for our choice of the ellipticity characterizing the fully stacked dimer, θ_s.

Most workers assume θ_s to be independent of both temperature and solvent, and iterate for the value of θ_s which best fits the van't Hoff equation to a straight line. The iterated choice of θ_s is ambiguous because the van't Hoff plot becomes more linear as θ_s increases, and thus depends on the criteria adopted for the best straight-line fit. Because of the ambiguity associated with an iteration we chose to use the theoretically calculated values of θ_s of Johnson and Tinoco (1969) for the CD of fully stacked dinucleotides. For θ_u, the ellipticity of the unstacked dimer, the sum of the ellipticities of the constituent mononucleotides was chosen. In the case of AMN we used the θ_s of UpA (since no theoretical data were available) and θ_u was set equal to the ellipticity of adenosine.

It should be stressed that the thermodynamic values listed in Tables I–IV have little quantitative significance because of the uncertainty in θ_s. The theoretical values of this parameter may be poor, but their use seems to us to be less arbitrary than iterated values of θ_s. It is incorrect to quantitatively compare the results obtained for one dinucleotide with another since the values of ΔH, ΔS, and $T_{1/2}$ critically depend on the choice of θ_s and θ_u. However, the relative values of these parameters were found to be independent of θ_s and θ_u over a wide range of values; thus the trends in these parameters for a particular dinucleotide in a variety of different solvents are meaningful.

The results presented in Figure 4 demonstrate that neutral salts destabilize purine–purine or purine–pyrimidine base–base stacking in 3′–5′-linked dinucleotide phosphates in a pattern roughly similar to that observed for DNA (von Hippel and Schleich, 1969a,b). Previous studies have shown that salt additives and increasing temperature do not appreciably alter the conformational features of nucleosides and nucleotides (Schleich *et al.*, 1972); consequently, conformational perturbants such as the neutral salts and increasing temperature must exert their effects at the dinucleotide or higher oligomer structural level. The results of the present study show that the

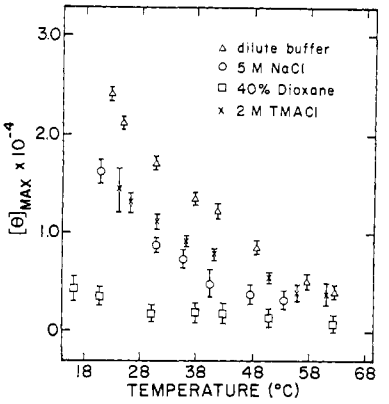

FIGURE 7: Ellipticities at 272 nm of ApA in the presence of neutral salt additives or dioxane as a function of temperature at pH 7.0.

dinucleotide is the minimum structural unit necessary for temperature- and additive-induced conformational changes. Additionally, both base composition and sequence influence the response to salt additives. Whether the dinucleotides maintain their sequence and composition-dependent salt response in polynucleotides is unknown. If they do, then a solution of NH_4Cl might be expected to induce stacking in one part of the polymer while causing unstacking in another portion relative to the salt-free case.

The polar additives unstacked the dinucleotides according to the Hofmeister series (Figure 4); AMN was stacked by these additives but to the same extent in all cases (Figure 3). In contrast to the polar salts, nonpolar solvents such as methanol, dioxane, and the long-chained tetraalkylammonium salts unstacked both the dinucleotides and AMN whereas additives with both polar and nonpolar character (such as urea and GdmCl) evoked an intermediate response. Although AMN has no phosphate backbone, fewer bonds between heterocyclic rings, and fewer hydrogen-bonding sites than the dinucleotides, the structural requirements necessary for nonpolar additives to disrupt the conformation are evidently present in AMN. The polar additives apparently require some additional structural feature in order to unstack dinucleotides. These results are evidence that additives which alter the con-

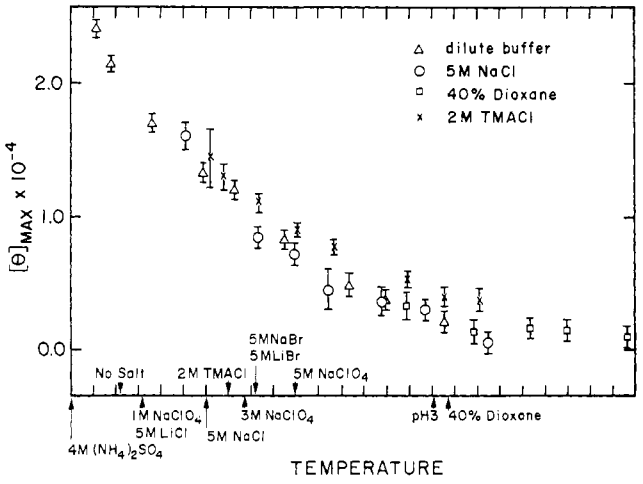

FIGURE 8: Superimposition of the temperature-dependent ellipticities of ApA (data taken from Figure 7). The units of the abscissa are temperature with 2° between each division. The arrows on the abscissa indicate the required value of the temperature offset (relative to no salt) to achieve melting curve superimposition in the presence of the specified additive.

formation of dinucleotides act by at least two different mechanisms.

Figure 5 shows the similarities between the CD spectrum of UpcA, the high-temperature spectrum of UpA and the maximum CD intensity of an equimolar mixture of adenosine and uridine. The magnitude of the UpcA CD was insensitive to temperature and also remained unchanged at 5° even in the presence of 4 M $(NH_4)_2SO_4$, an additive which has been shown to enhance stacking in dinucleotides. We conclude from these observations that UpcA is unstacked in solution.

The UpcA results indicate that the phosphodiester linkage is necessary for base stacking in UpA and possibly other 3′–5′-linked dinucleoside phosphates as well. This may be due to preferred conformations about the phosphodiester backbone. *Ab initio* molecular orbital calculations indicate a high torsional potential about the P–O bonds in dimethyl phosphate (Newton, 1973). Molecular orbital treatments of polynucleotide conformation likewise predict minima in the potential energy function for rotation about P–O bonds (Govil, 1973; Perahia *et al.*, 1973).

We suggest a mechanism by which the P–O bond might influence the conformation of dinucleoside phosphates. It is apparent from the bond lengths of phosphates and phosphoric acid that the P–O bonds of these compounds have over 50% double-bond character (Pauling, 1960). Similarly, the theoretical stability of the P–O ester bond in AMP depends significantly on the existence of a π bond between the phosphorus 3d orbitals and the oxygen 2p orbitals (Boyd and Lipscomb, 1969). Such a π bond is consistent with the X-ray structures of a variety of phosphate esters including nucleotides (Corbridge, 1966; Sundaralingam, 1969); the values of the C–O–P bond angles cluster about 120 ± 5° which is characteristic of sp^2 hybridization at the oxygen. Inspection of CPK space-filling models reveals that replacement of the 5′-oxygen in the adenosine portion of UpA by a methylene does not sterically hinder base stacking. However, a π bond to the phosphorus atom is not possible in the phosphonate derivative; hence we suggest that rotation about the P–O bonds of dinucleoside phosphates in solution is hindered as a consequence of this π bond. In UpA this rotational restriction is evidently necessary for the formation of the stacked incipient helical conformation. Additionally, our observation that neutral polar salt additives unstack dinucleotides but not AMN raises the intriguing possibility that these additives affect the conformation of 3′–5′-linked dinucleoside phosphates by easing rotational restrictions about the phosphodiester backbone, while nonpolar additives only modify the solvophobic base–base interactions.

Using ultraviolet difference spectroscopy, Simpkins and Richards (1967) concluded that low pH induced unstacking in ApA, ApU, and UpA. However, hypochromism and optical rotatory dispersion experiments (Warshaw and Tinoco, 1966) as well as our CD results indicate low pH only elicits unstacking in ApA and ApU, UpA remaining unaffected. The origin of the low pH induced unstacking of ApA, ApU, and AMN and the indifference of UpA conformation to changes in pH is uncertain. The CD spectra of UpA and ApU both shift to higher energy at low pH; evidently both molecules experience similar electronic perturbations as a result of protonation. However, the magnitude of the CD changes only for the latter. If we assume an incipient right-handed anti–anti RNA-type helix geometry for the various dimers, the location of the site of protonation, the adenine N(1), relative to the other base suggests a simple explanation for the pH-induced unstacking. In ApA the two sites of protonation seem sufficiently close for effective charge–charge repulsion to separate the chromophores. In ApU the adenine N(1) is covered by the plane of the neighboring base, and protonation places a charged group near the uracil. The free energy gained by solvating the protonated adenine could be the driving force for unstacking in ApU. AMN may unstack for similar reasons. The adenine N(1) in UpA, however, appears maximally exposed to solvent, thereby minimizing differential solvation effects.

In this simple form the above explanation is incomplete. Recently, Jordan and Sostman (1972) calculated the ground-state wave functions for protonated 8-methyladenine and found the positive charge was not localized but rather spread around the purine ring. They also found that protonation drastically increased the Coulombic repulsion between the bases. Whether Coulombic interactions alone are sufficient to account for the pH-induced unstacking of dinucleoside phosphates in the presence of solvent–dinucleotide interactions is a matter of conjecture at this point.

Conclusions

This study of the CD spectral changes of ApA, ApU, UpA, AMN, and UpcA induced by neutral salt additives, organic solvents, pH changes, and temperature has led to several conclusions. The alteration in dinucleoside phosphate conformation induced by neutral salts more or less follows the Hofmeister series. Our results combined with earlier studies demonstrate that the dinucleotide is the minimum structural unit necessary for additive induced conformation changes in nucleic changes in nucleic acid structures. Not all of the perturbants which unstacked dinucleoside phosphates also unstacked AMN; nonpolar additives caused AMN to unstack but the polar electrolytes induced stacking. This suggests that these two classes of additives act by different mechanisms. Both ΔH and ΔS (for unstacked → stacked) were negative and decreased in absolute magnitude in the presence of denaturing agents, whereas stabilizing salts had the opposite effect. Replacing the 5′-ester oxygen in 3′–5′-linked UpA with a methylene group destroys the stacked structure of the molecule, thus demonstrating the importance of an intact phosphodiester linkage for the maintenance of the stacked conformation. Apparently the solvophobic stacking interaction present in UpA is insufficient to induce stacking in the absence of the normal phosphodiester linkage.

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