Proton and Phosphorus-31 Nuclear Magnetic Resonance Study on the Stabilization of the Anti Conformation about the Glycosyl Bond of 8-Alkylamino Adenyl Nucleotides†

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ABSTRACT: A ^1H and ^{31}P NMR investigation was carried out on a collection of 8-alkylamino nucleotides in aqueous solution in order to help determine why some of these compounds were oriented preferentially into the anti conformation about the glycosyl bond. Correlations were found between backbone conformation, molecular structure, and the presence of the anti rotamer. The conformation of the nucleotides 8-amino-, 8-methylamino-, and 8-ethylamino-AMP was unusual because in addition to being in the anti form, the ribose ring and the C4′–C5′ bond were almost entirely in the C2′-endo and gauche–gauche forms. As a result, the position 8 substituent was close to the $-CH_2OP$ part of the ribose moiety. Removal of the 5′-phosphate group did not have much influence on the gauche–gauche or anti populations. However, locking the nucleotide into the alternate trans–gauche conformation about C4′–C5′ by 3′,5′-cyclization resulted in a predominant syn conformation about the glycosyl bond. Furthermore, dialkylation to form 8-$N(CH_3)_2$-AMP destabilized both the anti and gauche–gauche conformations. It was concluded that the proximity of the ribose 5′-oxygen and the 8-NH of the base was necessary for stabilization of the anti conformation. These data were consistent with the formation of an intramolecular hydrogen bond. Removal of this interaction destabilized the anti form in favor of the usual syn form found in other 8-substituted nucleotides. An intramolecular interaction between the negatively charged phosphate group and the 8-amino moiety was also detected. Dephosphorylation caused a small but significant decrease in the C2′-endo ribose ring population. In addition, the C5′–O5′ bond was in the gauche′–gauche′ domain, but the angle ϕ was preferentially greater than 180°, which contributed to an unusually large chemical shift difference of the ribosyl 5′-methylene protons because of the deshielding from the phosphate group. The alkyl group tended to destabilize the interaction with the base. The unique intramolecular interactions present in 8-alkylamino nucleotides were discussed with respect to the conformation of other nucleotides.

It has been generally accepted that substituents at the 8 position of the purine base of β-nucleosides and β-nucleotides induce steric and electrostatic interactions which destabilize the normal anti conformation about the glycosyl bond in favor of the syn conformation. This change had been observed for 8-fluoro (Ikehara et al., 1972), 8-iodo (Schweizer & Robins, 1973), 8-bromo (Tavale & Sobell, 1970; Ikehara et al., 1972; Sarma et al., 1974), 8-oxo (Schweizer & Robins, 1973), 8-methylmercapto (Sarma et al., 1974; Schweizer & Robins, 1973), 8-isopropyl (Ikehara et al., 1972), and 8-methyl (Schweizer & Robins, 1973) substitutions. More recently, the 8-alkylamino nucleotides 8-NH_2-AMP[1] and 8-$NHCH_3$-AMP were found to adopt the anti conformation instead of the expected syn form (Evans & Kaplan, 1976). This led to the suggestion that an attractive force between the sugar phosphate moiety and the 8 position substitutent could stabilize the anti conformation. It was proposed that an intramolecular hydrogen bond between the 8-amino hydrogen and the ribose 5′-oxygen may be the stabilizing interaction (Evans & Kaplan, 1976; Evans et al., 1978). Quantum mechanical calculations on 8-NH_2-AMP predicted the presence of the hydrogen bond (Pohorille et al., 1978) and in addition indicated the presence of an electrostatic interaction between the negatively charged phosphate group and 8-NH_2 protons. In this paper the ^1H and ^{31}P NMR results on the aqueous solution conformations of a collection of 8-alkylamino nucleosides and nucleotides are discussed with respect to base–phosphate and base–ribose interactions.

Experimental Procedure

NMR measurements were carried out on a Varian HR 220 or a JEOL PS-100 spectrometer. Both were equipped with Nicolet Fourier transform systems. Me_3SiP was used as an internal reference for aqueous solution studies. Me_2SO-d_5 was used as an internal reference when "100%" Me_2SO-d_6 was the solvent. Chemical shifts were converted to parts per million downfield from Me_4Si by assigning the Me_2SO-d_5 resonance as 2.51 ppm. Peak assignments, chemical shifts, and coupling constants were fit by computer simulations using the Nicolet ITRCL program and the HR 220 plotting system.

The reliability of ^{31}P NOE measurements was increased by appropriate gating conditions. Low power irradiation of protons at a single frequency applied during the interval between pulses was combined with high power noise modulation during data acquisition. For minimization of temperature cycling, the high power irradiation was not gated off during the interval between acquisitions but instead moved 200 KHz off resonance and switched from noise modulation to contin-

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[1] Abbreviations used: 8-NH_2-AMP, 8-aminoadenosine 5′-monophosphate; 8-$NHCH_3$-AMP, 8-(methylamino)adenosine 5′-monophosphate; 8-$N(CH_3)_2$-AMP, 8-(dimethylamino)adenosine 5′-monophosphate; 8-NH-CH_2-cAMP, 8-aminoadenosine cyclic 3′,5′-monophosphate; 8-$NHCH_3$-A, 8-(methylamino)adenosine; 8-$NHCH_2CH_3$-3′,5′-ADP, 8-(ethylamino)adenosine 3′,5′-diphosphate; NOE, nuclear Overhauser enhancement; Me_3SiP, trimethylsilyl propionate; Me_2SO, dimethyl sulfoxide.

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Table I: Ribose Proton Coupling Constants[a]

compd	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	$J_{5'5''}$	$J_{4'P}$	$J_{5'P}$	$J_{5''P}$
8-NH_2-AMP	7.9	5.8	2.1	1.8	2.1	−11.7	2.0	2.5	5.3
8-$NHCH_3$-AMP	8.0	5.9	2.1	2.1	2.2	−11.7	1.9	2.8	5.9
8-$NHCH_2CH_3$-AMP	8.0	5.9	2.2	2.2	2.2	−11.7	1.7	2.8	6.5
8-$NHCH_2CH_3$-3′,5′-ADP	8.5	5.5	1.4	2.3	2.0	−11.7	2.0	2.8	6.2
8-$NHCH_3$-AMP (pD 5.0)	7.8	5.9	2.2	2.0	2.5	−11.5	1.9	3.0	5.9
8-$NHCH_3$-A	7.4	5.7	2.4	2.2[b]	2.3[b]	−11.7			
8-$N(CH_3)_2$-AMP	6.7	6.3	4.3	4.4	6.1	−11.5	ND[c]	6.0	6.1
8-$N(CH_3)_2$-AMP (pD 5.0)	6.5	5.9	4.1	4.4	5.6	−11.4	ND[c]	6.4	6.4
AMP	6.0	5.1	3.5	3.2	3.3	−11.8	1.7	4.6	4.6

[a] Coupling constants are accurate to ±0.2 Hz. The measurements were carried out at 0.01 M concentration except for 8-$NHCH_3$-A (0.005 M). The pD values were 8.5, unless indicated otherwise. The pD of 8.5 results in a doubly ionized phosphate group. At pD 5.0 the phosphate group is in the monanionic form and the base carries a partial positive charge due to the relatively high pK_a (5.0) of the 8-alkylamino adenyl base (Evans et al., 1978). [b] Only the sum $J_{4'5'} + J_{4'5''}$ is significant because the ribose methylene protons exhibit no chemical shift difference. [c] ND = not detected.

Table II: Nonexchangeable Ribose Proton Chemical Shifts[a]

compd	proton 1′	2′	3′	4′	5′	5″
8-NH_2-AMP	6.10	4.81	4.49	4.38	4.03	4.08
8-$NHCH_3$-AMP	6.05	4.69	4.48	4.35	3.97	4.06
8-$NHCH_2CH_3$-AMP	6.05	4.83	4.49	4.35	3.98	4.09
8-$NHCH_2CH_3$-3′,5′-ADP	6.09	4.84	4.71	4.54	3.98	4.13
8-$NHCH_3$-A	5.98	4.79	4.41	4.28	3.90	3.90
8-$N(CH_3)_2$-AMP	5.80	5.31	4.50	4.22	4.13	4.06

[a] Chemical shifts are given in parts per million downfield from internal Me_3SiP. The solution conditions are given in Table I.

Table III: Computed Conformational Populations of Ribose Moiety[a]

compd	C4′–C5′ (method I) *gg* (%)	C4′–C5′ (method I) *gt*/*tg* (%)	C4′–C5′ (method II) *gg* (%)	C4′–C5′ (method II) *gt*/*tg* (%)	ring ^2E (%)	ring ^3E (%)
8-NH_2-AMP	(101)	0	91	9	80	20
8-$NHCH_3$-AMP	97	3	87	13	80	20
8-$NHCH_2CH_3$-AMP	96	4	86	14	80	20
8-$NHCH_2CH_3$-3′,5′-ADP	97	3	87	13	85	15
8-$NHCH_3$-AMP (pD 5.0)	95	5	85	15	80	20
8-$NHCH_3$-A	95	5	85	15	75	25
8-$N(CH_3)_2$-AMP	33	67	25	75	60	40
8-$N(CH_3)_2$-AMP (pD 5.0)	38	62	30	70	60	40
AMP	74	26	65	35	65	35

[a] Computation by method I (Lee & Sarma, 1976) results in approximately a 10% higher *gg* population than that by method II (Wood et al., 1973) due to use of a different H-C-C-H Karplus relationship. The ribose ring conformation was calculated according to the basic approach of Altona & Sundaralingam (1973) as described in Sarma et al. (1974). Again, the absolute error is at least 10%, but conformational trends are significant.

uous wave. During acquisitions, the frequency was moved back on resonance and the noise modulation switched on. Both a proton-decoupled ^{31}P resonance and a selective NOE were obtained. The standard gating technique of irradiation during data acquisition only has been used previously in ^{31}P studies to obtain decoupled spectra without a NOE (Evans & Kaplan, 1977).

The 8-alkylamino 5′-nucleotides were synthesized according to published procedures (Evans & Kaplan, 1976; Evans et al., 1978). The nucleoside 8-$NHCH_3$-A was prepared by enzymatic dephosphorylation of the 5′-nucleotide. 8-$NHCH_2CH_3$-3′,5′-ADP was prepared in the same manner as the other nucleotides except that 3′,5′-ADP (commercial product) was used as starting material. Samples were lyophilized from 99.8% D_2O and then dissolved in commercial "100%" D_2O. The pD values were corrected from pH meter readings by addition of 0.4 unit. For Me_2SO measurements, dry samples were directly dissolved in 100% Me_2SO-d_6.

Results

NMR parameters from the 220-MHz ^1H NMR spectrum of several 8-alkylamino nucleosides and nucleotides have been measured by matching computer-simulated spectra with the experimental results (Tables I and II. This procedure also allowed unambiguous assignments, with the exception of the distinction between the ribosyl 5′- and 5″-methylene protons.

The conformation of the ribose ring (Altona & Sundaralingam, 1973; Sarma et al., 1974) and the C4′–C5′ bond (Wood et al., 1973; Lee & Sarma, 1976) was computed from time-averaged proton–proton coupling constants according to the standard procedures. The conformation about the C5′–O5′ bond was determined from proton–phosphorus coupling constants (Lee & Sarma, 1976). The results for the ribose ring and C4′–C5′ bond are expressed in terms of conformer populations (Table III). Computations of this type were approximate in nature but were especially useful in discerning trends in conformational changes.

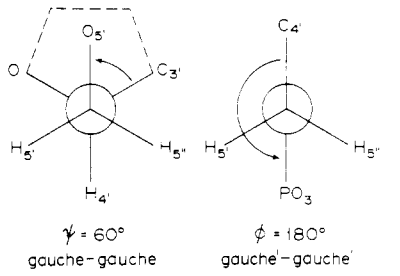

FIGURE 1: Newman projections defining the angle ψ for rotation about C4′–C5′ (left) and the angle ϕ for rotation about C5′–O5′ (right). The classical gauche–gauche (*gg*) and gauche′–gauche′ (*g′g′*) orientations are shown.

The distribution of conformations of the ribose ring was identical for 8-NH_2-, 8-$NHCH_3$-, and 8-$NHCH_2CH_3$-AMP (Tables I and III).[2] Each had 80% ^2E and 20% ^3E popula-

[2] The conformation of the ribose moiety of 8-$N(CH_3)_2$-AMP differed substantially from that of all the other 8-alkylamino nucleotides because it was the only nucleotide which was predominantly in the syn conformation about the glycosyl bond. The conformation of 8-$N(CH_3)_2$-AMP was similar to that previously reported for other syn purine nucleotides (Sarma et al., 1974).

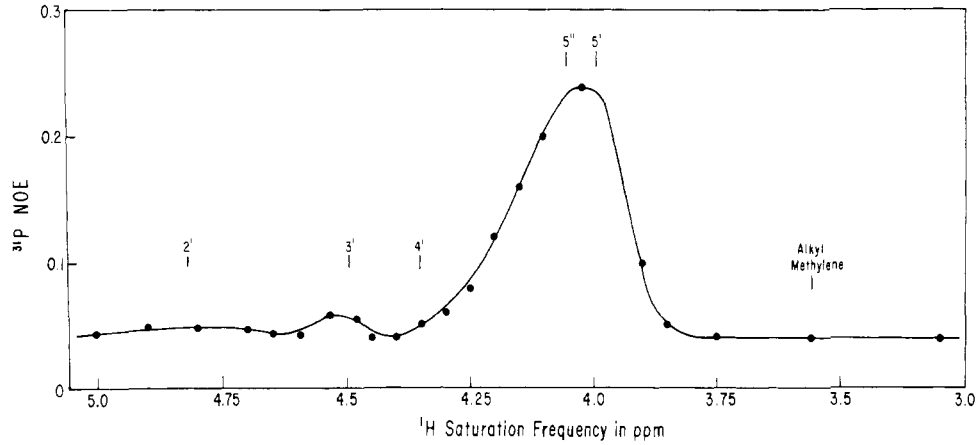

FIGURE 2: Magnitude of the ^{31}P NOE following low power selective ^{1}H irradiation for a number of different ^{1}H frequencies is greatest for irradiation of the ribose methylene protons. High power broad-band proton saturation causes an NOE of 1.68. Samples contained EDTA and were degassed with He.

tions. Removal of the 5′-phosphate group caused approximately a 5% decrease in the ^{2}E form, and 3′-phosphorylation caused approximately a 5% increase in the ^{2}E form.[3] The 85% ^{2}E population for the nucleoside 3′,5′-diphosphate was the highest ^{2}E population ever found for a flexible nucleotide. It has been suggested that the ^{2}E conformation reduces unfavorable steric interactions between the sugar and the 8-alkylamino substituent (Evans et al., 1978).

The C4′–C5′ bond was 85–100% in the *gg* form (Figure 1) for the above nucleotides, and the various phosphorylations had no detectable effect on the conformation (Table III). Increases in the length of the alkyl chain caused a very small decrease in the *gg* population. The magnitude of $J_{4'5'}$ was equal to that of $J_{4'5''}$ in each case. The C5′–O5′ bond was predominantly *g′g′* in all cases. According to a standard $J_{5'P}$ and $J_{5''P}$ analysis (Lee & Sarma, 1976), the *g′g′* populations were ~80% (Figure 1). The values $J_{5'P}$ and $J_{5''P}$ were unequal, and the difference between them increased with increasing alkyl chain length (Table I). A further description of the C5′–O5′ conformation is given under Discussion. It may be concluded that both the C4′–C5′ and C5′–O5′ bonds had conformational flexibility.

The chemical shift difference of the ribosyl methylene protons of the 5′-mononucleotides depended on the length of the alkyl chain. The chemical shift difference ranged from 0.05 ppm for 8-NH_2-AMP to 0.11 ppm for 8-$NHCH_2CH_3$-AMP (Table II). The chemical shift difference correlated to the difference between the coupling constants $J_{5'P}$ and $J_{5''P}$ (Table I). The ribose ring and C4′–C5′ conformation did not depend appreciably on alkyl chain length. These data strongly suggested that the orientation about the C5′–O5′ bond had some influence on the chemical shift difference between the methylene protons. This was consistent with a deshielding effect from the 5′-phosphate group.[4] Further, there was no detectable chemical shift difference for the ribosyl methylene protons of the nucleoside (Table II).

The assignment of the methylene proton (Table II) was based on the deshielding from a nearby 3′-phosphate group as had been described previously (Remin & Shugar, 1972; Lee & Tinoco, 1977) and also was supported by an independent technique (DeLeeuw et al., 1977). The high *gg* population about C4′–C5′ made 8-alkylamino nucleotides well suited to this assignment method.

The chemical shift of H2′ in purine nucleosides and nucleotides has been used as an indicator of the conformation about the glycosyl bond (Sarma et al., 1974; Evans & Kaplan, 1976; Schweizer & Robins, 1973; Pless et al., 1978; Giessner-Prettre & Pullman, 1977a,b). This accounted for the downfield chemical shift of H2′ in 8-$N(CH_3)_2$-AMP compared to other 8-amino nucleotides (Table II). The similarity in the chemical shift of H2′ in 8-$NHCH_3$-A to that of the anti 5′-nucleotides (Table II) showed that the 8-alkylamino nucleosides also were preferentially in the anti conformation.

The chemical shift of the exchangeable 8-amino proton of 8-$NHCH_3$-A was measured in Me_2SO-d_6. The chemical shift of 7.01 ppm from Me_4Si was within a range characteristic of aromatic amines.

^{31}P NOE measurements were carried out on 8-$NHCH_2CH_3$-AMP (Figure 2) to help determine the orientation of the phosphate group. The maximum NOE observed for broad-band proton saturation was 1.68, which was typical for biological phosphorus compounds reported at 40 MHz (Evans, 1979). Most of the NOE resulted from saturation of the ribosyl methylene protons. The other ribose protons as well as the alkylamino methylene protons did not contribute significantly to the NOE. This indicated that the ribosyl methylene protons were the nearest protons to the phosphate group. The upfield ^{31}P chemical shift of 8-alkylamino nucleotides compared to most other nucleotides was consistent with a Coulombic attraction between the phosphate group and the base (Evans & Kaplan, 1979).

Discussion

The unusually high population of *gg* for 8-NH_2-, 8-$NHCH_3$-, and 8-$NHCH_2CH_3$-AMP correlated with the anti conformation reported earlier (Evans & Kaplan, 1976). Most other nucleotides, including 8-substituted ones, had a different distribution of conformations for the ribose moiety (Davies & Danyluk, 1974; Sarma et al., 1974; Evans & Sarma, 1974). Insofar as the NH was spatially close to the $-CH_2OP$ part of the ribose phosphate moiety and this was only true in a simultaneous anti *gg* conformation, an interaction between these two groups which stabilized the anti conformation was implicated. A test of this hypothesis was in the conformation about the glycosyl bond of 8-$NHCH_3$-cAMP. Cyclization locked C4′–C5′ into the alternate trans–gauche conformation for which case the NH and $-CH_2OP$ groups would not interact significantly because the distance between them is too far.

[3] The increase in the ^{2}E population at the expense of ^{3}E manifests in an increase of $J_{1'2'}$ and a corresponding decrease in $J_{3'4'}$ (Evans & Sarma, 1974).

[4] The chemical shift difference of the ribosyl methylene protons need not be zero if ϕ was exactly 180° and the *gg* population was 100%.

Instead, there should be unfavorable steric and electrostatic interactions between the 8 position substituent and the ribose which would destabilize the anti conformation in favor of the syn form. One may observe that 8-$NHCH_3$-cAMP existed in the syn conformation (Schweizer & Robins, 1973), which was consistent with our hypothesis. Additional support came from the conformation of 8-$N(CH_3)_2$-AMP. This nucleotide apparently adopted the syn conformation (Evans & Kaplan, 1976) because of the absence of an NH group. All the above data were consistent with the suggestion made earlier that an intramolecular hydrogen bond between the NH of the base and the 5′-oxygen of the ribose stabilized the anti conformation (Evans & Kaplan, 1976). Molecular orbital calculations have provided direct evidence for hydrogen bonding in this novel class of nucleotides (Pohorille et al., 1978). The data here further show that there were stringent backbone conformational requirements for this interaction. If these requirements were not met or if the proton donor was unavailable, the flexible 8-alkylamino nucleotides adopted the syn conformation characteristic of other 8-substituted nucleotides.

Molecular orbital calculations on 8-alkylamino nucleotides have also indicated the existence of a Coulombic attraction between the negatively charged phosphate group and the 8-amino proton(s)[5] (Pohorille et al., 1978). Our studies supported this conclusion too. Dephosphorylation of an 8-alkylamino-AMP caused a reduction in the ^2E ribose ring population (Table III), whereas no such change occurred between the parent compounds AMP and adenosine (Evans & Sarma, 1976). Furthermore, as will be discussed next, the conformation about the C5′–O5′ bond was sensitive to interactions between the phosphate group and the 8 position substituent.

The large difference between the magnitude of $J_{5'P}$ and $J_{5''P}$ in the 8-alkylamino nucleotides (Table I) was a consequence of an unusual conformation for the C5′–O5′ bond. It was probable that this occurred while C4′–C5′ was in the gauche–gauche conformation because the 8-alkylamino nucleotides were almost entirely in the gauche–gauche conformation (Table III). Furthermore, in the one nucleotide with a low gauche–gauche population [8-$N(CH_3)_2$-AMP, Table III), the magnitude of $J_{5'P}$ equaled that of $J_{5''P}$ (Table I).

To help interpret the proton–phosphorus coupling constant data, an analysis of NOE and chemical shift measurements was made. The ^{31}P NOE data (Figure 2) indicated that the protons nearest to the phosphate moiety were those of the ribose methylene protons. This was inconsistent with the $t'g'$ conformation about C5′–O5′ because there would be a closer approach of the phosphate to the 3′ proton than to the methylene group. The r^{-6} dependence of dipolar relaxation would then result in a significant NOE upon selective irradiation of 3′, but this was not observed (Figure 2). A $g't'$ conformation was inconsistent with chemical shift data involving the deshielding effect of a phosphate group (Prado et al., 1978). In the $g't'$ conformation, the phosphate group would be nearest to 5′. However, 5′ was upfield of 5″. Only a predominant $g'g'$ conformational domain was consistent with all the chemical shift data and the NOE data. Thus, the higher value of $J_{5''P}$ indicated that ϕ (Figure 1) was preferentially greater than 180°. This may be due to an interaction between the 8 position substituent and the phosphate group. An attempt was made to define the average of ϕ from $J_{5'P}$ and $J_{5''P}$ by a Karplus analysis. Solutions could only be reached for models in which ϕ oscillated. A Karplus analysis showed that the greater the amplitude of oscillation in the $g'g'$ domain, the greater the time-averaged sum ($J_{5'P} + J_{5''P}$). According to this model, the longer alkyl chains produced more perturbation in the C5′–O5′ conformation. Insofar as the gg population about C4′–C5′ decreased somewhat with chain length, it appeared that the alkyl chain partially destabilized the interaction between the 8-amino moiety and the ribose phosphate group.

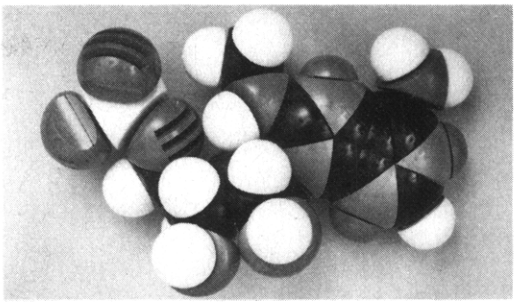

FIGURE 3: CPK molecular model of 8-$NHCH_3$-AMP in the anti, gg, $g'g'$, ^2E conformation illustrates the formation of a hydrogen bond between the NH of the 8-alkylamino base and the 5′-oxygen of the ribose moiety.

The question arose as to the relative importance of the proposed intramolecular hydrogen-bonding interaction compared to the electrostatic interaction involving the negatively charged phosphate group in stabilizing the anti conformation. Some information on this can be deduced from the conformation of the nucleoside (Table III). Dephosphorylation had no detectable effect on the gg population, and the anti form was still preferred in the nucleoside (vide supra). This was consistent with a hydrogen-bonding interaction as illustrated in Figure 3.

It was of interest to compare the conformation of 8-alkylamino nucleotides to trends which had been observed previously in other nucleosides and nucleotides. In structurally related pyrimidine nucleosides, increases in gg population were accompanied by increases in the ^3E population (Hruska et al., 1974). An inverse correlation existed for purine nucleosides (Hruska et al., 1977; Westhof et al., 1975). Some correlations had also been observed in nucleotides. In GMP a simultaneous increase in ^3E and gg populations followed protonation of the base (Hruska et al., 1974; Westhof et al., 1977). In 8-alkylamino nucleotides, the relatively strong interaction between the base and the ribose phosphate moiety dictated a different backbone conformation. It appeared that interrelationships between the ribose ring and the exocyclic conformation were brought about by relatively weak interactions and thus did not always control the final conformation. The data here also show the potential importance of a single relatively strong interaction in governing nucleotide conformation.

Formation of an intramolecular hydrogen bond between an amino group on the base and the ribose 5′-oxygen may take place in the other 8-alkylamino purine nucleosides and nucleotides as well since similar considerations would be involved. Furthermore, molecular models of pyrimidine nucleotides indicated that a hydrogen bond was structurally possible if an amino group resided at either the 2 or 6 position of a pyrimidine base. This type of interaction may be representative of a general principle concerning the conformation of modified nucleosides and nucleotides.

Added in Proof

The X-ray crystal structure of 9-β-D-arabinofuranosyl-8-(*n*-butylamino)adenine revealed that the anti conformation was stabilized by an intramolecular hydrogen bond between the

[5] The 8-alkylamino base of these nucleotides does not carry a formal positive charge in aqueous solution except under acidic conditions (Evans et al., 1978).

8-amino hydrogen and the sugar 5′-oxygen (Neidle et al., 1979).

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Helical Conformation of Glucagon in Surfactant Solutions†

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ABSTRACT: On the basis of circular dichroism (CD), glucagon in dilute acidic or alkaline solutions (e.g., less than 1 mg/mL) has little secondary structure (the polypeptide is sparingly soluble between pH 4 and 9). High peptide concentration, low temperature, and high ionic strength slightly enhance the helical conformation. In a 25 mM sodium dodecyl sulfate ($NaDodSO_4$) solution at pH below 4, about one-half of the glucagon molecule adopts a helical conformation that is independent of the polymer concentration. Sodium decyl sulfate is equally effective, but dodecylammonium chloride, dodecyltrimethylammonium chloride, and dodecyl heptaoxyethylene ether are slightly less effective in promoting the helicity of glucagon. Of the trypsin-digested glucagon fragments, only the C-terminal fragment (residues 19–29) can adopt a partially helical conformation in both ionic and nonionic surfactant solutions, but the N-terminal fragment (residues 1–12) and the middle fragment (residues 13–17) remain unordered in the presence of surfactant micelles. The results support our working hypothesis that surfactant micelles cluster around the polypeptide chain and hydrophobic interaction among the amphiphiles stabilizes the induced conformation that is related to the structure-forming potentials of the amino acid sequence. Charged side groups can interact with the amphiphile heads having charges of opposite sign, but charges of the same sign can destabilize the induced ordered conformation.

The anionic surfactant $NaDodSO_4$[1] has been widely used for studying protein subunit structures and for determining the molecular weights of proteins by gel electrophoresis (Weber & Osborn, 1969). It is known to alter the helical content of many proteins (Visser & Blout, 1971; Jirgensons, 1973; Mattice et al., 1976), but the mechanism of such interaction is not fully understood. The binding of $NaDodSO_4$ by proteins is believed to be stoichiometric on a weight basis (Reynolds & Tanford, 1970).

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[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; $NaDecSO_4$, sodium decyl sulfate; $DodNH_3Cl$, dodecylammonium chloride; $Dod(CH_3)_3NCl$, dodecyltrimethylammonium chloride; $C_{12}E_7$, dodecyl heptaoxyethylene ether; T-1, T-2, and T-3, glucagon fragments of residues 1–12, 13–17, and 19–29, respectively; CD, circular dichroism.

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