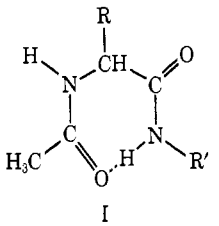
Circular Dichroism of Intramolecularly Hydrogen-Bonded Acetylamino Acid Amides†

John R. Cann

With the Technical Assistance of Mr. Robert O. Coombs

ABSTRACT: Circular dichroism (CD) and other spectroscopic measurements have been made on the intramolecularly hydrogen-bonded configuration of representative *N*-acetylamino acid amides, *N*-acetylamino acid *N'*-methylamides, and two tripeptides in the nonpolar solvent, dioxane. Aside from acetylprolinemethylamide, the hydrogen-bonded *N'*-methylamides and tripeptides examined exhibit CD, optical rotatory dispersion (ORD), and ultraviolet absorption characteristics reminiscent of α-helical polypeptides. Application of the CD and ORD of intramolecularly hydrogen-bonded acetylamino acid amides and *N'*-methylamides as an aid in interpreting the CD and ORD spectra of larger molecules is illustrated with bradykinin whose CD is reinterpreted as being indicative of a secondary structure having some order, most probably due to an internally hydrogen-bonded proline residue.

Recently, the circular dichroism (CD) and ultraviolet absorption difference spectra of *N*-acetyl-L-tyrosinamide produced by acetate buffer referred to methanol have proved useful in interpreting the changes in CD and ultraviolet absorption of ribonuclease accompanying interaction of the protein with acetic acid (Cann, 1971). These experiments have now been extended to include CD and other spectroscopic measurements on representative *N*-acetyl-L-amino acid amides, *N*-acetyl-L-amino acid *N'*-methylamides, and two tripeptides in the nonpolar solvent, p-dioxane. Dioxane was chosen for this study since it is known to promote formation of α helix and β structure in macromolecules (Iizuka and Yang, 1965, 1966). Moreover, as predicted theoretically (Madison and Schellman, 1970a) acetylamino acid methylamides form the intramolecularly hydrogen-bonded configuration (I) in nonpolar solvents like CCl_4 as revealed by



I

infrared spectroscopy on solutions sufficiently dilute that intermolecular hydrogen bonding is negligible (Tsuboi *et al.*, 1959).

As will be demonstrated shortly, knowledge of the CD characteristics of intramolecularly hydrogen-bonded acetylamino acid amides (and methylamides) is a valuable aid for interpretation of the CD spectra of polypeptides, proteins and supramolecular structure like cell membranes. Recently, Schellman and his coworkers (1967, 1970a,b) examined aspects of the CD and optical rotatory dispersion (ORD) spectra of acetylalaninamide, acetylprolinamide, and acetylprolinemethylamide in water and in nonpolar solvents including dioxane. They found that these compounds exhibit well-developed n–π^* Cotton effects in the neighborhood of 230 mμ in nonpolar solvents as contrasted to poorly resolved n–π^* Cotton effects at about 212 mμ in water, and concluded that these differences are conformationally related. Where our respective observations overlap, agreement is good.

Materials and Methods

The *N*-acetylamino acid amides, *N'*-methylamides, Gly-Tyr-Gly, and Gly-Phe-Gly (CP, Mann's analyzed or supplied with assay) were obtained from Cyclo Chemical, Schwarz/Mann, or Calbiochem, and were used without further purification. Eastman's *p*-dioxane was purified by passage through a column of activated aluminum oxide (Woelm basic, activity grade 1) and stored under nitrogen in the dark. In later experiments, the purified dioxane was stored over a small amount of the aluminum oxide.

CD and ORD spectra were recorded on a Cary Model 60 spectropolarimeter with a Model 6001 CD attachment. Measurements were made at 27.0°. Slits were programmed to yield a 15-Å bandwidth at each wavelength. Path lengths were dictated by the limited solubility of most of the compounds and by the absorbancy of dioxane in the far-ultraviolet region. In general, a 0.1-cm cell permitted reliable measurements down to a wavelength of 210 mμ with 0.03% solutions in dioxane. Reduced molar rotations, [*M'*], (deg cm²)/dmole, and ellipticities, [θ], (deg cm²)/dmole, were calculated in the usual fashion. Each spectrum is an average of at least two, usually three, and sometimes four determinations.

Ultraviolet absorption difference spectra were recorded on a Cary Model 14 spectrophotometer. Integral tandem absorption cells were employed. Near-infrared spectra (3150–3500 cm^{-1}) were recorded on a Beckman Model IR7 spectrophotometer with scale expansion to cover 90–100% transmission. The sample (1.4×10^{-3} M acetyltyrosinamide or 1.9×10^{-3} M

† From the Department of Biophysics and Genetics, University of Colorado Medical Center, Denver, Colorado 80220. *Received February 7, 1972.* Supported in part by Research Grant HE AI 13909-20 from the National Heart and Lung Institute, National Institutes of Health, U. S. Public Health Service. This publication is No. 468 from the Department of Biophysics and Genetics, University of Colorado Medical Center, Denver, Colo.

acetylprolinamide in dioxane) was contained in a liquid cell with NaCl windows spaced at 0.1 mm. A wedge was used to compensate for the blank. The author thanks Dr. Oscar K. Reiss for his kind permission to use these two instruments.

Results

CD, ORD, and ultraviolet absorption measurements have been made on derivatives of two aromatic amino acids, proline, and a few other aliphatic amino acids in 100% dioxane as compared to water or 50% dioxane–water mixture. The results of these measurements are presented below under headings corresponding to the forementioned order of amino acids.

Aromatic Amino Acids. In Figure 1A the CD spectrum of *N*-acetyl-L-tyrosinamide dissolved in 100% dioxane is compared to its spectrum in 50% dioxane–water mixture. The spectrum in 50% dioxane, which is essentially the same as in water or in 10^{-2} M HCl except for a small (2–3 mμ) red shift accompanied by a diminution of 5–15% in intensity in the mixed solvent, shows a strong positive band ([θ] 16,800°) at 228 mμ. In contrast, the spectrum in 100% dioxane shows three bands: a relatively weak, negative band whose wavelength of maximum intensity ([θ] −2600°) is 242 mμ; a positive band ([θ] 3300°) at 231 mμ; and a considerably stronger, negative band ([θ] −8200°) at 220 mμ. This drastic spectral change caused by a change from polar to nonpolar solvent is reversible. Thus, when a solution of the amide in 100% dioxane was diluted in half with water, the spectrum reverted to that shown in 50% dioxane by amide never exposed to pure dioxane. The CD difference spectrum of acetyltyrosinamide produced by 100% dioxane referred to 50% dioxane is presented in Figure 1B. The difference spectrum is characterized by a strong negative band (Δ[θ] −19,600°) centered at 224 mμ.

The effect of dioxane on the CD spectrum of acetyltyrosine amide finds its counterpart in the ORD spectra displayed in Figure 2A. Thus, the positive Cotton effect with a crossover at about 227 mμ shown in 50% dioxane–water mixture is red shifted and weakened by changing the solvent to 100% dioxane. The difference ORD spectrum produced by 100% dioxane referred to 50% dioxane (Figure 2B) is characterized by a negative Cotton effect with a trough (Δ[*M'*] −6400°) at 232 mμ; a crossover at 224.5 mμ; and a peak (Δ[*M'*] 8700°) in the region of 217 mμ.

Since it was anticipated that these results might have applications to polypeptides and proteins, it appeared desirable to extend our observations to include the tripeptide, Gly-Tyr-Gly. The CD spectrum of Gly-Tyr-Gly in 2×10^{-2} M HCl is much the same as that of acetyltyrosinamide (Figure 1A), exhibiting a strong positive band ([θ] 24,500°) centered at 226 mμ with a minimum ([θ] 12,500°) at 214 mμ. The spectrum in 98% dioxane–water mixture containing 4×10^{-4} M HCl shows a slightly red-shifted band ([θ] 21,900°) at 229 mμ, whose far-wavelength edge is relatively hypersharp, and a deep minimum ([θ] 3200°) at 219 mμ. Although the fraction of peptide molecules intramolecularly hydrogen bonded in 98% dioxane is evidently not as large as in the case of acetyltyrosinamide in pure dioxane (Figure 1A), the difference spectrum of the peptide produced by dioxane is similar to the difference spectrum of acetyltyrosinamide (Figure 1B) in that it shows a strong, negative band (Δ[θ] −15,000°) centered at 220 mμ.[1]

[1] This band would no doubt be centered closer to that of the amide had the difference spectrum been referred to 50% dioxane, which would be expected to cause a red shift of the positive CD band shown in water.

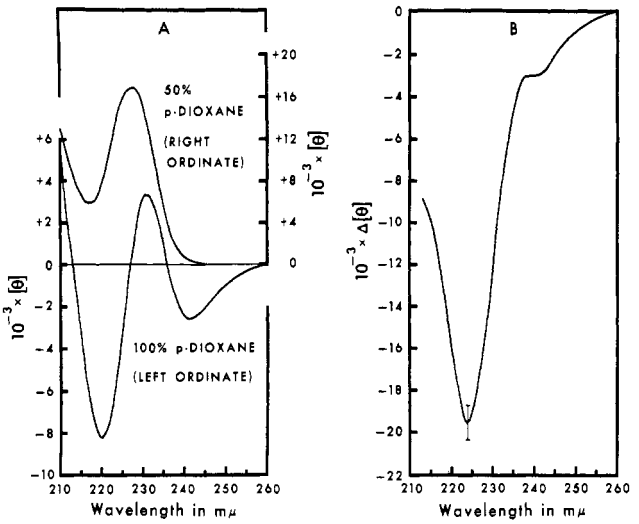


FIGURE 1: The CD behavior of *N*-acetyl-L-tyrosinamide. (A) Comparison of the CD spectra in 50% dioxane and in 100% dioxane; (B) CD difference spectrum produced by 100% dioxane referred to 50% dioxane. Error bar in this and the following figures indicates mean deviation.

There is also a weaker, positive band (Δ[θ] 2700°) at 230 mμ, which merely reflects the forementioned red shift.

Several different kinds of experiments were made in order to elucidate the molecular basis of the effect of dioxane described above. The first set of experiments explored the CD behavior of various tyrosine derivatives in 100% dioxane as contrasted to water, 10^{-2} M HCl or 50% dioxane–water mixture. In the case of *N,O*-diacetyl-L-tyrosinamide the behavior is very similar to that of *N*-acetyltyrosinamide. Thus, its CD spectrum in the two aqueous solvents or the 50% dioxane–water mixture shows a strong, positive band centered at about 220 mμ (221 mμ with [θ] 16,600° for 50% dioxane). In contrast, the spectrum in 100% dioxane shows two negative bands—one at 237 mμ ([θ] −5100°) and the other at 217 mμ ([θ] −11,500°). The difference CD spectrum shows a strong, negative band (Δ[θ] −26,700°) centered at 219 mμ. Likewise, the ORD spectrum of diacetyltyrosinamide is affected by dioxane in much the same way as in the case of acetyltyrosinamide; the difference ORD spectrum shows a trough (Δ[*M'*] −4900°) at 228 mμ, a crossover at 221.5 mμ, and a peak (Δ[*M'*] 12,800°) at 213 mμ. It can be concluded, therefore, that the phenolic hydroxy group of acetyltyrosinamide does not play a determining role in the effect of dioxane. On the other hand, the amide group is definitely required for the effect. Thus, *N*-acetyl-L-tyrosine methyl ester, *N*-acetyl-L-tyrosine, and *N,O*-diacetyl-L-tyrosine all exhibit essentially the same CD spectra in 100% dioxane (or 99.9% dioxane containing 10^{-2} M HCl) as in water or 10^{-2} M HCl; namely, a strong positive band centered at about 225 mμ in the first two cases and about 220 mμ in the third case. The role of the *N*-acetyl group was explored using L-tyrosinamide; but, unfortunately, the CD behavior of this compound is somewhat of an engima in that its spectrum exhibits a positive band in aqueous solvents (225 mμ) or 50% dioxane (229 mμ) and a single negative band (232 mμ) with about the same intensity in 100% dioxane. Thus, it appears that dioxane simply inverts the CD band, which would be a distinctly different effect from that described above for acetyltyrosinamide and diacetyltyrosinamide.

The second set of experiments inquired into possible molec-

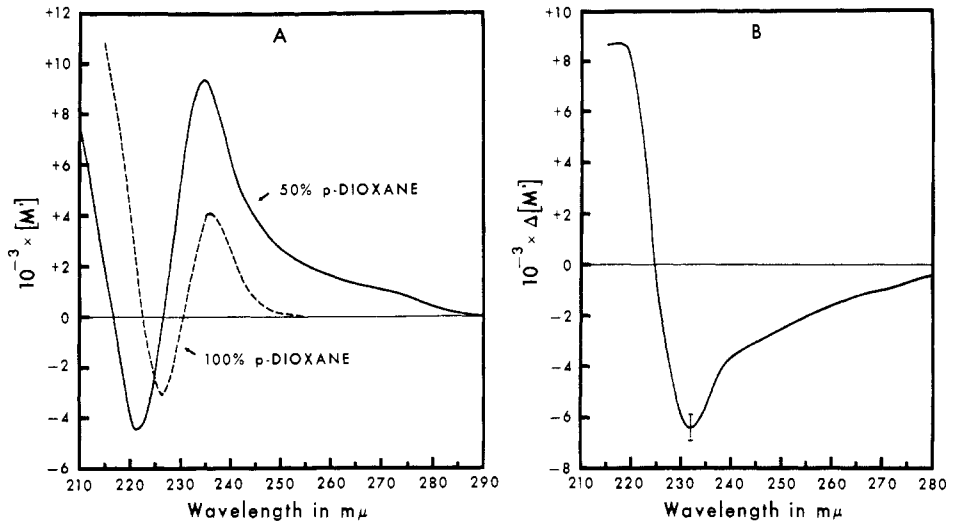


FIGURE 2: The ORD behavior of *N*-acetyl-L-tyrosinamide. (A) Comparison of the ORD spectra in 50% dioxane and in 100% dioxane; (B) ORD difference spectrum produced by 100% dioxane referred to 50% dioxane.

ular association of acetyltyrosinamide in dioxane. It is well known that amides and diamides at sufficiently high concentration in CCl_4 associate through intermolecular hydrogen bonding (Mizushima *et al.*, 1952; Klotz and Franzen, 1962). On the other hand, association of *N*-methylacetamide is much weaker in dioxane than in CCl_4 (Klotz and Franzen, 1962). As for acetyltyrosinamide, the following observations eliminate molecular association as the origin of the effort of dioxane on its CD behavior. (1) Its CD spectrum in dioxane (Figure 1A) is the same within experimental error at a concentration of 6.75×10^{-4} M as at 1.35×10^{-3} M. (2) Its ultraviolet absorption spectrum in dioxane shows bands at 285.5, 279, and 225 mμ whose absorbances strictly obey Beer's law over the 100-fold concentration range, 8.49×10^{-6} to 9.00×10^{-4} M, in the first two instances and the 10-fold concentration range tested, 8.49×10^{-6} to 8.49×10^{-5} M, in the third instance.

Finally, the near-infrared spectrum of acetyltyrosinamide in dioxane is similar to that reported by Tsuboi *et al.* (1959) for low concentrations of acetylamino acid methylamides in CCl_4 and shows a stretch band at 3300 cm^{-1} characteristic of the hydrogen-bonded NH group and another at 3420 cm^{-1} characteristic of the nonhydrogen-bonded NH. The molar absorption coefficients are about the same as reported for the methylamides in CCl_4.

These several observations justify the conclusion that the effect of dioxane on the CD spectrum of acetyltyrosinamide finds its origin in the formation of the intramolecularly hydrogen-bonded configuration I by at least a large fraction of the molecules in dioxane as contrasted to an extended, nonhydrogen-bonded configuration in water. Accordingly, the difference CD spectrum (Figure 1B) is interpreted to be the $n-\pi^*$ transition of the intramolecularly hydrogen-bonded diamide, which is shifted red relative to the extended configuration. This interpretation is in keeping with that of Madison and Schellman (1970a,b) with respect to proline derivatives.

The CD spectra of *N*-acetyl-L-phenylalaninamide and the tripeptide, Gly-Phe-Gly, are affected by dioxane in a manner similar to the corresponding tyrosine derivatives. The spectra of acetylphenylalanin amide are displayed in Figure 3. The difference spectrum produced by 100% dioxane relative to 50% dioxane shows a strong, negative band ($\Delta[\theta]$ −17,000°) centered at 218 mμ (217 mμ when referred to water). The spectra and difference spectrum obtained at a concentration of 1.45×10^{-4} M are the same within experimental error as those at 1.45×10^{-3} M. The CD spectrum of Gly-Phe-Gly in 98% dioxane containing 4×10^{-4} M HCl[2] shows a negative band ([θ] −2500°) at 237 mμ and a positive one ([θ] 6400°) at 221 mμ as contrasted to a single positive band ([θ] 18,700°) at 217 mμ in 2×10^{-2} M HCl; the difference spectrum shows a negative band ($\Delta[\theta]$ −14,000°) centered at 216 mμ.[1]

Proline. The CD and ORD spectra of *N*-acetyl-L-proline-*N'*-methylamide in dioxane and in water are compared in Figure 4A. The markedly red-shifted CD and ORD spectra in dioxane are also compared to the ultraviolet difference spectrum produced by dioxane referred to water: the negative

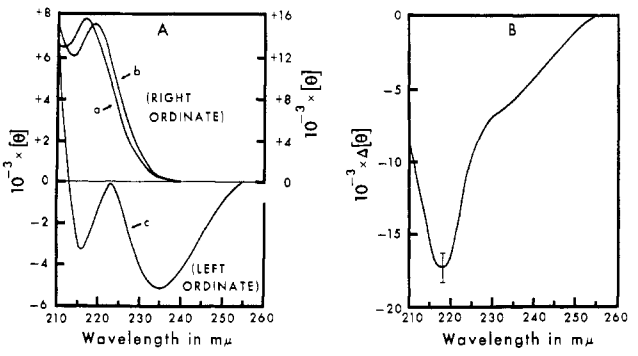


FIGURE 3: The CD behavior of *N*-acetyl-L-phenylalaninamide. (A) Comparison of the CD spectra in water (curve a) and in 50% dioxane (curve b) with the spectrum in 100% dioxane (curve c); (B) CD difference spectrum produced by 100% dioxane referred to 50% dioxane.

[2] Solutions of Gly-Phe-Gly in 98% dioxane were prepared by dilution with dioxane of a solution of the peptide in 2×10^{-2} M HCl. The peptide eventually crystallizes from this medium, but the CD spectra were recorded before crystallization ensued. The results were confirmed qualitatively by experiments on solutions containing only 90% dioxane from which the peptide does not crystallize; the difference spectrum relative to 2×10^{-2} M HCl showed a negative band ([θ] −8600°) at 216 mμ.

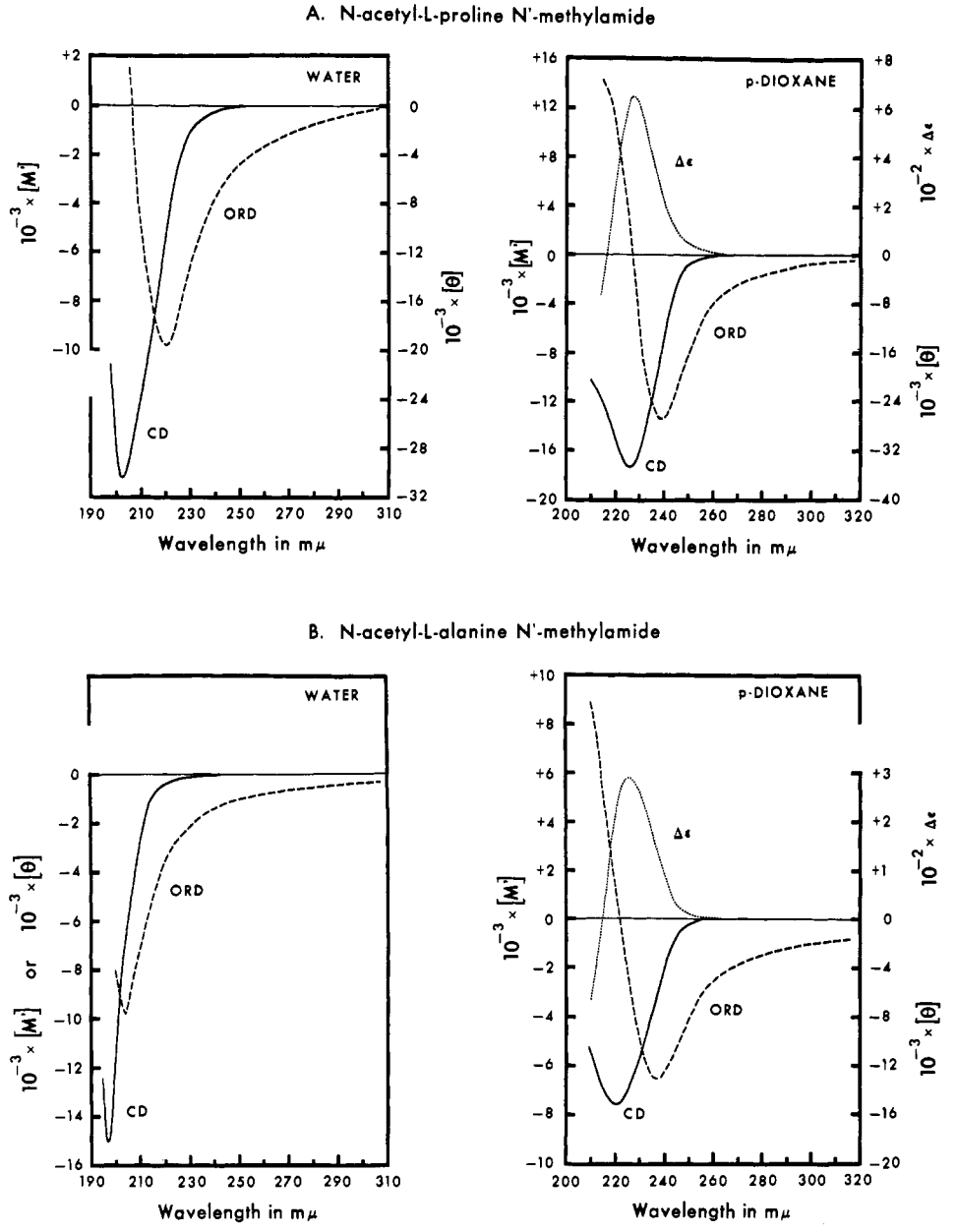


FIGURE 4: Comparison of the optical properties of aliphatic acetylamino acid methylamides in water with those in dioxane. (A) *N*-Acetyl-proline-*N'*-methylamide; $\Delta\epsilon$ is the difference molar extinction coefficient produced by dioxane referred to water. Only the positive ordinate for $10^{-2} \times \Delta\epsilon$ is shown; when reading negative values, extend the scale below zero assigning the value, -2, to each interval. (B) *N*-Acetyl-L-alanine-*N'*-methylamide; when reading negative values of $10^{-2} \times \Delta\epsilon$, extend scale below zero assigning the value, -1, to each interval.

CD band is centered at 226 mμ; the ORD trough is at 239 mμ with a crossover at 227 mμ; and the wavelength of maximum difference-absorbance is 227.5 mμ. Thus, as noted previously by Madison and Schellman (1970a), the optically active band is virtually isolated. Moreover, both the CD spectrum and the ultraviolet absorption difference spectrum are the same within experimental error at a concentration of 1.67×10^{-4} M as at 1.67×10^{-3} M, which eliminates molecular association of the amide as the source of the effect of dioxane. Acetylprolinamide behaves similarly. In dioxane it exhibits a negative CD band at 230 mμ; an ORD trough and crossover at 242 and 231 mμ, respectively; and a wavelength of maximum difference-absorbance at 229 mμ. Once again, its CD spectrum and absorbance difference spectrum is the same over a 10-fold concentration range; and its near-infrared spectrum shows a broad band at 3300 cm^{-1}.

Other Aliphatic Amino Acids. The optical properties of *N*-acetyl-L-alanine-*N'*-methylamide in water and in dioxane are displayed in Figure 4B. The striking features in dioxane are the negative CD band centered at 221 mμ; the ORD trough and crossover at 236.5 and 222 mμ, respectively; and the wavelength of maximum difference absorbance referred to water at 225.5 mμ. Virtually the same behavior is exhibited by acetylleucinemethylamide; although the intensity of the several bands are weaker, their position and the position of the ORD crossover are the same to within 1 mμ. It is interesting that in contrast to acetylalaninemethylamide, acetylalaninamide in dioxane shows an ORD trough above 240 mμ with a crossover at about 228 mμ (Schellman and Nielsen, 1967). We find that the same is true for acetylvalinamide whose ORD in dioxane shows a trough at 244 mμ with a crossover at 229 mμ. It exhibits a negative CD band at 224.5 mμ and a

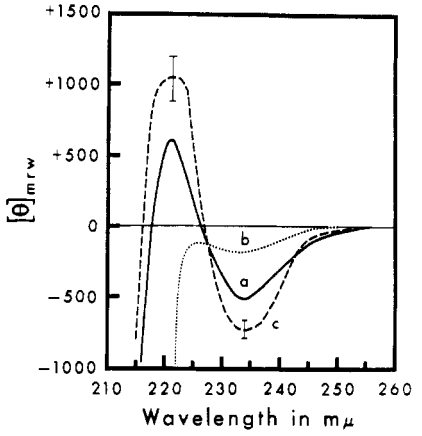


FIGURE 5: Comparison of the CD spectrum of bradykinin (curve a) with the spectrum of its analog [Leu[5,8]Gly[6]]-bradykinin (curve b) and with a spectrum simulated for bradykinin as described in the text. The spectrum of bradykinin is from Brady *et al.* (1971) as confirmed by J. R. Cann and J. M. Stewart, unpublished data; bradykinin analog from J. R. Cann and J. M. Stewart, unpublished data.

wavelength of maximum difference-absorbance referred to water at 230 mμ. Its difference CD spectrum shows a negative band at 231 mμ and its difference ORD, a trough at 246 mμ with a crossover at 234 mμ. The CD spectra and the ultraviolet absorption difference spectrum obey Beer's law over the 10-fold concentration range, 1.90×10^{-4} to 1.90×10^{-3} M.

Discussion

Two generalizations emerge from the results presented above. The first one is that the well-developed n–π* Cotton effect exhibited by intramolecularly hydrogen-bonded *N*-acetylamino acid amides in dioxane is red shifted with respect to that shown by hydrogen-bonded *N*-acetylamino acid *N'*-methylamides, and tripeptides. Thus, for example, the Cotton effect shown by acetylalaninamide is centered at about 228 mμ while that shown by acetylalaninemethylamide is at 222 mμ. Likewise, acetylprolinamide shows its Cotton effect at 231 mμ as contrasted to 227 mμ for acetylprolinemethylamide. The second generalization is that intramolecularly hydrogen-bonded acetylprolinemethylamide is unique in that its Cotton effect occurs at a longer wavelength than that shown by the other hydrogen-bonded *N'*-methylamides and tripeptides examined. In the case of the latter compounds, the Cotton effect is centered near 220 mμ. This observation calls to mind the 220–222-mμ n–π* Cotton effect of the α helix. Indeed, without implying any underlying relationship between the intramolecularly hydrogen-bonded *N'*-methylamides and tripeptides and the α-helical conformation of polypeptides, we note several parallelisms between their CD, ORD, and ultraviolet absorption difference spectra in the wavelength region under consideration. . Compare, for example, the optical properties of hydrogen-bonded acetylalaninemethylamide (Figure 4B) with those of the α-helical conformation of poly(L-Glu) and poly(L-Lys) (Fasman, 1967; Glazer and Rosenheck, 1962). (In the case of the α helix, Δε is referred to the random-coil conformation.) Although these parallelisms may be of limited theoretical significance, they have practical implications for the interpretation of CD and ORD spectra of naturally occurring polypeptides, proteins, and supramolecular structures like cell membranes. It is possible, for example, that hydrogen-bonded configurations of the sort shown by acetylamino acid methylamides in nonpolar solvents might form between adjacent, linked peptide units in the hydrophobic interior of a protein molecule. In the nomenclature of Venkatachalam (1968) such hydrogen bonding is of the type 3→1, which Ramachandran *et al.* (1966) showed is possible for a small range of dihedral angles. The contribution of such configurations to the CD and ORD of the protein would ape that of the α helix, which casts further doubts on estimates of α-helical content from the amplitudes of the 220-mμ CD band or the 233-mμ ORD trough.

That 3→1 hydrogen-bonded configurations may occur even in relatively small polypeptides in aqueous solution is illustrated by the nonapeptide, bradykinin. The following reinterpretation of its CD spectrum exemplifies how knowledge of the CD characteristics of intramolecularly hydrogen-bonded acetylamino acid amides can be used as an aid in interpreting the CD spectra of more complex molecules.

The CD spectra of bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and its analog, [Leu[5,8],Gly[6]]-bradykinin (Arg-Pro-Pro-Gly-Leu-Gly-Pro-Leu-Arg), in the wavelength interval, 260–210 mμ, are displayed in Figure 5. The spectrum of bradykinin (curve a) shows two weak bands, a negative one centered at 234 mμ and a positive one at 221 mμ. Because of its similarity ot that of the random-coil configuration of ionized polypeptides, the CD spectrum of bradykinin has previously been interpreted as indicating that the peptide exists in solution as a random coil (Brady *et al.*, 1971). Meyer (1969) has concluded, however, that the 238-mμ minimum in the CD curves of poly(L-Glu) and poly(L-Lys) near-neutral pH does not correspond to a spectral transition but rather is an artifact arising from the summation of a small amount of α-helical structural contribution (2–5%) to a predominantly random-coil spectrum. Moreover, one notes that bradykinin contains two phenylalanine residues which in model compounds in aqueous solution have intense, positive CD bands centered at 217 mμ. We have found an alternative interpretation of the CD spectrum of bradykinin which takes cognizance of these observations and allows for possible intramolecular hydrogen bonding. In light of the results described above for intramolecularly hydrogen-bonded acetylphenylalaninamide (Figure 3, curve c) and Gly-Phe-Gly, ones first inclination is to hypothesize that one of the phenylalanine residues of the peptide is internally hydrogen bonded, *i.e.*, bridged by a 3→1 hydrogen bond to give a structure analogous to configuration I. To test this idea, the CD spectrum of bradykinin was simulated by adding to the CD spectrum of the bradykinin analog devoid of phenylalanine (curve b in Figure 5) the normalized spectrum of intramolecularly hydrogen-bonded acetylphenylalaninamide (curve c in Figure 3A with molar ellipticities multiplied by one-ninth) and the normalized spectrum of the nonhydrogen-bonded configuration of the amide (curve a in Figure 3A multiplied by one-ninth). As anticipated, the simulated spectrum has a negative band centered at 236 mμ and a positive one at 223 mμ with amplitudes only slightly greater (by about 200°) than the observed values. Also, the simulated and observed values of $[\theta]_{236}/[\theta]_{223}$ agree to within experimental error. This simulation supports our contention that the secondary structure of bradykinin in aqueous solution has some order. In retrospect, however, the hypothesis that it is a phenylalanine residue which is internally hydrogen bonded can be faulted in that the bradykinin analog also shows a shallow band at 233 mμ even though it contains no phenylalanine.

A more realistic hypothesis might be that one of the proline residues in the analog spends a small portion of its time in the

internally hydrogen-bonded configuration; and that in bradykinin it spends more time in that configuration. In other words, the secondary structure of these peptides as revealed by CD is a time average of two rapidly interconnecting structures—a disorder structure and a partially ordered one in which a proline residue is internally hydrogen bonded. Given this hypothesis, the CD spectrum of bradykinin was simulated assuming that one of its proline residues spends a quarter of its time internally hydrogen bonded over and above the time spent in that configuration in the analog. Simulation was accomplished by adding to the CD spectrum of the analog the normalized spectrum of two moles of acetylphenylalaninamide in its nonhydrogen-bonded configuration and one-fourth of the normalized difference spectrum of acetylprolinemethylamide produced by dioxane referred to water as calculated from the curves presented in Figure 4A. The simulated spectrum (curve c of Figure 5) is in striking agreement with the observed one (curve a) with respect to both the location of the bands and crossovers and the ratio of amplitudes of the bands ($[\theta]_{234}/[\theta]_{221} = 0.7 \pm 0.1$ for simulated and 0.89 ± 0.03 for observed spectrum), even though Gly replaces Ser in the analog.

In order to test these ideas experimentally and to define more precisely the secondary structure of bradykinin in solution, the author in collaboration with Dr. John M. Stewart has initiated an investigation of the CD behavior of bradykinin, its analogs, its peptide fragments, and model compounds. Results obtained to date are in accord with the views expressed above.

References

Brady, A. H., Ryan, J. W., and Stewart, J. M. (1971), *Biochem. J. 121*, 179.

Cann, J. R. (1971), *Biochemistry 10*, 3713.

Fasman, G. D. (1967), Poly-α-Amino Acids. Peptide Models for Conformational Studies, New York, N. Y., Marcel Dekker, Inc., Chapters 6 and 7.

Glazer, A. N., and Rosenheck, K. (1962), *J. Biol. Chem. 237*, 3674.

Iizuka, E., and Yang, J. T. (1965), *Biochemistry 4*, 1249.

Iizuka, E., and Yang, J. T. (1966), *Proc. Nat. Acad. Sci. U. S. 55*, 1175.

Klotz, I. M., and Franzen, J. S. (1962), *J. Amer. Chem. Soc. 84*, 3461.

Madison, V., and Schellman, J. (1970a), *Biopolymers 9*, 511.

Madison, V., and Schellman, J. (1970b), *Biopolymers 9*, 569.

Meyer, Y. P. (1969), *Macromolecules 2*, 624.

Mizushima, S., Shimanouchi, T., Tauboi, M., and Souda, R. (1952), *J. Amer. Chem. Soc. 74*, 270.

Ramachandran, G. N., Venkatachalam, D. M., and Krimm, S. (1966), *Biophys. J. 6*, 849.

Schellman, J. A., and Nielsen, E. B. (1967), *in* Conformation of Biopolymers, Ramachandran, G. N., Ed., New York, N. Y., Academic Press, p 109.

Tsuboi, M., Shimanouchi, T., and Mizushima, S. (1959), *J. Amer. Chem. Soc. 81*, 1406.

Venkatachalam, C. M. (1968), *Biopolymers 6*, 1425.

Physical Studies on Deoxyribonucleic Acid after Covalent Binding of a Carcinogen†

Robert Fuchs and Michel Daune*

ABSTRACT: DNA from various sources was reacted with *N*-acetoxy-*N*-2-acetylaminofluorene and the physical properties of modified DNA were studied. A simple and rapid spectrophotometric method for the determination of fixed carcinogen on native DNA is proposed. Circular dichroism and melting curve analysis showed that modified bases are shifted outside the double helix, while the fixed carcinogen is inserted. Viscosity and light-scattering studies indicated that the fixation of *N*-acetoxy-*N*-2-acetylaminofluorene induces hinge points in the DNA molecule. The existence of cross-links in DNA reacted with carcinogen was demonstrated by the helix–coil transition and light scattering. The stability of these cross-links was studied as a function of pH and temperature. It was found that cross-links were destroyed at high pH values and elevated temperature. The importance of varying amounts of guanine in different DNA samples on cross-link formation is also demonstrated.

It is well established that several hepatic carcinogens bind *in vivo* to liver nucleic acids, proteins and carbohydrates (Miller and Miller, 1967; Farber *et al.*, 1967). AAF[1] is a potent liver carcinogen which, after metabolic activation, binds to liver tRNA, rRNA, DNA, and proteins when administered *in vivo* (Miller *et al.*, 1961; Marroquin and Farber, 1962; Henshaw and Hiatt, 1963; Marroquin and Farber,

† From the Centre de Recherches sur les Macromolecules, 67 Strasbourg, France. *Received October 4, 1971.* Supported by a grant of Ligue Nationale Française contre le Cancer.

[1] Abbreviations used are: AAF, *N*-2-acetylaminofluorene; *N*-OH-AAF: *N*-hydroxy-*N*-2-acetylaminofluorene; *N*-AcO-AAF: *N*-acetoxy-*N*-2-acetylaminofluorene; T_m^{260} and T_m^{305}, melting temperature at 260 and 305 nm, respectively; $(H_{DNA}^{260})_c$, DNA hyperchromicity of variously reacted DNA at 260 nm; $(H_{DNA}^{260})_c$, is a function of the percentage of modified bases; H_{DNA+c}^{260}, total hyperchromicity at 260 nm; A_c^{260}, A_c^{305}, fixed carcinogen absorbances at 260 and 305 nm; A_{DNA+c}^{260}, A_{DNA+c}^{305}, total absorbances of carcinogen reacted DNA at 260 and 305 nm; $(A_{DNA}^{260})_c$, contribution of the DNA in absorbance at 260 nm, when carcinogen is bound to DNA; ΔA_{DNA+c}^{260}, total absorbance increase at 260 nm for reacted DNA samples due to thermal helix–coil transition; $(\Delta A_{DNA}^{260})_c$, contribution of the DNA to the thermal-induced increase of absorbance at 260 nm, when the carcinogen AAF is bound to DNA; index $c = 0$ is relative to nonreacted DNA.