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900 VETERAN AVENUE
UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA 90024

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THE ORIGINS OF CIRCULAR DICHROISM BANDS
IN BOWMAN-BIRK SOYBEAN TRYPSIN
INHIBITOR

Ernest Kay

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Mail proofs to: Ernest Kay
Laboratory of Nuclear Medicine
and Radiation Biology, UCLA
900 Veteran Avenue
Los Angeles, California 90024

Ernest Kay

Laboratory of Nuclear Medicine and
Radiation Biology, University of
California, Los Angeles, California 90024

RUNNING TITLE: CD OF BOWMAN-BIRK INHIBITOR

SUMMARY

The spectral properties of Bowman-Birk soybean trypsin inhibitor (BBI)¹ were investigated by analyzing difference absorption spectra and difference CD spectra and by comparing them with those of tyrosyl model compounds. The O-acetylation of tyrosyl side chains showed that the ultraviolet CD bands of BBI above 225 nm originates from disulfide side chains and tyrosyl phenolic groups; phenylalanyl residues do not give rise to detectable CD in BBI in this wavelength region. The results of the tyrosyl ionization experiment were consistent with this interpretation.

A broad negative CD band centered around 280 nm in BBI arises mainly from disulfide bonds ($\epsilon_L - \epsilon_R = -0.83 \text{ M}^{-1} \text{ cm}^{-1}$ per disulfide).¹ Each of two tyrosyl residues gives rise to negative CD at this region; together they contribute approximately 10% of the total CD intensity at 277 nm ($\epsilon_L - \epsilon_R = -0.36 \text{ M}^{-1} \text{ cm}^{-1}$ per tyrosyl). Disulfide bonds in BBI also have a broad positive CD band centered around 240 nm ($\epsilon_L - \epsilon_R = 0.9 \text{ M}^{-1} \text{ cm}^{-1}$ per disulfide). Tyrosyl side chains give rise to a sharp positive peak at 231 nm, overlapping with the positive disulfide CD. Dimerization of monomeric BBI did not alter the CD profile.

One of two tyrosyl phenolic groups is relatively exposed to solvent and can be O-acetylated by 100 to 1500-fold molar excess of N-acetylimidazole. The other is inaccessible to the reagent even in the presence of 8 M urea, but can be acetylated in the presence of 6 M guanidine hydrochloride. Fully acetylated BBI has the near

ultraviolet disulfide CD and the far ultraviolet polypeptide CD very similar to those of the native inhibitor, indicating that O-acetylation of two tyrosyl side chains did not induce much conformational change in BBI.

The near ultraviolet CD of BBI was altered in the presence of 8 M urea or 6 M guanidine hydrochloride, with a greater change brought about by the latter. Dithiothreitol (20 mM) completely abolished the tyrosyl and disulfide CD in this region.

BBI was first described by Bowman in 1944 as an antitryptic material which was soluble in alcohol and precipitated with acetone (1). Since then, several trypsin inhibitors of different molecular weights but very similar amino acid compositions have been isolated from soybean (2-5). They were shown to be the same protein by Frattali (5) and the difference in molecular weight was explained by the concentration dependent self association of BBI (6,7).

The amino acid composition of BBI is characterized by a high disulfide content (7 disulfides/7975 daltons) and the lack of tryptophan and glycine. Aromatic amino acids consist of 2 tyrosyl and 2 phenylalanyl residues (5,8,9). The lack of tryptophanyl moiety makes spectral analyses of BBI in the near ultraviolet region relatively simple. An earlier study of the protein using the CD and ORD techniques revealed that BBI was nonhelical. Only one tyrosyl residue was considered to give rise to a large negative CD centered around 280 nm. A positive peak at 234 nm was attributed to tyrosyls. Aromatic amino acids (tyrosyls and phenylalanyls) and/or disulfide bonds were thought to originate the 245 nm shoulder (10).² However, the 280 nm CD band reported is too large for a tyrosyl residue only and relatively intense CD above 300 nm is suggestive of a sizable disulfide contribution (11).

In this study, the origins of the ultraviolet CD bands of BBI above 225 nm were identified, using model compounds to analyze the changes in absorption and CD spectra accompanying O-acetylation and ionization of tyrosyl phenolic groups in the protein.

EXPERIMENTAL PROCEDURE

Absorption spectra were recorded on a Cary Model 15 spectrophotometer. CD spectra were measured with an instrument described elsewhere (12-14). To obtain difference CD spectra, each CD spectrum was stored in a signal averager and recorded separately before subtracting one spectrum from the other.

Crude BBI was extracted from defatted soybean flour (Nutrisoy 7-B) (Archer Daniels Midland, Palos Verdes, Calif.) with ethanol and precipitated with acetone according to the Bowman's procedure (15) modified by Birk (16). The crude inhibitor was chromatographed on a CM-cellulose column (2.8 x 22 cm) (2), then on an anion exchange column (1.9 x 9 cm) and eluted with an exponential gradient of sodium acetate pH 5.0 from 0.15 M to 0.25 M (1 liter each) (4). DEAE Sephadex A50 substituted for DEAE-cellulose. The inhibitor was further purified by gel filtration on a Sephadex G-100 column (3 x 43 cm) (4). The purified BBI was dialyzed against cold water, lyophilized and stored at 4° until used. The final yield was 367 mg BBI from 1.5 kg defatted soybean flour. Homogeneity of the purified preparation was ascertained with the polyacrylamide gel disc electrophoresis (17).

Acetylation of BBI (18,19) was performed as described previously (20). Reaction media were dialyzed against cold water to terminate the acetylation and to remove reagents. Spectrapor 3 membrane (Spectrum Medical, Los Angeles) with an approximate molecular weight cutoff of 3500 daltons was used for the dialysis so that acetylated BBI could be recovered quantitatively.

The concentration of native BBI was estimated spectrophotometrically using the values of $M_r = 7975$ (5) and $A_{280}^{1\%} = 4.4$ (4,5).

Trypsin (EC 3.4.4.4.) (Worthington) activity was determined from the rate of hydrolysis of p-toluenesulfonyl-L-arginine methyl ester (Sigma) by the increase of absorbancy at 247 nm at 25° (21). The reaction buffer consists of 40 mM Tris-Cl pH 8.1 and 10 mM CaCl_2 . Trypsin inhibitor activity was assayed by mixing BBI with trypsin in the above buffer and incubated for one minute before adding the substrate.

N-acetyl-L-tyrosinamide (Sigma) was purified on a Sephadex G-10 column (1.5 x 55 cm), eluting with water. N,O-diacetyl-L-tyrosinamide was prepared by acetylating N-acetyl-L-tyrosinamide with 50-fold molar excess of N-acetylimidazole. After two hours at room temperature, acetylation was terminated by chromatography on a Sephadex G-10 column (1.5 x 55 cm) with water. Molar extinction coefficients of N-acetyl-L-tyrosine and N,O-diacetyl-L-tyrosine (18, 22) were used for N-acetyl-L-tyrosinamide ($\epsilon_{275} = 1370$) and N,O-diacetyl-L-tyrosinamide ($\epsilon_{260} = 260$).

Other chemicals used were of analytical grade. Double distilled water was used throughout.

RESULTS

Absorption Spectra of Acetylated BBI - Native BBI has an absorption maximum at 274.5 nm, a shoulder around 282 nm, and fine structure at 268, 264, and 257 nm (Fig. 1A). The 282 shoulder and 275 peak arise respectively from 0-0 and 0-800 cm^{-1} vibronic transitions of tyrosyl phenolic groups (23, 24). The fine structure can be assigned to phenylalanyl transitions (25). The other two traces in Figure 1A show the decrease in near ultraviolet absorption upon acetylating tyrosyl side chains with N-acetylimidazole. Treating the inhibitor with increasing amounts of N-acetylimidazole resulted in a progressive loss of the near ultraviolet absorption. However, no additional decrease of the absorption could be realized beyond the 100-fold molar excess of the reagent. Therefore, spectra of modified BBI indicate incomplete acetylation of tyrosyl side chains by N-acetylimidazole alone as the tyrosyl bands at 282 and 275 nm are still very evident. Approximately one tyrosyl residue was modified by the 100 to 1500-fold molar excess of N-acetylimidazole. 8 M urea did not enhance the tyrosyl acetylation by the 1500-fold excess of the reagent. When acetylating in the presence of 6 M guanidine hydrochloride, however, the tyrosyl transitions at 282 and 275 nm were almost completely eliminated. The residual tyrosyl absorption in the sample acetylated by the 1500-fold molar excess of N-acetylimidazole in the presence of 6 M guanidine hydrochloride, estimated by extrapolating disulfide absorption from the region above 295 nm, was less than 0.07 tyrosyl per BBI. The decrease in ϵ was 2240 at 282.5 nm and 2560 at 274.5 nm in BBI having both tyrosyl side chains O-acetylated. The absorption spectrum of native inhibitor was restored fully when the modified BBI was deacetylated with hydroxylamine (18,19).

The wavelength profiles of the difference absorption spectrum between the native and fully acetylated BBI (Fig. 1B) and that between N-acetyl-L-tyrosinamide and N,O-diacetyl-L-tyrosinamide (Fig. 2) are very similar. When both tyrosyls in BBI were fully acetylated, the decrease in ϵ was 2500 at 276 and 8000 at 228 nm, values comparable to the loss of ϵ when N-acetyl-L-tyrosinamide was O-acetylated, 1300 at 274 and 5000 at 226 nm. Thus, the loss of the near ultraviolet absorption upon acetylating BBI can be accounted for by the absorption change in the tyrosyl side chain transitions; a blue-shifting and hypochromism of 1L_b vibronic bands and a blue-shifting of 1L_a transition.

CD Spectra of Acetylated BBI - Figures 3A and 3B present the CD spectra corresponding to the absorption spectra shown in Figure 1. The near ultraviolet CD spectra of BBI is dominated by a broad intense negative band centered around 280 nm, a sharp positive peak at 231 nm and a pronounced shoulder around 245 nm (Fig. 3A). Acetylating 1 or 2 tyrosyl side chains caused only a slight decrease in the intensity of the 280 CD band. The positive peak at 231 nm, however, diminished greatly upon acetylation. The shoulder at 245 nm was relatively unaffected by this treatment. Deacetylation of the modified BBI fully restored the native CD spectrum.

The spectrum profile of difference CD between the native BBI and the fully acetylated derivative (Fig. 3B) matches very well with that of difference absorption spectra shown in Figures 1B and 2. Double peaks at 284 and 277 nm are characteristic of tyrosyl 1L_b transition and the positive peak at 231 nm corresponds well with the 1L_a band of the tyrosyl absorption spectrum.

The assignment of the lost CD in acetylated BBI to tyrosyl side chains was reinforced by the CD of N-acetyl-L-tyrosinamide and its O-acetylated derivative. As in the absorption change (Fig. 2), the modification resulted in a drastic reduction in 1L_b CD accompanied by a hypsochromic shift of about 10 nm (Fig. 4A). The change observed in 1L_a band was less drastic, about 15% reduction of intensity and 6-7 nm blue shift at the maximum.

A good matching of the difference CD spectrum profiles between the proteins (Fig. 2B) and the model compounds (Fig. 4B) suggests that the observed CD changes in acetylated BBI are due mainly to O-acetylation of tyrosyl phenolic groups.

The Origins of BBI CD above 230 nm - Based on the above analysis, the origins of BBI CD above 231 nm are assigned to disulfide bonds and tyrosyl phenolic groups. The broad negative CD centered around 280 nm is largely due to cystinyl, with approximately 10% of the intensity at 277.5 nm arising from the 1L_b transition of tyrosyl side chains. Disulfide bonds also give rise to a broad positive band around 240 nm. The intensity of this CD at its maximum is approximately the same or slightly larger than that of the 280 nm band. The tyrosyl 1L_a transition gives rise to a sharp positive CD at 231.5 nm, overlapping with the broad disulfide band.

CD Spectra of Ionized BBI - As the pH of BBI solutions was raised progressively from neutrality, the 231 nm positive peak decreased and disappeared completely when tyrosyl phenolic groups were fully ionized (Fig. 5). The intensity of the 245 nm shoulder decreased with the partial ionization then increased with the full ionization of tyrosyl side chains. The 280 nm negative CD band decreased slightly with

rising alkalinity. At pH where both tyrosyls were fully ionized (pH 12.8), approximately 20% reduction in the CD intensity at 275 nm was observed.

The disappearance of the 231 nm peak and the increase in the 245 nm CD can be accounted for by the bathochromic shift in the tyrosyl 1L_a band by about 15 nm (Fig. 1B, 6, 7). The positive CD at 245 nm in the ionized BBI, therefore, must be the sum of contributions from disulfide and ionized tyrosyl side chains.

The decrease of 245 nm CD at an intermediate pH (pH 10) indicates that the alkali condition also affects disulfide CD. A noticeable reduction of CD intensity above 300 nm at pH 12.8 cannot be accounted for by the change of tyrosyl CD and must be attributed to an alteration in disulfide CD because tyrosyl side chains do not have CD in this region either at neutral or alkali condition (Fig. 7).

Effect of Urea, Guanidine Hydrochloride and Dithiothreitol on BBI -

Urea (8 M) elicited some change in tyrosyl CD without much affecting the disulfide CD (Fig. 8). Guanidine hydrochloride (6 M) caused a greater change in tyrosyl CD as well as some alteration in disulfide CD. This is consistent with the result of acetylation experiments where complete acetylation of tyrosyl side chains by 1500-fold molar excess of N-acetylimidazole was achieved only in the presence of 6 M guanidine hydrochloride (Fig. 1A). Only one tyrosyl residue could be acetylated by the reagent alone or with 8 M urea.

Reducing disulfide bonds with dithiothreitol completely eliminated the disulfide and tyrosyl CD bands. In addition a change in polypeptide CD in the far ultraviolet region was observed, as evidenced by the onset of an intense negative CD above 240 nm.

Effect of BBI Polymerization on CD - BBI in solution has been shown to exist in monomer, dimer, and perhaps, trimer forms (5-7). High protein concentration and high ionic strength promote self-aggregation. The aggregation is also more favorable at pH 5 than pH 7 (6, 7). Because of this property, CD of BBI at 0.09 and 1.63 mg/ml was measured in three different buffer systems: (a) 50 mM sodium phosphate pH 7.0, (b) 50 mM sodium phosphate pH 7.0, 100 mM NaCl, (c) 50mM sodium acetate pH 5.0, 100 mM NaCl. Estimated average molecular weight is 8250 for 0.09 mg/ml protein at pH 7 and 12500 for 1.63 mg/ml at pH 5 (7). Since BBI has molecular weight of 7975 daltons (5), the monomeric form is predominant in the former, while it is intensively polymerized in the latter. CD spectra recorded under these diverse conditions were essentially identical and represented in Figure 3A (—). Apparently, self-association of BBI does not induce conformational change and tyrosyls and disulfides giving rise to BBI CD probably are not situated at aggregating surfaces.

DISCUSSION

This study shows that ultraviolet CD spectrum of BBI above 225 nm consists of cystinyl and tyrosyl origins. Superimposed on a broad negative disulfide CD band from 330 to 258.5 nm are negative tyrosyl fine structure bands at 284 and 277 nm. Tyrosyl side chains contribute approximately 10% of CD intensity at its maximum at 277.5 nm. Around 240 nm, disulfide bonds have a broad positive CD of comparable maximum intensity. A sharp positive CD arises from tyrosyls at 231 nm. The assignment of tyrosyl CD bands are based on: (a) difference CD spectrum between native and acetylated BBI possessing transitions and vibronic structure characteristic of the tyrosyl side chain, (b) reappearance of tyrosyl CD upon deacetylating O-acetyltyrosyls with hydroxylamine, (c) an analogy with the CD of L-tyrosyl model compounds, and (d) bathochromic shift of the positive CD arising from tyrosyl 1L_a band upon ionization of tyrosyl phenolic hydroxyl groups.

The reduction of the 280 nm negative band upon acetylation is suggestive of tyrosyl contribution in this region. However, it can be argued that coupled interactions between tyrosyl residues and disulfide transition may affect the CD contribution of the latter and be responsible for the decreased CD in the acetylated derivative. A positive assignment of the reduced CD to tyrosyl side chains was made possible because of the characteristic vibronic fine structure of tyrosyl phenolic groups in the difference CD spectrum (Fig. 3B).

Acetylation experiments also revealed that the side chain of one tyrosyl residue is more accessible to solvent than the other (Fig. 1A). The 1L_b transition of both tyrosyl residues gives rise to negative CD.

At 277.5 nm, two tyrosyls together have CD intensity of $0.72 \text{ M}^{-1} \text{ cm}^{-1}$. The exact contribution of each tyrosyl to the 280 CD band could not be determined with confidence.

Tyrosyl ${}^1\text{L}_a$ CD band is expected to occur around 230 nm, (Fig. 2, 4) (26). However, a definitive assignment of CD bands in this region in proteins has not been easy because tryptophanyl and cystinyl transitions also occur in the same region (27). Thus, tyrosyl residues were thought to give rise to a positive CD peak at 226 nm in Kunitz soybean trypsin inhibitor (28) and tryptophanlys at 228-232 nm in avidin and streptavidin (29). In proteins lacking tryptophanlys, such a band has been ascribed to either tyrosyl residues, e.g. limabean trypsin inhibitor at 233 nm (10), RNase A and S at 240 nm (26), ovomucoid at 243 nm (30), and ovine lutinizing hormone at 235-238 nm (31, 32), or disulfide bonds, e.g. limabean trypsin inhibitor at 248 nm (30), and bovine thyrotropin and lutinizing hormone at 230-235 nm (33). In human carbonic anhydrase B and C which are devoid of disulfide bond, a positive peak centered around 235-242 nm has been attributed to tyrosyl side chains (34).

In BBI, the absence of tryptophanyl eliminated it from consideration. The assignment of the 231 nm CD peak to tyrosyl phenolic groups is strongly supported by the observation that the acetylation of BBI induced almost 50% reduction in CD intensity at 231 nm (Fig. 3). This is in good agreement with the result of the model compound study where O-acetylation of tyrosyl phenolic group caused about 50% decrease in CD at 228 nm due to a blue shift in ${}^1\text{L}_a$ CD band (Fig. 4). This conclusion is in accordance with the results of ionization experiments (Fig. 5-7).

The intensity and wavelength position of disulfide CD bands are highly conformation dependent, determined by the dihedral angle, bond angle, and vicinal interactions of disulfide side chains (35). The longest wavelength transition of cystinyl side chain gives rise to a broad CD band centered around 255-290 nm. The second transition has a broad CD band centered around 230-255 nm (36).

Identification of disulfide CD bands in BBI is based on the CD spectrum of fully acetylated BBI (Fig. 3A) and an analogy with known disulfide CD bands. In the fully acetylated BBI, the only source of CD above 280 nm is the disulfide bond. Likewise, the disulfide bond is the only possible source of CD around 240 nm in acetylated BBI because O-acetylated tyrosyl side chains do not have transition in this region (Fig. 4). The lowest energy cystinyl band proposed for BBI has about the shape and intensity as the corresponding band in limabean trypsin inhibitor (10, 30), ovomucoid (20), and neurophysins (37, 38) ($\epsilon_L - \epsilon_R = -0.83 \text{ M}^{-1} \text{ cm}^{-1}$ per disulfide at 280 nm).

Disulfide contribution around 245 nm has been suggested in limabean trypsin inhibitor (30) and neurophysins (37, 38) for proteins and oxytocins for polypeptides (39). In ovomucoid (10) a similar band has been thought to have originated from aromatic amino acids or disulfide bonds or both. In BBI, CD around 245 nm arising from disulfides was clearly distinguishable from tyrosyl CD by O-acetylating tyrosyl side chains. The intensity of this disulfide CD at 240 nm is $0.9 \text{ M}^{-1} \text{ cm}^{-1}$, comparable to that found in neurophysin I (37).

The exact rotatory strength and maximum intensity of the tyrosyl positive band at 231 nm cannot be estimated accurately because of overlapping CD with the opposite sign below 230 nm. The onset of very intense negative polypeptide CD around 230 nm (Fig. 9) could have truncated the positive CD below 231 nm and distorted its actual size and shape. Without an interference from the polypeptide negative CD, 1L_a tyrosyl CD could possibly be larger and peaked at a shorter wavelength than observed.

The CD of phenylalanyl is expected to occur between 260 to 270 nm (25). No characteristic fine structure was discernible in either native or acetylated inhibitor, however, suggesting very little, if any, phenylalanyl CD in BBI. Whether neither of them gives rise to measurable CD or CD from both cancels each other is not known. 1L_b transition of O-acetylated tyrosyl side chains, expected around 250-280 nm (Fig. 4), also does not have measurable CD in this region (Fig. 3).

The CD spectrum of BBI in 6 M guanidine hydrochloride indicates that there is a considerable alteration in disulfide CD (Fig. 8). However, the CD profile of fully acetylated BBI differ only slightly from that of native BBI above 280 and from 255 to 240 nm where only cystinyls have CD in either protein (Fig. 3A). Apparently, on removing guanidine hydrochloride, disulfide bonds reverted to their native configuration and very little, if any, conformational change was induced by the O-acetylation of tyrosyl side chains in BBI. The virtual identity of the far ultraviolet CD between the native and fully acetylated BBI (Fig. 9) is also consistent with this observation.

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FOOTNOTES

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1. Abbreviations used were: BBI, Bowman-Birk soybean trypsin inhibitor; $\epsilon_L - \epsilon_R$, molar extinction coefficient for left circularly polarized light minus that for right circularly polarized light.
2. Ikeda et al (10) thought that BBI they had isolated had molecular weight of 16,400. The 280 nm negative CD was attributed solely to one of four tyrosyls per 16,400. Disulfide CD was poorly understood then and the possibility of its contribution to the near ultraviolet region (~ 280 nm) was not considered in BBI.

LEGENDS TO FIGURES

FIG. 1A. Absorption spectra of BBI, acetylated BBI, and deacetylated BBI in 50 mM sodium phosphate pH 7.0. —, native or acetylated and deacetylated BBI; ----, acetylated with 1500 molar excess of N-acetyl-imidazole in 50 mM sodium borate pH 7.5 for 2 hours, with or without 8 M urea; -----, acetylated with 1500 molar excess of N-acetylimidazole in 6 M guanidine hydrochloride for 2 hours; ·····, extrapolated disulfide absorption.

FIG. 1B. Difference absorption spectra of BBI produced by subtracting the spectrum of fully acetylated derivative (-----, Fig. 1A) from native BBI spectrum (—, Fig. 1A).

FIG. 2. Absorption spectra of N-acetyl-L-tyrosinamide and N,O-diacetyl-L-tyrosinamide in water and their difference spectrum. ----, N-acetyl-L-tyrosinamide; -----, N,O-diacetyl-L-tyrosinamide; —, N-acetyl-L-tyrosinamide spectrum minus N,O-diacetyl-L-tyrosinamide spectrum.

FIG. 3A. CD spectra of BBI, acetylated BBI, and deacetylated BBI in 50 mM sodium phosphate pH 7.0. Symbols are the same as those in Fig. 1A except that ····· indicates baseline.

FIG. 3B. Difference CD spectra of BBI produced by subtracting the spectrum of fully acetylated derivative (-----, Fig. 3A) from native BBI spectrum (—, Fig. 3A) (16 scans each). Bar on baseline indicates peak-to-peak noise level for ---- trace around 230 nm.

FIG. 4A. CD spectra of N-acetyl-L-tyrosinamide and N,O-diacetyl-L-tyrosinamide in water. ----, N-acetyl-L-tyrosinamide; -----, N,O-diacetyl-L-tyrosinamide; ·····, baseline.

FIG. 4B. Difference CD spectrum produced by subtracting N,O-diacetyl-L-tyrosinamide CD spectrum (-----, Fig. 4A) from N-acetyl-L-tyrosinamide CD spectrum (----, Fig. 4A).

FIG. 5. Effect of alkali on BBI CD. —, 100 mM NaCl pH 7.4; -----, 100 mM glycine pH 9.9; ----, 10 mM NaOH, 100 mM NaCl pH 11.8; ·····, 100 mM NaOH pH 12.8.

FIG. 6. Effect of alkali on BBI absorption. —, BBI absorption in 50 mM sodium phosphate pH 7.0 or in 100 mM NaOH, pH 12.8; ----, difference absorption spectrum produced by subtracting pH 7.0 spectrum from pH 12.8 spectrum.

FIG. 7. Effect of alkali on N-acetyl-L-tyrosinamide CD. —, in water, pH 7.0; ----, in 100 mM NaOH, pH 12.8; ·····, baseline (100 mM NaOH pH 12.8).

FIG. 8. CD spectra of BBI in 30 mM sodium phosphate pH 7.0 buffer in the presence of urea, guanidine hydrochloride, or dithiothreitol. —, buffer only; ----, buffer and 6 M guanidine hydrochloride; -----, buffer and 8 M urea; ·····, buffer and 20 mM dithiothreitol.

FIG. 9. Far ultraviolet CD of BBI and acetylated derivative in 50 mM sodium phosphate pH 7.0. —, native; -----, acetylated in 1500 molar excess of N-acetylimidazole in 50 mM sodium borate pH 7.5 for 2 hours in the presence of 6 M guanidine hydrochloride.

FIGURE 1

FIG. 1A. Absorption spectra of BBI, acetylated BBI, and deacetylated BBI in 50 mM sodium phosphate pH 7.0. —, native or acetylated and deacetylated BBI; ----, acetylated with 1500 molar excess of N-acetylimidazole in 50 mM sodium borate pH 7.5 for 2 hours, with or without 8 M urea; -.-.-.-, acetylated with 1500 molar excess of N-acetylimidazole in 6 M guanidine hydrochloride for 2 hours; ·····, extrapolated disulfide absorption.

FIG. 1B. Difference absorption spectra of BBI produced by subtracting the spectrum of fully acetylated derivative (-.-.-.-, Fig. 1A) from native BBI spectrum (—, Fig. 1A).

FIGURE 2

FIG. 2. Absorption spectra of N-acetyl-L-tyrosinamide and N,O-diacetyl-L-tyrosinamide in water and their difference spectrum. ----, N-acetyl-L-tyrosinamide; - · - · - ·, N,O-diacetyl-L-tyrosinamide; —, N-acetyl-L-tyrosinamide spectrum minus N,O-diacetyl-L-tyrosinamide spectrum.

FIGURE 3

FIG. 3A. CD spectra of BBI, acetylated BBI, and deacetylated BBI in 50 mM sodium phosphate pH 7.0. Symbols are the same as those in Fig. 1A except that indicates baseline.

FIG. 3B. Difference CD spectra of BBI produced by subtracting the spectrum of fully acetylated derivative (-----, Fig. 3A) from native BBI spectrum (—, Fig. 3A) (16 scans each). Bar on baseline indicates peak-to-peak noise level for ----- trace around 230 nm.

FIGURE 4

FIG. 4A. CD spectra of N-acetyl-L-tyrosinamide and N,O-diacetyl-L-tyrosinamide in water. ----, N-acetyl-L-tyrosinamide; - · - · - ·, N,O-diacetyl-L-tyrosinamide; · · · · ·, baseline.

FIG. 4B. Difference CD spectrum produced by subtracting N,O-diacetyl-L-tyrosinamide CD spectrum (- · - · - ·, Fig. 4A) from N-acetyl-L-tyrosinamide CD spectrum (----, Fig. 4A).

FIGURE 5

FIG. 5. Effect of alkali on BBI CD. —, 100 mM NaCl pH 7.4;
 - - - - , 100 mM glycine pH 9.9; - · - · - , 10 mM NaOH, 100 mM NaCl pH 11.8;
 · · · · · , 100 mM NaOH pH 12.8.

FIGURE 6

FIG. 6. Effect of alkali on BBI absorption. —, BBI absorption in 50 mM sodium phosphate pH 7.0 or in 100 mM NaOH, pH 12.8; ----, difference absorption spectrum produced by subtracting pH 7.0 spectrum from pH 12.8 spectrum.

FIGURE 7

FIG. 7. Effect of alkali on N-acetyl-L-tyrosinamide CD. —, in water, pH 7.0; ----, in 100 mM NaOH, pH 12.8; ·····, baseline (100 mM NaOH pH 12.8).

FIGURE 8

FIG. 8. CD spectra of BBI in 30 mM sodium phosphate pH 7.0 buffer in the presence of urea, guanidine hydrochloride, or dithiothreitol.
 —, buffer only; ----, buffer and 6 M guanidine hydrochloride;
 - · - · - ·, buffer and 8 M urea; · · · · ·, buffer and 20 mM dithiothreitol.

FIGURE 9

FIG. 9. Far ultraviolet CD of BBI and acetylated derivative in 50 mM sodium phosphate pH 7.0. —, native; ----, acetylated in 1500 molar excess of N-acetylimidazole in 50 mM sodium borate pH 7.5 for 2 hours in the presence of 6 M guanidine hydrochloride.