Conformational Studies of Secreted Mouse Pituitary Prolactin†

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ABSTRACT: A secreted form of mouse pituitary prolactin has been shown to contain only a single tryptophan residue. All previously reported prolactins contain two tryptophans. Circular dichroism spectra indicate that secreted mouse prolactin is conformationally similar to stored forms of prolactin previously isolated from several other species, including its α-helix content (65%). However, like secreted rat prolactin, secreted mouse prolacin shows no tryptophan signal in its circular dichroism spectrum. All stored forms of prolactin studied to date display distinct tryptophan signals. This suggests the possibility that secretion of prolactin may be accompanied by modification of the protein's tertiary structure.

It has been widely demonstrated that pituitary prolactin is of crucial importance to mammalian reproduction and to mammary gland tumorigenesis [for reviews, see Elias (1980), Clarke & Bern (1980), and Clifton & Furth (1980)]. However, estimates of circulating levels of prolactin through bioassay, radioimmunoassay, and radiomembrane binding assay (the so-called radioreceptor assay) are not always consistant (Kuo & Gala, 1972; Nicoll, 1975; Nicoll et al., 1976; Asawaroengchi & Nicoll, 1975; Leung, 1980). It has been suggested that these discrepancies may arise from structural differences between the secreted and stored forms of the hormone (Farmer et al., 1976; Markoff et al., 1981; Colosi & Talamantes, 1981).

A comparison of secreted and stored rat prolactin (r-PRL)[1] indicated no demonstrable differences in molecular weight, amino acid composition, or electrophoretic behavior, although slight differences in biological and immunological potencies were found (Farmer et al., 1976). CD spectra of the two forms showed a significant difference in the local conformations of the tryptophan residues, although other conformational details were found to be indistinguishable.

Recently, secreted forms of prolactin have been isolated from organ cultures of chinchilla, hamster, and mouse pituitaries (Kohmoto, 1975; Shoer et al., 1978; Jibson & Talamantes, 1978; Colosi et al., 1981). Secreted m-PRL appeared to be of particular interest since initial data indicated it might contain only a single tryptophan (Colosi & Talamantes, 1981). All other mammalian prolactins studied to date contain two tryptophan residues (Wallis, 1978; Cooke et al., 1980; Li, 1980).

We have now established that secreted m-PRL does contain only one tryptophan and, further, that its tryptophanyl CD spectrum is analogous to that of secreted r-PRL. In other respects, the conformation of secreted m-PRL is similar to that of the stored prolactins isolated from most other mammalian species.

Materials and Methods

The preparation, criteria of purity, amino acid composition, and biological characterization of secreted m-PRL have been presented elsewhere (Shoer et al., 1978; Markoff et al., 1981; Colosi & Talamantes, 1981; Markoff & Talamantes, 1980). The state of aggregation of a typical lyophilized preparation was determined by exclusion chromatography on Sephadex G-100 in 0.1 M Tris-HCl buffer (pH 8.2). Only the monomeric fraction was used in these studies.

Thermolysin was obtained from Calbiochem, lot no. 73326; *o*-nitrobenzenesulfenyl chloride, *N*-acetyl-L-tryptophanamide, *N*-acetyl-L-phenylalanine ethyl ester, and L-cystine were obtained from Sigma Chemical Co. These and all other chemicals were used without further purification.

CD spectra were taken at 27 °C on a Cary Model 60 spectropolarimeter equipped with a Model 6002 circular dichroism attachment according to Bewley et al. (1972). The method of Chen et al. (1972) was used to estimate α-helix contents. The mean residue weight was taken to be 115.

Protein concentrations were determined spectrophotometrically. All spectra were corrected for light scattering as described by Beavan & Holiday (1952). The absorptivity (E_M and $E^{0.1\%}_{1.0cm}$) of m-PRL was determined by a novel procedure (Bewley, 1982). Absorption spectra were recorded at 25 °C in stoppered, quartz cuvettes on a Perkin-Elmer Model 552 double-beam spectrophotometer equipped with temperature control units, as well as background correction and second derivative accessories. Spectra were scanned from 360 to 235 nm at either 20 nm/min (zero-order spectra) or 60 nm/min (second-order spectra). The slit width was set for a 1.0-nm band-pass in all cases. The wavelength positions of all second-order minima were corrected as previously described (Bewley, 1982). Difference spectra, produced by neutral solvent perturbation with 40% (v/v) methanol, were obtained according to Herskovits & Laskowski (1962).

Thermolysin digestion was performed at 25 °C in 0.1 M Tris-HCl buffer (pH 8.2), at an enzyme to substrate ratio of 1:100 (w/w). The digestion was followed spectrophotometrically by the difference spectral technique. Difference spectra were scanned at appropriate time intervals until a stable spectrum was obtained.

Modification of tryptophan with *o*-nitrobenzenesulfenyl chloride was attempted both in 1.0 M sodium acetate buffer

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[1] Abbreviations: r-PRL, rat pituitary prolactin; m-PRL, mouse pituitary prolactin; o-PRL, ovine pituitary prolactin; w-PRL, fin whale pituitary prolactin; NPS-Cl, *o*-nitrobenzenesulfenyl chloride; NPS-m-PRL, the *o*-nitrobenzenesulfenyl derivative of m-PRL; ΔE, change in optical density; E_M, molar extinction coefficient; ΔE_M, change in molar extinction; $E^{0.1\%}_{1cm}$, absorptivity of a 1.0 mg/mL solution through a 1.0-cm optical path; V_e/V_0, ratio of elution to void volume in exclusion chromatography; w/w, weight in weight; v/v, volume in volume; CD, circular dichroism; Tris, tris(hydroxymethyl)aminomethane.

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Table I: Second-Order Spectral Minima (nm)[a]

native m-PRL (pH 8.2)	digested m-PRL (pH 8.2)	digested m-PRL (pH 1.5)	mix-A (pH 1.5)	chromophore[b] assignment
289-290[c]	288.4	288.4	288.2	Trp
284.1[d]	282.1	281.7	281.7	Tyr + Trp[d]
278.7[d]	275.4	275.1	275.0	Tyr + slight Trp[d]
268.6[d]	267.5	267.3	267.4	Phe + slight Tyr[d]
264.7	263.7	263.5	263.6	Phe
258.8	257.8	257.4	257.7	Phe
253.0	251.9	251.8	251.8	Phe
248.6	247.3	246.9	247.0	Phe

[a] Corrected as described in text. [b] From comparisons with spectra in Beavan & Holiday (1952), Wetlaufer (1962), and Balestrieri et al. (1978, 1980). [c] Appears only as a shoulder in native protein. [d] Composite band containing unresolved contributions from more than one type of chromophore. Major contributor is listed first.

(pH 3.6) and in 50% acetic acid, as previously described for o-PRL (Kawauchi et al., 1973). The modified protein was purified on Sephadex G-100 in the Tris-HCl buffer. The extent of modification was determined spectrophotometrically (Scoffone et al., 1968). The fluorescence emission spectrum of NPS-m-PRL was taken in the Tris-HCl buffer on a Hitachi Perkin-Elmer spectrofluorometer, Model MPF-2A, as described elsewhere (Kawauchi et al., 1973).

Two model compound mixtures, designed to reproduce the optical absorption of m-PRL at a concentration equivalent to 1.0 mg/mL protein, were prepared from appropriate derivatives of the chromophoric amino acids plus cystine, dissolved in the Tris-HCl buffer at pH 1.5. In both mixtures, the molar ratios of cystine, phenylalanine, and tyrosine were 3:7:6, respectively, as indicated from the amino acid composition of m-PRL (Colosi & Talamantes, 1981). In addition, "mix-A" contained 1.0 mol of tryptophan, while "mix-B" contained 2.0 mol, relative to the other chromophores. The second-order and neutral solvent perturbation spectra of these model mixtures were compared to analogous spectra of the thermolysin digest of m-PRL at the same pH.

Results

State of Aggregation. Exclusion chromatography of a lyophilized sample of m-PRL displayed a single, major peak with an elution position (V_e/V_0 = 2.0) that is similar to that of o-PRL monomer on the same column and that is consistent with the monomer molecular weight of ~23 000 reported elsewhere (Colosi & Talamantes, 1981) for m-PRL.

Absorption Spectra of Intact m-PRL. The zero-order absorption spectrum of intact, secreted m-PRL is shown in Figure 1A. The unusually smooth absorption envelope displays a single maximum at 278.0 ± 0.1 nm. The only visible fine structure consists of weak vibronic bands, typical of phenylalanine residues, appearing as shoulders between 270 and 250 nm. The second-order spectrum (Figure 1B, Table I) displays a weak shoulder between 290 and 285 nm, distinct minima at 284.1 and 278.7 nm, an extremely weak shoulder near 271–270 nm, and five distinct minima between 268.6 and 248.6 nm.

Absorption Spectra of Thermolysin-Digested m-PRL. Figure 1 contains the zero-order and second-order spectra of the thermolysin digest of secreted m-PRL. Digestion produces two striking effects: a pronounced blue shifting of all absorption bands and a definite hypochromism. The zero-order spectrum of the digest exhibits a maximum at 274.0 nm, with

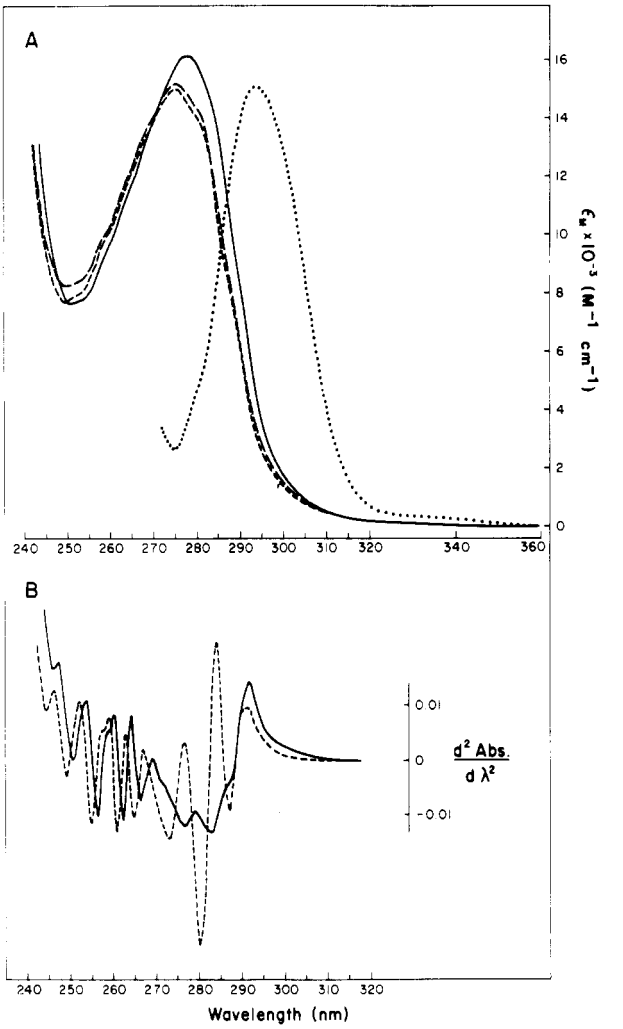

FIGURE 1: (A) Zero-order absorption spectra of (—) native m-PRL, pH 8.2, (– –) thermolysin-digested m-PRL, pH 8.2, and (- - -) thermolysin-digested m-PRL, pH 1.5, and (···) difference ionization spectrum taken between two aliquots of thermolysin-digested m-PRL at pH 1.5 (reference) and pH >13 (sample). (B) Second-order absorption spectra of (—) native m-PRL, pH 8.2, and (- - -) thermolysin-digested m-PRL, pH 1.5.

obvious shoulders near 290 and 280 nm arising from tryptophan absorption. Note (Table I) that the various minima in the second-order spectrum of the digest at pH 1.5 are blue shifted to the same wavelengths as found for mix-A. In fact, as further shown in Figure 2, the *entire* second-order spectrum of the digest is almost perfectly congruent with the spectrum of mix-A. In contrast, the spectrum of the digest is not even closely congruent with the spectrum of mix-B in the region of tryptophan absorption between 289 and 270 nm.

The spectral congruency of the digest and mix-A can also be demonstrated from zero-order spectra if each is perturbed with 40% (v/v) methanol and submitted to difference spectroscopy. All three samples (Figure 3) show intense, positive, red-shift difference bands near 289, 284, and 277 nm. The 289-nm tryptophan band appears as a shoulder in both the m-PRL digest and mix-A. In contrast, this band in mix-B is a much more intense and resolved maximum.

E_M and $E^{0.1\%}_{1.0cm}$. Absorptivity values were obtained from the zero-order spectrum of the native protein at pH 8.2 and an ionization difference spectrum generated between aliquots of a thermolysin digest of the protein, one at pH 1.5 and the other at pH >13. Both spectra are shown in Figure 1A. These measurements, whose rationale is presented elsewhere (Bewley,

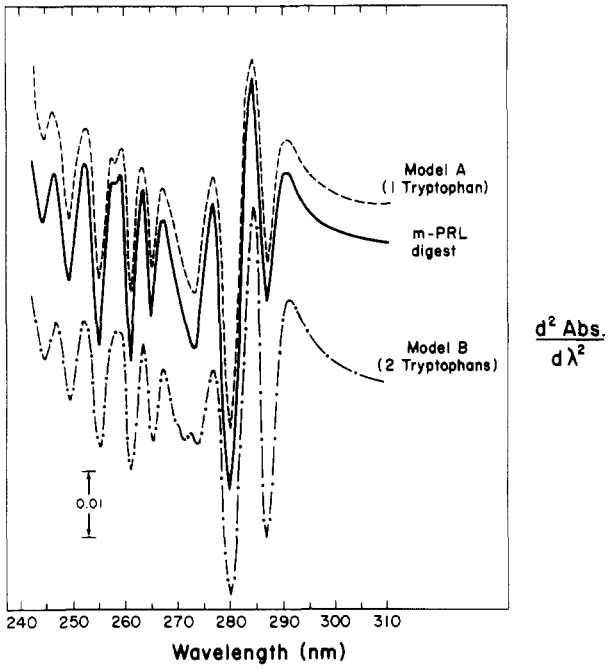

FIGURE 2: Second-order absorption spectra of (—) thermolysin digest of m-PRL at pH 1.5, (--) model compound mix-A containing Trp, Tyr, Phe, and Cys in a molar ratio of 1:6:7:3, respectively, at pH 1.5, and (-·-) model compound mix-B containing Trp, Tyr, Phe, and Cys in molar ratio of 2:6:7:3, respectively, at pH 1.5. The concentration of each sample was adjusted to be equivalent to 1.0 mg/mL of intact m-PRL. The three spectra have been arbitrarily offset on the vertical axis for ease of comparison.

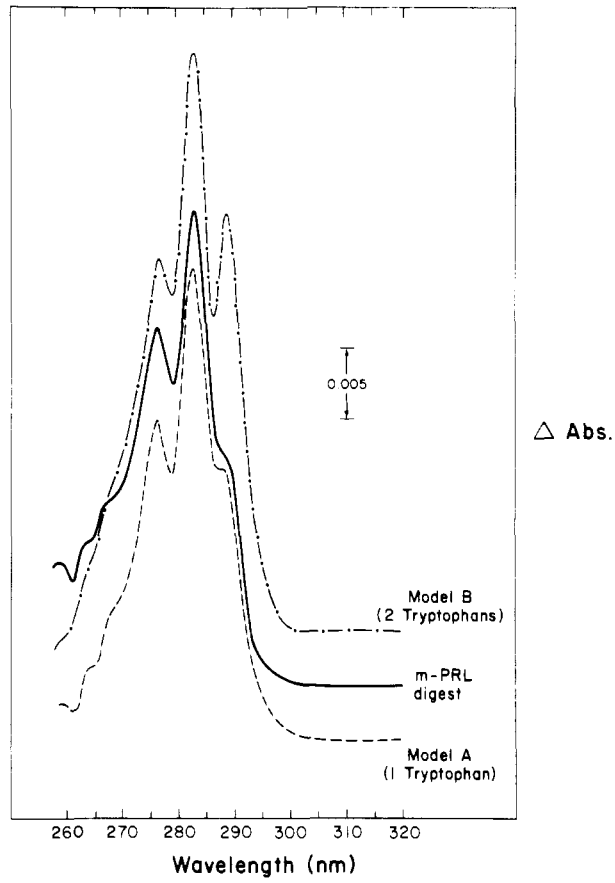

FIGURE 3: Neutral perturbation difference spectra produced by 40% (v/v) methanol (pH 1.5) for (—) thermolysin digest of m-PRL, (--) model compound mix-A containing Trp, Tyr, Phe, and Cys in a molar ratio of 1:6:7:3, respectively, and (-·-) model compound mix-B containing Trp, Tyr, Phe, and Cys in a molar ratio of 2:6:7:3, respectively. Concentrations were adjusted to be equivalent to 1.0 mg/mL of intact m-PRL. The spectra have been arbitrarily offset on the vertical axis for ease of comparison.

1982), indicate values of E_M = 16 000 ± 240 M^{-1} cm^{-1} and $E^{0.1\%}_{1.0cm}$ = 0.717 ± 0.010, at 278.0 nm, for mildly alkaline solutions of the intact hormone. It may be mentioned that perturbation of the tyrosyl ionization spectrum by new, charged termini in the enzyme digest has either been eliminated or strongly minimized by the experimental design (Bewley, 1982).

Changes in Absorption Spectrum during Thermolysin Digestion. Figure 4 presents a series of difference spectra obtained during thermolysin digestion of secreted m-PRL. During the first half of the digestion, well-resolved blue-shift maxima appear at 291.5, 286.5, and 279 nm. As digestion progresses, however, the 291.5-nm band degenerates into an unresolved shoulder. Also during the later stages, small blue-shift bands due to phenylalanine appear between 265 and 250 nm, superimposed on the more intense positive limbs of those blue-shift bands whose negative limbs occur above 270 nm. Of special interest is the presence of an apparent isosbestic point near 271 nm throughout the entire digestion.

Reaction with NPS-Cl. Following an attempt to modify secreted m-PRL with NPS-Cl in 1.0 M sodium acetate (pH 3.6), absorption spectra of the purified protein indicated a complete lack of any reaction (data not shown). However, when the modification was carried out in 50% acetic acid, the purified product was found to contain 1.06 ± 0.07 mol of covalently bound NPS/mol of m-PRL. A fluorescence emission spectrum of NPS-m-PRL displayed a single, weak maximum at ≃220 nm, with no evidence of tryptophan emission between 360 and 335 nm (data not shown). Exclusion chromatography of NPS-m-PRL (Figure 5) indicates that >90% of the modified protein elutes as a monomer (V_e/V_0 = 2.0).

Circular Dichroism Spectra. Below 250 nm, secreted m-PRL displays the two intense, negative CD bands at 221 and 209 nm characteristic of the α helix (Figure 5A). The α-helix content is found to be 65% at pH 8.2 and 55% at pH 3.6. In the region of side-chain absorption (Figure 5B), neither spectrum displays any significant dichroism above 287 nm. However, at either pH value, a poorly resolved region of negative dichroism containing barely discernible negative maxima near 279 and 272 nm can be seen. In addition, somewhat more intense and better resolved negative maxima appear at 269 and 261 nm. At pH 3.6 there is a slight loss in overall negative intensity, with an enhancement of the maxima at 268 and 261 nm, along with the appearance of a weak shoulder near 255 nm. Back-titration of the protein from pH 3.6 to pH 8.2 produces essentially complete return of the entire CD spectrum to that of the native protein.

Discussion

As described in reviews by Wallis (1978) and Farmer & Papkoff (1979) all mammalian prolactins studied to date contain two tryptophan residues and three disulfide bonds. Initial characterizations of m-PRL indicated that it too contained three disulfide bonds but only a single tryptophan (Colosi & Talamantes, 1981). Since the initial evidence suggesting a single tryptophan in m-PRL was spectroscopic in nature and, as discussed below, the absorption spectrum of mPRL is rather unusual, it seemed wise to carefully reinvestigate this aspect, especially in view of the potential importance and uniqueness of the result.

Absorption Spectra of m-PRL. The zero-order spectrum of secreted m-PRL (Figure 1A) shows an unusually smooth and symmetrical absorption envelope, curiously devoid of the

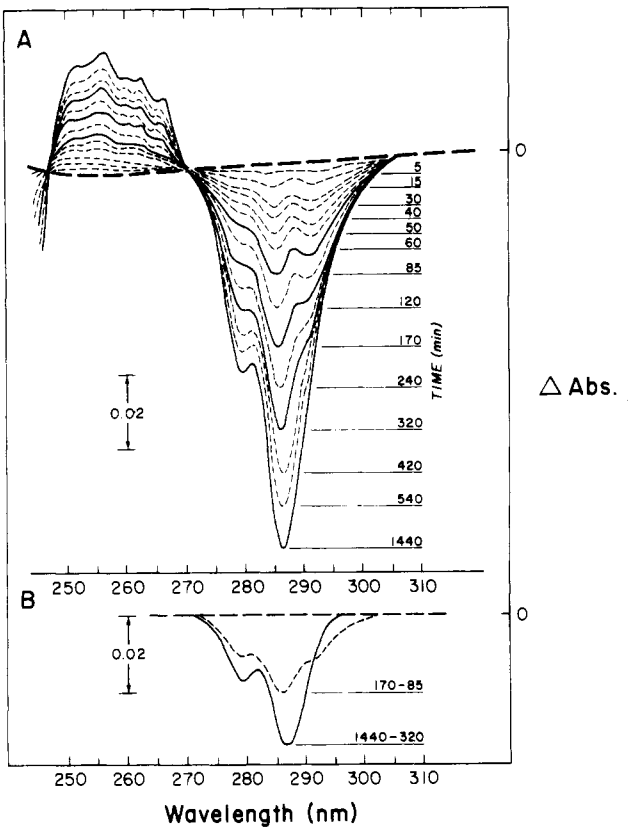

FIGURE 4: (A) Difference absorption spectra obtained during thermolysin digestion of m-PRL at 25 °C and pH 8.2. The reference cell contained the protein at 0.721 mg/mL, while the sample cell contained the same concentration of protein plus 1/1000 (w/w) thermolysin. The time interval (min) after addition of the enzyme, at which each scan was begun, is noted. (B) Difference–difference absorption spectra obtained by (---) subtraction of the 85-min difference spectrum in (A) from the 170-min spectrum and (—) subtraction of the 320-min difference spectrum in (A) from the final, 1440-min difference spectrum.

shoulders near 290 and 280 nm typical of tryptophan-containing proteins (Beavan & Holiday, 1952; Wetlaufer, 1962; Donovan, 1969).

Apparently, in secreted m-PRL, the conformation of the protein has produced an abnormally large red shift in some, or all, of the tyrosine absorption, relative to approximately "normal" red shifting of the tryptophan absorption. As a result, the indole absorption bands near 290 and 280 nm are masked in the zero-order spectrum. Only a faint hint of the lowest energy tryptophan band at 290 nm can be seen in the second-order spectrum (Figure 1B). This interpretation can easily be tested since it predicts that any process that destroys the protein's conformation should thereby release the tyrosyl side chains from their red-shifting environments. The resulting change in refractive index would blue shift (normalize) their absorption back to the well-known wavelength positions typical of tyrosines in small, conformationless peptides (Wetlaufer, 1962; Donovan, 1969). Moreover, since red shifting is usually accompanied by some degree of hyperchromic effect, destruction of the conformation should also produce a decrease in the molar extinction of the protein (Wetlaufer, 1962). Similar blue-shift and hypochromic effects have been shown to occur during thermolysin digestion of seven typical globular proteins (Bewley, 1982).

We have digested m-PRL with thermolysin in conjunction with a determination of the E_M of the protein. The zero-order and second-order spectra of the digest (Figure 1 and Table I) unequivocally show the predicted effects. The hypochromism is best seen from a comparison of the zero-order spectra (Figure 1A), while the blue shifts are more clearly seen from the second-order spectra (Figure 1B and Table I). The wavelength positions of the second-order minima of the digest at pH 1.5 (Table I) and their near equivalence with the positions of the model compound mixtures indicate that virtually all of the original conformation has been destroyed by the combined effects of digestion and titration to acidic conditions (Bewley, 1982).

Tryptophan Content of m-PRL. The normalized absorption of the digest at pH 1.5 can be used to establish the tryptophan content of the native protein. To accomplish this, two model compound mixtures, containing different amounts of tryptophan, were prepared as described under Materials and Methods. In the absence of conformational effects, the *relative intensities* of the various second-order minima are directly

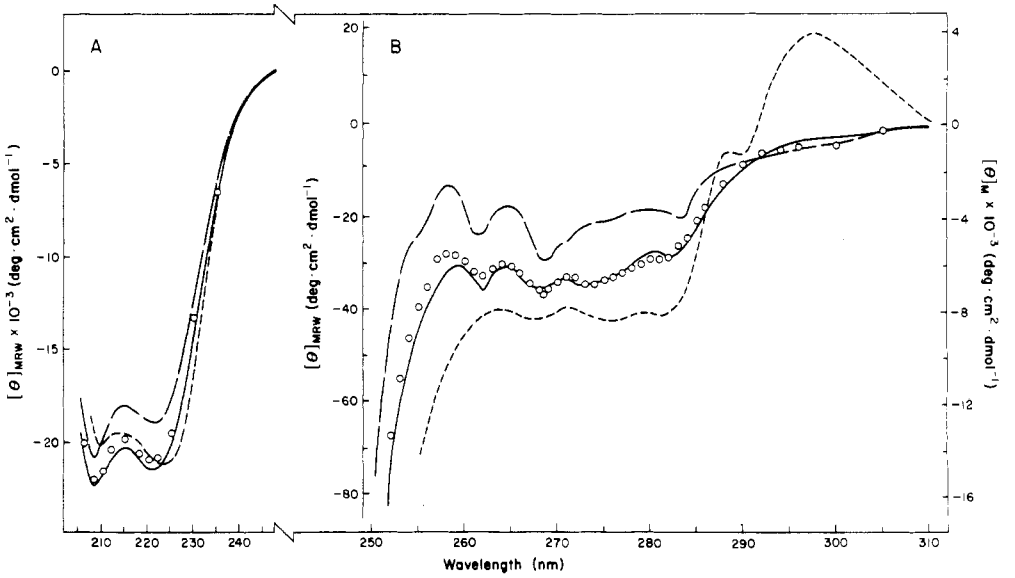

FIGURE 5: (A) Circular dichroism spectra in region dominated by amide bond absorption for intact m-PRL at (—) pH 8.2, (--) pH 3.6, and (O) after back-titration from pH 3.6 to pH 8.2. (B) Circular dichroism spectra in region of side-chain absorption. Symbols are the same as in part A. In both (A) and (B), the CD spectrum of the stored form of o-PRL (---) has been added for comparison [taken from Bewley & Li (1972)].

proportional to the molar ratios of the chromophoric amino acids (Balestrieri et al., 1978, 1980). The near perfect congruency of the entire second-order spectrum of the m-PRL digest with that of mix-A (Figure 2) and the obvious lack of congruency with mix-B in the crucial 290–285-nm indole ring absorption region directly and unequivocally establishes the existence of a single tryptophan residue in secreted m-PRL.

This result is confirmed by solvent perturbation difference spectra (Figure 3), whose band intensities are also directly proportional to the chromophoric amino acid compositions of the samples (Herskovits & Laskowski, 1962; Herskovits & Sorensen, 1968). The congruency between the perturbation difference spectrum of the m-PRL digest and that of mix-A, especially in the region of the 291-nm tryptophan band, is noteworthy.

Finally, direct modification of intact m-PRL with NPS-Cl in 50% acetic acid shows that only 1 mol of NPS chromophore can be covalently incorporated. The NPS-m-PRL derivative exhibits no detectable fluorescence emission from excess, unreacted tryptophan, indicating quantitative modification of the total tryptophan content of the protein.

Conformational Properties of m-PRL Deduced from Chemical Modification. In the absence of primary structure information, it is uncertain which of the two tryptophan residues, characteristic of other mammalian prolactins, m-PRL contains. In o-PRL, Trp-150 is relatively exposed to the external solvent and can be selectively modified with NPS-Cl in 1.0 M sodium acetate at pH 3.6 (Kawauchi et al., 1973). In contrast, Trp-90 in o-PRL is buried within the interior of the molecule and cannot be modified without partial denaturation in 50% acetic acid. Similar experiments with secreted m-PRL indicate no reactive tryptophan in 1.0 M sodium acetate (pH 3.6) but complete modification in 50% acetic acid. This suggests that the tryptophan in secreted m-PRL is buried, in analogy with Trp-90 in o-PRL. It does not, however, prove their equivalence.

Conformational Properties of m-PRL Deduced from Absorption Spectra. Further evidence indicating that the tryptophan in secreted m-PRL is buried within the nonpolar interior of the folded molecule can be seen from difference spectra taken during thermolysin digestion (Figure 4). These spectra show intermediate stages in the progressive blue-shift peaks produced by destruction of the protein's conformation. The total blue shift (peak intensity) for each type of chromophore (Trp, Tyr, or Phe) will depend both on its content and on its average environment in the folded form. The entire 291-nm band and a small portion (<10%) of the 286-nm band are produced by collapse of the local conformation around the tryptophan residue (Wetlaufer, 1962; Donovan, 1969). The fact that the tryptophan absorption is blue shifted by an amount ($\Delta E_{M_{291.5nm}} = -1600\ M^{-1}\ cm^{-1}$) that is typical for globular proteins (Donovan, 1964, 1969) strongly supports the hypothesis of a buried indole ring.

Most of the 286-nm band and virtually all of the 278-nm band are produced by conformational collapse around the tyrosine residues. Unfortunately, it is not possible to resolve the tyrosyl blue shift into individual contributions from each of the six tyrosine residues. Nevertheless, the large total red shift in tyrosyl absorption, characteristic of the intact protein and suggested earlier as an explanation for the peculiar shape of the zero-order spectrum of the native molecule, is consistent with the intense blue shift ($\Delta E_{M_{286.5nm}} = -3345\ M^{-1}\ cm^{-1}$) in the major tyrosyl band produced by digestion.

During the first third of the digestion, the rates of exposure of tryptophan and tyrosine, as evidenced by the growth of their respective blue-shift maxima, are approximately equal. In contrast, during the last half of the reaction, the tryptophan peak at 291 nm seems to stop increasing. Ultimately, it is overwhelmed by the very intense tyrosyl blue-shift peak at 286.5 nm. This can be seen more clearly in Figure 4B. When the difference spectrum taken at 85 min is subtracted from the difference spectrum taken at 170 min, a "difference–difference" spectrum is generated. The three blue-shift bands in this latter spectrum demonstrate that *both* tryptophan and tyrosine are being exposed during this time period. However, if a difference–difference spectrum is produced by subtraction of the 320 min spectrum from the 1440 min spectrum, it is evident from the lack of a band at $\simeq$290 nm that very little if any transfer of tryptophan is occurring during this later portion of the digestion. Clearly, the loss of conformation is a multistep process, occurring at different rates in different parts of the m-PRL molecule.

Figure 4A also shows an apparent isosbestic point at 271 nm. This is formed at the crossover (through $\Delta E = 0$) of the negative and positive limbs of the blue-shifted tyrosine and tryptophan difference absorption bands (Donovan, 1969). In principle, the tyrosyl and tryptophanyl contributions should each have a separate and characteristic crossover point. However, because the difference spectrum of m-PRL is dominated by tyrosine, the position of the composite crossover appears as a single, sharp isosbestic point. This point is of great practical utility since its absorptivity ($E_{M_{271nm}} = 14\,500\ M^{-1}\ cm^{-1}$) is nearly independent of pH (at least in the acidic and weakly alkaline range where tyrosyl ionization is not possible). Even more surprising is the fact that this absorptivity value appears to be effectively independent of the conformation of the protein. This would allow for precise determination of the protein's concentration, regardless of the conformational state of the molecule.[2] A second, apparent isosbestic point can also be seen near 248 nm (Figure 4A). However, its extinction is significantly lower ($E_{M_{248nm}} = 7700\ M^{-1}\ cm^{-1}$) and more subject to light scattering error.

Conformational Properties of m-PRL Deduced from Circular Dichroism Spectra. In the region dominated by amide bond absorption, the CD spectrum of secreted m-PRL (Figure 5A) is quite similar to that previously reported for the stored forms of o-PRL (Bewley & Li, 1972), p-PRL (Bewley & Li, 1975), and w-PRL (Kawauchi & Tubokawa, 1979). When calculated according to Chen et al. (1972), the α-helix content of m-PRL is found to be 65% at pH 8.2 and 55% at pH 3.6. Both values are equivalent[3] to those previously reported for the stored forms of o-PRL and p-PRL (Bewley & Li, 1972, 1975).

In the region of side-chain absorption (Figure 5B), the CD spectrum of secreted m-PRL, at both pH values, contains additional elements that are characteristic of stored prolactins. These include the broad, poorly resolved negative dichroism between $\simeq$285 and 270 nm, as well as the increase, upon acidification, of the relative intensity and resolution in the negative phenylalanine peaks at 269, 261, and 255 nm (Bewley & Li, 1972, 1975). Thus, in many aspects of its secondary

[2] This presumes that the solvent itself does not affect the normalized absorption of the protein. For instance, if the solvent contained urea, guanidine, or nonpolar additives, the apparent isosbestic point might occur at a slightly different wavelength, with a slightly different extinction coefficient.

[3] Previous estimates of the α-helix contents of o-PRL and p-PRL (Bewley & Li, 1972, 1975) show slightly lower values. This discrepancy is due to a difference in calculation method. The CD spectra of m-PRL, o-PRL, and p-PRL indicate that all three contain nearly the same amount of α helix.

and tertiary structures, secreted m-PRL closely resembles the stored forms of prolactin isolated from other species.

However, all stored forms of prolactin studied to date exhibit distinct tryptophanyl dichroism above 288 nm. Secreted m-PRL shows no evidence for any tryptophanyl CD band(s) at either pH value. This is analogous to the lack of tryptophanyl CD reported for secreted r-PRL (Farmer et al., 1976). Stored r-PRL shows a distinct positive tryptophanyl CD band, comparable to that seen in Figure 5B for stored o-PRL. Sufficient amounts of stored m-PRL are not yet available for comparison, but it will be of great interest to determine whether the stored form of m-PRL, which should also contain only a single tryptophan, shows a visible tryptophanyl CD signal. Identification of the amino acid that has replaced the second tryptophan in m-PRL will be of equal interest.

Acknowledgments

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