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Conformational Prediction for Snake Venom Toxins and Laser Raman Scattering of a Cardiotoxin from Taiwan Cobra (*Naja naja atra*) Venom†

Tzong-Hsiung Hseu,* Yin-Chang Liu, Chung Wang, Hua Chang, Dah-Min Hwang, and Chen-Chung Yang

ABSTRACT: Secondary structure regions in snake venom toxins were predicted using the prediction method of Chou and Fasman (Chou, P. Y., and Fasman, G. D. (1974), *Biochemistry 13,* 222) and an averaging scheme assuming structural homology in each type of toxins. The results indicate that, in general, snake toxins contain only some β-sheet regions and β bends. The content of secondary structures thus predicted does vary to some extent. The predicted results correlate well with conclusions from physicochemical studies. Interestingly, β-bend regions predicted for the two types of neurotoxins, short-neurotoxin-type and long-neurotoxin-type, are primarily located in the middle of disulfide loops in spite of large differences in primary sequences. Comparisons between predicted results and the crystal structure of erabutoxin b determined at 2.75 Å resolution suggest that the two types of neurotoxin are both sequencely and conformationally related while cardiotoxins could have an entirely different molecular topology. The Raman spectrum of a Taiwan cobra cardiotoxin indicates that the content of β-pleated-sheet structure could be greater than that in neurotoxins.

Toxins from venom of snake of *Proteroglyphae* suborder are small basic polypeptides devoid of enzymatic activity. One group of these toxins which block the nicotinic acetylcholine receptor of the muscle motor end plate is known as curarimimetic neurotoxins (Lee, 1972; Yang, 1974; Condrea, 1974). Another group, cardiotoxins or cytotoxins, has a relatively low toxicity to animials. This group does induce a variety of effects which are exerted primarily on cellular membrane leading to a disturbance of its organization and function (Condrea, 1974).

Much work has been done over the past decade toward clarification of the structure-functional relationship of these toxins. This work includes sequence determination, chemical modification studies, x-ray structure analysis, and other physicochemical investigations. To date there have been over 62 amino acid sequences determined (Figure 1). These toxins are among the smallest proteins known (60–74 amino acid residues). They are cross-linked by four or five disulfide bonds. Although they are homologous to one another in sequence, the pharmacological and serological properties differ greatly from neurotoxins to cardiotoxins. There are only 11 invariant amino acid residues found from sequence alignment including 8 half-cystines in invariant disulfide bridges. Therefore, these snake venom toxins provide particularly favorable conditions for comparative structural studies, e.g., through conformational prediction, laser Raman scattering, and CD and ORD[1] spectra measurements.

The main purpose of the present work was to predict secondary structure regions in snake venom toxin proteins and to

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[1] Abbreviations used: CD, circular dichroism; ORD, optical rotatory dispersion.

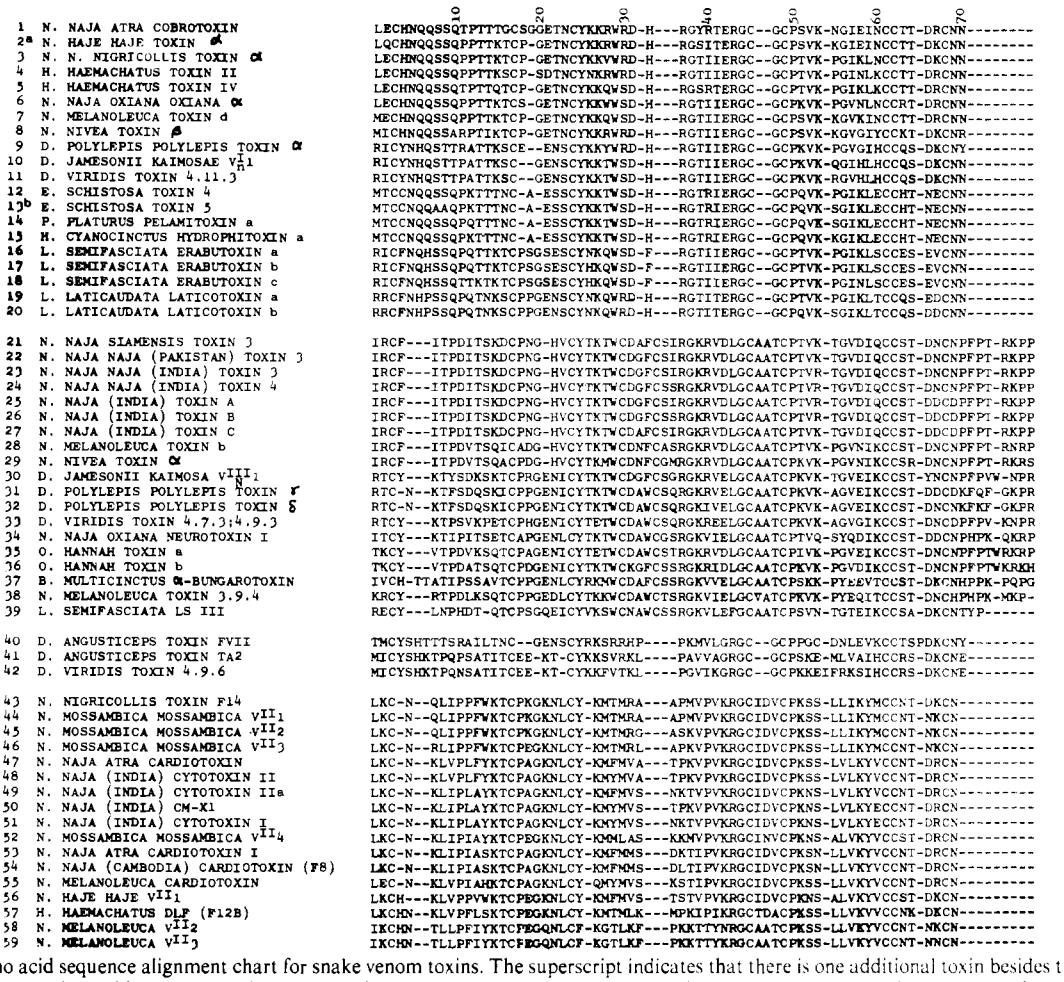

FIGURE 1: Amino acid sequence alignment chart for snake venom toxins. The superscript indicates that there is one additional toxin besides the one shown which has the same amino acid sequence: a (*N. nivea* toxin δ); (b) (*H. cyanocinctus* hydrophitoxin b). The amino acid sequences shown are from: 1 (Yang et al., 1969); 2 (Botes and Strydom, 1969); 2a (Botes et al., 1971); 3 (Eaker and Porath, 1967); 4, 5 (Strydom and Botes, 1971); 6 (Arnberg et al., 1974; Grishin et al., 1973); 7 (Botes, 1972); 8 (Botes, 1971); 9 (Strydom, 1972); 10 (Strydom, 1973); 11 (Banks et al., 1974); 12 (Fryklund et al., 1972); 13 (Sato, 1974); 13b (Liu et al., 1973); 14 (Liu et al., 1975); 15 (Liu and Blackwell, 1974); 16, 17 (Sato and Tamiya, 1971); 18 (Tamiya and Abe, 1972); 19 (Sato and Tamiya, 1971); 20 (Sato, 1974); 21 (Karlsson et al., 1972); 22, 23, 24 (Karlsson, 1974); 25 (Nakai et al., 1971); 26 (Ohta and Hayashi, 1973); 27 (Hayashi, 1974); 28 (Botes, 1972); 29 (Botes, 1971); 30 (Strydom, 1973); 31, 32 (Strydom, 1972); 33 (Banks et al., 1974); 34 (Grishin et al., 1974); 35, 36 (Joubert, 1973); 37 (Mebs et al., 1972); 38 (Shipolini et al., 1974); 39 (Maeda and Tamiya, 1974); 40 (Viljoen and Botes, 1973); 41 (Viljoen and Botes, 1974); 42 (Shipolini and Banks, 1974); 43 (Botes, 1974b); 44, 45, 46 (Louw, 1974a); 47 (Hayashi et al., 1976); 48 (Takechi and Hayashi, 1972); 49, 50 (Takechi et al., 1973); 51 (Hayashi et al., 1971); 52 (Louw, 1974b); 53 (Hayashi et al., 1975); 54 (Botes, 1974); 55 (Carlsson and Joubert, 1974); 56 (Botes, 1973); 57 (Fryklund and Eaker, 1973); 58, 59 (Carlsson, 1974).

correlate the predicted conformation with results from chemical modification and some physicochemical studies.

Raman spectra of a number of neurotoxins have recently been reported (Yu et al., 1975; Harada et al., 1976). Raman spectra of proteins are sensitive to conformation. As an aid to interpretation of predicted results, laser Raman spectra of a cardiotoxin from Taiwan cobra (*Naja naja atra*) venom was obtained.

After much of the present study was completed, it came to the authors' attention that the crystal structures of two short neurotoxins, erabutoxin b (Low et al., 1976) and a neurotoxin from the sea snake *Laticauda semifasciata* (Tsernoglou and Petsko, 1976), have been successfully determined. Inasmuch as our predicted results are made without any prior knowledge of these x-ray results (Liu, 1976), comparisons between predicted and experimentally determined results provide a test of the validity of the prediction method as well as some insights into relations between sequence homology and structure homology.

Materials and Methods

Conformational Prediction. The method of Chou and Fasman (1974a,b) was used for conformation prediction. This method was devised from statistical analysis of x-ray results of 15 proteins and is primarily based on the stabilizing effect of local interactions. It has succeeded in elucidation of secondary structure in proteins with a 77% accuracy (Chou and Fasman, 1975). In the present work, the conformational parameters for the 20 amino acids and the assignments of the amino acid residues as former, breaker, or indifferent to helix and β-sheet formation were obtained from the original tables (Chou and Fasman, 1974b, 1975). These parameters and assignments were then given to the amino acid residues of the toxins to be studied. The predictions were then made following the rules as given (Chou and Fasman, 1974b).

Also, assuming each type of toxins is structurally homologous, an averaging scheme was used for prediction of secondary structures for each type, i.e., short neurotoxins, long neurotoxins, and cardiotoxins. The averaging scheme was based on the following procedures. (1) The amino acid sequences of 20 short neurotoxins, 19 long neurotoxins, and 17 cardiotoxins were first aligned (see Figure 1). (2) The corresponding conformational parameters (P_α, P_β, P_t) and values of f_i, f_{i+1}, f_{i+2}, and f_{i+3} for each type of toxin and for every alignment position

were calculated. P_α, P_β, and P_t are the conformational parameters for helix, β-sheet, and the β-bend, respectively. f_i, f_{i+1}, f_{i+2}, and f_{i+3} are the frequencies of occurrence for residues at 1st, 2nd, 3rd, and 4th position of a β bend, respectively. For example, for short neurotoxins, the distribution of amino acid residues at the N terminus is Arg:Leu:Met[2] = 8:6:6. The P_α at this position is then calculated as $p_\alpha = 0.4P_\alpha(\text{Arg}) + 0.3P_\alpha(\text{Leu}) + 0.3P_\alpha(\text{Met})$. (3) Based on the calculated parameter values, regions of secondary structure were located following the rules given by Chou and Fasman (1974b).

Materials and Laser Raman Spectra Measurements. Cardiotoxin was isolated from the venom of Taiwan cobra (*Naja naja atra*) as previously described (Lee et al., 1968; Narita and Lee, 1970). The Raman spectra of this toxin were obtained on a Spex 1401 spectrometer and an SSR photon counter with the 514.5-nm line of a Spectra-Physics Model 170 argon ion laser as the exciting source. The protein solution was prepared at concentration of 100 mg/mL in 0.01 M acetic acid solution (pH 3.5). A Lindemann-glass tube (2 mm id) with a thin cover glass on one end and containing about 25 μL of the solution was used for Raman measurement.

Results and Discussion

A Raman spectrum of the cardiotoxin from Taiwan cobra venom is reproduced in Figure 2. Predicted regions of secondary structure in three short neurotoxins [cobrotoxin, toxin α (*N. haje haje*), and erabutoxin a], three long neurotoxins [toxin A (*N. naja*), α-bungarotoxin, and toxin α (*N. nivea*)], and two cardiotoxins [cardiotoxin (*N. naja atra*) and cytotoxin I (*N. naja*)] are shown in Table I. Results from predictions based on the averaging scheme for 20 short neurotoxins, 19 long neurotoxins, and 17 cardiotoxins are given in Table II. It should be noted that conformation of 15 snake toxins has recently been independently predicted using the same method (Chen et al., 1975). Since different parameter values (f_i, f_{i+1}, f_{i+2}, and f_{i+3}) were used, their conclusions regarding the number and location of secondary structures are somewhat different from those given here for the same toxins. Therefore, their results will not be used in this discussion.

Predicted α Helix. Table II shows that the helical structure is absent in the snake toxins. The low helicity could be foreseen from the average helical forming potentials of these molecules (Table III). The helical structure is also not predicted in all types of toxins using the averaging scheme. Previously, Lim (1974a) predicted that cobrotoxin had a helical region in the segment[3] 25–37 and erabutoxin a had a helical region in 26–40. The disagreement may be related to the difference in the principles upon which the two methods are based. The long-range interaction for stabilizing conformation of large and compact globular proteins receives the major emphasis in Lim's algorithms (Lim, 1974b), whereas the stabilizing effects primarily from the short and middle range interactions are stressed in the model of Chou and Fasman (1974b).

From the results of some physicochemical investigation, it appears that the cobra and sea snake venom toxins are devoid of helical structure. Recently, laser Raman spectra of neurotoxins from *L. hardwickii, E. schistosa,* and *L. semifasciata* contained the amide I and III band frequencies which indicate that random coil and β structure are dominant in these toxins

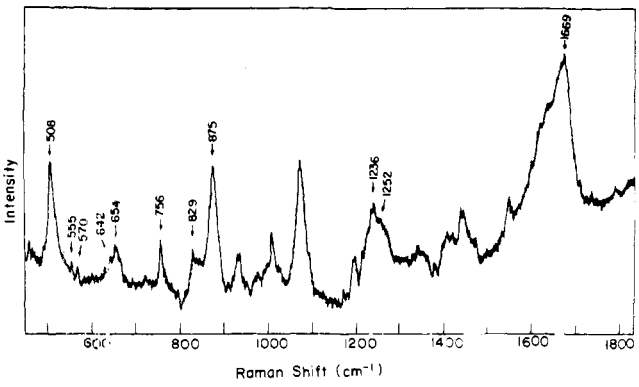

FIGURE 2: Raman spectrum of cardiotoxin (*N. naja atra*). Spectral slit width, 4 cm^{-1}; scale, 10^4 counts/s; rate of scan, 1 cm^{-1}/s; standard deviation, 1 2%; the laser power, 300 mW at 514.5 nm.

(Yu et al., 1975; Harada et al., 1976). Furthermore, cobrotoxin gave a positive ORD peak at 233 nm and a positive CD band at 228 nm (Yang et al., 1968). These are very unusual peaks. Similar results were also found in spectra of other snake toxins, for example, erabutoxin a and b (Menez et al., 1976), α-neurotoxin (Menez et al., 1976), toxin A (Nakai et al., 1971), α-bungarotoxin (Hamaguchi et al., 1968), and cytotoxin I and II (Takechi and Hayashi, 1972). Generally, proteins having right-handed α-helical structures show negative troughs at these wavelengths.

Therefore, the predicted results and some physicochemical investigations indicate that these toxins have no or very little, if any, helical structure.

Predicted β Structure. Some β structure, but not much, may be present in short neurotoxins and long neurotoxins. Cardiotoxins and cytotoxins have more β structure. Results predicted by the averaging scheme are essentially consistent with those from predictions for individual toxins except short neurotoxins (Tables I and II). There are some variations in the predictions of β structure among short neurotoxins. Lim (1974a) predicted that cobrotoxin contained β strand in residues 57–61 and erabutoxin a in 1–6.

Based on the method of Chou and Fasman, Low et al. (1976) predicted that erabutoxin b had three β-strand regions (2–6, 12–16, and 37–41). Erabutoxin a differs from erabutoxin b only by one residue. The work reported herein predicts no β strand in the region 2–6 in erabutoxin a. This fact deserves some additional discussion. The method of Chou and Fasman provides three procedures for predicting the three types of secondary structure regions. Using this method, it is easier to distinguish a helix from a β-strand region. It provides no criteria for choosing a β bend from a helix or β-strand region when these two types of secondary structures simultaneously satisfy the rules given by the method. The region 5–8 in erabutoxin a was predicted as β bend with the preconception that the accuracy of the β-sheet prediction is lower than that of β-bend prediction.

The accuracy of β-structure prediction was discussed recently (Schulz et al., 1974) and it was concluded to be less accurate than the helix and β-bend predictions. This might be attributed to the fact that β sheets are predominantly formed by interaction between residues that are far apart along the polypeptide chain (long-range interaction), whereas helices and β bends are caused by interaction between neighbors along the chain (local interaction). Also, the snake venom toxins are small proteins containing a large number of disulfide bridges which may render the results of predictions inaccurate.

[2] Abbreviations for amino acid residues are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (*Biochemistry 11*, 942 (1972); *J. Biol. Chem. 247*, 323 (1972)).

[3] Residue number given is homologous alignment sequence number (Figure 1).

TABLE I: Predicted Secondary Structures in Snake Toxins.[a]

Toxin	α helix	β sheet	$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$	β bend
Cobrotoxin (*N. naja atra*)	No	13-17 61-66	0.75 0.83	1.14 1.16	7-10, 17-20 18-21, 22-25 33-39, 49-52 66-69
Toxin α (*N. haje haje*)	No	61-66	0.83	1.16	7-10, 11-14 12-15, 17-21 18-22, 22-25 33-39, 43-48 49-52, 66-69
Erabutoxin a (*L. semifasciata*)	No	12-17 31-41	0.91 0.86	1.14 1.16	5-8, 7-10 17-20, 18-21 20-23, 24-27 43-48, 53-57
Toxin A (*N. naja*)	No	1-6 51-62	0.92 0.99	1.31 1.36	9-12, 14-17 17-20, 22-25 26-29, 31-34 36-39, 63-67 64-68, 69-72 72-76
α-Bungarotoxin (*B. multicinctus*)	No	1-10 28-34 45-49	1.00 1.07 1.05	1.27 1.22 1.15	10-13, 17-20 18-21, 34-37 35-38, 36-39 49-52, 50-53 63-67, 66-69 70-73, 71-75
Toxin α (*N. nivea*)	No	1-9 12-17 41-49	0.92 1.02 1.03	1.30 1.18 1.14	9-12, 17-20 22-25, 36-39 63-67, 64-68 66-69, 69-72 72-76
Cardiotoxin (*N. naja atra*)	No	12-17 23-36 44-49 55-62	0.96 1.07 0.87 1.02	1.17 1.30 1.24 1.30	17-20, 18-21 20-23, 49-52 50-53, 63-67 66-69
Cytotoxin I (*N. naja*)	No	12-17 23-31 44-49 55-59	1.01 0.99 0.86 1.10	1.12 1.35 1.24 1.22	17-20, 18-21 20-23, 49-52 50-53, 63-67 66-69

[a] Residue number given is homologous alignment sequence number (Figure 1).

The results of physicochemical investigations suggest that some β structures are present in snake toxins. Laser Raman spectra of some neurotoxins of sea snakes indicated that conformation of these toxins was mainly "anti-parallel pleated-sheet configuration" (Yu et al., 1975; Harada et al., 1976). Cobrotoxin was suggested to contain β-sheet structure because the CD spectra had a negative maximum at around 217 nm which is in agreement with the observations of CD spectra of proteins having β structures (Ikeda et al., 1968; Izuka and Yang, 1966). Similarly, α-bungarotoxin (Hamaguchi et al., 1968), erabutoxin b, and toxin α (*N. nigricollis*) (Menez et al., 1976) were also suggested to contain β structure based on the same observation (negative band at 216 nm). Therefore, the results of the present predictions appear to agree with the conclusions of physicochemical studies.

Predicted β Turns. It appears that a large proportion of β turns are present in snake venom toxins. β turns or bends have been proposed as a mechanism for tertiary folding of globular proteins (Lewis et al., 1971; Kuntz, 1972). These bends or turns, consisting of only four amino acid residues, enable a polypeptide chain to reverse itself by nearly 180° with hydrogen bonding between the C=O group of residue *i* and the NH group of residue *i*+3.

A comparison of the predicted positions of β turns among snake venom toxins shows a remarkable conservation of this feature. Therefore, it might be assumed that β turns play an important role in the maintenance of biologically active conformation of these snake toxins. It is also consistent with the pH-dependent rate of reactivation of reduced cobrotoxin; the rate of reactivation of reduced cobrotoxin increases as the pH of the medium increased (Yang, 1967). It is likely that in lowering the pH condition the formation of β bends is retarded through protonation of the electron-donating group.

Implication from Results of Chemical Modification and Physicochemical Studies. Results of chemical modification of amino acid residues of snake venom toxins performed during the past years provide the following conclusions. (a) The invariant Tyr-25 may be essential for toxicity, and it may be buried in the interior part of short neurotoxins (Yang, 1974). This was further supported by spectrophotometric titration (Chang et al., 1971; Chicheportiche et al., 1972) and laser Raman spectroscopic studies (Yu et al., 1975; Harada et al.,

TABLE II: Secondary Structures Predicted Using Averaging Scheme for the Three Types of Snake Toxins.[a]

Toxins	α helix	β sheet	$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$	β bend
Short neurotoxins	No	No			6-9, 7-10 11-14, 14-17 17-20, 18-21 22-25, 33-39 43-48, 53-57 63-67, 66-69
Long neurotoxins	No	1-9	0.86	1.15	9-12, 17-20
		23-30	0.90	1.22	18-21, 30-33
		43-49	1.01	1.11	35-38, 36-39 49-52, 63-67 66-69, 69-72 71-74
Cardiotoxins	No	12-17	0.96	1.12	17-20, 18-21
		23-36	1.02	1.25	20-23, 49-52
		44-49	0.87	1.21	50-53, 63-67
		55-62	1.00	1.26	

[a] Residue number given is homologous alignment sequence number (Figure 1).

TABLE III: $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_t \rangle$ of Snake Toxins.[a]

Toxin	$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$	$\langle P_t \rangle$
Cobrotoxin	0.87	0.95	1.12
Toxin α (*N. haje haje*)	0.81	0.96	1.11
Erabutoxin a	0.90	0.96	1.07
Toxin A (*N. naja*)	0.87	1.05	1.04
α-Bungarotoxin	0.94	0.99	1.02
Toxin α (*N. nivea*)	0.91	1.05	1.03
Cardiotoxin (*N. naja atra*)	0.94	1.08	0.95
Cytotoxin I (*N. naja*)	0.94	1.06	0.99

[a] These are the average forming potentials of α helix, β sheet, and β bend of whole molecule of a toxin.

1976). (b) The invariant Trp-29 is partially buried. Also, it is essential for biological activity of short neurotoxins (Yang, 1974). This fact is further supported by fluorescence (Seto et al., 1970; Bukolova-orlova et al., 1974) and laser Raman data (Yu et al., 1975). (c) The invariant Lys-53 in short neurotoxins may be functionally important. This residue was proposed (Yang, 1974) to offer a positive site for covalent bonding with the negative site of a receptor. (d) In short and long neurotoxins, the invariant Arg-36 may be involved in the active sites of toxin molecules similarly to the involvement of Lys-53 (Yang, 1974). (e) Glu-21 which is preserved in almost all short neurotoxins except toxin II from *H. haemachatus* may also be important for biological activity (Yang, 1974; Chicheportiche et al., 1975).

Based on chemical evidence such as given above and physicochemical behavior of 27 snake toxins, Ryden et al. (1973) constructed a model of the three-dimensional structure of the toxin α (*N. nigricollis*). The assumption that a hydrophobic core exists in the molecule and the classification of the amino acid residues as "inside" or "outside" were the principal guidelines for the construction. The importance of β bends as a mechanism for tertiary folding of globular proteins was also realized and was considered in construction of the model. Two β bends, 53-57 and 63-67, were predicted. The model was obviously intended to serve as a summary of the available data for these toxins and was largely intuitive. However, the model did not successfully account for all the data available.

Laser Raman Scattering of a Cardiotoxin from Taiwan Cobra (N. naja atra) Venom. The amide I band of the spectrum (Figure 2) is observed at 1669 cm^{-1}. This indicates a large fraction of β-sheet conformation (Yu and Lin, 1972). The amide III bands are at 1236 (strong) and 1252 cm^{-1} (shoulder). These data indicate that β-sheet structure is dominant in the molecule (Yu and Lin, 1972). A comparison between the amide I and III frequencies of this cardiotoxin and those of erabutoxin b (Harada et al., 1976) suggests that the cardiotoxin may contain more β-sheet structure than erabutoxin b. This agrees with the predicted results from this study (Table I).

A doublet at about 850 and 830 cm^{-1} is generally assigned as a tyrosine band. The intensity ratio of the two peaks is correlated with the environment and interaction of tyrosyl residues in a protein (Yu et al., 1973). The spectrum of this cardiotoxin in this region differs significantly from those found in neurotoxins (Yu et al., 1975; Harada et al., 1976). This difference is expected, however, as the cardiotoxin has two more tyrosyl residues (Tyr-14 and Tyr-59) in addition to Tyr-25 which is common to all toxins (Figure 1). The spectrum of the cardiotoxin in this region is difficult to interpret because there is a sharp strong peak at 875 cm^{-1}. The two tyrosyl residues, Tyr-14 and Tyr-59, are expected to be in different environments. Chemical modification indicates that Tyr-14 and Tyr-25 are exposed while Tyr-59 may be buried (Hung et al., 1976). Note that Tyr-25 in neurotoxins was suggested to be buried both from chemical modification (Yang, 1974) and laser Raman spectroscopic studies (Yu et al., 1975; Harada et al., 1976). Tyr-25 was also experimentally determined to be in a relatively inaccessible position in erabutoxin b (Low et al., 1976).

The S-S stretching vibration is observed at 508 cm^{-1} (Figure 2). It is similar to those observed for neurotoxins (Yu et al., 1975; Harada et al., 1976). Thus, Raman spectrum of the cardiotoxin has an appearance quite similar to those of other neurotoxins. However, the position of amide I and amide III peaks indicates that the conformation of the cardiotoxin may be different from that of neurotoxins.

Comparison with X-Ray Results. The predicted secondary structures and the experimentally determined ones in erabutoxin b are compared in Figure 3. Erabutoxin b is a short

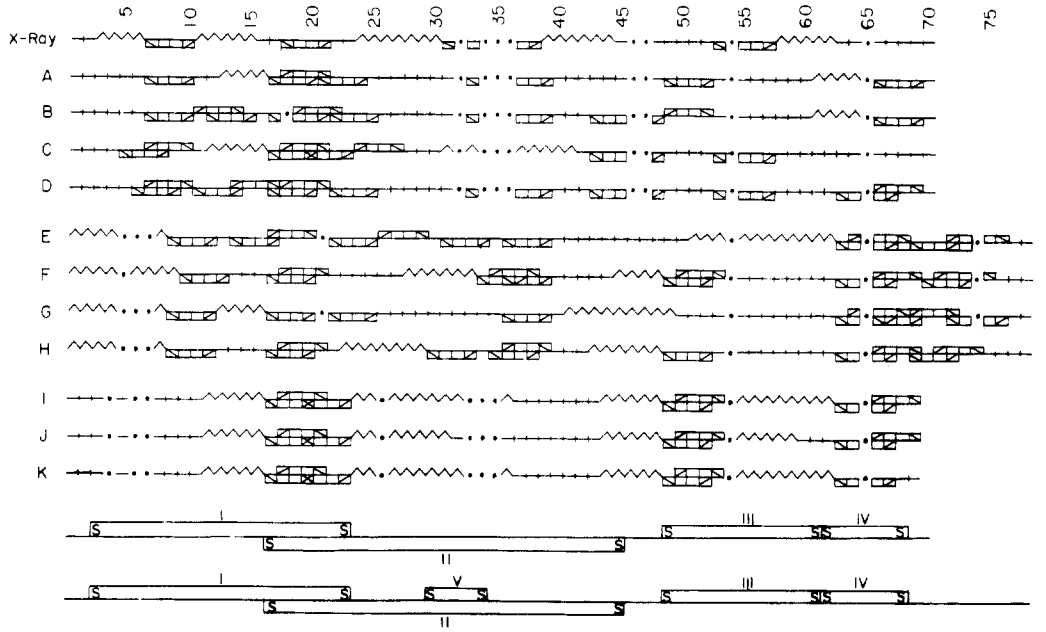

FIGURE 3: Comparison of predicted and experimentally determined secondary structure in snake venom toxins. The experimental data (x-ray are from Low et al., 1976). Predictions A–D are for short neurotoxins: (A) cobrotoxin; (B) toxin α (*N. haje haje*); (C) erabutoxin a; (D) all short neurotoxins by averaging scheme. Predictions E–H are for long neurotoxins: (E) toxin A (*N. naja*); (F) α-bungarotoxin; (G) toxin α (*N. nivea*); (H) all along neurotoxins by averaging scheme. Predictions I–K are for cardiotoxins: (I) cardiotoxin (*N. naja atra*); (J) cytotoxin I; (K) all cardiotoxins by averaging scheme. Disulfide loops are designated at bottom of the figure for 4 disulfide bridges and 5 disulfide bridges situations. Symbols: β-sheet (∧∧∧); coil residues (+++); β-turn tetrapeptide (,); deletion introduced in sequence alignment (•).

neurotoxin. It differs greatly from cobrotoxin and toxin α in sequence, i.e., by 29 and 24 amino acid residues, respectively, as shown in Figure 1. Nevertheless, consistent predictions are obtained for most β bends (see also Gabel et al., 1976). Also the absence of α helix has been correctly predicted. The prediction using the averaging scheme even correctly locates all experimentally determined β-bend regions (Figure 3). Helices and β bends are caused by interaction between neighbors along a polypeptide chain. The consistent prediction of these secondary structures also suggests that the prediction is better for secondary structures caused by local interaction than those caused by long-range interaction (Schulz et al., 1974).

The accuracy of the β-strand prediction is rather low. None were predicted using the averaging scheme. It is doubtful, therefore, that the predicted secondary structures could lend itself to a three-dimensional model. Nevertheless, it is interesting to note that both x-ray and predicted results give β-bend regions in the middle of loops I, II, and III and the overlapping region of loop I and II (Figure 3). The predicted results imply that conformation of short neurotoxins is primarily anti-parallel β-pleated sheet. This agrees with the conclusion based on Raman spectroscopic studies (Yu et al., 1975; Harada et al., 1976). The consistent prediction of β bends in these regions also suggests that short neurotoxins are conformationally as well as sequencely homologous proteins.

For long neurotoxins the predicted results are similar to those of short neurotoxins except in the region of loop V which is predicted most likely to be a β bend. These two types of neurotoxins have similar pharmacological activities but differ in immunochemical properties (Botes, 1972). Sequencely, toxin A differs from α-bungarotoxin and toxin α (*N. nivea*) by 39 and 17 amino acid residues and α-bungarotoxin differs from toxin α by 37 residues. These three long neurotoxins all have only 14 residues in common with short neurotoxins. However, the close similarities in number as well as in location of the predicted β-bend regions in these two types of neurotoxins suggest that long neurotoxins have conformations similar to that of short neurotoxins. If indeed this is the case, then the extra disulfide bridge (loop V) of long neurotoxins, which is located in the region hypothesized (in light of x-ray results) as the reactive site of erabutoxin b (Low et al., 1976), might be responsible for the lower toxicity and different immunochemical behavior of long neurotoxins. It should be noted that the results of chemical studies have indicated that loop V is not important for toxicity and is on the exterior portion of the molecule (Botes, 1974a; Chicheportiche et al., 1975).

Cardiotoxins have entirely different biological activities (Viljoen and Botes, 1973). The predicted secondary conformation also differs from that of neurotoxins (Figure 3); only two β-bend regions are predicted (excluding that in loop IV). A recent study showed that positions of the disulfide bonds in cardiotoxin (*N. naja atra*) are the same as that in cobrotoxin (Cheng et al., 1976). Selective modification of the three tyrosyl residues it contains further suggests that Tyr-14 and Tyr-25 are exposed while Tyr-59 may be buried (Hung et al., 1976). Laser Raman spectrum of the same cardiotoxin indicates that it may have a higher content of β sheet than neurotoxins have, as described earlier. Although the predicted results as well as the conclusions from CD and ORD measurements (Hung and Chen, 1976) and laser Raman scattering data suggest that the cardiotoxin contains mainly β sheet and β bend, the location and number of predicted secondary structures differ from those of neurotoxins. Moreover, the predicted β bends and β-sheet structures in cardiotoxins clearly suggest that they are of anti-parallel pleated-sheet configuration differing from that in erabutoxin b (Low et al., 1976). The construction of a simple wire model based on the predicted results suggests that cardiotoxins could have an alternative molecular topology besides the one observed for erabutoxin b. The alternative model has a distorted short rod-like shape. The peptide chain coils spirally and the disulfide bridges secure the coil and prevent unwinding. The model has the indication that Tyr-59 in the smaller loop

III region is relatively inaccessible. The sequences of the amino acid residues in the overlap region of loop I and II are relatively conserved in all cardiotoxins while they vary in neurotoxins. The conformation of the β bend predicted in this region, therefore, is structurally very important in determining the molecular topology of cardiotoxins according to the alternative model. Difference in molecular topology between cardiotoxins and neurotoxins could be responsible for their differences in pharmacological activities and serological properties.

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Characterization and Comparative Aspects of the Serum Very Low and Low Density Lipoproteins and Their Apoproteins in the Chicken (*Gallus domesticus*)†

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ABSTRACT: Sera from young laying chickens, found to be hypertriglyceridemic by serum lipid and lipoprotein analyses, were fractionated by ultracentrifugation into very low (d < 1.006 g/mL) and low density (d 1.006–1.063 and 1.024–1.045 g/mL) lipoproteins (VLDL and LDL). The purity of these lipoprotein fractions was evaluated by electrophoretic, immunological, and electron microscopic techniques; their chemical and physical properties were subsequently determined and compared with those of the corresponding human fractions. While an overall resemblance was evident between each chicken fraction and its human counterpart, minor differences were detected in surface charge, chemical composition, and particle size. Both chicken VLDL and LDL exhibited low surface charge upon electrophoresis; the triglyceride content and particle size of the chicken LDL fractions were greater than those of the corresponding human preparations. Immunological studies revealed a partial identity between the VLDL and between the LDL of chicken and man; quantitative microprecipitation showed the cross-reactivity of chicken and human LDL to amount to about 10%. The chicken lipoproteins possessed a common antigenic determinant and reacted strongly to an antiserum to human apolipoprotein B. The presence of an apolipoprotein B like component in chicken VLDL and LDL was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis of their total apoproteins, which revealed a major component of high molecular weight (>250 000). This component was isolated in the void volume as fraction I upon gel filtration chromatography on Sephadex G-200; the behavior of fraction I on sodium dodecyl sulfate–polyacrylamide gel and its amino acid composition indicate that it is a counterpart of apolipoprotein B in man. Low-molecular-weight components were detected in apo-VLDL and apo-LDL by electrophoretic procedures in polyacrylamide gel; gel filtration chromatography facilitated their isolation as fraction II and showed them to constitute some 50% of apo-VLDL, but rather less of apo-LDL (d 1.006–1.063 g/mL, about 25%; d 1.024–1.045 g/mL, about 20%). The basic character of the fraction II apoproteins was revealed by amino acid analysis and by their low electrophoretic mobility in polyacrylamide gel at pH 8.9. Fraction II contained up to seven components, the most prominent exhibiting molecular weights of 27 000, 18 500–21 400, 13 500–14 000, 8500, and 4000. These data indicate that the major protein component of chicken VLDL and LDL is a counterpart of human apolipoprotein B, although the lower molecular weight components of these avian lipoproteins appear to be more distinct from those of man.

In recent years, avian species have attracted considerable interest as animal models in which to study the mechanisms of estrogen-induced hyperlipidemia (Hillyard et al., 1956; Kudzma et al., 1973; Luskey et al., 1974) and of diet-induced hypercholesterolemia (Hillyard et al., 1956; Kruski and Narayan, 1972). Several aspects of lipid metabolism in birds appear, however, to differ substantially from those typical of man. In particular, avians absorb exogenous fat as VLDL[1] via

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[1] Abbreviations used: VLDL, very low density lipoproteins of d < 1.007 g/mL; LDL, low density lipoproteins, density as defined; HDL, high density lipoproteins, d 1.063–1.21 g/mL; apo-B, apolipoprotein B; EDTA, ethylenediaminetetraacetic acid; SEM, standard error of the mean.