Secondary Structure Prediction of Chicken Egg White Ovomucoid

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The secondary structure of chicken egg white ovomucoid (186 residues) was predicted from the amino acid sequence by the method of Chou and Fasman (P. Y. Chou and G. D. Fasman, *Biochemistry*, **13**, 222 (1974)). The ovomucoid α -helix regions were predicted by the method of Lewis *et al.* (P. N. Lewis *et al.*, *Proc. Natl. Acad. Sci. U.S.*, **65**, 810 (1970)). The relative composition of the secondary structure obtained by the former method was: α -helix (7 regions) 27%, β -structure (10 regions) 33%, β -turn (11 regions) 23% and random coil 17%. In the predicted conformation, there were some α -helical and β -structure segments that tended to be segregated along the polypeptide chain. These results indicate that the ovomucoid may be classified into the $\alpha + \beta$ type protein group and that the reactive site (Arg⁸⁹↓Ala⁹⁰) of trypsin inhibition is probably situated at the C-terminal β -structure end. In comparisons of the predicted structures of the three domains in ovomucoid, domains I and II were suggested to be partially homologous to one another and not to domain III.

Chicken egg white ovomucoid is a glycoprotein with a trypsin inhibitory activity.¹⁾ The amino acid sequence of the ovomucoid has been determined by Kato *et al.*,²⁾ and it was demonstrated that the ovomucoid molecule consisted of three well separated domains, each being cross-linked by three disulfide bonds, and that amino acid sequences of the three domains were homologous to each other.^{2,3)} The reactive site of trypsin inhibition was also reported to be Arg⁸⁹↓Ala⁹⁰ in domain II.⁴⁾ It is of interest to predict the secondary structure of the ovomucoid in the light of the reactive site and the sequence homology among the three domains. We have already reported the composition of the secondary structure of the ovomucoid and its three domains calculated from circular dichroism (CD) data, though the CD data provided only the percentage of each structural element.⁵⁾

During the last several years, a number of predictive methods for the secondary structure of proteins have been reported.⁶⁾ In this paper, we present the ovomucoid secondary structure as predicted essentially according to the method of Chou and Fasman,⁷⁾ then refined by the method of Lim,⁸⁾ and the helix probability profile obtained by the method

of Lewis *et al.*⁹⁾ and Lewis and Scheraga,¹⁰⁾ and discuss the conformational characteristics of the ovomucoid, the secondary structure at the reactive site and the structural homology among the three domains.

METHODS

The amino acid sequence of the ovomucoid used for conformational predictions, with the carbohydrate-attachment sites, was as shown by Kato *et al.*²⁾ and Kato³⁾ (Fig. 1).

Method of Chou and Fasman

1) α -Helix and β -structure regions. α -Helix and β -structure regions were essentially determined by the predictive rules of Chou and Fasman.⁷⁾ For the conformational assignments of individual amino acids and the calculation of average conformational potentials, $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$, the data of Fasman *et al.*¹¹⁾ were used. When there were overlapping predictions, the segments predicted in duplicate were determined to be in a helical or β -structure conformation according to their average conformational potentials. The minimum numbers of residues for α -helix and β -structure predictions were usually set at six and five, respectively.

2) β -Turn regions. The relative probability, P_t , that a tetrapeptide forms a β -turn was given by Lewis *et al.*¹²⁾

$$P_t = f_i \cdot f_{i+1} \cdot f_{i+2} \cdot f_{i+3}$$

In this expression, the f 's represent the frequency of

The predicted α -helix and β -structure regions of the ovomucoid are summarized in Table I. There were 7 helical regions containing 51 residues and 10 β -structure regions containing 62 residues. Region 144~151 with a $\langle P_{\alpha} \rangle$ value of 1.00 does not satisfy the condition that any segment predicted as a helical conformation must have a $\langle P_{\alpha} \rangle$ value above 1.03. However, an α -helix was predicted for this segment because Pro¹⁴⁴, Asp¹⁴⁵ and Arg¹⁵¹ are good indicators of a helical formation; Pro and Asp prefer the N-terminal helical end and Arg prefers the C-terminal helical end, and in such cases an I_{α} assignment is given to these

TABLE I. CONFORMATIONAL PREDICTION OF OVOMUCOID COMPUTED FOR α -HELIX AND β -STRUCTURE REGIONS: $\langle P_\alpha \rangle$ AND $\langle P_\beta \rangle$ VALUES

Ovomucoid (186 residues)				
	Region*	Assignments	$\langle P_\alpha \rangle^\dagger$	$\langle P_\beta \rangle^\dagger$
α -Helix	11 ~ 18 (8)	H ₂ h ₂ I ₂ iB	1.08	0.71
	55 ~ 59 (5)	HhI ₂ i	1.09	0.66
	61 ~ 66 (6)	H ₃ h ₂ i	1.13	0.93
	110 ~ 115 (6)	H ₂ h ₃ I	1.21	0.94
	117 ~ 124 (8)	Hh ₂ I ₃ i ₂	1.05	0.86
	128 ~ 137 (10)	H ₄ h ₃ Ii ₂	1.16	0.82
	144 ~ 151 (8)	H ₂ I ₄ ib	1.00	0.77
β -Structure	19 ~ 23 (5)	H ₂ h ₂ i	0.94	1.36
	35 ~ 39 (5)	H ₂ hi ₂	0.78	1.23
	41 ~ 48 (8)	H ₂ h ₄ ib	0.97	1.20
	50 ~ 54 (5)	Hh ₂ ib	0.86	1.16
	73 ~ 77 (5)	Hh ₂ i ₂	0.89	1.11
	83 ~ 92 (10)	H ₂ h ₄ i ₄	1.06	1.19
	100 ~ 104 (5)	H ₂ hib	0.85	1.16
	106 ~ 109 (4)	h ₄	0.96	1.25
	165 ~ 172 (8)	H ₂ h ₃ i ₃	0.93	1.22
	177 ~ 183 (7)	h ₅ ib	1.00	1.14
α -Helix: 7 regions, 51 residues, 27%				
β -Structure: 10 regions, 62 residues, 33%				

* The predicted α -helix and β -structure regions are based on the rules of Chou and Fasman⁷⁾ with conformational parameters updated for 29 proteins.¹¹⁾

† $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ are, respectively, the average conformational potential for the computed region to be in the helical and β -structure conformation.

FIG. 2. Probability of Tetrapeptide β -Turns in Ovomucoid.

Of the peaks above the cut-off value (2.33×10^{-4}), 7 were excluded (residue numbers shown in parentheses) as belonging to other predicted regions.

residues.⁷⁾ The $\langle P_\beta \rangle$ value of 1.14 for region 177 ~ 183 is higher than the $\langle P_\alpha \rangle$ value of 1.00. A β -structure is predicted for this region though α -helicity can not be ruled out completely.

The relative probabilities that a tetrapeptide will form a β -turn were calculated with the equation described above and are depicted in the probability profile shown in Fig. 2. Eight-

TABLE II. CONFORMATIONAL PREDICTION OF OVOMUCOID COMPUTED FOR β -TURN REGIONS

Residues	Tetrapeptide*	P_t §	$\langle P_t \rangle^\dagger$	$\langle P_\alpha \rangle^\dagger$	$\langle P_\beta \rangle^\dagger$
8~11	Phe-Pro-Asn-Ala	3.64×10^{-4}	1.09	0.95	0.91
23~26	Asn-Lys-Asp-Leu	3.47×10^{-4}	1.12	1.01	0.87
31~34	Gly-Thr-Asp-Gly	4.78×10^{-4}	1.38	0.75	0.81
67~70	Pro-Met-Asn-Cys	3.09×10^{-4}	1.25	0.85	0.92
70~73	Cys-Ser-Ser-Tyr	4.51×10^{-4}	1.28	0.73	1.04
78~81	Ser-Glu-Asp-Gly	3.85×10^{-4}	1.29	0.97	0.60
92~95	Asn-Pro-Val-Cys	2.66×10^{-4}	1.22	0.75	1.08
96~99	Gly-Thr-Asp-Gly	3.63×10^{-4}	1.38	0.75	0.81
138~141	Cys-Ser-Glu-Tyr	3.63×10^{-4}	1.15	0.92	0.95
156~159	Ser-Asp-Asn-Lys	3.31×10^{-4}	1.31	0.90	0.73
173~176	Glu-Ser-Asn-Gly	3.64×10^{-4}	1.31	0.88	0.69

β -Turns: 11 regions, 43 residues, 23%

§ P_t is the probability of β -turn occurrence obtained by the method of Lewis *et al.*¹²⁾ with conformational parameters update for 29 proteins.¹³⁾

† $\langle P_t \rangle$, $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ are, respectively, the average conformational potential for the computed region to be in the β -turn, helical and β -structure conformation.

* A β -turn is predicted for a tetrapeptide if the following conditions are met: $\langle P_\alpha \rangle < \langle P_t \rangle$; $\langle P_\beta \rangle < \langle P_t \rangle$; $P_t > 2.33 \times 10^{-4}$.

FIG. 3. Schematic Diagram of Predicted Secondary Structure of Ovomucoid.

Residues are represented in their respective conformational states: helical (■), β -structure (□), β -turn (▨) and random coil (—). Identical amino acid residues in two or three domains (▤) are also shown below the diagram.

teen peaks were above the determined cut-off value of 2.33×10^{-4} . Of these peaks, 11 were predicted as β -turns, while the rest were excluded (residue numbers shown in parentheses in Fig. 2) as belonging to other predicted regions. The predicted β -turn regions of the ovomucoid are presented in Table II.

The predicted fraction of the ovomucoid secondary structure was as follows: α -helix 27%, β -structure 33%, β -turn 23% and random coil 17%. This composition was in relatively good agreement with that calculated from the CD data (α -helix 26%, β -structure 46%, β -turn 10% and random coil 18%),⁵⁾ though there were some discrepancies. The Chou-

Fasman prediction is not perfect; it sometimes gives overlapping predictions for α -helices and β -structures and there are some ambiguities in their rules.¹⁵⁾ The interpretation of the CD data in terms of a backbone conformation is not a straightforward matter either as described previously.⁵⁾ Hence, some discrepancy will presumably be unavoidable with these methods.

A schematic diagram of the predicted secondary structure of ovomucoid is presented in Fig. 3. There were some α -helix and β -structure segments that tended to be segregated along the polypeptide chain. According to this characteristic, it was predictable that the

ovomucoid is a protein of the $\alpha+\beta$ type like lysozyme, papain, *etc.*¹⁸⁾ Each pair of two β -structure regions (73~77 and 83~92, and 165~172 and 177~183) had a predicted β -turn between them, that is, 78~81 and 173~176, respectively, which might favor anti-parallel β -structure formation in these regions.

Lim introduced the predictive approach based on the analysis of the hydrophobicity and size of the residue side chains, and the characterization of β -structure segments into "internal", "semi-surface" and "surface" β -structures.⁸⁾ Based on this method an "internal" β -structure was predicted for region 82~89, which consisted of five large hydrophobic, two large hydrophilic and one small hydrophilic residues. This result was in good accordance with that of the Chou-Fasman prediction (β -structure region 83~92). Thus, it was suggested that the reactive site (Arg⁸⁹↓Ala⁹⁰) was situated at the C-terminal β -structure end.

Previously, Aubert *et al.* found that 37 carbohydrate peptide linkages including *O*-glycosidic (*N*-acetylgalactosamine→Ser/Thr) and *N*-glycosidic (*N*-acetylglucosamine→Asn) bonds, characterized in 19 glycoproteins, were situated predominantly in β -turn regions.¹⁷⁾ Beeley determined that four glycosylated Asn residues in the ovomucoid were very close to groups of amino acids that occurred with high frequency in β -turns.¹⁸⁾ Our finding of five glycosylated Asn residues in this work also indicated that tetrapeptides with high frequency in β -turns were closely related to all the sites of glycosylation except one (Asn⁵³), that is, three (Asn¹⁰, Asn⁸⁹ and Asn¹⁷⁵) of the five carbohydrate attachment sites were situated in β -turn regions and the other one (Asn⁷⁵) was very close to a β -turn region.

The predicted structures of the three domains in the ovomucoid are compared with each other in Fig. 3. Judging from our prediction, the three domains in the ovomucoid did not seem to be homologous to each other. The proportion of identical amino acid residues between domains I and II was 48%. Moreover, in the light of the conformational potential of the secondary structure, the pro-

portion of residues of identical conformational assignment (former; *H*, *h*, *I*, or indifferent; *i*, or breaker; *B*, *b*) reached 72% for α -helix and 75% for β -structure regions. However, domains I and II were predicted to be only partially homologous in regard to the secondary structure. For example, for region 45~54, the average conformational potentials were $\langle P_\alpha \rangle = 0.98$ and $\langle P_\beta \rangle = 1.08$, and the conformational assignments were $(H_2h_3i_2b_2B)_\alpha$ and $(H_3h_2i_2b_2B)_\beta$, whereas for region 110~119, the average conformational potentials were $\langle P_\alpha \rangle = 1.11$ and $\langle P_\beta \rangle = 0.96$, and the conformational assignments were $(H_3h_4IiB)_\alpha$ and $(H_2hi_3b_3B)_\beta$. Thus, the predicted structures of region 45~54 in domain I and the corresponding region, 110~119, in domain II were clearly different. The proportions of identical amino acid residues between domains I and III, and between domains II and III were no more than 38% and 32%, respectively. In addition, the proportions of residues of identical conformational assignment were no more than 70% both between domains I and III, and between domains II and III. These facts also suggested that the structural homology of domain III to the other domains might be inferior to that between domains I and II.

Lewis *et al.* prediction.

The helix probability profile of the ovomucoid obtained by the method of Lewis *et al.*^{9,10)} is shown in Fig. 4-A. The mean helix probability, θ_H , is represented by a horizontal line which intersects the ordinate. If the helix probability for the *i*-th residue, $P_H(i)$, equals or exceeds the mean helix probability, the residue is considered to be in the α -helix conformation. Plots of the quantities $P_{ch}(i)$ and $P_{hc}(i)$ are shown in Fig. 4-B. The magnitude of peaks reflects the probability that a helix begins or ends at the *i*-th position. The prediction whether or not a particular residues is α -helical was determined by, first, whether or not $P_H(i)$ exceeded θ_H and, second, whether or not this residue lay in the regions defined by $P_{ch}(i)$ and $P_{hc}(i)$. The helical regions predicted in this manner are also shown as horizontal

FIG. 4. Helix Probability Profile and Plots of $P_{ch}(i)$ and $P_{hc}(i)$ for Ovomuroid.

A: Helix probability profile. The horizontal line is the calculated values of the overall mean helix probability, θ_H , for the ovomucoid.

B: Plots of $P_{ch}(i)$ (—) and $P_{hc}(i)$ (-----).

The predicted helical regions, utilizing the $P_H(i)$, $P_{ch}(i)$ and $P_{hc}(i)$ information, are shown as horizontal bars (□). The helical regions predicted by the method of Chou and Fasman (■) are also shown for comparison.

FIG. 5. Comparison of Helix Probability Profiles for Domains I (—), II (---) and III (···) of Ovomuroid.

bars in Fig. 4-A. On comparison between the locations of helical regions determined by the Lewis *et al.* and Chou-Fasman methods as shown in Fig. 4, these two predictions were found to be in relatively good agreement.

Lenstra evaluated the secondary structure predictions for proteins obtained by the recently published methods (the joint histogram method of Argos *et al.*,¹⁹⁾ the stereochemical method of Lim,⁸⁾ the statistical mechanical method of Tanaka and Scheraga,¹⁵⁾ and the statistical method of Nagano²⁰⁾) using the data of 33 proteins.⁹⁾ The comparison showed that the method of Tanaka and Scheraga,¹⁵⁾ which was essentially the same as that of Lewis *et al.*^{9,10)} for α -helix prediction, yielded the

least reliable results of the four. However, it should be noted that if the objects of prediction are limited to α -helices in $\alpha+\beta$ proteins like insulin, papain, *etc.*, the method of Tanaka and Scheraga is not less reliable than the others.⁶⁾ The Chou-Fasman prediction and the CD analyses⁵⁾ on the ovomucoid suggested that the ovomucoid was probably an $\alpha+\beta$ protein as described above. Hence, it seems reasonable to predict only α -helix regions in the ovomucoid by the method of Lewis *et al.*

The helix probability profiles of the three domains in the ovomucoid are compared in Fig. 5. The profiles of domains I and II are similar to one another in their shape but do not necessarily correspond in their intensities.

As seen in Fig. 5, on the N-terminal side the intensity in domain I is stronger than in domain II, whereas on the C-terminal side the intensity in domain II is stronger than in domain I. The profile of domain III was evidently different from the others in both shape and intensity. Moreover, the helix probability profiles of domains I and II did not show the same extent of similarity as seen in the several species of Cytochrome C proteins,¹⁰⁾ which were reported to be structurally homologous.

Consequently, the predicted secondary structure of the ovomucoid suggested that the ovomucoid molecule was an $\alpha+\beta$ protein and its domains I and II were only partially homologous to one another and not to its domain III.

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