Problem Session

2021/9/4 Kensuke Miura

Question 1. Drawing CD spectra

1-1. Raw data of CD spectra and baseline data were sent (Sheet 1-4). Please draw the CD spectrum of myoglobin and carboxypeptidase A on excel using these data.

* The raw data were cited from Protein Circular Dichroism Data Bank (PCDDB)

1-2. The unit of the obtained raw data (θ) is mdegree. Please convert θ of the CD spectrum you drew in Q1-1 into $[\theta]$ using the following formulas and values.

θ: ellipticity (mdegrees)

$[\theta]$:molar ellipticity (degrees$\cdot$cm$^2\cdot$dmol^{-1})

c_p: concentration of protein (mg/mL)

M: mean residual weight (g/mol)

C: mean residue concentration (mol/L)

L: cell path length (cm)

$$[\theta] = \theta/10CL,\ C = c_p/M$$

conditions of experiment

myoglobin	carboxypeptidase A
M: 111.5, c_p: 11.44, L: 0.00063	M: 112.2, c_p: 2.14, L: 0.0022

Question 2. Predicting content of secondary structure of protein using CD spectra of a model polypeptide.

Spectra of 100% content of a-helix, b-structure, and random coil were obtained respectively by varying the secondary structure of polyl-L-ysine (Figure 1). The values of $[\theta]$ (degrees$\cdot$cm$^2\cdot$dmol^{-1}) at each wavelength of poly-L-lysine were sent (Sheet 5, from 208 to 240 nm).

Please predict the content of each secondary structure (f_α, f_β, f_R) of myoglobin and carboxypeptidase A using these values ($[\theta]_{lys\alpha}$, $[\theta]_{lys\beta}$, $[\theta]_{lysR}$).

The calculated intensity of the spectrum is expressed as follows using the content and the value of the reference spectrum.

$$[\theta]_{calc} = f_\alpha[\theta]_{lys\alpha} + f_\beta[\theta]_{lys\beta} + f_R[\theta]_{lysR}$$

f_α: percentage of α-helix ($0 \le f_\alpha \le 1$)

f_β: percentage of β-form ($0 \le f_\beta \le 1$)

f_R: percentage of random coil ($0 \le f_R \le 1$), $f_\alpha + f_\beta + f_R = 1$

Please calculate f_α, f_β, and f_R that minimize $\Sigma\varepsilon^2$.

$\varepsilon = [\theta]_{obs} - [\theta]_{calc}$

$\Sigma\varepsilon^2 = \Sigma([\theta]_{obs} - [\theta]_{calc})^2$

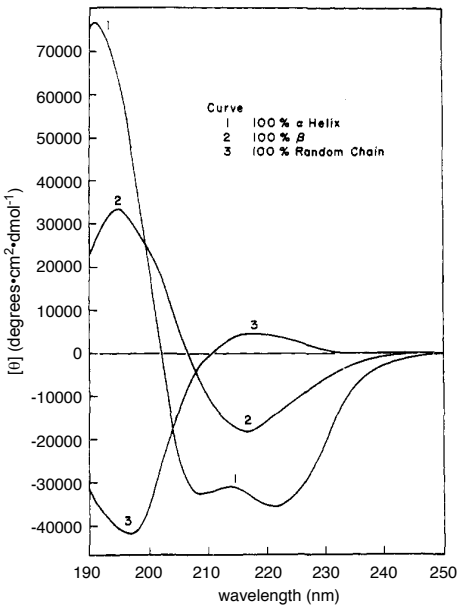


Figure 1

Question 3. The PDB (Protein Data Bank) ID of the given proteins are 1ymb and 5cpa respectively. Please compare the obtained result of question 2 with the experimental results. If possible, please explain the problem of using CD spectra of polypeptides as reference spectra when calculating the content of secondary structures.

Answer

Topic: Prediction of secondary structure of proteins using CD spectrum

0. Introduction

0-1. Determination of secondary structure of proteins

Protein secondary structure is the three dimensional form of local segments of proteins. Determination of secondary structure is the basis of protein structural analysis at the atomic level and is important in structural biology. The following methods have been used to analyze the secondary structure.

0-1-1. X-ray

The secondary structure in the sequence is determined by observing X-rays of the crystallized protein and identifying the coordinates of each atom.

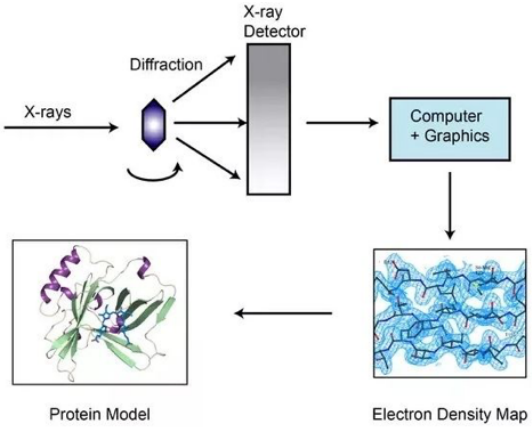


Figure 1. Overview of analysis using X-ray

pros: determination of the three dimensional positions of each atom in a protein,

cons: only for crystalized protein

0-1-2. NMR

The measurement is performed according to the following procedure.

1) The $^{13}C/^{15}N$-labeled target protein is expressed in large quantities using genetic engineering.

2) Obtaining high-resolution multidimensional NMR spectra of labeled proteins.

3) Analyzing the spectra to obtain dihedral angle information and hundreds of interatomic distances.

4) The structure that satisfies the obtained information is obtained by calculation.

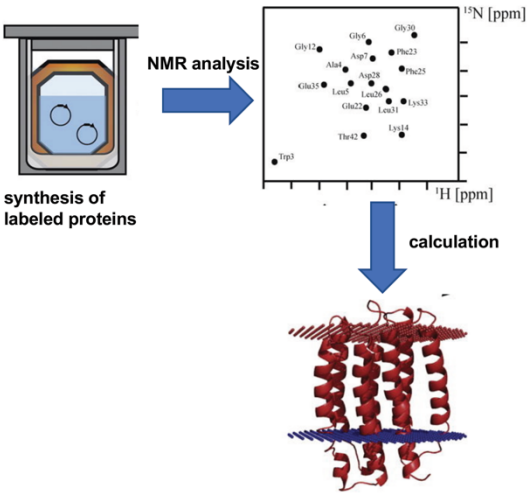


Figure 2. Overview of analysis using NMR

pros: determination of the three dimensional positions of each atom in a protein, no need for crystallization

cons: need for labeling, limitation of molecular weight (~40 kDa)

The above methods require a large amount of protein for measurement. In addition, labeling and crystallization are required, which makes it time-consuming to prepare the proteins. The method using CD spectra described below does not require crystallization or labeling, and requires only a small amount of protein for measurement.

CD spectrum

pros: small amount of samples

cons: not direct structural information

Table 1. Comparison of the amount of protein required for each analysis

	amount of proteins needed for experiment
X-ray	1-10 mg (for investigating the conditioin of crystalization)
solution NMR	2-5 mg (labeled proteins)
CD spectrum	0.01-0.1 mg

0-2. Introduction of CD spectrum

0-2-1. Circular dichroism (CD)[2)]

Planar polarized light consists of left- and right-handed circular polarized light with the same amplitude. When these lights are absorbed by the medium in different proportions, the amplitudes of the left- and right-handed circular polarized light become different, and the resultant light changes to elliptically polarized light (Figure 1).

This phenomenon of unequal absorption is called circular dichroism (CD). Circular dichroism is represented by the ellipticity θ. The following equation shows the relationship between the minor and major axes of an ellipse

$$\tan\theta = (A_L - A_R)/(A_L + A_R)$$

The θ that satisfies the above is called the ellipticity.

A plot of the ellipticity at each wavelength is called the CD spectrum.

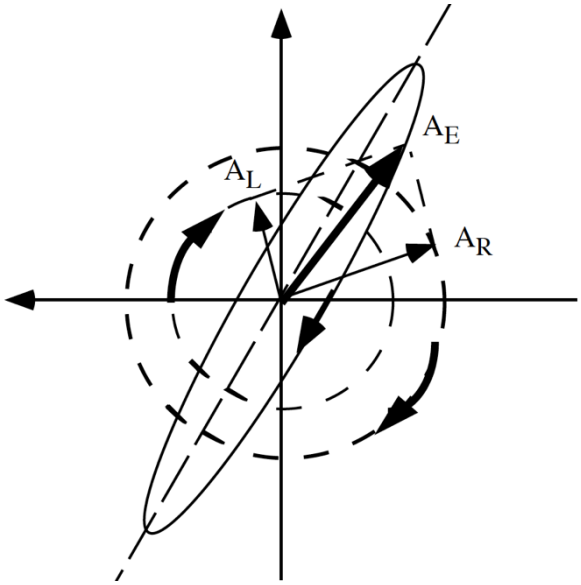


Figure 3. Generation of elliptically polarized light

0-2-2. Application of CD spectrum for analysis of proteins

1. Measuring the thermal denaturation of proteins

2. Prediction of content of secondary structure (in this problem)

Circular dichroism (CD) spectroscopy can reliably assign the proportion of α-helices and β-sheets in a protein based on differences in the spectral properties of their backbone. Although this method does not provide detailed information about the secondary structure, it is convenient because it allows measurements related to content information in a short time. Comparing the CD spectrum of a protein of unknown structure with the CD spectrum of a protein of known structure can give a good interpretation.

0-2-3. Measurement of CD spectrum

The CD spectra are measured in the same way as the measurements of optical rotation, only the solvent is measured and the baseline data is subtracted from the sample data in order to cancel the absorption of solvent. The unit of the obtained raw data (θ) is mdegree.

protocol

1) measurement of baseline (solvent only)

2) measurement of sample

3) Subtract the baseline value from the observed value

0-2-4. Database of CD spectra of proteins with known structure

Protein Circular Dichroism Data Bank (PCDDB)[3)4)]

The raw data in this problem (myoglobin[5)], carboxypeptidase A[6)]) was downloaded from PCDDB.

Answer

Question 1. Drawing CD spectra

1. Solution to Question 1

1-1. Data processing

Procedure

1) Subtract the baseline value from the observed value

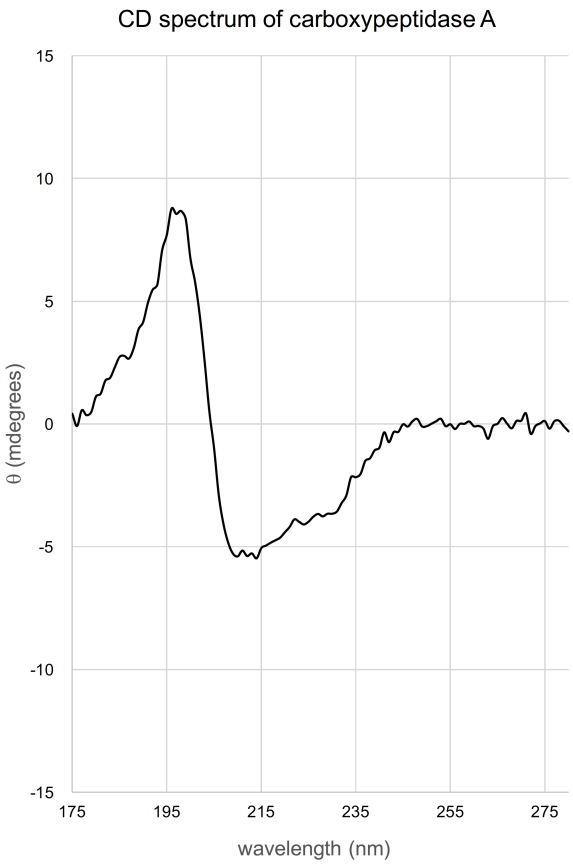
in the case of myoglobin

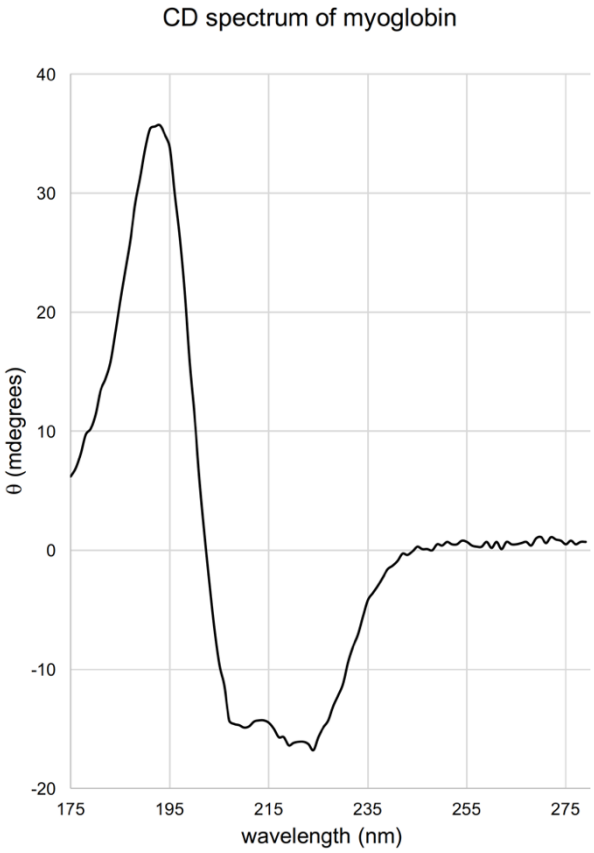
raw data			baseline	
wavelengs(nm)	θ (mdegrees)		wavelengs(nm)	θ (mdegrees)
279	1.10E+00		279	4.00E-01
278	1.10E+00		278	4.00E-01
277	7.00E-01		277	2.00E-01
276	1.30E+00		276	5.00E-01
275	7.00E-01		275	2.00E-01
274	1.00E+00		274	2.00E-01
273	9.00E-01		273	0.00E+00
272	1.20E+00		272	1.00E-01

Data processing

processed data	
wavelengs(nm)	θ(obs)-θ(baseline)
279	7.00E-01
278	7.00E-01
277	5.00E-01
276	8.00E-01
275	5.00E-01
274	8.00E-01
273	9.00E-01
272	1.10E+00

2) draw CD spectrum using resultant value





1-2. normalizing the observed value

$$[\theta] = \theta/10CL,\ C = c_p/M$$

conditions of experiment

myoglobin	carboxypeptidase A
M: 111.5, c_p: 11.44, L: 0.00063	M: 112.2, c_p: 2.14, L: 0.0022

Substitute the respective values into the above equation.

myoglobin

$$[\theta] = \frac{\theta}{10CL} = \frac{\theta}{10\times\frac{11.4}{111.5}\times 0.00063} = 1552\theta$$

carboxypeptidase A

$$[\theta] = \frac{\theta}{10CL} = \frac{\theta}{10\times\frac{2.14}{112.2}\times 0.0022} = 2383\theta$$

For drawing normalized CD spectra

myoglobin

wavelengs(nm)	θ(obs)-θ(baseline)	
279	7.00E-01	
278	7.00E-01	
277	5.00E-01	
276	8.00E-01	
275	5.00E-01	
274	8.00E-01	
273	9.00E-01	
272	1.10E+00	

× 1552

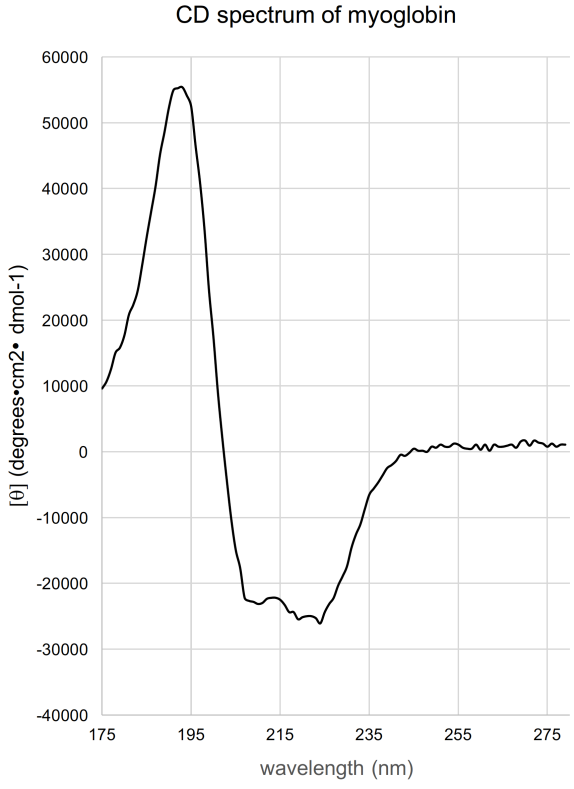
wavelengs(nm)	[θ] (degree•cm2•dmol-1)
279	1086.4
278	1086.4
277	776
276	1241.6
275	776
274	1241.6
273	1396.8
272	1707.2

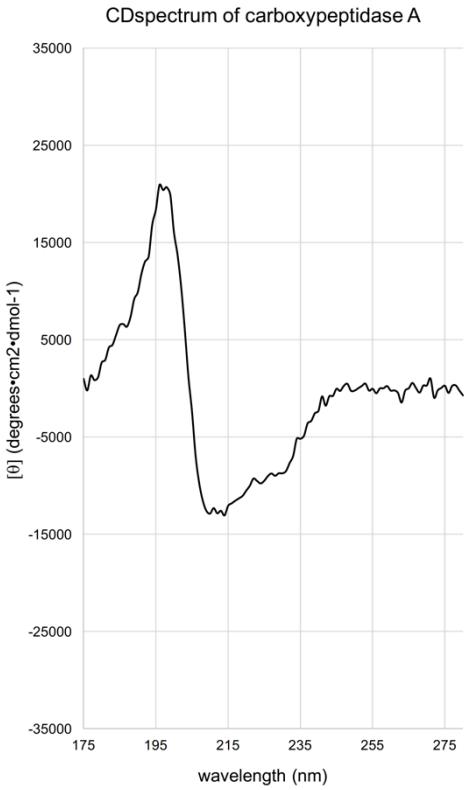
carboxypeptidase A

wavelengs(nm)	θ(obs)-θ(baseline)	
280	-3.03E-01	
279	-9.59E-02	
278	1.26E-01	
277	1.04E-01	
276	-1.96E-01	
275	1.11E-01	
274	1.86E-02	
273	-8.87E-02	
272	-4.03E-01	

× 2383

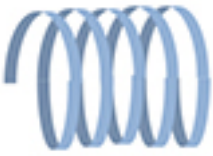
wavelengs(nm)	[θ] (degree•cm2•dmol-1)
280	-722.270619
279	-228.472508
278	299.70991
277	248.127492
276	-466.772508
275	265.32322
274	44.22848
273	-211.27678
272	-960.570619





Question 2. Predicting content of secondary structure of protein using CD spectra of a model polypeptide.

2-1. Model polypeptide (poly-L-lysine)



α-helix
pH: 11.1
temp: 22 °C

β-sheet
pH: 11.1
temp: 52 °C

random coil
pH: 5.7
temp: 22 °C

Poly-L-lysine was selected as a model peptide because it can change its structure from a-helix to b-sheet to random coil by changing the solution conditions[5]. The CD spectrum of the form containing 100% secondary structure is observed and used as a reference spectrum to calculate the prediction.

As for other polypeptides, it has been reported that poly-L-glutamic acid can change its secondary structure depending on the conditions. In the case of poly-L-glutamic acid, it has been reported that the structure of either a-helix or random coil can be changed depending on the solution conditions[6].

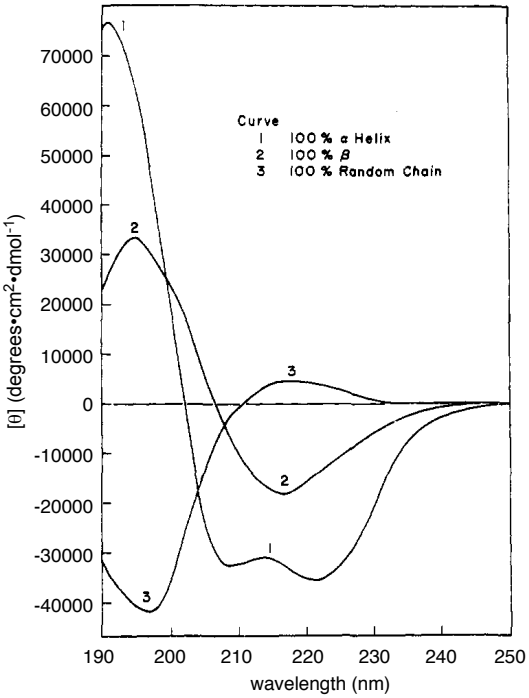


Figure 4. CD spectra of poly-L-lysine in the α-helical, β and random conformation.

2-2. Choice of the region of wavelength (208 to 240 nm)

The 208-240 nm region was chosen for the following reasons

1) The circular dichroism spectra of the three reference structures empirically seem less sensitive to non-chromophoric side-chain variation and solvent than the region below 208 nm.

2) Chromophores other than amides have minimal effect in this region

2-3. Solution of Questioin 2

given formula

$[\theta]_{calc} = f_\alpha[\theta]_{lys\alpha} + f_\beta[\theta]_{lys\beta} + f_R[\theta]_{lysR}$

f_α: percentage of α-helix ($0 \le f_\alpha \le 1$)

f_β: percentage of β-form ($0 \le f_\beta \le 1$)

f_R: percentage of random coil ($0 \le f_R \le 1$), $f_\alpha + f_\beta + f_R = 1$

$\varepsilon = [\theta]_{obs} - [\theta]_{calc}$

$\Sigma\varepsilon^2 = \Sigma([\theta]_{obs} - [\theta]_{calc})^2$

$\Sigma\varepsilon^2 = \Sigma([\theta]_{obs} - [\theta]_{calc})^2$

$= \Sigma([\theta]_{obs} - f_\alpha[\theta]_{lys\alpha} - f_\beta[\theta]_{lys\beta} - f_R[\theta]_{lysR})^2$ (describing $\Sigma\varepsilon^2$ using f_α, f_β, f_R, $[\theta]_{lys\alpha}$, $[\theta]_{lys\beta}$, and $[\theta]_{lysR}$)

$= \Sigma\{[\theta]_{obs} - f_\alpha[\theta]_{lys\alpha} - f_\beta[\theta]_{lys\beta} - (1-f_\alpha- f_\beta)[\theta]_{lysR}\}^2$ ($f_\alpha + f_\beta + f_R = 1$)

$= \Sigma\{([\theta]_{obs} [\theta]_{lysR})- f_\alpha([\theta]_{lys\alpha}-[\theta]_{lysR})- f_\beta([\theta]_{lys\beta}-[\theta]_{lysR})\}^2$

$= \Sigma(A - f_\alpha\alpha - f_\beta\beta)^2$ ($A=[\theta]_{obs} [\theta]_{lysR}$, $\alpha=[\theta]_{lys\alpha}-[\theta]_{lysR}$, $\beta=[\theta]_{lys\beta}-[\theta]_{lysR}$)

$= \Sigma (A^2 - 2f_\alpha\alpha A - 2f_\beta\beta A + 2f_\alpha f_\beta\alpha\beta + f_\alpha^2\alpha^2 + f_\beta^2\beta^2)$

Since $\Sigma\varepsilon^2$ is expressed as a quadratic function of a and b, a and b where this quadratic function is minimized need to be calculated. Therefore, a and b that satisfy the following equations is calculated.

$$\frac{\partial}{\partial \square_\alpha} = -2\Sigma\alpha A + 2\square_\alpha\Sigma\alpha\beta + 2\square_\alpha\Sigma\alpha^2 = 0$$

$$\frac{\partial}{\partial \square_\beta} = -2\Sigma\beta A + 2\square_\beta\Sigma\alpha\beta + 2\square_\beta\Sigma\beta^2 = 0$$

First, the values of $\Sigma\alpha A$, $\Sigma\beta A$, $\Sigma\alpha^2$, $\Sigma\beta^2$,and $\Sigma\alpha\beta$ are calculated respectively, then f_α and f_β that satisfy the equation are calculated. The value of f_R is calculated using the f_α and f_β and the formula $f_\alpha + f_\beta + f_R = 1$.

for myoglobin

	[θ] (degrees•cm2•dmol-1)			
	α-helix	β-structure	random coil	[θ]obs
208	-32600	5700	-3400	-22659.2
210	-32400	-4700	-1400	-23124.8
211	-32100	-10800	0	-22969.6
214	-31000	-12100	3500	-22193.6
215	-31400	-16400	4100	-22504
217	-33100	-17900	4600	-24366.4
220	-35300	-18400	4400	-25142.4
222	-35700	-15700	3900	-24987.2
225	-32400	-13800	2700	-24366.4
230	-21900	-11400	800	-17382.4
234	-11400	-6400	0	-8691.2
238	-4300	-3600	-140	-3569.6
240	-3300	-1400	-150	-2017.6

$f_\alpha = 0.69$, $f_\beta = 0.11$, $f_R = 0.20$

α-helix: 69%, β-sheet: 11%, random coil: 20%

calculation of $\Sigma\alpha A$, $\Sigma\beta A$, $\Sigma\alpha^2$, $\Sigma\beta^2$,and $\Sigma\alpha\beta$

	α	β	A	α^2	β^2	αβ	αA	βA
208	-2.92.E+04	9.10.E+03	-1.93.E+04	8.53.E+08	8.28.E+07	-2.66.E+08	5.62.E+08	-1.75.E+08
210	-3.10.E+04	-3.30.E+03	-2.17.E+04	9.61.E+08	1.09.E+07	1.02.E+08	6.73.E+08	7.17.E+07
211	-3.21.E+04	-1.08.E+04	-2.30.E+04	1.03.E+09	1.17.E+08	3.47.E+08	7.37.E+08	2.48.E+08
214	-3.45.E+04	-1.56.E+04	-2.57.E+04	1.19.E+09	2.43.E+08	5.38.E+08	8.86.E+08	4.01.E+08
215	-3.55.E+04	-2.05.E+04	-2.66.E+04	1.26.E+09	4.20.E+08	7.28.E+08	9.44.E+08	5.45.E+08
217	-3.77.E+04	-2.25.E+04	-2.90.E+04	1.42.E+09	5.06.E+08	8.48.E+08	1.09.E+09	6.52.E+08
220	-3.97.E+04	-2.28.E+04	-2.95.E+04	1.58.E+09	5.20.E+08	9.05.E+08	1.17.E+09	6.74.E+08
222	-3.96.E+04	-1.96.E+04	-2.89.E+04	1.57.E+09	3.84.E+08	7.76.E+08	1.14.E+09	5.66.E+08
225	-3.51.E+04	-1.65.E+04	-2.71.E+04	1.23.E+09	2.72.E+08	5.79.E+08	9.50.E+08	4.47.E+08
230	-2.27.E+04	-1.22.E+04	-1.82.E+04	5.15.E+08	1.49.E+08	2.77.E+08	4.13.E+08	2.22.E+08
234	-1.14.E+04	-6.40.E+03	-8.69.E+03	1.30.E+08	4.10.E+07	7.30.E+07	9.91.E+07	5.56.E+07
238	-4.16.E+03	-3.46.E+03	-3.43.E+03	1.73.E+07	1.20.E+07	1.44.E+07	1.43.E+07	1.19.E+07
240	-3.15.E+03	-1.25.E+03	-1.87.E+03	9.92.E+06	1.56.E+06	3.94.E+06	5.88.E+06	2.33.E+06
			Σ	1.18.E+10	2.76.E+09	4.93.E+09	8.69.E+09	3.72.E+09

for carboxypeptidase A

	[θ] (degrees•cm2•dmol-1)			
	α-helix	β-structure	random coil	[θ]obs
208	-32600	5700	-3400	-11556
210	-32400	-4700	-1400	-12868
211	-32100	-10800	0	-12306
214	-31000	-12100	3500	-13055
215	-31400	-16400	4100	-12067
217	-33100	-17900	4600	-11539
220	-35300	-18400	4400	-10517
222	-35700	-15700	3900	-9274
225	-32400	-13800	2700	-9495
230	-21900	-11400	800	-8729
234	-11400	-6400	0	-5169
238	-4300	-3600	-140	-3346
240	-3300	-1400	-150	-2307

$f_\alpha = 0.34, \quad f_\beta = 0.12, \quad f_R = 0.54$

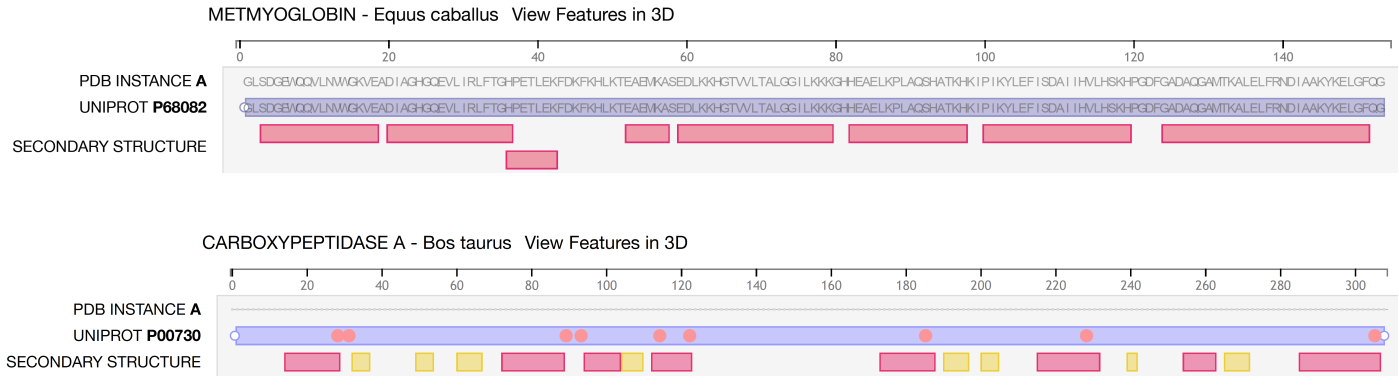
α-helix: 34%, β-sheet: 12%, random coil: 54%

calculation of ΣαA, ΣβA, $\Sigma\alpha^2$, $\Sigma\beta^2$,and Σαβ

	α	β	A	α^2	β^2	αβ	αA	βA
208	-2.92.E+04	9.10.E+03	-8.16.E+03	8.53.E+08	8.28.E+07	-2.66.E+08	2.38.E+08	-7.42.E+07
210	-3.10.E+04	-3.30.E+03	-1.15.E+04	9.61.E+08	1.09.E+07	1.02.E+08	3.56.E+08	3.78.E+07
211	-3.21.E+04	-1.08.E+04	-1.23.E+04	1.03.E+09	1.17.E+08	3.47.E+08	3.95.E+08	1.33.E+08
214	-3.45.E+04	-1.56.E+04	-1.66.E+04	1.19.E+09	2.43.E+08	5.38.E+08	5.71.E+08	2.58.E+08
215	-3.55.E+04	-2.05.E+04	-1.62.E+04	1.26.E+09	4.20.E+08	7.28.E+08	5.74.E+08	3.31.E+08
217	-3.77.E+04	-2.25.E+04	-1.61.E+04	1.42.E+09	5.06.E+08	8.48.E+08	6.08.E+08	3.63.E+08
220	-3.97.E+04	-2.28.E+04	-1.49.E+04	1.58.E+09	5.20.E+08	9.05.E+08	5.92.E+08	3.40.E+08
222	-3.96.E+04	-1.96.E+04	-1.32.E+04	1.57.E+09	3.84.E+08	7.76.E+08	5.22.E+08	2.58.E+08
225	-3.51.E+04	-1.65.E+04	-1.22.E+04	1.23.E+09	2.72.E+08	5.79.E+08	4.28.E+08	2.01.E+08
230	-2.27.E+04	-1.22.E+04	-9.53.E+03	5.15.E+08	1.49.E+08	2.77.E+08	2.16.E+08	1.16.E+08
234	-1.14.E+04	-6.40.E+03	-5.17.E+03	1.30.E+08	4.10.E+07	7.30.E+07	5.89.E+07	3.31.E+07
238	-4.16.E+03	-3.46.E+03	-3.21.E+03	1.73.E+07	1.20.E+07	1.44.E+07	1.33.E+07	1.11.E+07
240	-3.15.E+03	-1.25.E+03	-2.16.E+03	9.92.E+06	1.56.E+06	3.94.E+06	6.79.E+06	2.70.E+06
			Σ	1.18.E+10	2.76.E+09	4.93.E+09	4.58.E+09	2.01.E+09

Question 3. The PDB (Protein Data Bank) ID of the given proteins are 1ymb and 5cpa respectively. Please compare the obtained result of question 2 with the experimental results. If possible, please explain the problem of using CD spectra of polypeptides as reference spectra when calculating the content of secondary structures.

3-1. Comparison between calculated and experimental result



From these data, the content of secondary structure of given proteins are

myoglobin: α-helix: 85%, β-sheet: 0%, random coil: 15%

carboxypeptidase A: α-helix: 38%, β-sheet: 15%, random coil: 47%

This result shows that the prediction of secondary structure content using poly-L-lysine is not very accurate. The problems are mentioned below.

3-2. difference between model peptide and proteins

The secondary structure formed by the model peptide does not reproduce the real protein for the following reasons.

3-2-1. α-helix

While the length of the a-helix of the polypeptide used in the model is infinite, the length of the a-helix observed in the protein is relatively short, ranging from 5 to 30. rotational strength of the n-π* transition at 222 nm of a-helix was calculated to be chain length dependent[7]. This difference in the length of the secondary structure is the reason for the difference between the calculated and experimental CD spectra.

3-2-2. β-sheet

There are various forms of β-structure, and the CD spectrum differs depending on whether it is twisted, parallel, or anti-parallel. (Figure 5)[8]. This time, the poly-L-lysine only consisted of parallel counterparts (Figure 5B). The β-structure found in proteins was not reflected in the model, and the CD spectrum of β-sheet was not further classified, which may have reduced the accuracy.

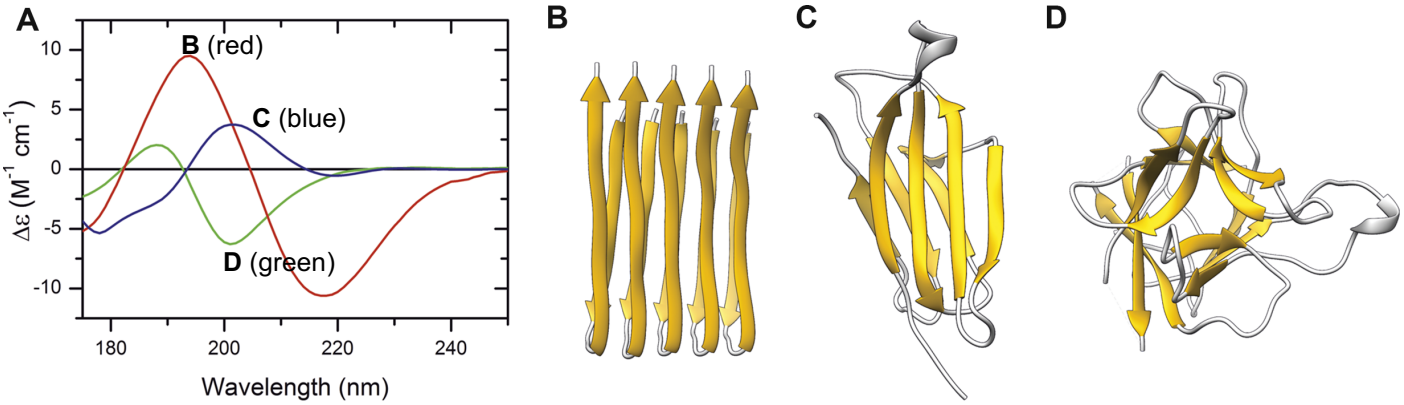


Figure 5. (A) CDspectra of β-amyloid (1–42) fibrils (red), native β2-microglobulin (b2m,blue) and soybean trypsin inhibitor (SBTI, green) downloaded from PCDDB. (B) Solid-state NMR model (PDB ID: 2BEG) of amyloid-fibrilsconsisting of parallel β-sheets, (C) relaxed (slightly right-hand twisted) antiparallel β-sheets of b2m (PDB ID: 2YXF) and (D) highly right-handtwisted antiparallel β-structure of SBTI (PDB ID: 1BA7).

3-2-3. random coil

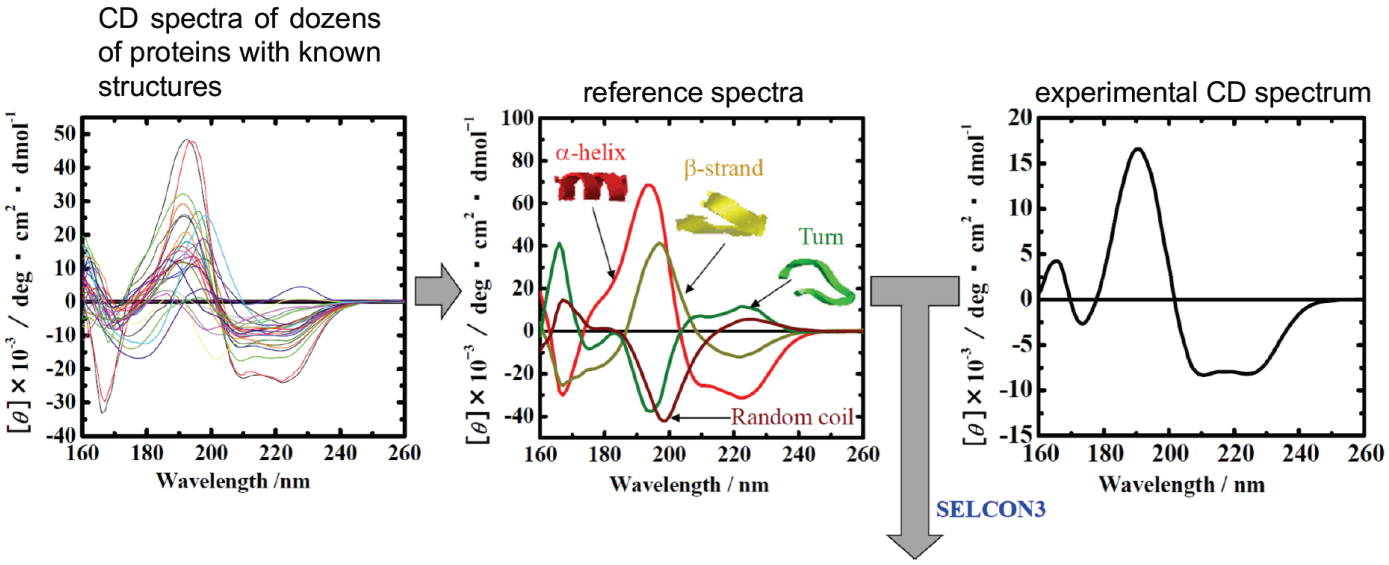
random coil of poly-L-lysine (in pH5.7) is extended form avoiding electrostatic repulsion (Figure 6A). On the other hand, real random coils found in proteins are more contracted (Figure 6B)[9]. This difference affect the CD spectra.

Figure 6. (A) The structure of random coil of poly-L-lysine, (B) comparison of contracted and extended random coil.

3-3. revised method for calculating the content of secondary structure of proteins

At present, reference spectra of secondary structures are prepared from CD spectra of dozens of proteins with known structures and secondary structure content data. Then, based on the reference spectrum, the content of the secondary structure is estimated from the CD spectrum obtained in the experiment.

As described above, a secondary structure analysis program (SELCON3, CDSSTR, CONTIN)[10)11)] is used to predict the secondary structure content from CD spectra. There are online tools such as Dichro Web[12)] and BESTSEL[13)] that can predict secondary structures.



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