

## Secondary structural analysis of Lysozyme

**Introduction:** It is well known that secondary structures of proteins are sensitive to changes of pH value of solution, temperature of it, and host molecules located about the proteins. This feature causes the chemical reaction in the organism, where appropriate conformations constructed by several secondary structures. Therefore, to know weights of secondary structures of proteins is one of the keys to reveal the appropriate conformation in the organism. Concerning to the secondary structural analysis, helix, b-sheet, turn, and random structures are important factors, so JASCO has been produced the secondary structural analysis program package JWSSE-480, which based on Dr. Yang's concept describing CD spectra by using CD reference of such four structures [1]. Here, we introduce the application JWSSE-480 and show the several functions of it.

**Measurement and calculation:** We employed phosphoric buffer (pH=7.0) as solvent in order to avoid large absorption below 200 nm. The solution was set under the room temperature. The CD spectrum of lysozyme was obtained by using J-810 spectropolarimeter. After the measurement, we carried out the structural analysis using four structures, helix, b-sheet, turn, and random structures, which are installed on JWSSE-480 program package.

**Result:** Fig.1 and Table.1 show the CD spectrum of lysozyme and the calculated weights of the secondary structures, respectively.  $CD_R$  and  $CD_N$ , depicted in Table.1 are the weights of the secondary structures under the contribution including other structures and neglecting the effects. The RMS values are very small in both cases, but  $CD_R$  is much smaller than  $CD_N$ . This small variation causes the similarity between the calculated and experimental spectra (Fig.1). And also, the smaller RMS value causes the good agreement with the X-ray result. Considering these facts, it is suggested that lysozyme in pH-7.0 buffer is constructed by mainly helix, random, and b-sheet, where the weights' ratio is 9:6:4 and other structures.

Table 1. Calculation result of Lysozyme by JWSSE-480 (%)

	helix	$\beta$ -sheet	turn	random	RMS
$CD_N^{[1]}$	59.4	6.6	0.	34.0	5.3
$CD_R^{[2]}$	46.7	22.8	0.	30.5	3.4
X-ray <sup>[1]</sup>	41.0	16.0	37.0	20.0	-

1) Normalized 2) Re-normalized

[1] Yang et al., *Anal. BioChem.* **91**, 13-31 (1978).

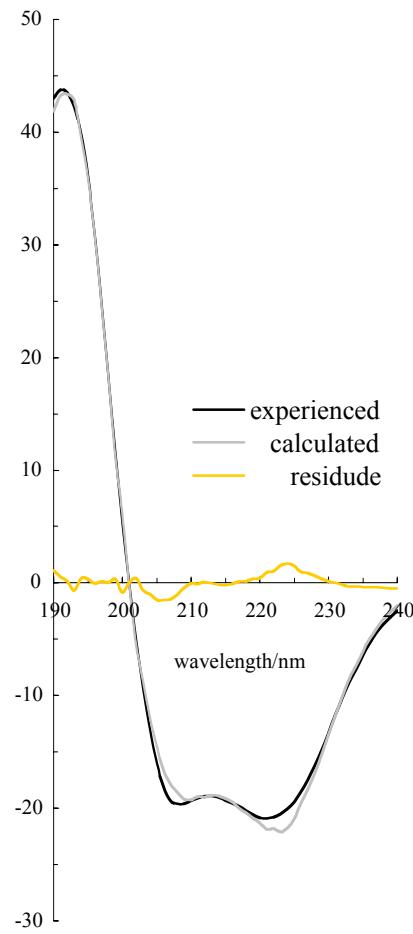


Fig. 1 Comparison of experience and calculated spectrum