Irreversible Nature of the Stacked β-Pleated Sheets of a Model Polypeptide

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Synopsis. A mode of association of β-sheets, most probably the stacking of the pleated sheets, has been studied by circular dichroism and light scattering on the 5×10^{-2} mol dm^{-3} NaCl solutions of poly(*S*-carboxymethyl-L-cysteine). It was found that the association is highly irreversible with respect to concentration change at constant pH. Relevance of the study of synthetic polypeptides is discussed in relation to the association mode found in proteins.

It has been frequently reported that aggregation of polypeptide chains takes place when the β-structure is formed.[1-10] Association of extended chains, observed on short chains,[2,11-14] couples with the formation of the β-structure. In this case, the β-content from circular dichroism (CD) parallels the aggregation number. In low ionic strength media, dissociation of β-aggregates occurs very slowly.[11,12,14] On addition of a salt, however, reversible association-dissociation takes place depending on the concentration of the salt.[12,13] Another well-defined association process was found recently,[15] in which the extent of aggregation incerases with concentration accompanied by a negligible change of the β-content, as indicated by a nearly constant residue ellipticity. An edge-to-edge association of the folded-chain-pleated sheets was suggested for this process,[15] mediated by hydrogen bonds between peptide groups of different chains. This edge-to-edge association of β-sheets has been proposed for the monomer-monomer interface within a dimer of concanavalin A.[16]

There are many facts concerning the aggregation of the β-structure of synthetic polypeptides which cannot be understood consistently in terms of these two types of association and their mixtures. Different lines of evidence have been accumulated for different polypeptides that a face-to-face association, or stacking, of the pleated sheets also occurs in addition to the above edge-to-edge association of pleated sheets. Various diagnostic means have been used for the detection of the stacking, including fluorescence (hydrophobic probe),[3,17-19] hydrodynamic properties,[6,7] potentiometric titration,[7,20,21] counterion activity,[20] and isodichroic point associated with the β-coil conversion.[10] Recently, it was indicated that increase of aggregation number is enormous when stacking occurs and hence this could be used as another diagnostic tool for the detection of the stacking.[15]

In the present study, circular dichroism (CD) and aggregation number by light scattering were measured on 5×10^{-2} M (mol dm^{-3}) NaCl solutions of different concentrations of poly(*S*-carboxymethyl-L-cysteine)-(poly[Cys(CH_2COOH)]) at a constant pH of 4.86±0.02 with special reference to the reversibility of the association. The aggregation is expected of face-to-face type according to the results from the above mentioned diagnostic means.

Experimental

The weight average molecular weight ($\bar{M}_w$) and degree of polymerization (DP_w) of the sample were 5.8×10^4 and 360, respectively. Light scattering was measured on a Chromatix low-angle light scattering photometer KMX-6 at room tmperature (24±2 °C). A value of 0.220 was used for the refractive index increment,[15] since it could not be measured at the pH used (4.86±0.02) due to the low solubility of the polypeptide in the β-structure. Apparent aggregation number m was evaluated at each concentration, assuming nonideal contribution to be negligible. CD spectra were taken on a Jasco J-40A Circular Dichrograph at 25±0.1 °C using cells of 0.5, 1, 2, 5, 10, and 20 mm light paths. Four to eight scans were accumulated. Measurements were made on the solutions about 24 hours after their preparation.

Results and Discussion

In Fig. 1, the concentration dependence of the apparent aggregation number m is shown on a double logarithmic plot. At the lowest polypeptide concentration C_p of about 1×10^{-4} residue M, aggregation already occurs for the chain of DP_w=360, which is consistent with the previous result on the

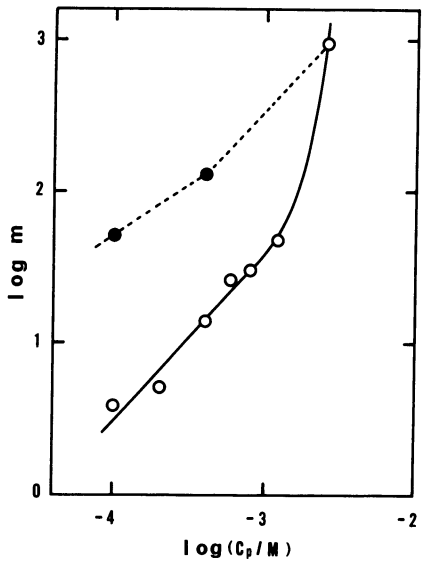

Fig. 1. Concentration dependence of apparent aggregation number m. Filled circles refer to solutions prepared by diluting the solution of the highest concentration.

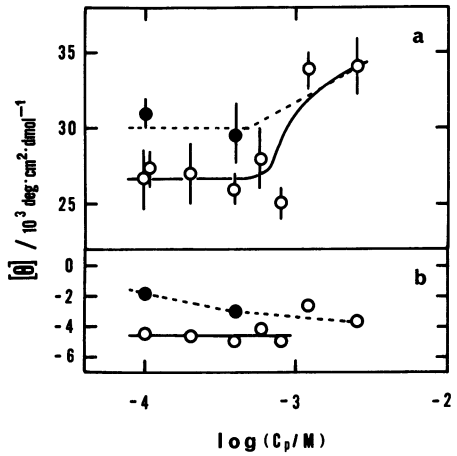

Fig. 2. Concentration dependence of residue ellipticities at 200 nm (a) and 218 nm (b). Filled circles refer to the solutions prepared by diluting the solution of the highest concentration.

same sample[15] but is in contrast with the previous result on a sample of DP_w=630 that approximately unassociated folded-chain β-structure exists at this concentration.[15] Upon increasing the concentration, the aggregation increases steadily. At C_p=2.48×10^{-3} M, however, the aggregation number increases enormously, suggesting that the stacking of pleated sheets occurs. Each solution corresponding to an open circle was prepared from each solution of the same concentration at neutral pH by the addition of HCl. At neutral pH, polypeptides were molecularly dispersed as random coils irrespective of the concentration.

In Fig. 2(a), the concentration dependence of the residue ellipticity at 200 nm, $[\theta]_{200}$, is given as a measure of the β-content. In a concentration range below about 1×10^{-3} M, $[\theta]_{200}$ remains nearly unchanged in spite of the increase of the aggregation number m. It is likely that edge-to-edge association of pleated sheets occurs in this concentration range, consistent with previous result.[15] When C_p exceeds about 1×10^{-3} M, $[\theta]_{200}$ increases sharply, which seems to correspond to the stacking process, although the correspondence between m and $[\theta]_{200}$ is not complete enough.

It has been shown that an isodichroic point around 218 nm, associated with the β-coil conversion of poly[Cys(CH_2COOH)], disappeared when the stacking of pleated sheets was expected to occur.[10] In Fig. 2(b), values of $[\theta]_{218}$ are plotted against log C_p. The presence of an isodichroic point around 218 nm is suggested in the concentration range lower than about 1×10^{-3} M. Disappearance of the isodichroic point is clear at C_p=1.2×10^{-3} M.

Two solutions were prepared by diluting the solution at C_p=2.48×10^{-3} M. The results on these two solutions are indicated by filled circles in Figs. 1 and 2. In Fig. 1, aggregation numbers found on these solutions are greater by nearly an order of magnitude than those found on the corresponding solutions of the same concentration prepared by the addition of HCl to the solution of neutral pH. On dilution, values of $[\theta]_{200}$ decrease but remain significantly greater than those of the solutions containing negligible amount of stacked aggregates. The observed slight decrease of $[\theta]_{200}$ may indicate the dissociation of either a small amount of stacked aggregates or unstacked portion of aggregates. A clearer indication of irreversible change at $[\theta]_{200}$ than shown here was reported previously. (Figure 5 of Ref. 11). Disappearance of the isodichroic point is clearly indicated for the solutions prepared by dilution. Therefore, dissociation of stacked aggregates by dilution is largely inhibited even in the presence of excess salt in the present case.

It is to be stated that essentially identical behavior to that is shown in Figs. 1 and 2 was obtained in three independent sets of experiments. However, all these results could not be put together into a single figure, since residue ellipticities differed appreciably for different sets due to slight differences in pH (smaller than 0.04) among them.

In conclusion, a mode of association which most likely corresponds to face-to-face association, or stacking, of pleated sheets is shown to be irreversible in the present study. It is to be noted that the (assumed) stacked aggregates can be easily dissociated by charging the side chains, i.e., by raising pH in the present case.

It is pertinent to ask whether this face-to-face association of β-sheets occurs in proteins. A good counterpart is found in the dimer of Streptomyces subtilisin inhibitor (SSI), which has been indicated to be formed by the stacking of two β-sheets belonging to each subunit.[22] Consistent with the present result, dimeric SSI does not dissociate into monomer on dilution, showing strong adhesive interaction.[23] The dissociation rate constant was obtained recently by clever means.[24]

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