STRUCTURAL STUDIES ON THE FREEZING-POINT-DEPRESSING PROTEIN OF THE WINTER FLOUNDER PSEUDOPLEURONECTES AMERICANUS†

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SUMMARY

The freezing-point-depressing protein from the winter flounder, Pseudopleuronectes americanus has been shown from circular dichroism measurements to possess a large proportion (~85%) of the α-helical conformation in aqueous solution (pH 8.0) at -1°C. The helical content decreases as the temperature is raised. Viscosity data at -1°C indicate an asymmetric shape for the protein molecule compatible with its high helical content. Thus, the secondary and tertiary structure of this freezing-point-depressing protein as well as its primary structure (reported elsewhere), are found to be different from its counterpart glycoproteins isolated from the Antarctic fish.

INTRODUCTION

The survival of certain marine fishes under sub-zero temperatures (< -1°C) has been shown to be due to the presence of an unique class of proteins that depress the freezing point of their serum [1,2]. Detailed studies made on the proteins isolated from an Antarctic fish Trematomus borchgrevinki have shown that these proteins are a family of glycoproteins of different molecular weights, all consisting of repeating alanyl-alanyl-threonyl tripeptide units with a disaccharide moiety attached to the threonyl residue [1,2]. Hew et al. (3) and Duman and DeVries [4,5] have demonstrated the presence of similar freezing-point-depressing proteins (FPDP) in the winter flounder Pseudopleuronectes

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Abbreviations - FPDP (freezing-point-depressing protein) CD (circular dichroism)

americanus which resides in the northern Atlantic coast of America and Canada. Flounder's FPDP has a molecular weight of about 10,000 and contains eight amino acids in its composition. The N-terminal 28 amino acid sequence of this protein has been determined [6]. Flounder's FPDP is not a glycoprotein and does not possess the repeating tripeptide sequence seen in the primary structure of the Antarctic fish proteins [1,2]. However, one common feature between these two classes of proteins is the relatively large content (over 60%) of alanyl residues in their amino acid composition.

The freezing-point-depressing activity of the glycoproteins from the Antarctic fish has been ascribed to the presence of the sugar moieties as well as to the extended configuration of the polypeptide chain in these proteins [1,2]. Inasmuch as the FPDP from the winter flounder possesses a different primary structure, it was of interest to us to find out the similarity or otherwise of the secondary and tertiary structures of these two proteins. Our results presented below show that the FPDP from the flounder has a relatively large α-helical content (∿85%) and possibly possesses an asymmetric (rod-like) shape in contrast to the random coil, extended conformation reported for the Antarctic fish glycoproteins [1,2].

Experimental

FPDP was isolated and purified according to the procedure described elsewhere [3,6]. The lyophilized protein was dissolved in 0.05M NH_4HCO_3 to yield concentrations between 0.05 and 0.5% (w/v). The protein concentration was determined from the optical density of the solution of 230 nm which was calibrated by means of micro-kjeldahl nitrogen analysis (7). The pH of the protein solutions was 8.0 ± 0.1.

Circular dichroism (CD) measurements were made using a Jasco J-20 spectropolarimeter. Water jacketed cells of 0.1 and 0.01 cm path lengths were used. Temperature control was achieved by circulating thermostated water from a Lauda K2-R bath through the cells. The CD data are expressed in terms of the ellipticity, [θ], in deg.cm^2.$dmole^{-1}$, using a mean residue weight of 86.4 for the protein (3,6). Viscosity measurements on a 0.25% protein solution were made using Ostwald-and Ubbelohde-type viscometers.

RESULTS AND DISCUSSION

The CD spectra of FPDP in 0.05M NH_4HCO_3 (pH 8.0) obtained at -1°, 25° and 80°C are shown in Fig. 1. Comparison of these spectra with those obtained for model polypeptides and other proteins [8,9] reveals that the spectrum at

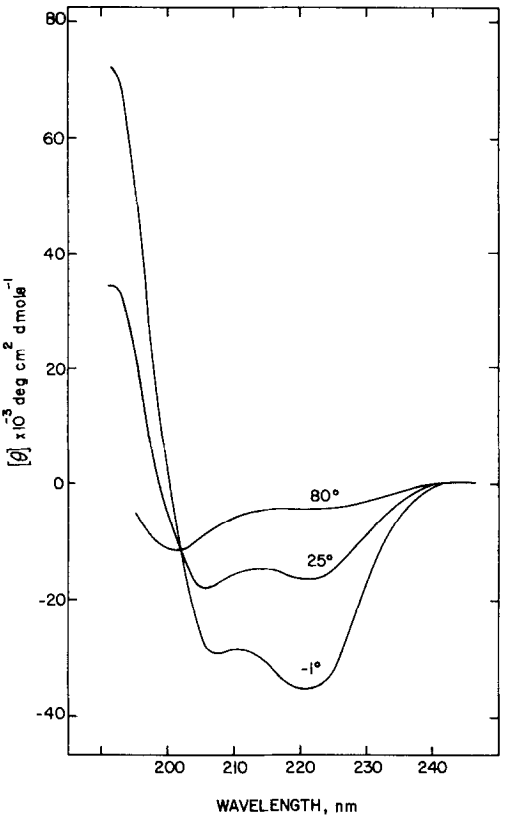

Fig. 1 CD spectra of flounder FPDP in 0.05M NH_4HCO_3 (pH 8.0) at the indicated temperatures (°C).

-1°C arises from a predominantly α-helical conformation, while at 80°C, the spectrum resembles those obtained for polypeptides [8,9] and proteins [10] in essentially the random coil form. The wavelength at which all the three spectra overlap as well as the magnitude of [θ] at this wavelength correspond to those calculated for mixtures of the α-helix and random coil conformation with very little, if any, of the β-structure [8,9].

An estimate of the α-helical content of the protein at the three temperatures was made using the equation [8]:

$$\% \text{ α-helix} = \frac{[\Theta]_{208} - 4000}{29{,}000} \times 100$$

where $[\Theta]_{208}$ is the ellipticity at 208 nm. The FPDP is found to possess 87%

α-helix at -1°C, 47% at 25°C and 9% at 80°C. These estimates should be taken as approximate owing to the limitations involved in the use of the model compound data [8,9]. It is, however, interesting to note the preponderence of the α-helical conformation in the protein at -1°C which is close to the temperature of its biological function. This conformation appears to be stable since we find that taking the protein solution to the higher temperatures (up to about 100°C) and cooling it back to lower temperatures restores the CD spectra back to those obtained before heating. Changing the pH of the solution from 2 to 10 did not change the CD spectrum. It is worthwhile mentioning here that a predominantly α-helical conformation has been observed earlier in aqueous solutions of polypeptides containing large proportions of alanine distributed randomly in binary copolymers, especially at low temperatures [11].

An insight into the tertiary structure of FPDP was sought to be obtained from viscosity measurements on dilute (0.25%) solutions of the protein (pH 8.0) at -1° and 20°C. The values of the reduced viscosity, $\eta_{\blacktriangledown}$, at these temperatures are, 5 ± 1 and 11 ± 1 respectively. Comparison of these values with the intrinsic viscosity data obtained with poly-L-glutamic acid [12] (of molecular weights 5000 - 10,000) indicate that the value of $\eta_{\blacktriangledown}$ at -1°C (ignoring any concentration dependence for the moment) is compatible with an asymmetric (rod-like) hydrodynamic shape of the FPDP molecule at this temperature, while at the higher temperature (20°C), it might exist in a more expanded shape due to the admixture of the random coil. In contrast, an intrinsic viscosity of about 20 was obtained at 0.5°C and 17°C by DeVries et. al. [13] for the glycoprotein of molecular weight 21,000 isolated from the Antarctic fish. On the basis of this and the CD data, these authors concluded that the Antarctic fish glycoproteins have no ordered structure and exist as extended random coils. The entirely different secondary and tertiary structure of flounder's FPDP i.e., high α-helical content and an asymmetric shape, is most likely to arise out of the absence of conformational requirements imposed by the disaccharide moieties present in the Antarctic fish proteins.

Both the flounder's FPDP and the Antarctic fish glycoproteins depress the freezing point of water by a mechanism different from the usual colligative properties of solute molecules (1,2,5). Although these proteins are different in their amino acid sequence as well as in their secondary and tertiary structures as reported in this communication, the presence of large proportion (over 60%) of alanine residues in both proteins may lead us to detect a common structural basis for their function. A detailed study of the physical chemical aspects of this problem is now in progress in our laboratory.

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