Effect of temperature on the circular dichroism spectra of β_2-microglobulins

When the temperature was lowered from 25 to 5°C dramatic changes were observed in the near-ultraviolet circular dichroism spectra of bovine and caprine but not human β_2-microglobulin. Comparison of the protein sequences suggests that the conformational change occurs in the amino-terminal 24 residues and that a tyrosine residue located on a potential β-turn acts as a reporter group. Because ΔH° is small (-22 kcal · mol^{-1}), such conformational changes, possibly not readily observed, may occur at low temperatures in other proteins having potential β-turns in otherwise aperiodic regions of sequence.

β_2-Microglobulin *Near-UV CD* *Temperature effect* *Tyrosine* *β-Turn*

1. INTRODUCTION

β_2-Microglobulin (β_2-m), a subunit of the class 1 histocompatibility antigens, is found membrane bound and free in many body fluids [1]. Human β_2-m (M_r 11730) consists of a single polypeptide chain of 99 amino acid residues [2], while bovine [3] and probably caprine [4] β_2-m contain 98 (residue 49 is deleted in bovine β_2-m). Comparisons of β_2-m from several species [2,3] show that the sequence is highly conserved, including the single disulfide bond linking cysteines 25 and 80 of human β_2-m and tryptophyl residues 60 and 95. The number of tyrosyl residues is variable among the species, but human, bovine, and caprine β_2-m each contain 6. During a study of oligomer formation by bovine β_2-m [5], an unexpected temperature dependence of the CD spectrum was observed. The different effects of temperature on the near-UV CD spectra of bovine, caprine, and human β_2-m were investigated.

2. EXPERIMENTAL

Bovine and caprine β_2-m were isolated from colostrum as previously described [3,4]. Human β_2-m was kindly provided by M.D. Poulik, William Beaumont Hospital, Royal Oak, MI. Solutions of β_2-m ($A_{280\text{nm}}$ = 1.0–1.2) in pH 6.0 buffer (0.02 M citrate, 0.07 M phosphate) were filtered through a 0.4 μm polycarbonate membrane into a cylindrical 1-cm pathlength jacketed cell. CD spectra were recorded on a Jasco 41-C spectropolarimeter using the data processor to accumulate and average 8 scans per spectrum. An external circulating bath was used to heat or cool the sample. CD spectra were acquired after the temperature had stabilized, a 5°C change in temperature required 15 min. The temperature was read both before and after a scan using a microthermistor inserted into the solution. CD results are expressed as θ_M in units of deg · cm^2 · dm^{-1}. Protein concentrations were calculated using $\epsilon_{280\text{nm}}$ = 19200 M^{-1} · cm^{-1} [7]. The secondary structures of the proteins were predicted from their amino acid sequences by the Chou-Fasman method [7], and plotted using a modification of the computer program of Corrigan and Huang [8].

3. RESULTS AND DISCUSSION

At 25°C the near-UV CD spectra of human, bovine, and caprine β_2-m were similar and in reasonable agreement with published spectra of the

human protein in the pH 7–8 range [9–11]. The spectra are relatively complicated, but details are qualitatively the same for all three species. As the temperature was lowered from 25 to 5°C, the only changes noted in the spectrum of human β_2-m (fig.1A) were small shifts in the positions of band maxima and a slight increase in intensity of the 294 nm band, which could be predicted from changes in solvent polarity and the slowing of the molecular motion as the temperature is lowered. In contrast, when solutions of bovine or caprine β_2-m were cooled to 5°C, dramatic changes in their near-UV CD spectra occurred (fig.1B). Because these changes were nearly identical, only the bovine spectra are shown. The 286 nm band was greatly diminished, and became a shoulder on the strong negative band appearing at 275 nm, whereas the 294 nm band remained nearly constant. Within the 25–5°C range, the changes were completely reversible.

Changes in the CD spectrum are caused by a conformational change in the protein. The apparent equilibrium constant, K_{eq}, and ΔG^o for this change were calculated from the variation of θ_M at 275 nm with temperature assuming a two-state model (table 1). Van 't Hoff plots were linear and were used to obtain ΔH^o and ΔS^o, these values are compared with published values for other proteins in table 2. The signs of ΔH^o and ΔS^o are consistent with the formation of hydrogen bonds as the temperature is lowered. The magnitudes of ΔH^o and ΔS^o for low temperature changes are smaller and opposite in sign to those for the complete unfolding of ribonuclease at 30°C [12]. The conformational change appears to be localized, and not to involve a major change in the secondary structure as no large changes were observed in the far-UV CD spectrum.

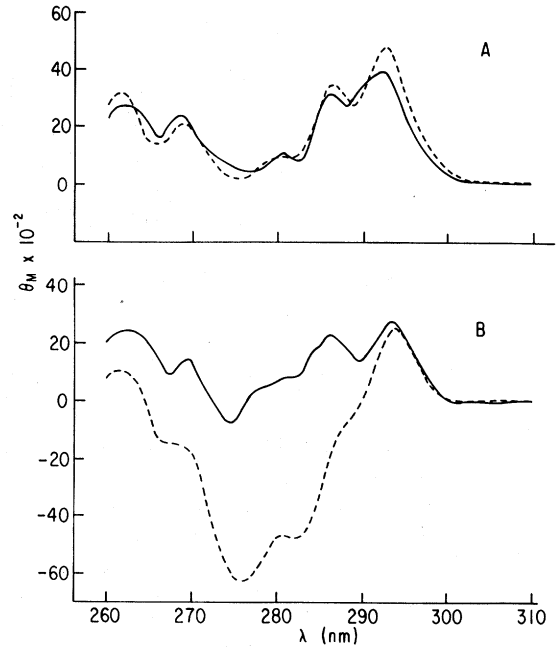

Fig.1. Near-UV CD spectra at 25°C (——) and 5°C (– – –) of (A) human and (B) bovine β_2-m (spectra of caprine β_2-m, not shown, are nearly identical to those of bovine β_2-m). All solutions in 0.02 M citrate, 0.07 M phosphate buffer (pH 6.0).

Table 1

CD and equilibrium parameters for the low temperature transition of β_2-m

T (K)	Bovine			Caprine		
	θ_M (275 nm)	K_{eq}	ΔG^o (kcal·mol^{-1})	θ_M (275 nm)	K_{eq}	ΔG^o (kcal·mol^{-1})
303	−500			−1500		
298	−780	0.04	1.9	−3200	0.4	0.54
293	−2900	0.53	0.36	−4600	1.0	−0.03
288	−4000	1.1	−0.03	−5400	1.8	−0.34
283	−5000	1.8	−0.34	−6000	3.2	−0.64
278	−6400	4.8	−0.86	−6500	4.9	−0.88
273	−7500			−7500		

$$K_{eq} = (\theta T - \theta_{303} T)/(\theta_{273} T - \theta T)$$

Table 2

Thermodynamic parameters for conformational changes in proteins

Proteins (conditions)	ΔH^o (kcal·mol^{-1})	ΔS^o (cal·deg^{-1} mol^{-1})	Reference
Ribonuclease (thermal denaturation) (pH 2.5, 30°C)	+57	+185	[9]
Cryoglobulin, λ chain (pH 5.2, 26–0°C)	−74	−260	[10]
β-Lactoglobulin (pH 8.2, 15–5°C)	−8	−31	[a]
Bovine β_2-m (pH 6.0, 25–5°C)	−22	−78	[b]
Caprine β_2-m (pH 6.0, 25–5°C)	−20	−68	[b]

[a] Calculated from data in [14]
[b] This study

Low temperature mediated conformational changes in proteins have been reported previously. For example, the light chain of the cryoglobulin (DoIgG) [13] which shows homology in its constant domain with β_2-m, has a similar near-UV CD spectrum which is also temperature dependent. The spectral changes reported for DoIgG are more complicated than those observed for β_2-m and result in larger negative values for ΔH^o and ΔS^o. β-Lactoglobulin also undergoes a small conformational change with a decrease in temperature in mildly alkaline solution; the van 't Hoff plot for this conformational change exhibits a minimum at 15°C [14].

Fig.2 shows the conformations predicted for human and bovine β_2-m by the Chou-Fasman method [7]. Although only the first 32 residues of caprine β_2-m have been sequenced, it has a 96% compositional homology with bovine β_2-m, and presumably the same conformation. The only significant differences in the predicted conformations of the three proteins are located on the amino-terminal side of the disulfide bond where relatively little structure other than β-turns is predicted. Of the periodic structures shown, pleated β-strands incorporated into sheets are the most stable, whereas β-turns, which represent points of minimum resistance, are least stable.

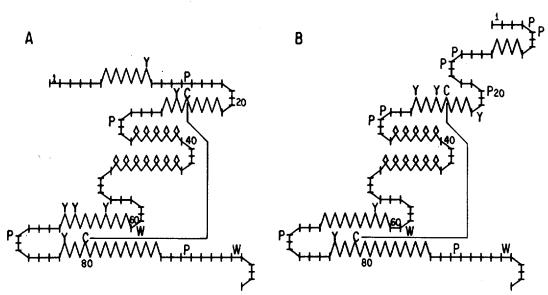

Fig.2. Schematic diagrams of the predicted secondary structures of (A) human and (B) bovine β_2-m. Conformational states are represented as helical (⅄), β-sheet (⅄), unordered (——), or β-turn (⅄). The locations of tyrosyl (Y), tryptophyl (W), prolyl (P), and cysteinyl residues (C) are shown, as is the disulfide bond.

Tryptophyl, tyrosyl, and cystinyl residues are the major contributors to the CD spectrum in the 260–300 nm range. The 294 nm band can be attributed to one or both tryptophyl residues, and the small decrease in this band (fig.1B) may be due to the decrease in the 286 nm band or possibly the large increase in negative ellipticity near 275 nm. A disulfide bond may contribute a broad band across the 250–310 nm region. Since the disulfide loop encompasses more than half the amino acid residues of β_2-m, it would not be expected to greatly restrict motion among the included residues. However, most of those residues are predicted to be in stable α-helical or β-pleated sheet structures. Reduced and carboxymethylated bovine β_2-m was not soluble enough to study, however, in the presence of 0.03 M dithiothreitol the temperature effects on the near-UV CD spectrum of bovine

β_2-m were reduced only slightly, suggesting that the conformational change occurs outside the disulfide loop. Tyrosyl residues may have bands near 286 and/or 275 nm. The similarity of the near-UV CD spectra of the three proteins at room temperature, suggests similar environments for the tyrosyl residues under physiological conditions.

Bovine and caprine β_2-m have a larger number of prolyl residues in the amino terminal portion (fig.2) and thus more potential β-turns than does human β_2-m. Rearrangement of these predicted β-turn regions along with their possible stabilization through hydrogen bonds is a thermodynamically reasonable event. Tyrosyl residue 22 of bovine and caprine β_2-m located on a potential β-turn with prolyl residue 20 would be an excellent reporter residue.

Changes in the conformation of a flexible tail may precede oligomer formation, the cryoglobulin [10] is a case in point. Of the β_2-m's, only the bovine protein which tetramerizes [5], has been crystallized [15] in a form suitable for X-ray studies [16]. The existence of such a flexible tail could be a contributing factor to the difficulties in refining the crystal structure [16]. The amino terminal portion of human β_2-m may be sufficiently different from the ruminant proteins, so that the conformation of this protein is not temperature dependent. Alternatively, it is possible that similar changes do occur, but the lack of a suitably located reporter residue prevents their being monitored. If, as is suggested by the data of table 2, small conformational changes are a common response when the temperature of a protein is lowered, these changes should be considered in the physiological interpretation of enzymological studies at subzero temperatures as proposed by Fink [17].

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