HYDROPHOBIC CORE AND SURFACE CHARGES OF HUMAN β_2-MICROGLOBULIN PROBED BY CD MEASUREMENTS

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The roles of hydrophobic bonding and charge electrostatics in the stabilization of human β_2-microglobulin have been probed by variations in solution conditions and monitored by circular dichroism in the near and far UV regions. Sodium perchlorate initially gives a decrease in intensity of the positive 234 nm peak in the near UV followed by a shift of this peak to negative ellipticity at high perchlorate concentration. These 234 nm changes indicate a new environment for a tyrosyl chromophore(s). A conformational rearrangement of the β-sheet sandwich must occur since all six tyrosines of human β_2-microglubulin are located in the two β-sheets of this sandwich. A slight decrease in intensity for the 200 nm positive peak in the far UV indicates a less close packing of the β-sheets at high perchlorate. In other experiments, enthalpy and entropy values have been calculated from thermal unfolding studies at 50 and 180 mM NaCl for pH values 6·0 and 8·0. Larger enthalpy values are obtained at higher NaCl concentration consistent with salt shielding of predominantly unfavorable charge interactions. These enthalpy differences are relatively large suggesting that charge electrostatics are energetically significant in stabilization of human β_2-microglobulin.

β_2-Microglobulin (β_2m) is a small protein (m.w. 12 000) present in abnormally high levels in urine of patients with tubular-type proteinuria[1]. β_2m has the basic fold of an immunoglobulin constant domain[2,3] where two antiparallel β-sheets are closely packed, face to face, forming a sandwich. Antiparallel β-sheets are flexible structures, being able to undergo conformational rearrangements within a β-sheet by variations of β-strand twist and extension[4]. In this paper, we study conformational rearrangements of human β_2m induced by water structure disordering using a chaotropic salt, sodium perchlorate. In addition, surface charge shielding by a nonchaotropic salt, sodium chloride, has been determined at two pH values. The method used to monitor protein conformation is circular dichroism in near and far UV. Urine of patients suffering from Balkan nephropathy, a tubular-proteinuric disease was a source of β_2m, which was isolated according to the procedure of Berggard and Bearn[1].

RESULTS AND DISCUSSION

The near UV CD spectra of human β_2m at various sodium perchlorate concentra-

tions are shown in Fig. 1. The plots of mean residue ellipticity for peaks at 234, 242, 275, 288 and 293 nm versus perchlorate concentration are given in Fig. 2.

The peak positions at 288 and 293 nm are nearly invariant with perchlorate. As seen in Figs 1 and 2, significant changes occur for the peaks at 275 and 288 nm and the conformation most different from the native one occurs at an intermediate perchlorate concentration of 145 mmol l^{-1}. These changes at 275 and 288 nm indicate a change for a Tyr chromophore(s) in a hydrophobic environment, quite possibly located in the interior of the β-sheet sandwich. The biggest spectral change occurs for the 234 nm peak which is strongly positive in the absence of perchlorate. This 234 nm peak becomes less positive at low perchlorate concentrations, then shifts to negative ellipticity at 145, 213 and 351 mM $NaClO_4$. The peak at 242 nm also becomes more negative in ellipticity at high perchlorate concentrations. However, the change at 242 nm is smaller in magnitude than at 234 nm and is questionable due to overlap of the positive 234 and negative 242 nm peaks. In initial stages, β_2m denaturation by either temperature[5] or acid pH (ref.[6]) is much different in that

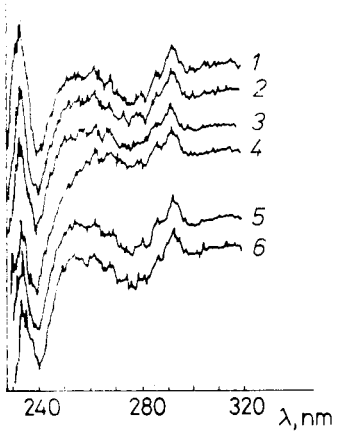

FIG. 1

Near UV CD spectra at 22°C of human β_2m at various sodium perchlorate concentrations. Conditions are: 2·1 mM sodium phosphate buffer, pH 7·8, in absence of sodium chloride. Sodium perchlorate concentrations (mmol/l): *1* 0, *2* 41, *3* 78, *4* 145, *5* 213, *6* 351

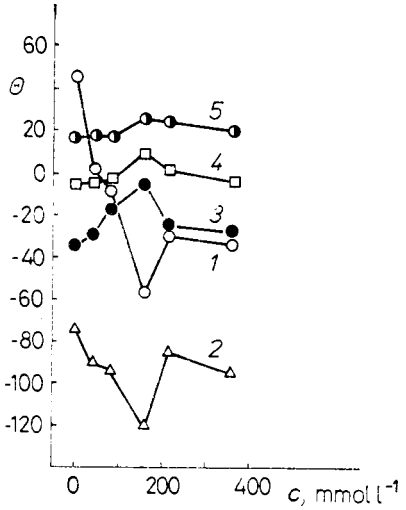

FIG. 2

Mean residue ellipticity (Θ, deg cm^2/dmol) for the near UV peaks of human β_2m plotted versus sodium perchlorate concentration (c mmol/l). Conditions are given in Fig. 1. λ, nm: *1* 234, *2* 242, *3* 275, *4* 288, *5* 293

a much stronger shift to negative ellipticity occurs below 250 nm with obliteration of the peaks at 234 and 242 nm. However, perchlorate gives a selective change of the 234 nm peak which is contributed by a Tyr residue(s). This change at 234 nm can be assigned to a rearrangement within the β-sheet sandwich since all six Tyr residues of human β_2m are located in the two β-sheets forming the immunoglobulin sandwich structure[7,8]. The 234 and 242 nm peaks are both probably due to Tyr pairing. Selective modification of either the disulfide or Tyr phenolic side chains would fail to assign any peaks since Tyr residues 66 and 78 are both in proximity to the single disulfide[7,8]. The interpretation of any specific modification would be complicated by the possibility that an altered chromophore could change the environment of another chromophore in proximity or a distant one via a conformational change[9].

Perchlorate effects in the far UV are shown in Fig. 3. All spectra are about the same indicating no major disruption of secondary structure. The slight decrease in positive ellipticity at 200 nm at high perchlorate concentration is expected if there

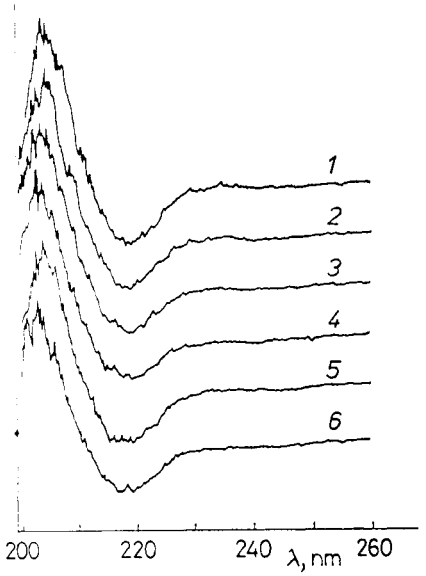

FIG. 3

The far UV CD spectra at 22°C of human β_2m at various sodium perchlorate concentrations (mmol/l) *1* 0, *2* 41, *3* 78, *4* 145, *5* 213, *6* 351. Conditions are given in Fig. 1

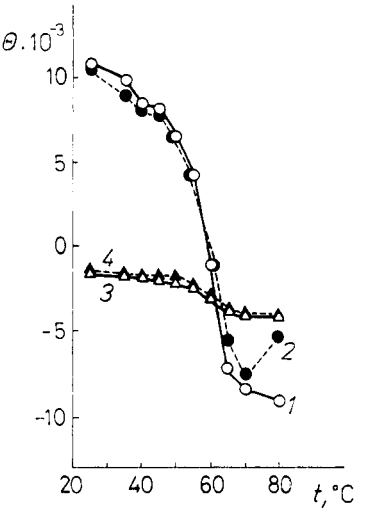

FIG. 4

The mean residue ellipticity (Θ, deg cm^2/dmol) of the far UV peaks at 201 and 219 nm versus temperature (t, °C) for thermal unfolding of human β_2m in the presence of 3·5 mM sodium phosphate buffer, pH 8·0, λ (nm), c_{NaCl} (mmol/l): *1* 201, 50; *2* 202, 180; *3* 219, 50; *4* 219, 180

is a less close β-sheet packing[10]. However, a shift to more negative ellipticity in the far UV could also be due to partial denaturation since random coil can give large negative contribution in the far UV.

TABLE I

Enthalpy (ΔH) and entropy (ΔS) values for thermal unfolding of human β_2m for various solution conditions

pH	Salt concentration	ΔH kJ/mol	ΔS J/mol deg
6	50 mM NaCl	167	492
8	50 mM NaCl	190	574
6	180 mM NaCl	203	613
8	180 mM NaCl	256	773
8	381 mM $NaClO_4$	162	499

TABLE II

Close charge interactions on the surface of the β-sheet sandwich of human β_2m

β-Sheet	Opposite charges		Like charge repulsions
	positive	negative	
	Aligned positions of adjacent β-strands		
I	Lys 48	Glu 69	Arg 3, His 31
II	Arg 81	Asp 38	
	1–3 Positions within a single β-strand		
I	Lys 48[a]	Glu 50	Arg 3, Lys 6
II			Asp 34, Glu 36 Glu 36, Asp 38[a]
	Diagonal positions in adjacent β-strands		
I			Lys 6, His 31 Glu 50, Glu 69[a]
II	Arg 81[a]	Glu 36	

[a] These charges could be neutralized by salt bridging of opposite charges located at aligned positions of adjacent β-strands due to their proximity.

Hydrophobic interactions are weakened by the large perchlorate anion by disordering water structure[11]. Perchlorate appears to result in a rearrangement of β-sheet packing which is mediated primarily by hydrophobic side chain interactions. Ionic strength variations due to perchlorate appear of minor importance since similar spectral changes are observed in the presence of 128 mM NaCl (results not shown) as in the absence of NaCl. Thus, perchlorate primarily acts to weaken hydrophobic bonding while shielding effects are of minor importance.

In other experiments, surface charges have been shielded by a nonchaotropic salt, NaCl. The near and far UV CD spectra of β_2m were identical at 50 and 180 mM NaCl for pH 6·0 and 8·0. Spectral changes with thermal unfolding were seemingly identical for all four conditions with a midpoint at about 60°C. Plots of the mean residue ellipticity for peaks at 200 and 219 nm at pH 8·0 versus temperature are shown in Fig. 4. The 219 nm peak was suitable for calculation of equilibrium constants for various temperatures. The enthalpy, ΔH, and entropy, ΔS, values have been calculated assuming a two-state denaturation mechanism. The enthalpy values given in Table I indicate that human β_2m is more stable at higher NaCl concentration. These enthalpy differences are surprisingly large suggesting that electrostatic inter-

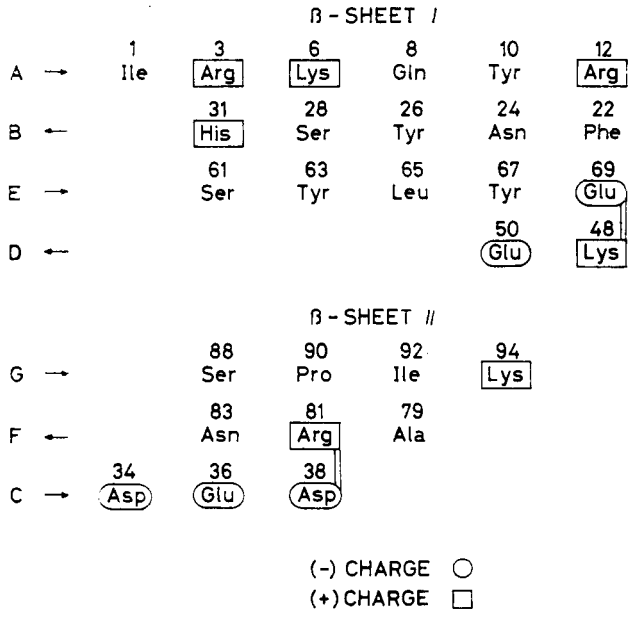

FIG. 5

The distribution of positive and negative charges on the sandwich surface of human β_2m. The sandwich is formed by face to face packing of two β-sheets[7,8]

actions between surface charges are important for β_2m stability. Salt stabilization indicates that the charge interactions being shielded are primarily unfavorable between like charges. A summary of near side chain charges of human β_2m is given in Fig. 5 and Table II. Nearest charges are in side chains at aligned positions of adjacent β-strands. Next-nearest charges are at 1,3-positions within a β-strand or nearest diagonal positions of adjacent strands. Nearest charges at pH 8·0 are exclusively stabilizing, opposite-charge pairs. Since human β_2m is not destabilized at higher salt concentrations, nearest charges seem insensitive to these salt variations possibly due to proximity and salt bridging. However, shielding of next-nearest charges can explain salt stabilization of β_2m since there is an excess of three like--charge relative to opposite-charge pairs. Thus, salt shielding of like-charge interactions can explain higher enthalpies and greater stability observed at higher NaCl concentration.

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