COMPARATIVE CIRCULAR DICHROISM STUDIES ON HUMAN β_2-MICROGLOBULIN

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1. Introduction

Human β_2-microglobulin is a 11 800 mol. wt protein first isolated from the urine of patients with severe renal tubular malfunction [1]. This protein appears only in small quantities in normal body fluids, but has been shown to be an important normal membrane component of many human cell types and is closely associated with HLA antigens [2,3]. The amino acid sequence of human β_2-microglobulin displays a partial homology with human immunoglobulin domains, notably with the Cγ3 domain, and the molecule contains no carbohydrate component [4]. No evidence for any molecular polymorphism in human β_2-microglobulin has been reported.

Previous circular dichroism (CD) studies on human β_2-microglobulin have shown that this protein exhibits marked similarity to isolated immunoglobulin domains in protein secondary and tertiary structure [5,6]. The purpose of this study was to investigate any physiochemical differences between separate β_2-microglobulin preparations that may be manifest in their CD spectra.

2. Materials and methods

2.1. *β_2-microglobulin preparations*

Two human β_2-microglobulin preparations (I and III) were prepared as in [1]. These proteins were both shown to be homogeneous by an agar gel electrophoretic procedure [7] and SDS–polyacrylamide gel electrophoresis [8]. When large amounts of these proteins were assayed by SDS–polyacrylamide gel electrophoresis, a faint component amounting to <1–2% of the total protein and with an app. mol. wt 20 000–22 000 could be observed. A third β_2-microglobulin preparation (II) was kindly supplied by Dakopatts A/S. The purity of this preparation had been shown by immunodiffusion and immunoelectrophoretic analyses. The β_2-microglobulin preparations I and II had been isolated from pooled urine, whereas preparation III was isolated from the urine of a single patient.

2.2. *Circular dichroism*

CD measurements were performed on a Cary 61 recording spectropolarimeter as in [9,10]. All β_2-microglobulin preparations were filtered through a sterile Millipore filter (0.45 μm) immediately prior to CD analyses. Protein concentrations were determined spectrophotometrically at 280 nm directly after the CD spectra had been obtained, using $E^{1\%}_{1\,cm} = 16.8$ for β_2-microglobulin [1]. A mean residue weight of 109 was assumed in all calculations, and CD measurements expressed as the mean residue ellipticity (θ) in deg. cm^2/dmol.

3. Results and discussion

The CD spectra, between 210 nm and 305 nm, for three separate human β_2-microglobulin preparations in phosphate-buffered isotonic saline (pH 7.5) are shown in fig.1. In agreement with [5,6], these β_2-microglobulin preparations each demonstrated a

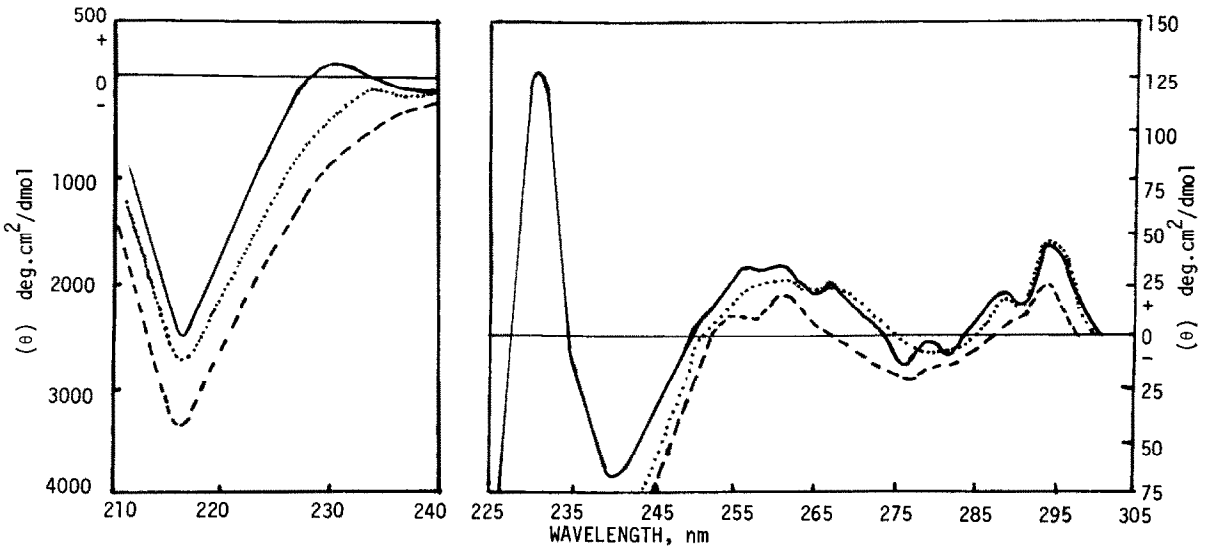

Fig.1. Circular dichroism spectra, between 210 nm and 305 nm, of human β_2-microglobulin preparation numbers (- - - -), II (· · ·) and III (——) in phosphate-buffered isotonic saline (pH 7.5). The ellipicity scale is expanded in the right hand figure to show greater spectral detail between 225 nm and 305 nm.

large negative A_{217} max, attributable to peptide bonds in β-pleated sheet protein secondary structure [11]. At wavelengths >240 nm, CD spectra appear as a series of overlapping adsorption maxima resulting from the asymmetric environment of disulphide and aromatic chromophores. The CD spectrum of each of the three human β_2-microglobulin preparations were closely similar to each other in this region (fig.1), and agreed well with the spectra in [5,6].

However, only one of the β_2-microglobulin preparations (no. III) demonstrated a marked positive A_{232} max (fig.1), as has been reported for other human β_2-microglobulin preparations [5,6]. The occurrence of an analogous positive CD absorption maximum in this wavelength region has been observed in several studies on human immunoglobulin molecules, notably some IgG [9,11] and IgD [12,13] myeloma proteins as well as κ light chains and its constant region domain [14,15]. It is interesting that separate β_2-microglobulin preparations would appear to share a similar variability with myeloma immunoglobulin molecules in the expression of a clear positive CD $A_{232-235}$ max. This transition has been thought to derive from the asymmetric environment of a tyrosine residue in the constant region of some immunoglobulin light chains resulting from the interaction between light chain variable and constant regions, and is enhanced on interaction of immunoglobulin light and heavy chains [11,13]. Such an explanation is difficult to extrapolate to β_2-microglobulin, despite its partial homology to individual immunoglobulin domains [4], unless the 232 nm transition is postulated to reflect an asymmetric environment of the corresponding tyrosine residue resulting from the interaction between separate β_2-microglobulin molecules on dimerisation.

The assignment [5] of the positive CD A_{232} max for β_2-microglobulin to a tyrosine residue was suggested from its behaviour on alkaline titration since this transition was shown to disappear and a new positive A_{248} max appeared. Similar alkaline titration experiments were performed by addition of NaOH to the three human β_2-microglobulin preparations described here. Each β_2-microglobulin preparation showed different conformational alterations on alkaline titration as manifest in their CD spectra in the range 230–310 nm (fig.2). A positive CD A_{248} max became evident at pH 11.5 for all three β_2-microglobulin preparations. However, the intensity of this transition varied markedly between separate prepara-

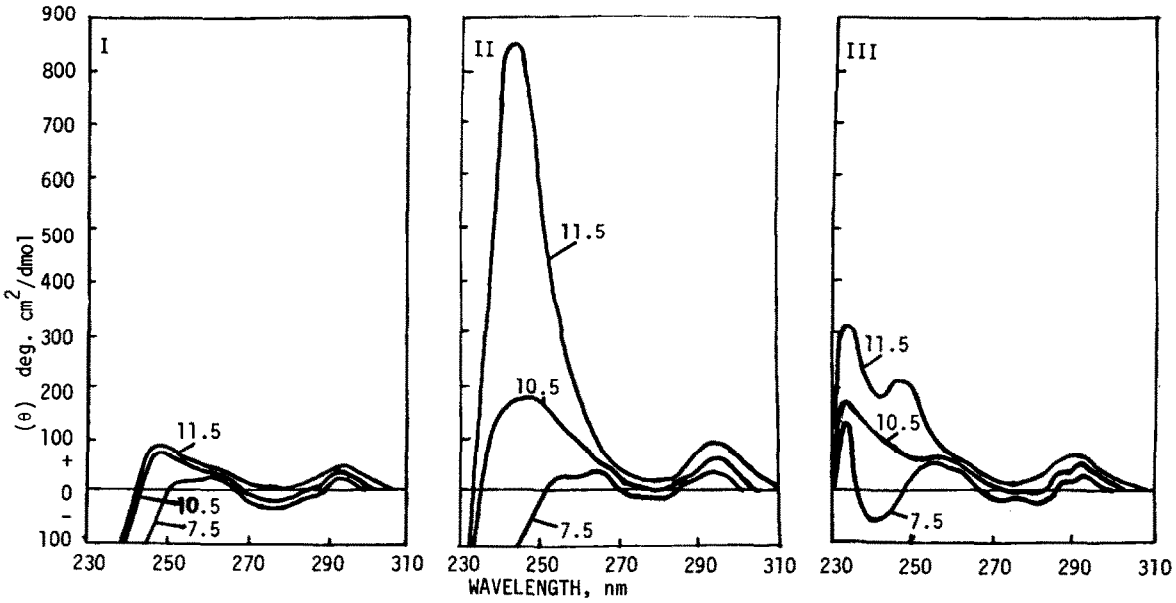

Fig.2. Circular dichroism spectra, between 230 nm and 310 nm, of three separate human β_2-microglobulin preparations (I, II and III) each at pH 7.5, 10.5 and 11.5.

tions ($\theta_{248\ nm}$ = 93, 860 and 201 deg. cm^2/dmol at pH 11.5 for preparations I, II and III, respectively). There was little alteration in the CD spectra above 270 nm for each β_2-microglobulin preparation (fig.2), whereas the 217 nm negative absorption maxima at neutral pH was always replaced at pH 11.5 by an intense negative ellipticity centred below 210 nm and thought to represent the unfolded form of β_2-microglobulin [6]. No indication of the appearance of any CD spectral transition at 232 nm for the β_2-microglobulin preparations I and II was observed during alkaline titration (fig.2). However, the positive CD A_{232} max at neutral pH for the β_2-microglobulin III was not reduced on alkaline titration, as noted [5], but instead was enhanced. It would appear inappropriate to assign this transition at 232 nm to a tyrosine residue that may be analogous to a similar transition observed in the CD spectra of some human immunoglobulin molecules, but instead this transition appears to reflect some variable structural feature within separate human β_2-microglobulin preparations.

In conclusion, despite close similarities elsewhere in the CD spectra, the variation in positive A_{232} max for separate β_2-microglobulin preparations at neutral pH noted here and by comparison [5,6], as well as the different conformational susceptibility to alkaline titration, suggests important differences between individual isolated human β_2-microglobulin preparations. Whether this reflects (a) an, as yet unidentified, molecular polymorphism of human β_2-microglobulin, (b) a predominant characteristic of any trace impurities, or (c) the degree of dimerisation in individual β_2-microglobulin preparations remains to be determined.

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