CIRCULAR DICHROISM STUDIES ON HUMAN CHORIONIC GONADOTROPIN AND ITS SUBUNITS*

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1. Introduction

During the past three years several investigators have obtained highly purified human chorionic gonadotropin (HCG) [1–4]. These preparation used to be inhomogeneous in disc electrophoresis in presence of urea [4]. By the technique of preparative isoelectric focusing it was possible to separate six main fractions, which differed in their biological activities, IP's and contents of *N*-acetylneuraminic acid [5, 6].

Swaminathan and Bahl [7] and Morgan and Canfield [8] separated two subunits, α and β, from a highly purified HCG, with different molecular weights, amino acid and sugar composition.

The present communication reports circular dichroism (CD) measurements of native HCG, of the fractions isolated by isoelectric focusing and of the α and β subunit of HCG. It provides additional information about their conformation.

2. Material and methods

Crude HCG with a biological activity of 3300 IU/mg was purchased from Schering AG, Berlin. The purified material had a biological activity of 13 000 IU/mg as determined with the modified rat prostate test [4]. Isoelectric focusing (IEF) on a stabilized Sephadex gel thin-layer was performed as previously described [5]. The α and β subunits of HCG were obtained by the method of Morgan and Canfield [8]. Disc electrophoresis was carried out as described earlier [5]**.

CD measurements between 270 and 185 nm were performed in 0.02 M phosphate buffer, pH 7.5 at 20° using a Jouan Dichrograph II model. Solutions of 2 mg/ml and cuvettes of a path length of 0.1 mm were used, determining the base line on the buffer solution. The CD spectra expressed in terms of $\Delta\epsilon = (\epsilon_l - \epsilon_r)$ instead of the more usual elipticity. The molar elipticity can be calculated by multiplying $\Delta\epsilon$ with the factor 3300.

The calculation of $\Delta\epsilon$ was based on a molecular weight of 40 000 for native HCG as obtained by ultracentrifuge measurements [4]. When determined on a Sephadex G-200 sf thin-layer, the fractions isolated by IEF showed molecular weights similar to that of native HCG**. For this reason a molecular weight of 40 000 was used for all of them. The molecular weights of the HCG α and β subunits were taken from Morgan and Canfield [8].

3. Results

3.1. *Disc electrophoretic analysis*

Highly purified HCG (13 000 IU/mg) was separated by disc electrophoresis into 6 bands (see fig. 1). The fractions of the IEF showed single bands with an increasing mobility from fraction 1 to 6. In the presence of urea these fractions split, yielding several bands (see fig. 1). Fraction 2, for example, migrated to the anode with two main bands. It had the highest biolog-

* Part IX of a series on human chorionic gonadotropin. For part VIII see [5].

** A detailed report on further experimental data is in preparation.

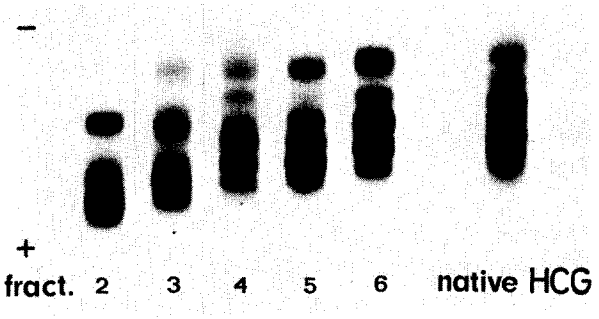

Fig. 1. Disc electrophoresis of native HCG and HCG fractions 2–6 isolated by isoelectric focusing in a 12.5% polyacrylamide gel, pH 9.3, in presence of 6 M urea.

ical activity (16 000 IU/mg) and the highest NANA content (10.5%).

In agreement with the results published by Morgan and Canfield [8] the α and β subunits of HCG revealed several bands (see fig. 2).

3.2. *Circular dichroism measurements*

The CD spectra of all preparations had a negative dichroistic absorption in the far ultraviolet region. In addition the CD spectra of native HCG show two negative Cotton effects at 207 and 196 nm (see fig. 3 and table 1). The CD spectra of the IEF fractions are similar to that of native HCG. However the maxima increase from fraction 1 to 5 (see figs. 4, 5 and table 1).

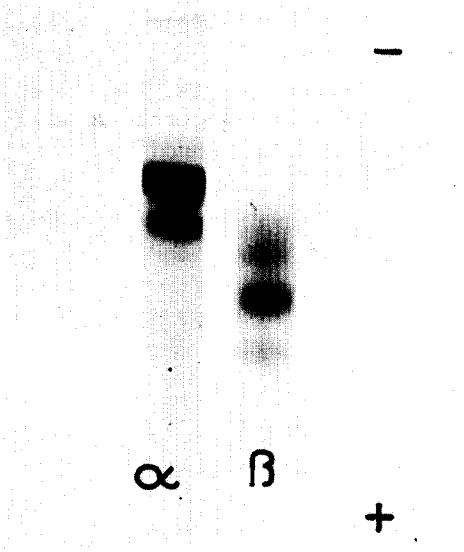

Fig. 2. Disc electrophoresis of the α and β subunits of HCG in a 12.5% polyacrylamide gel, pH 9.3, in presence of 6 M urea.

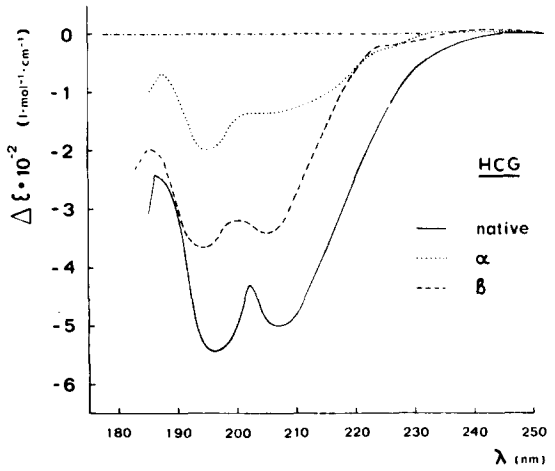

Fig. 3. Far ultraviolet CD spectra of native HCG and the α and β subunits in a 0.02 M phosphate buffer, pH 7.5 at 20°.

Furthermore significant differences in the CD spectra of native HCG and the α and β subunits could be detected (see fig. 3 and table 1). While the CD spectrum of the β subunit is similar to that of native HCG, the α subunit has only one Cotton effect at 195 nm and a plateau between 205 and 202 nm.

4. Discussion

By the technique of IEF it was possible to separate 6 different fractions which all possess biological activity. The first 3 fractions showed higher biological activity than the starting material and the other IEF fractions [9]. In disc electrophoretic analysis with urea the fractions of the IEF revealed a similar band pattern. However a decrease in the intensity of the faster moving bands and an increase of the slower moving bands were observed from fractions 1 to 6.

By circular dichroistic measurements it is possible to make a statement about the conformation of proteins. All preparations examined so far have little, if any, α-helix or β-structure. They do not exhibit a negative Cotton effect at 220 nm, not even a shouder, and

Table 1
Circular dichroism measurements of native HCG, the α and β subunits and the HCG fractions, isolated by isoelectric focusing, expressed in terms of $\Delta\epsilon = (\epsilon_1 - \epsilon_r)$ (1 × mole^{-1} cm^{-1}).

	HCG			Fraction no.					
	Native	α	β	1	2	3	4	5	6
λ_1 max	207	205	205	207	206	206	207	207	207
$\Delta\epsilon$	−500	−135	−338	−358	−412	−483	−455	−490	−420
λ min	202	202	200	201	202	201	202	202	201
$\Delta\epsilon$	−430	−135	−318	−305	−341	−441	−385	−425	−358
λ_2 max	196	195	195	195	195	195	195	195	195
$\Delta\epsilon$	−540	−198	−364	−374	−444	−501	−516	−575	−450

no positive Cotton effect can be detected in the far ultraviolet region.

For the case of native HCG this result was already discussed by Mori [10]. We have found in addition, that native HCG, its fractions from IEF and the β subunit display 2 Cotton effects, whereas the α subunit has only one at 195 nm and a plateau between 205 and 202 nm.

There are striking similarities between the CD spectra of the IEF fractions, even of those with little biological activity, as for example fractions 5 and 6. Furthermore a far reaching conformity with the CD spectrum of native HCG could be observed. Therefore one might assume that native HCG and the IEF fractions have the same conformation.

No direct correlation between the extent of the Cotton effect and the biological activity could be observed. Fraction 2 with the highest biological activity displayed no outstanding absorption features.

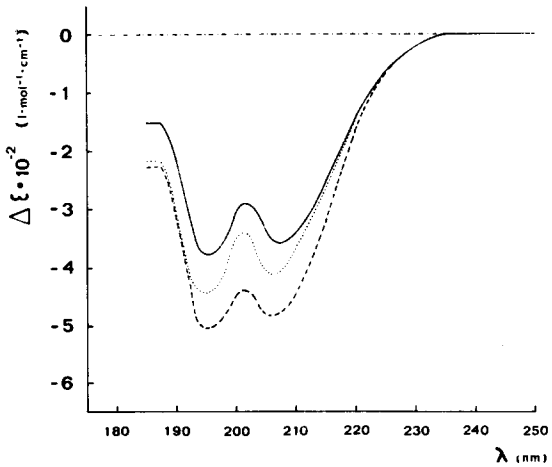

Fig. 4. Far ultraviolet CD spectra of HCG fraction 1 (——), 2 (· · · ·) and 3 (- - - -), isolated by isoelectric focusing in 0.02 M phosphate buffer, pH 7.5 at 20°.

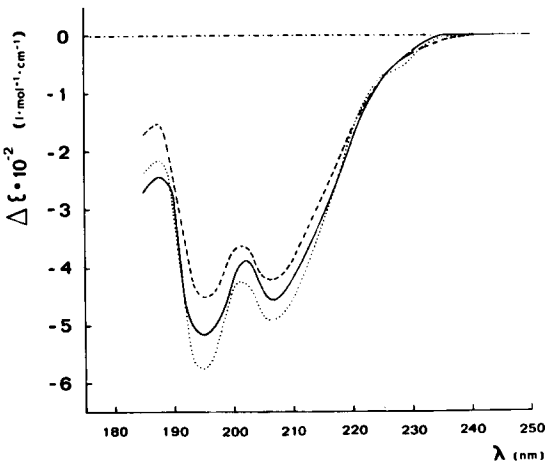

Fig. 5. Far ultraviolet CD spectra of HCG fraction 4 (——), 5 (· · · ·) and 6 (- - - -), isolated by isoelectric focusing in 0.02 M phosphate buffer, pH 7.5 at 20°.

The differences in the spectra of the α and β subunits of HCG were to be expected, as it is known that they have different molecular weights, amino acid and carbohydrate contents [7, 8]. The significance of the two Cotton effects of the native HCG, the IEF fractions and the HCG subunits is not yet clear. Efforts are currently made in this laboratory towards a better understanding of these results.

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