

THE MICROHETEROGENEITY OF HUMAN CHORIONIC GONADOTROPIN (hCG) REFLECTED IN THE β -SUBUNITS

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1. Introduction

It is now evident that human chorionic gonadotropin (hCG) is not a single molecular entity but consists of a number of different biologically active components [1–4]. By means of gel isoelectric focusing 6 hCG forms recently could be isolated and characterized [2–4]. Furthermore it is well established that hCG is composed of 2 subunits: hCG- α (common to other proteohormones) and hCG- β (hormone specific) [5, 6]. The correlation, however, between the microheterogeneity of the intact hormone and the subunits was not clear. We therefore wish to present data about the interrelationship between the hCG isohormones, the subunits, and sialic acid-free hCG.

2. Material and methods

Crude hCG with biological potencies of 2,100 IU/mg and 2,660 IU/mg, respectively, was obtained from N.V. Organon (Holland) and Schering (Berlin). Purification was performed by chromatography on CM-Sephadex C-50 using 0.1 M ammonium acetate buffer, pH 5.0 with continuous increasing sodium chloride molarity up to 0.7, essentially according to van Hell et al. [1].

Isolation of the isohormones was done by preparative flat bed isoelectric focusing in polyacrylamide gel

Abbreviations:

hCG = human chorionic gonadotropin;
SDS = sodium dodecyl sulfate;
pI = isoelectric point.

as described elsewhere [3, 7]. Isolation of the subunits was achieved by chromatography on DEAE-Sephadex as described by Morgan and Canfield [8] followed by gel filtration on Sephadex G-100, and by column isoelectric focusing in sucrose density gradient in the presence of 4 M urea. Analytical gel electrofocusing was carried out as described earlier [9]. For polyacrylamide gel electrophoresis the Davis system (pH 8.9, 7.5%) [10], and the Reisfeld system (pH 4.3, 7.5%) [11] were used. SDS disc electrophoresis was performed according to Weber and Osborne [12].

Sialic acid was removed by neuraminidase from *Vibrio cholerae* (Behring-Werke, Germany). Sialic acid was determined according to Warren [13]. Biological activity was measured in the ovarian ascorbic acid depletion assay of Karg [14] and Parlow [15].

3. Results

3.1. *The purified hCG complex* displays one zone on gel electrophoresis at pH 8.9 whereas it separates into 6 bands on gel electrofocusing corresponding to isohormones in the pI range 3.8–5.4 [3]. The isolated isohormones however appear on gel electrophoresis as sharp bands of slightly differing mobility. On gel filtration on Sephadex G-100 the isohormones show identical behaviour.

3.2. *The isolated subunits* from the hCG complex yielded 20% for hCG- α and 55% for hCG- β . The biological activities of both were found to be less than 1% of the native hormone.



3.3. *HCG- α* , isolated from the hormone complex, shows two components (α_1, α_2) on gel electrophoresis at pH 8.9 (fig. 1) appearing too, when the different intact isohormones are treated with 10 M urea and subjected to gel electrophoresis in the presence of urea. The two α -components are common to all isohormones revealing however different intensity ratios. There was found an increasing intensity of α_1 (pI: 8.5 as determined by column electrofocusing) corresponding to the isohormones with increasing pI's from which it is derived. An inverse ratio was found for the α_2 -component as illustrated in fig. 2. *HCG- β* , isolated from the hormone complex, reveals 5 compo-

Fig. 1. Disc electrophoresis (pH 8.9, 7.5%) of the native hCG complex (position 1), hCG- α (pos. 2) and hCG- β (pos. 3).

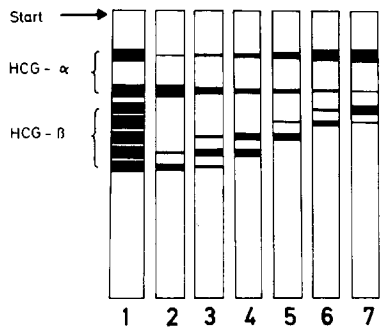


Fig. 2. Schematic representation of disc electrophoresis (pH 8.9, 7.5%) of urea treated hCG complex (pos. 1) and of urea treated six isolated isohormones of hCG (pos. 2-7).

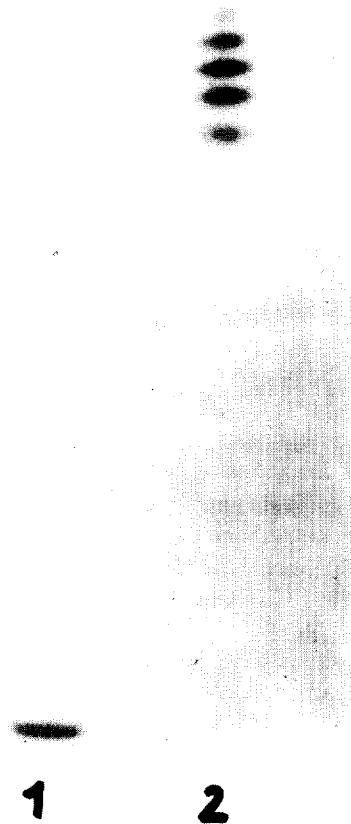


Fig. 3. Analytical thin-layer isoelectric focusing in polyacrylamide gel, pH range 3-10, of sialic acid-free hCG (pos. 1) and of native hCG complex (pos. 2).

nents (pI range 3.7–5.0) on gel electrophoresis at pH 8.9 (fig. 1). The individual isohormones yielded 2 β -components each when treated with urea and examined on gel electrophoresis (fig. 2). Whereas the two α 's have the same migration velocities for all isohormones, the 2 β -components in pairs differ in migration correlating to the isohormone they are derived from. These findings could be confirmed by gel electrofocusing.

3.4. *On SDS disc electrophoresis* the isolated hCG subunits migrate as single bands with molecular weights of approx. 18,000 for hCG- α and 32,000 for hCG- β . The native hCG complex as well as the individual isohormones identically show 2 components, coincident in migration with the α - and β -subunit, respectively.

3.5. *Sialic acid-free hCG* obtained from the hCG complex by treatment with neuraminidase appeared as homogeneous on gel electrophoresis in the Reisfeld system and also shows one band on gel electrofocusing, having an unusually basic pI of about 9.5 as demonstrated in fig. 3.

4. Discussion

The 6 hCG isohormones obtained by preparative gel electrofocusing differ in their isoelectric point, sialic acid content, migration velocity on disc electrophoresis and biological potencies [3]. On SDS disc electrophoresis, however, the different forms identically show two components of molecular weight in the order of 18,000 (hCG- α) and 32,000 (hCG- β) which agrees well with the molecular weights as reported by Morgan and Canfield [5] obtained from the hormone complex. In addition, the 6 forms showed identical behaviour during gel filtration on Sephadex G-100. From these observations it seems evident that the isohormones at least are very similar in molecular size.

On the other hand, several authors [5, 6, 16] have described disc electrophoretic heterogeneity of samples of α - and β -subunits, which could be found by us, too. We got further information about the relationship between the microheterogeneity of the native hCG complex and the subunits by studying the urea treated individual isohormones on disc electro-

phoresis and gel electrofocusing. Thus, all of them share two common α -components only different in the intensity ratio, whereas the two β -components in mobility and pI correlate to the isohormone they are derived from. From these data it is obvious that the microheterogeneity of hCG resides in the β -subunits. An indication attributing the microheterogeneity of the native hCG complex to a different sialic acid content only, was the finding that the 6 isohormones could be converted into sialic acid-free hCG.

Acknowledgements

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