ISOLATION AND PARTIAL CHARACTERIZATION OF SEVERAL DIFFERENT CHORIONIC GONADOTROPIN (HCG) COMPONENTS

D. GRAESSLIN, H.C. WEISE and P.J. CZYGAN

Abteilung für klin. u. experimentelle Endokrinology der Universitäts-Frauenklinik, 2 Hamburg 20, Germany

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1. Introduction

Endeavours in purification of human chorionic gonadotropin (HCG) yielded highly potent glycoprotein hormone preparations [1–7]. The most active ones, however, displayed microheterogeneity in starch gel electrophoresis and in polyacrylamide gel electrophoresis with urea [4, 7]. Moreover it has been demonstrated that HCG contains several biologically active components [3, 4]. This microheterogeneity of HCG seems to be due to differences in the carbohydrate moiety, especially in the sialic acid residues.

The application of isoelectric focusing in polyacrylamide gel and sucrose density gradient in the present study offered the chance for the isolation of several different components, each possessing HCG activity.

2. Materials and methods

Crude HCG with a biological activity of 2,100 IU/mg was obtained from N.V. Organon, Holland. Ion exchange chromatography was done on CM-Sephadex C-50, using phosphate buffer for stepwise elution, as reported earlier [8], and on QAE-Sephadex A-50 with piperazine/HCl buffer, 0.02 M and 0.25 M, at pH 4.65.

Analytical isoelectric focusing in polyacrylamide gel was carried out essentially according to Awdeh et al. [9]. A direct staining procedure with Coomassie Brillant Blue R 250, selective for proteins in the presence of Ampholine was used [10]. Preparative thin layer isoelectric focusing in polyacrylamide gel was performed as described elsewhere [8].

Column isoelectric focusing (110 ml capacity) in a sucrose density gradient using Ampholine carrier ampholytes, pH 3–6, were performed as described in the Instruction Manual 1-8100-E 01 (LKB-Produkter, Stockholm). For disc electrophoresis in polyacrylamide gel a Davis system (pH 8.9, 7.5%) was used [11]. Biological activity was measured in the OAAD assay of Parlow [12], the radioimmunological potencies were determined by the dioxane method of Thomas et al. [13]. Agar immunoelectrophoresis was done according to Scheidegger [14]. Sialic acid (NANA) was determined by the Warren method [15].

3. Results

When crude HCG was subjected to CM-Sephadex chromatography at pH 5.2, 4 peaks were obtained. The third peak, which was released by a 0.25 M phosphate buffer, showed the highest biological (7,600 IU/mg) and radioimmunological (5,000 IU/mg) activity and was used for further investigation. This preparation revealed on disc electrophoresis only 1 broad zone. Gel isoelectric focusing in the pH range from 3–10, however, displayed 5 clearly separated bands with isoelectric points between 3.8–5.1.

For the isolation of these bands analytical gel isoelectric focusing was transferred to a preparative scale. In order to achieve better resolution of the adjacent protein bands, Ampholine of pH range of 3–6 was chosen.

By this technique, up to 20 mg HCG could be applied yielding 5 distinct components of 1.5–2 mg each. The total protein recovery was 60%. The homo-

Table 1
Biological, immunological and physicochemical properties of different HCG components.

Material	Potencies in IU/mg HCG (a)		Isoelectric point (pI)	Sialic acid (%)
	Bioassay (b)	Radioimmunoassay (c)		
HCG crude	2,100	2,100	–	6.4
CM-H_2C_3	7,700	5,000	3.8–5.1	7.5
HCG A	4,600	2,300	3.8	9.0
HCG B	18,500	3,150	4.3	8.8
HCG C	14,200	5,600	4.5	6.7
HCG D	12,500	4,400	4.9	5.5
HCG E	7,400	3,300	5.1	2.9

(a) in terms of II-IS-HCG; (b) OAAD test; (c) dioxane method.

geneity of each component is demonstrated by subjecting aliquots to analytical refocusing (fig. 1). All 5 protein bands possessed HCG activity (table 1). The highest biological activity of 18,200 IU/mg was found in HCG-B, with a pI of 4.3.

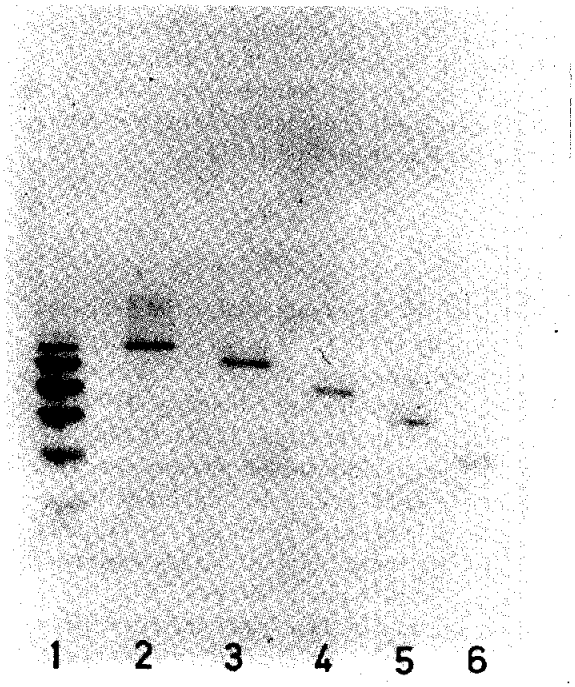

Fig. 1. Analytical gel isoelectric focusing pattern of CM fraction H_2C_3 of HCG (pos. 1) and 5 HCG components, isolated by preparative gel isoelectric focusing from H_2C_3 (pos. 2–6). 5% Polyacrylamide gel, 2% Ampholine, pH 3–6. Staining with Coomassie Brillant Blue. Anode at the top.

The NANA content of the HCG bands varied between 2.9–9.0 (table 1). After gel filtration on Sephadex G-100 all 5 HCG components emerged as a single symmetrical peak. The single components migrated on disc electrophoresis as sharp bands slightly differing in electrophoretic mobility. In immunoelectrophoresis each of them developed a single arc against rabbit antiserum to crude HCG.

In order to exclude artefacts due to the isolation procedure, crude HCG was purified by a second approach as outlined below: by anion exchange chromatography on QAE-Sephadex the active HCG fraction was eluted with 0.25 M piperazine buffer pH 4.65. For the following isoelectric focusing the column procedure in a sucrose density gradient, Ampholine pH 3–6, was chosen. The elution pattern showed again a separation of HCG into several distinct fractions of which the isoionic points and biological activities were in good agreement with those of the HCG bands isolated from gel slab.

4. Discussion

This report presents data of 5 isolated HCG components which showed differences in sialic acid content correlating with biological activity (table 1). The exception of the most acidic component in this respect and the varying radio-immunological potencies cannot be explained so far. All HCG fractions were homogeneous in gel isoelectric focusing and disc electrophoresis. In immunoelectrophoresis and agar diffusion they were found to be pure HCG without

antigenic differences. The differences in sialic acid content probably reflect heterogeneity in the carbohydrate portion and may be due to an incomplete biosynthesis or to partial degradation by kidney glycosidases as recently suggested [16].

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