PURIFICATION AND CHARACTERIZATION OF HUMAN CHORIONIC GONADOTROPIN*

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1. Introduction

Several investigators [2–7] have previously attempted to purify human chorionic gonadotropin (HCG). These methods proved to be either too time consuming, not reproducible, or limited to small quantities. In addition, these purified products are difficult to compare because of differences in the methodology of biological and physico-chemical measurements.

This paper describes a simple and fast chromatographic method for the preparation of this glycoprotein hormone in a highly purified form. Its characterization was achieved by physico-chemical, biological and immunological methods. The following report deals with the chemical modifications of HCG at specific sites. It provides some information about the importance of certain parts of the molecule for the biological and immunological activity.

2. Materials and methods

Chromatography: The starting material with a biological activity of 2660 IU/mg was purchased from Schering, Berlin. CM-Sephadex C-25 and Sephadex G-25 were obtained from Pharmacia, Uppsala. In this report the HCG was chromatographed twice on CM-Sephadex C-25 with an ammonium acetate buffer under identical conditions, similarly to the method described by Van Hell et al. [7].

Ultracentrifugation was carried out with a Beckman Model E at 56,000 rpm in 0.05 M phosphate buffer, pH 7.0 under different conditions as described in table 1. The molecular weight was calculated on the basis of the sedimentation coefficient by the method of Atassi [8].

Starch gel electrophoresis was performed according to Smithies [9], Poulik [10] and Ferguson [11] in discontinuous systems. A saturated solution of Amido Black B in methanol–water–acetic acid (5:5:1) served as staining reagent.

Disc electrophoresis in polyacrylamide was done as described by Davis [12] with modifications recommended by Reisfeld and Small [13], at pH 9.3 with a 12.5% gel with and without 6 M urea.

N-Acetyl neuraminic acid (NANA) was cleaved both with 0.1 N H_2SO_4 at 80° and enzymatically with *Vibro cholerae* neuraminidase (EC 3.2.1.18) obtained from Behring Werke, Marburg and determined according to Warren [14].

Biological activity was determined as described by Diczfalusy [15]. As diluent 0.9% NaCl containing 0.25% Haemaccel was used. Further characteristics of this test: a) so called symmetrical test arrangement (parallel line assay); b) duration of the test was 6 day; c) daily injection of 0.5 ml containing one fifth of total dosis subcutaneously; d) statistical evaluation after Borth et al. [16].

Antisera against HCG were prepared by immunization of rabbits and goats with either crude HCG or purified HCG.

Immunochemical analysis: double diffusion in agar-agarose gel was performed after Ouchterlony [17].

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Immuno-electrophoresis was carried out after Grabar and Williams [18] and quantitative precipitation after Kabat and Mayer [19].

3. Results

The first chromatography on CM-Sephadex C-25 at pH 5.0 with an ammonium acetate buffer of stepwise increasing molarity (0.05, 0.3 and 1.0 M) gave a clear separation into four distinct peaks. The highest activity was found in the second peak (9,570 IU/mg). After rechromatography of this product under identical conditions, the biological activity increased to 14,700 IU/mg. The overall recovery in respect to the starting material was 66% of total activity. Ultracentrifugation revealed symmetrical peaks each time (table 1). The highly purified HCG was tested by starch gel electrophoresis with different buffer systems. It always migrated as a single band. Only in discontinuous buffer systems [9–11] did the hormone move to the anode with several bands. For the best separation 5 bands using Ferguson buffer were obtained [11]. In polyacrylamide electrophoresis, purified HCG showed one broad smear without urea. Contrary to other authors a distinct separation into 7 bands using 6 M urea (see fig. 1) was obtained.

The second peak of the two chromatographic separations was analyzed for its NANA content. After the first chromatography, the purified fraction contained 8.1% NANA compared to 9.4% after the second chromatography (on the basis of a MW of 40,000). No NANA is destroyed under the test conditions as demonstrated in kinetic studies. Neuraminidase (8 IU/mg HCG) caused a nearly complete cleavage of NANA during a 10 hr incubation at 37°. The biological activity of the NANA-free HCG was entirely destroyed [20]. This product showed a sedimentation constant of $s_{20,w} = 2.9$ in the ultracentrifuge. In starch gel electrophoresis under the conditions described above, the NANA-free HCG moved in a broad smear to the cathode.

The goat antiserum against crude HCG showed one sharp line with the highest purified fraction in agar-agarose gel. In immunoelectrophoretic analysis, this material precipitated as a sharp line, which tended to diffuse at both ends. In quantitative precipitation about the same amount of rabbit antiserum against highly purified HCG was precipitated by HCG and by NANA-free HCG at the point of equivalence. However, about 50% less of the latter was needed to give maximal precipitation.

Table 1
Ultracentrifuge analysis of the rechromatographed fraction 2 at 56,000 rpm in 0.05 M phosphate buffer at pH 7.0.

Solution	$s_{20,w}$	MW
0.05 M phosphate buffer, pH 7.0	3.30	40,000
0.05 M phosphate buffer, pH 7.0 in 8 M urea	2.40	23,000
0.05 M phosphate buffer, pH 7.0 after removal of the urea	2.80	28,500

4. Discussion

In spite of the fact that we have obtained a highly purified hormone, which is homogeneous in the ultracentrifuge and in immunological analysis, in starch gel electrophoresis under certain conditions, the preparation showed several bands even in the absence of urea. Two of them are of equal intensity. Nevertheless

Fig. 1. Disc electrophoresis of highly purified HCG with 6 M urea in 12.5% polyacrylamide gel, pH 9.3.

we do not consider them subunits, but rather to represent a further example of microheterogeneity as is well known for glycoproteins. The additional bands in disc electrophoresis compared to starch gel electrophoresis could represent subunits formed in the presence of urea. It is obvious that the starting hormone represents a mixture of genetic variants.

Moreover, the original hormone could somehow have been altered during kidney passage. These points must be considered during any attempt to elucidate the structure of HCG.

Interestingly, according to the quantitative precipitation, NANA-free HCG proved to be a far better antigen then HCG itself. The reason for this difference in immunological properties is not yet clear. The different net charge or steric problems could be involved. Work towards the solution of these problems is currently in progress.

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