# Dissociation and Recombination of the Subunits of Human Chorionic Gonadotropin*

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SUMMARY

Human chorionic gonadotropin has been dissociated into two subunits by urea. They have been separated by chromatography on DEAE-Sephadex. The subunits are non-identical as evidenced by electrophoresis, amino and carbohydrate composition and reassociate under suitable conditions.

Leutenizing hormone (LH) from ovine (Papkoff and Ananthasamy, 1967) and bovine (Reichert et al, 1969) has been shown to consist of two nonidentical subunits. Since human chorionic gonadotropin (HCG) and LH resemble in their biological behavior, it strongly suggested that HCG might also be composed of two subunits. During our studies on the preparation of carboxamidomethyl derivative of desialyzed HCG, we fortuitously separated the two subunits. The carboxamidomethyl derivative of one of the subunits precipitated during dialysis. When carboxymethyl HCG was subjected to electrophoresis in polyacrylamide gel, two broad bands were observed and isolated (Canfield et al., 1969). These considerations prompted us to attempt the isolation of the subunits of native HCG. A simple procedure for the separation of

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subunits of HCG is reported in the present communication. The amino acid and carbohydrate composition and conditions for their recombination are also described.

## EXPERIMENTAL AND RESULTS

Separation of Subunits. The dissociation of HCG into two subunits was effected by 8 M urea. A sample (11.9 mg) of the purified HCG (Bahl, 1969) was dissolved in 100 µl of 8 M urea in 0.04 M Tris-phosphate buffer, pH 7.5, and incubated at 37° for sixty min. The incubation mixture was diluted to 250 µl with the above buffer and transferred to a column of DEAE-Sephadex A-50 (0.9 x 30 cm) pre-equilibrated with 0.04 M Tris-phosphate buffer, pH 7.5. The column was developed with a salt gradient and two protein peaks, designated as A and B in Fig. 1, were obtained. The fractions comprising each peak were pooled, exhaustively dialyzed against distilled water and lyophilized, thus giving subunits A and B.

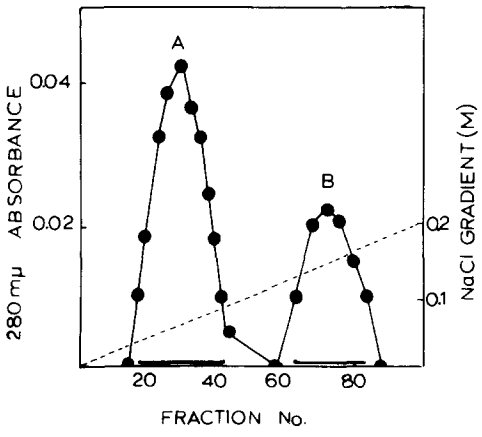


Fig. 1. DEAE-Sephadex chromatography of urea-treated HCG. The column (0.9 x 30 cm) was eluted with a continuous linear NaCl gradient (0.0 to 0.2 M) in 0.04 M Tris-phosphate buffer, pH 7.5. Fractions pooled, -----.

Recombination of HCG. The subunit A showed two major and one minor band whereas subunit B showed only one broad band, when subjected to electrophoresis in polyacrylamide gel (Fig. 2) in Tris-glycine buffer, pH 8.3 (Davis, 1964). When a mixture of 2.5 mg of each subunit in 4 ml of 0.02 M

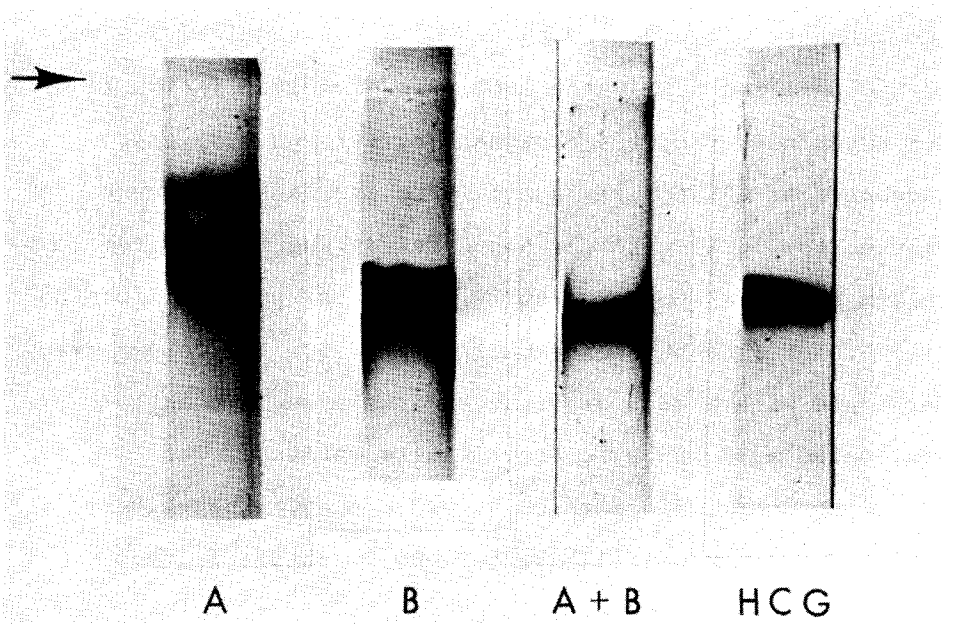


Fig. 2. Disc electrophoresis of subunits A and B, reconstituted HCG (A + B) and native HCG in polyacrylamide gel in glycine buffer, pH 8.3.

sodium phosphate buffer, pH 6.8 was incubated for 15-20 hours at 37° (Reichert et al, 1969), the subunits reassociated as evidenced by a single band on electrophoresis in polyacrylamide gel (Fig. 2). It may be noted that the dissociation of HCG into subunits by urea could also be shown by polyacrylamide electrophoresis prior to their separation by chromatography on DEAE-Sephadex.

Amino Acid and Carbohydrate Composition of the Subunits. The hydrolyses were carried out in 5.7 N HCl in evacuated

TABLE I

Amino Acid Composition* of Subunit of HCG

| Amino Acid | A % | B % | $\frac{A+B}{2}$ | HCG % |
|---|---|---|---|---|
| Lysine | 7.8 | 3.5 | 5.65 | 5.15 |
| Histidine | 3.9 | 1.1 | 2.50 | 2.40 |
| Arginine | 4.2 | 10.7 | 7.45 | 9.93 |
| Aspartic Acid | 7.3 | 9.7 | 8.50 | 8.46 |
| Threonine | 8.0 | 6.6 | 7.30 | 7.20 |
| Serine | 7.2 | 7.8 | 7.50 | 7.26 |
| Glutamic Acid | 12.7 | 8.9 | 10.80 | 9.79 |
| Proline | 6.9 | 13.8 | 10.35 | 11.16 |
| Glycine | 2.5 | 3.3 | 2.90 | 2.90 |
| Alanine | 3.3 | 3.9 | 3.60 | 3.69 |
| Half Cysteine | 9.4 | 7.4 | 8.4 | 8.28 |
| Valine | 7.0 | 6.6 | 6.80 | 6.88 |
| Methionine | 3.0 | 0.4 | 1.70 | 2.06 |
| Isoleucine | 0.9 | 2.9 | 1.90 | 2.32 |
| Leucine | 4.0 | 8.4 | 6.20 | 6.93 |
| Tyrosine | 5.9 | 3.0 | 4.45 | 4.28 |
| Phenylalanine | 5.8 | 2.2 | 4.0 | 3.61 |

* Values represent percent of the polypeptide content.

** Hydrolyses were carried out for 24 hours only and therefore no correction was made for destruction. Tryptophan was not determined.

sealed tubes at 110° for 24 hours. After the removal of the acid (Bahl and Smith, 1965), the hydrolysate was analyzed by a Spinco Automatic Amino Acid Analyzer. The amino acid composition of A and B as seen in Table I reflects significant differences. For instance, subunit A has much higher content of lysine, histidine, glutamic acid, methionine, tyrosine and phenylalanine and subunit B, on the other hand, is rich in arginine, proline, isoleucine, and leucine.

Neutral sugars were determined by gas chromatography using trimethylsilylether derivatives as previously described (Bahl, 1969). Hexosamines were determined by an

TABLE II

Carbohydrate Composition* of Subunits of HCG

| Sugar | A | B | $\frac{A+B}{2}$ | HCG |
|---|---|---|---|---|
| Galactose | 1.52 | 7.50 | 4.51 | 4.30 |
| Mannose | 5.40 | 4.80 | 5.10 | 4.30 |
| Fucose | 0.36 | 1.30 | 0.73 | 0.60 |
| N-Acetylglucosamine | 8.55 | 7.40 | 7.95 | 7.10 |
| N-Acetylgalactosamine | 0.19 | 2.00 | 1.09 | 1.70 |
| Sialic Acid | 3.90 | 10.20 | 7.00 | 7.20 |

* Uncorrected for water content of protein.

Automatic Amino Acid Analyzer (Bahl, 1969) and sialic acid by Warren's procedure (1959). The carbohydrate content of monosaccharides recorded in Table II shows that subunit B contains considerably higher amounts of galactose and sialic acid than A.

## DISCUSSION

The results presented above show clearly that HCG is composed of two subunits. Since the subunits are dissociable by urea, they must be attached noncovalently. Although previous studies based on amino and carboxy terminal analyses suggested that the two subunits might be identical (Bahl, 1969), the present findings on the electrophoretic mobilities, and the carbohydrate and amino acid composition of the subunits definitely establishes their nonidentity. Furthermore, the fingerprints of the tryptic digest of the carboxamidomethyl derivatives of the subunits were remarkably different (unpublished results).

Subunit A showed two major and one minor band whereas B showed only one broad band on electrophoresis in polyacrylamide gel. Since A and B reassociated to give a single band, it indicates the presence of microheterogeneity in HCG. Preliminary results on the amino acid content of the individual components of A, obtained by preparative electrophoresis, showed no significant differences. The sialic acid content was, however, different suggesting that sialic acid might be responsible for this microheterogeneity.

The procedure for separation of the subunits of HCG is simple and has been found to be suitable for their large scale preparation. It offers a potential method for studying the subunit structure of other gonadotropic hormones. Since a knowledge of the number of subunits or polypeptide chains and their separation is an essential prerequisite for the study of primary structure, the present investigation would, therefore, greatly facilitate the current work on the elucidation of the primary structure of the hormone.

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