The Heterogeneity of Human Gc-globulin

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SUMMARY

Gc-globulin or group-specific component, also known as the vitamin D-binding protein, was investigated by the combined use of electrofocusing and immunofixation. Serum of the Ge 2-2 type was found to contain a single protein band whereas serum of the Ge 1-1 type shows two bands with a lower isoelectric point. The Ge 1-2 type contains all three bands known as Gc-2 (pI 5.10), Ge-1slow (pI 5.03), and Ge-1fast (pI 4.95). Each apoprotein shows an anodal shift of about 0.07 pH unit after incubation with an excess of 25-hydroxycholecalciferol. After treatment with sialidase Ge-1fast focuses in the position of Ge-1slow, whereas the position of Ge-2 remains unchanged.

Human Gc-globulin, discovered in 1959 by Hirschfeld (1), shows three common phenotypes which are determined by a pair of co-dominant autosomal alleles (Ge' and Ge). Serum of people homozygous for Cc' (Gc 1-1) contains Gc migrating in the position of Gc-lslow, whereas the position of Gc-2 remains unchanged.

EXPERIMENTAL PROCEDURES

Isoelectric Focusing—Electrofocusing in polyacrylamide gels was performed with an LKB Multiphor electrofocusing apparatus cooled at 4°C. Ampholine PAGplates (11 × 12 cm, LKB) with a pH range from 4 to 6.5 were used. Usually, 15 μl of human serum, diluted 15-fold with distilled water, was applied using sample application pieces. A constant current of 10 mA was used, until a voltage of 700 V was reached. The voltage was then kept constant for the rest of the experiment (3 to 4 h). The pH was measured at 1-cm intervals on the gel surface using a contact electrode (Ingold). Gc-globulin was detected by immunofixation using cellulose acetate strips soaked in sodium acetate buffer, pH 5.8, containing 0.17 M NaCl and 0.02% NaN3. Control samples without sialidase were treated identically. Using the thiobarbituric acid assay of Warren (6), it was found that more than 90% of the sialic acid was released. Measurement of Gc-globulin by single radial immunodiffusion (7) indicated that sialidase treatment did not alter the Gc-globulin concentration.

RESULTS AND DISCUSSION

Phenotyping of Human Gc-globulin—Different serum samples of known Gc-globulin composition were focused on polyacrylamide gels, followed by immunofixation. Three different patterns were obtained, having one, two, or three protein bands, corresponding to Ge-2, Ge-1, and Ge-1, respectively. As shown in Fig. 1A, the distance between each protein is constant (about 0.07 pH unit). From pH measurements on the gel, the following isoelectric points were obtained by interpolation: Gc-2, 5.10; Gc-1slow, 5.03; Ge-1fast, 4.95. These results confirm the recent observations of Constans and collaborators (8, 9) on the superiori of isoelectric focusing and immunofixation for the phenotyping of Gc-globulin.

Effect of Vitamin D Binding—As shown in Fig. 1B, each Gc band moves anodically as a result of the binding of 25-OH-D3. The apoprotein, as present in serum, and the corresponding holoprotein, obtained after incubation with a 25-fold molar excess (10 μM) of 25-OH-D3, differ by 0.07 pH unit. Nonsaturating amounts of 25-OH-D3 produced two Gc bands, corresponding to the isoelectric position of the apo- and holoprotein. This is clearly demonstrated when a serum sample of the Ge-2 type is focused after addition of increasing amounts of 25-OH-D3 (Fig. 1C). Equimolar concentrations of Gc-globulin and 25-OH-D3 result only in about 50% conversion, probably due to lower affinity at this pH.

In another experiment, the influence of a 25-fold molar excess (10 μM) of 24,25-dihydroxycholecalciferol, 2α,25-dihydroxycholecalciferol, and vitamin D3 was investigated. The anodic shift was observed with 24,25-dihydroxycholecalciferol, partially with 2α,25-dihydroxycholecalciferol but not with vitamin D3. This probably reflects differences in affinity of these vitamin D metabolites for Gc-globulin.

A similar anodic shift of the vitamin D-binding protein of rat serum was observed by the addition of excess 25-OH-D3.

The molecular basis of the vitamin D-induced charge difference remains speculative and could be explained either by a conformational change or by the burying of (an) ionizable group(s) of the binding protein. A similar phenomenon has been described for the desialylated form of human transcortin which shows an anodic shift of 0.2 pH unit as the result of cortisol binding (10). The heterogeneity induced by 25-OH-D3 binding is unlikely to be detected in normal human serum since Gc-globulin circulates mainly as an apoprotein (7). However, in vitamin D-treated patients, a supplementary Gc band will be detected, as shown in Fig. 1C and recently described by Brissenden and Cox (11). This vitamin D-induced charge difference also implies that autoradiography of 25-OH-D3-labeled serum will detect the position of the holoprotein which differs 0.07 pH unit with the pattern detected by immunofixation. The present results also indicate that, as previously stated (10), trace amounts of radioactive ligands are not always good markers for the isolation of a binding
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As measured immunochemically, it is inferred that Gc-1Fast focuses in the position of Gc-1Slow. Addition of 25-OH-D$_3$ in 25-fold molar excess (10 $\mu$m) to sialidase-treated samples results in an anodic shift of about 0.07 pH unit of all components, showing that this treatment does not alter the vitamin D-binding properties of Gc-globulin.

Since Gc-1Fast and Gc-1Slow co-focus after sialidase treatment, they differ certainly in their carbohydrate composition. Whether they differ in amino acid composition remains to be determined.

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**Fig. 1.** Isoelectric focusing of human serum on polyacrylamide gels (pH 4 to 6.5) followed by immunofixation with rabbit antiserum against Gc-globulin. The isoelectric points are indicated at the right and the anode is at the top. For experimental details see the text. A, detection of the common Gc phenotypes (the “holes” are due to antigen excess). B, influence of a 25-fold molar excess (10 $\mu$m) of 25-OH-D$_3$. C, influence of increasing amounts of 25-OH-D$_3$. D, influence of sialidase treatment.

Indeed, the ligand-induced charge difference results in an altered physicochemical behavior especially on ion exchange chromatography (12).

**Effect of Sialidase Treatment**—Since it is known that Gc-globulin is a glycoprotein (13), the effect of sialidase treatment was also investigated. As shown in Fig. 1D, Gc-1Fast disappears in serum of the Gc 1-1 and Gc 1-2 phenotype. Since sialidase treatment does not alter the concentration of Gc-globulin, as measured immunochemically, it is inferred that Gc-1Fast focuses in the position of Gc-1Slow. Addition of 25-OH-D$_3$ in 25-fold molar excess (10 $\mu$m) to sialidase-treated samples results in an anodic shift of about 0.07 pH unit of all components, showing that this treatment does not alter the vitamin D-binding properties of Gc-globulin.
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