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RELATIONS BETWEEN VITAMIN D AND FATTY ACID BINDING PROPERTIES OF VITAMIN D-BINDING PROTEIN

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Summary. The interaction of fatty acids with bovine vitamin D-binding protein (DBP) was studied using a partition equilibrium method. This protein has one high affinity site for binding of fatty acids with an association constant $K_a = 7 \times 10^5 M^{-1}$ for palmitic acid and $K_a = 6 \times 10^5 M^{-1}$ for arachidonic acid. Competition experiments showed that palmitic acid hardly competes with 25-hydroxycholecalciferol for binding to DBP. However, arachidonic acid showed comparatively a stronger competition for binding to this protein. The great difference in competition of palmitic and arachidonic acids with 25-hydroxycholecalciferol may be related to changes in DBP conformation promoted by the binding of different ligands.

The transport of vitamin D metabolites in the plasma of vertebrates is mediated by a specific protein, namely vitamin D-binding protein (DBP) or group-specific component (Gc), with a molecular weight of about 58,000 (1). Its primary sequence has a high degree of homology corresponding to albumin and alpha-fetoprotein (2), and the placement of cysteine residues is also identical to that of albumin, indicating a similar secondary folding structure (3). However, DBP is smaller due to the lack of a large part of the domain III.

Fatty acid binding and transport is probably one of the main physiological roles of albumin (4) and alpha-fetoprotein (5,6). It has been recently reported that human (7,8) and bovine (8) DBP isolated from blood serum have fatty acids bound in proportions between 0.4 and 1.5 moles per mol of protein (8), meanwhile the binding of vitamin D and its metabolites is only about 0.04 moles per mol (9). This work is a first attempt to establish a possible relationship between the binding of fatty acids and vitamin D to DBP.

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MATERIALS AND METHODS

Materials. [^{14}C]palmitic acid, [^{14}H]arachidonic acid and [^{3}H]25-hydroxycholecalciferol (25-hydroxyvitamin D_3) were purchased from New England Nuclear (Berkeley, California). Solutions of these chemicals in heptane were washed three times with saline solution to eliminate any water-soluble contaminant. Unlabelled 25-hydroxycholecalciferol was a gift from Hoffmann-La Roche (Basle, Switzerland). Bovine DBP was isolated using Cibacron-Blue affinity chromatography as described in (8). Endogenous fatty acids of DBP were eliminated by incubation with heptane overnight.

Determination of apparent affinity constants. Binding constants and number of binding sites for the interaction between bovine DBP and fatty acids were determined by the two phase partition method (10) as described in detail in (11). The concentration of protein was around 4.5 mM determined by the method of Lowry, and the initial concentration of fatty acids in the heptane phase ranged between 6 mM and 5,000 mM. Binding parameters were calculated by the method of Scatchard. Unbound fatty acids in aqueous phase were calculated using the partition coeficients in the absence of protein.

Competition between vitamin D and fatty acids. Volumes of 500 ml of protein solution (2.1 mM) in 0.15 M NaCl, 0.01 M phosphate buffer, pH 7.4 were incubated with 500 ml of a 0.5 mM solution of 25-hydroxycholecalciferol in heptane, with a trace of [^{3}H]25-hydroxycholecalciferol; this concentration in the organic phase was chosen to achieve a 3% of DBP saturation, as in physiological conditions. Unlabelled palmitic or arachidonic acids were added to the organic phase at concentrations ranging between 50 mM and 15,000 mM. Radiactivity bound to protein was determined as described in (11).

RESULTS

Binding of fatty acids to DBP. The Scatchard plot obtained for the interaction of bovine DBP with palmitic acid (Fig.1) shows that DBP binds this fatty acid with an apparent affinity constant $K_a = 7 \times 10^5\ M^{-1}$. Assuming 58,000 as molecular weight for bovine DBP, the equilibrium partition method used indicates that this protein has only one high affinity binding site for fatty acids. The non-straight alignment of points corresponding to the highest concentrations of fatty acid suggests that other low affinity binding sites can also exist. For arachidonic acid the interaction pattern obtained by the same method indicates a $K_a = 6 \times 10^5\ M^{-1}$.

Competition between fatty acids and 25-hydroxycholecalciferol. Fig.2 shows the results obtained when DBP was incubated with [^{3}H]25-hydroxycholecalciferol and unlabelled palmitic or arachidonic acid. This experiment evidences a concentration-dependent inhibition of binding of vitamin D by fatty acids.

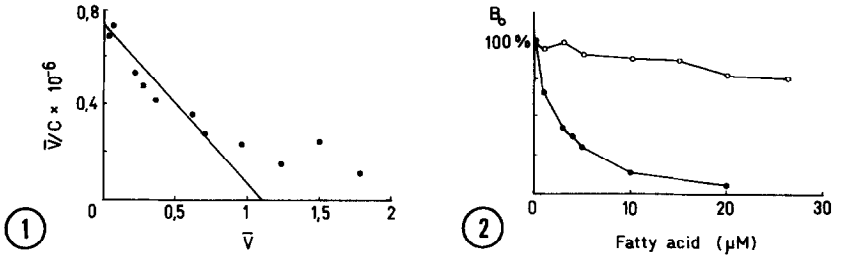

Figure 1. Scatchard plot obtained for the binding of palmitic acid to bovine vitamin D-binding protein. For this plot K_a = 7 X 10^5 M^{-1} and n = 1 sites/molecule.

Figure 2. Competition between palmitic acid (O—O) and arachidonic acid (●—●) and 25-hydroxycholecalciferol. The 25-hydroxycholecalciferol binding in absence of fatty acid was taken as the 100% binding capacity (B_O). Residual binding was plotted against the concentration of fatty acid present in the aqueous phase.

Palmitic acid affects only slightly the 25-hydroxycholecalciferol binding ability of DBP. An increase of palmitic acid in the aqueous phase from 0 to 25 μM decreases the binding only in a 25%. By contrast, arachidonic acid affects greatly the 25-hydroxycholecalciferol binding. A concentration of 3 μM decreases the binding in a 60%.

DISCUSSION

Serum albumin has three strong binding sites for fatty acids, with the primary binding site located in the loops 7 and 8 of the third domain (4). This region is lost in the DBP sequence (2,3). Our results show that because of the lack of this region bovine DBP is able to bind with high affinity only one molecule of fatty acid, with an equilibrium constant lower than that of albumin.

An initial explanation of the competition between arachidonic acid and 25-hydroxycholecalciferol for binding to DBP would be that the fatty acid binding site overlaped the vitamin D binding site. However, the apparent affinity constant of DBP for 25-hydroxycholecalciferol is about 7 X 10^8 M^{-1} (12), clearly greater than the calculated in this work for palmitic and arachidonic acids. Moreover, the great difference observed in the competition experiments between palmitic and arachidonic acid does not seem directly related to their respective affinity constants. It is well known that the binding of ligand to DBP induces a change in protein conformation (13) and in the ability to interact with other ligands (14). Probably the binding of arachidonic acid

could promote a change in DBP structure that diminishes its affinity for vitamin D metabolites.

From other reports (8,9), we can deduce that the levels of arachidonic acid seem to be greatly variable. The metabolic significance of fatty acid binding to DBP and the competition with vitamin D metabolites remain unclear. The binding of 25-hydroxycholecalciferol to DBP seems to decrease the accesibility of the vitamin to their specific cellular receptors (15). Therefore, we can suppose that an increase of arachidonic acid levels in plasma may contribute to the liberation of a part of vitamin D metabolites bound to DBP, modifiying the physiological activity of this vitamin. This hypothesis is under investigation in our laboratory.

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