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PREPARATION OF VARIANT-SPECIFIC ANTI-HUMAN PLACENTAL PHOSPHATASE ANTISERA BY IMMUNOABSORPTION

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Summary

Antiserum raised in rabbits sensitized with purified human placental alkaline phosphatase of the rare FD phenotype was absorbed on purified FF phenotype enzyme conjugated to Sepharose. The absorbed antiserum was not able to bind to the F-variant, but was still capable of binding to the D-variant enzyme, determined by electrophoretic retardation and gel filtration. It therefore appears that some allelic variants of placental phosphatase differ in their antigenic structure.

Introduction

Human placental alkaline phosphatase has been found to have a large number of electrophoretically defined allelic variants (1-3), particularly in comparison to a number of other enzyme loci which have been carefully studied (4). Variants have been implicated in spontaneous abortion (5,6) and complications of pregnancy (6). Enzymes which resemble the placental enzyme have been identified in cancer patients (7), and the suggestion has been made that variant enzymes identified in cancer may correspond to genetic variants of the placental phosphatase (8), although the criteria supporting this suggestion are open to some doubt (6).

Differences have been found in the sensitivity of several allelic variants to heat inactivation (9) and to specific amino acid and peptide inhibitors (10), but the principal criterion used to date to discriminate these variants has been starch gel electrophoresis. Studies on chemical composition of variant enzymes (11,12) have not yielded any information to date on structural distinctions among them.

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As an alternative approach to study structural variations among these allelic variants, we have absorbed antiserum raised against a mixed phenotype enzyme containing the rare L-leucine-sensitive D-variant (FD) (13,19) with a homozygous common enzyme phenotype (FF). The antiserum so absorbed is able to bind to the D-variant enzyme, but not to the common F-variant against which it was absorbed. This report is intended to document these observations.

Materials and Methods

Reagents. All substrates, 2-amino-2-methyl-1,3-propane diol, Triton X-100 detergent and agarose were purchased from Sigma (St. Louis, Mo.); 4-amino-antipyrene from Polysciences (Warrington, Pa.); variamine blue (4-amino diphenylamine, diazonium salt #5531) from Dajac Laboratories (Borden Chemical Co., Philadelphia, Pa.).

Enzyme assay was by the method of Fishman et al. (14) at 37°C with 18 mM phenyl phosphate in 50 mM sodium carbonate-bicarbonate buffer (pH 9.8), 10 mM $MgCl_2$. The unit of enzyme activity is micromoles of substrate hydrolyzed per minute per milliliter.

Protein assay was by the method of Lowry et al. (15) using Sigma protein standard (Sigma catalog #540-10).

Disc-gel electrophoresis was as previously described (16).

Enzyme staining was by the method of Angellis et al. (17), except that neither zinc nor magnesium was included in the buffer.

Enzyme preparations. Purification of homogeneous enzyme was as described previously (18).

Immunization protocol was as previously described (18). Antisera used in these studies were pooled from three rabbits and were obtained 6 months after primary immunization.

Absorption of antiserum. One thousand units of immunoabsorbent-purified FF enzyme was coupled to 5 ml of cyanogen-bromide-activated Sepharose 4B. Ten milliliters of antiserum containing 0.1% sodium azide was recycled through this column overnight at 24°C using a peristaltic pump.

Results

Figure 1 shows the electrophoresis of purified FD phenotype mixed with serial dilutions of absorbed antiserum before application to the gel. It is clear that the slow-migrating DD and the intermediate-migrating FD hybrid enzymes are both retarded while the FF enzyme is unaffected at the highest antiserum concentration. Upon dilution, the absorbed antiserum loses its ability to retard the electrophoretic mobility of the DD and FD enzymes, and the electrophoretic results are identical to those in the absence of antiserum.

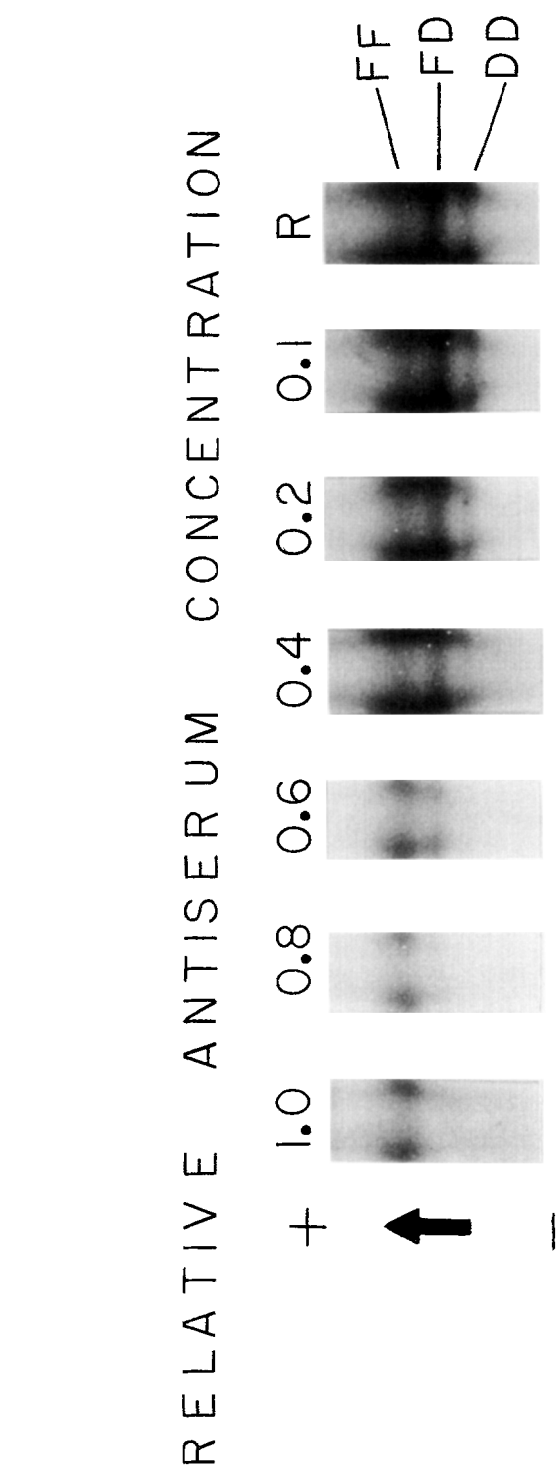

Figure 1. Disc-gel electrophoresis of FD phenotype human placental alkaline phosphatase in the presence of antiserum raised against this phenotype and absorbed with the FF phenotype. The enzyme was diluted to a final activity of 0.55 units·ml^{-1} in absorbed antiserum or into electrophoresis sample buffer, and the two were mixed in the proportions indicated, 1.0 indicating the sample of enzyme diluted into absorbed serum, R indicating the reference in the absence of antiserum.

Since it is reasonable to assume that one or only a few antigenic sites would be variant-specific, the size of immune complexes formed between the absorbed antiserum and purified FD variant enzyme was compared with the purified, nonreactive FF enzyme by gel filtration on Sepharose 6B columns. As seen in Figure 2, the absorbed antiserum does not alter the apparent molecular weight of the FF phenotype, since the elution profile is identical to that in normal rabbit serum. The unabsorbed antiserum yields very large complexes which elute in the void volume of the agarose column. For the FD phenotype, the profile in the presence of the absorbed antiserum is distinct both from that in normal rabbit serum and in unabsorbed antiserum. A broad peak with a molecular weight intermediate between that of free enzyme and the void volume of the column is obtained. Ninety-five percent of the enzyme is recovered in this peak, compared with 11% for the unabsorbed antiserum, indicating that there was not extensive inactivation or precipitation of the variant enzyme with the absorbed antiserum.

Measurement of the inhibition of the enzyme contained in the intermediate peak by 0.5 mM L-leucine was used as an index of the D-variant enzyme concentration (13,19). The inhibition varied from 68% near the void volume (tube 7) to 30% in tube 11, the peak tube in the absence of antiserum. This indicates that the D-variant enzyme, which is markedly more sensitive to L-leucine than the F-variant, is the predominant enzyme species in the antibody-enzyme complex.

Discussion

It is clear from these studies that electrophoretically defined allelic variants of human placental alkaline phosphatase do differ in antigenic structure.

The development of immunochemical approaches for study of these variant enzymes should yield structural criteria for their discrimination. It is hoped that this will make it possible to determine whether the enzyme found in cancer patients ("Regan isoenzyme") (7) which resembles the human placental isoenzyme is in fact identical to the placental enzyme.

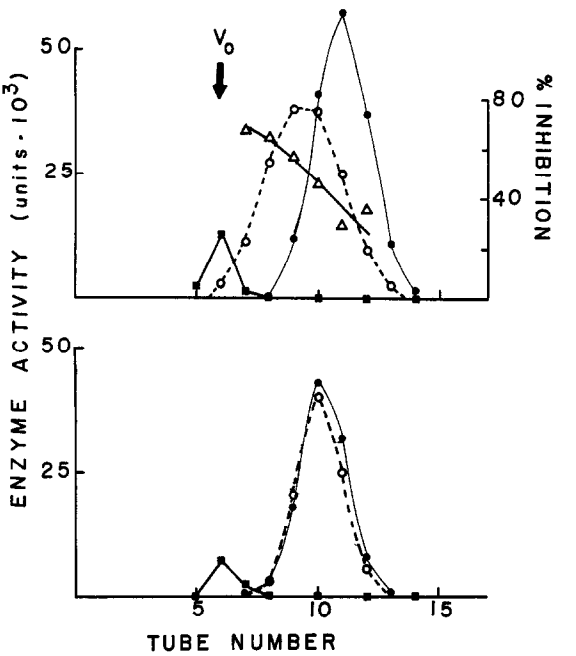

Figure 2. Sepharose 6B elution profiles of FD (top) and FF (bottom) phenotype enzyme incubated with normal rabbit serum (closed circles), rabbit antiserum to FD phenotype enzyme (closed squares) and rabbit anti-FD phenotype enzyme absorbed with FF enzyme (open circles). Two columns were used, both with the dimensions 0.7 x 27.5 cm; all samples containing FD enzyme were applied to one column, all samples of FF enzyme to the other, fraction size 0.6 ml. Open triangles - inhibition by 0.5 mM L-leucine.

Specific immunochemical assays for variant enzymes may also make it possible to discriminate these variants in tissues that contain only trace amounts of placental phosphatase. This could be of use in determining whether a correlation exists between placental phosphatase phenotypes and spontaneous abortion in the late-first and early-second trimester, as suggested previously (5,6).

These absorbed antisera may also be useful in elucidating the detailed chemical structure of the placental enzyme. If the antigenic determinant that is being recognized is principally due to a difference in primary sequence rather than enzyme conformation (20), antisera recognizing this specific sequence may be used to isolate a specific peptide which differentiates that genetic variant.

Acknowledgements

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