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DIFFERENT GENES CODE FOR ALKALINE PHOSPHATASES FROM HUMAN FETAL AND ADULT INTESTINE

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Alkaline phosphatases from human adult intestine and fetal intestine (meconium) were purified and compared. Electrophoresis in SDS showed one band of protein in the former. There were three bands of protein in the latter, all with essentially the same peptide map. Thus, two of the bands probably arose by proteolysis of the third, which was largest (M_r 73000). In gradient gels of polyacrylamide the alkaline phosphatase from fetal intestine showed only one band of protein coincident with the band of activity (M_r 151000). Radiolabeled mapping showed that the tryptic peptides of the alkaline phosphatase from fetal intestine were distinctly different from those of adult intestine and human liver, and placenta, indicating a gene distinct from the three that code for the enzyme in liver/kidney/bone, placenta, and adult intestine.

Genes at three loci have been identified that code for human alkaline phosphatases (EC 3.1.3.1.) [1]: for the liver/kidney/bone form, the intestinal form, and the placental form of the enzyme. The gene that codes for the placental form is known to have three common and numerous rarer alleles, whereas the liver/kidney/bone form has not been demonstrated to have any [1]. Some properties of alkaline phosphatase derived from the mucosa of adult intestine and from fetal intestine (mucosa and meconium) are different [2-9], but it is not known whether these differences represent the expression of different genes or post-translational modifications of a common precursor protein.

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Abbreviations: SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PMSF, Phenylmethylsulfonyl fluoride; MCA, Monoclonal antibody

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We now report evidence that the fetal and adult forms of the intestinal enzyme are coded for by different genes, and that the gene which codes for the fetal form is different from those for placental and liver alkaline phosphatases.

MATERIALS AND METHODS

Meconium (first stool passed by neonate) collected within 8 h of birth was obtained from the Department of Pediatrics of the University of Alberta Hospital and frozen immediately to -20°C. Samples were used individually or pooled to obtain approximately 10 g. Intestine of adults with no known gastrointestinal disorder was obtained at necropsy within 12 h of death. Purification was essentially as described for the other molecular forms of this enzyme [10], including butanol extraction, acetone fractionation, ion-exchange chromatography, chromatography on immobilized p-aminobenzylphosphonic acid, and gel-permeation chromatography. Samples of the purified enzyme were divided; some were processed immediately and others were stored at -20°C until used (up to 2 yr). For assay of enzyme activity the substrate was 10 mM p-nitrophenylphosphate in 1 M 2-(ethylamino)-ethanol (Aldrich, Milwaukee, WI) plus 1.5 mM $MgCl_2$, at pH 10.3. The rate of p-nitrophenol formation at 30°C was followed continuously at 405 nm; one unit of activity was defined as the amount of enzyme that hydrolyzed 1 µmol substrate/min.

The marker proteins (Bio-Rad, Richmond, CA) used for SDS-PAGE were: myosin, 200000; β-galactosidase, 116250; phosphorylase b, 97300; bovine serum albumin, 66200; and ovalbumin, 45000. The procedure was carried out as previously described [10], with one modification: 7% (w/v) acrylamide gels were used, as the glycoprotein migrates anomalously in SDS at low acrylamide concentration. The subunit M_r of the alkaline phosphatase was determined by boiling the enzyme in mercaptoethanol and SDS then mixing it with the appropriate M_r markers. With samples that had not been denatured, the activity remaining after electrophoresis in SDS was located by the fluorescence after hydrolysis of α-naphthol AS-MX phosphoric acid catalyzed by alkaline phosphatase [11]. The gels were photographed under ultraviolet light.

Native M_r was determined by electrophoresis on cylindrical gradient gels of polyacrylamide (2.5 - 27%). The marker proteins (Pharmacia, Uppsala, Sweden) consisted of: thyroglobulin, 669000; ferritin, 440000; catalase, 232000; lactate dehydrogenase, 140000; and albumin, 67000. The buffer was 0.09 M Tris with 0.08 M boric acid, pH 8.4. Marker proteins were mixed with the sample and a potential of 150 V was applied to the gels at 4°C for 24 h. After location of the enzyme activity as above and determination of the enzyme's R_f under ultraviolet light, the gel was stained to reveal the marker proteins. Subunit and native M_r values were determined from a plot of log M_r vs R_f.

Purified alkaline phosphatase was subjected to PAGE in SDS or gradient gels and stained for protein. Gel slices (~ 1 - 2 mm wide) containing the stained protein bands were excised and washed; the protein was radioiodinated, treated with trypsin, and lyophilized [12]. The trypsin digests were subjected to thin-layer electrophoresis followed by thin-layer chromatography in a second dimension. Radioactivity was detected by autoradiography (Kodak X-Omat AR film).

RESULTS

Electrophoresis. Study of the fetal intestinal enzyme by SDS-PAGE (Fig. 1) under non-denaturing conditions revealed several bands of protein (gel b), but

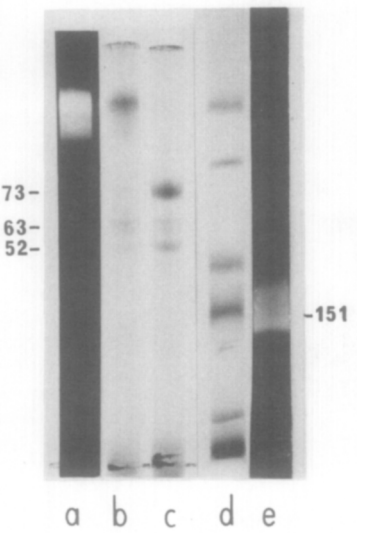

Figure 1. Electrophoresis of alkaline phosphatase from human fetal intestine. Gel a, non-denatured sample: SDS-PAGE followed by staining for activity; b, non-denatured sample: SDS-PAGE followed by protein staining; c, denatured sample: SDS-PAGE followed by protein staining; d, gradient-gel electrophoresis of marker proteins plus enzyme (151 kDa), followed by protein staining; e, gradient-gel electrophoresis of enzyme, followed by staining for activity. The M_r values in kDa on the left are applicable to gels a, b, and c, and that on the right to gels d and e.

only the slowest had enzymic activity (gel a). The completely denatured sample (gel c) had three major bands, with M_r values (mean ± SD) of 732000 ± 800, 62600 ± 420, and 51900 ± 100; in some preparations the intermediate band resolved into two components. Preparations from different samples demonstrated various proportions of the three bands, and storage of the enzyme resulted in conversion of the largest species into the 2 smaller ones. Purification of the enzyme with the addtion of the proteolytic enzyme-inhibitor PMSF still resulted in the appearance of three bands on the SDS gels but prevented conversion of the largest peptide into the smaller ones on storage. Electrophoresis in polyacrylamide-gradient gels of the native form of the enzyme from fetal intestine revealed only one band of protein (Fig. 1, gel d) and one band of activity (gel e). The native M_r was 151000 ± 800 (mean ± SD), confirming the protein's dimer nature.

Peptide Maps. Three protein bands were seen consistently on SDS-PAGE in several preparations of the fetal intestinal enzyme from individual

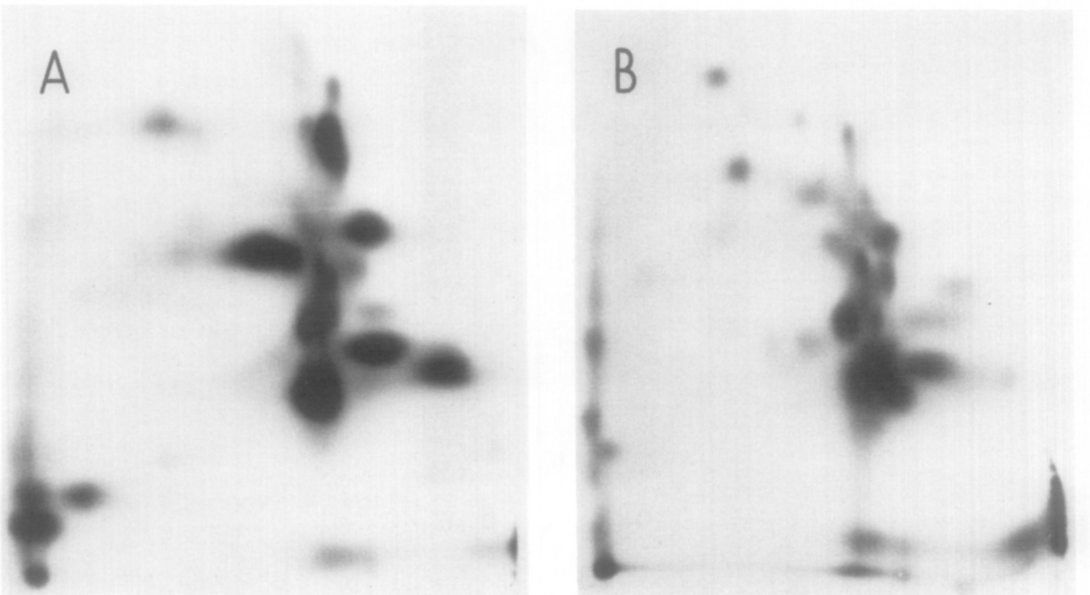

Figure 2. Radiolabeled-peptide maps of alkaline phosphatase from human fetal (A) and adult (B) intestine. The origin is at lower left. Electophoresis vertically was followed by chromatography horizontally.

specimens. Peptide maps of the individual bands from any one gel demonstrated almost identical patterns with each other and with the corresponding bands in other preparations (Fig. 2A). Further, the single protein band recovered from a gradient gel of polyacrylamide also gave the same pattern. These maps of the fetal enzyme revealed peptide composition markedly dissimilar from that of the adult intestinal enzyme (Fig. 2B).

DISCUSSION

Similarities of alkaline phosphatase from fetal intestinal mucosa and from meconium indicate that the proteins are identical and probably coded for by the same gene [2]. It has not been established, however, whether the enzymes in fetal and adult intestine are coded for by the same gene: as early as 1970 it was suggested that differences on electrophoresis may reflect the activity of different generic loci [4], but as recently as 1983 it was suggested that the same gene codes for both [13]. Developmental changes in intestinal alkaline phosphatase have been noted in human [2-4, 7] and other mammalian species [14-16].

The similarities between the fetal and adult intestinal alkaline phosphatases relate to inhibitors [2,6,7,17], K_m values [3,7,17], heat stability [7], SDS stability [17], electrophoretic mobility after neuraminidase [5,7], and immunologic reactivity to various polyclonal antibodies [5, 7,17]. Differences between the two enzymes include inhibition by $HgCl_2$ and $CdCl_2$ [17], isoelectric point [17], and electrophoretic mobility after neuraminidase treatment [2]; and, most recently [18,19], greatly differing reactivities with monoclonal antibodies (MCA). The findings with MCA indicate structural differences in the epitopes being detected, but it is not known whether these arise from post-translational modification or represent expression of different gene loci. The N-terminal amino acid sequences of the adult and fetal intestinal enzymes are different [8], and the fetal intestinal enzyme has a lower subunit molecular weight that the adult enzyme [5,9]. We have determined a subunit molecular weight of 79400 for the adult form [20], and the present experiments have 73200 for the fetal form; the latter form of the enzyme may be an already partly degraded peptide, but this is unlikely. The electrophoretic pattern of the fetal form after purification in the presence of PMSF indicated that the three proteins seen in SDS-PAGE arose as a result of proteolytic degradation and that the degradation had occurred before purification.

Behrens et al [13] presented evidence indicating that the fetal intestinal enzyme in a heterodimer composed of one subunit of placental alkaline phosphatase and one of the adult intestinal enzyme, and suggested that no 'new' structural genes or loci are involved in the transition from fetal to adult intestinal enzyme. We would resolve only one unique structural component for the fetal intestinal enzyme, a finding reported by others [2,6], and believe that the peptides of different molecular weights seen in our SDS polyacrylamide system are derived from the same protein. The single band of protein in the gradient gels of polyacrylamide may have remained intact, although perhaps cleaved by a proteolytic enzyme, until unfolded in SDS. Thus,

the finding reported by Behrens et al may relate to two peptides of different sizes but derived by proteolysis from the same protein.

The dissimilarity of the peptide maps of alkaline phosphatase from human fetal and adult intestine (Fig. 2) is unlikely to reflect differences in the carbohydrate composition or other post-translational modifications of the two proteins [12]. Further, the peptide maps of the enzymes from liver and placenta are distinct from both intestinal forms [12] and thus the fetal intestinal type has unique primary structural features. We conclude that alkaline phosphatase from human fetal intestine is coded for by a gene different from the 3 established ones that code for other forms of this enzyme.

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