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Inherited Variations of Human Serum α_1-Antitrypsin

Abstract. *The normal serum α_1-antitrypsin migrates as a three banded pattern when separated electrophoretically in starch gel with a sodium acetate–ethylenediaminetetraacetic acid buffer of pH 4.95. The results obtained when certain inherited variants of the serum α_1-antitrypsin are separated electrophoretically suggest that the previously described variations in the region preceding the albumin band represent inherited variations of the serum α_1-antitrypsin.*

The α_1-antitrypsin (α_1-at), one of the four known protease inhibitors in human serum, is of interest because of the possible deleterious consequences of an inherited deficiency of this protein (*1*). The genetically determined deficiency, as well as a recently discovered structural mutation, might serve as additional genetic markers in man. A large pedigree in which both mutations occur has been reported (*2*).

The method commonly used to detect a deficiency of the α_1-at is to measure the capacity of serum to inhibit the hydrolysis of the synthetic substrate N,α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) by trypsin (*3*). Electrophoretic variants have been identified by the antigen-antibody crossed electrophoresis as developed by Laurell (*4*). This method requires electrophoretic separation of serum in two dimensions, consumes relatively large amounts of specific antiserum, and is therefore not ideally suited for screening populations for genetic variants.

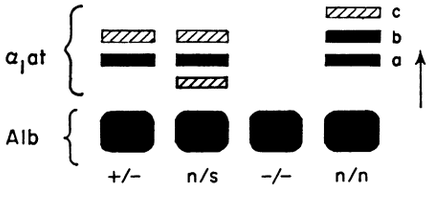

Fig. 1. Diagram of starch-gel electrophoresis of serums obtained from four individuals with different α_1-antitrypsin phenotypes. Buffers: Cathodal vessel contained 0.125*M* sodium acetate, 0.011*M* ethylenediaminetetraacetic acid (EDTA). Anodal vessel contained the same buffer used at half concentration. The gel buffer was 0.031*M* sodium acetate, 0.004*M* EDTA; *p*H of all buffers, 4.95. Electrophoresis for 9.5 hours at 250 volts. Stain: Amido black. *n/n*, normal phenotype; *n/s* heterozygous for slow variant; −/−, homozygous for deficiency gene; +/−, heterozygous for deficiency gene; $\alpha_1$at, α_1-antitrypsin; *Alb*, albumin; *a*, *b*, and *c* are the three major α_1-antitrypsin bands.

Between *p*H 8.0 and 9.0 the α_1-at migrates electrophoretically more slowly than albumin and forms a large part of the α_1-band in both paper electrophoresis and agarose-gel electrophoresis. Although under these conditions the α_1-at migrates as a single band, the antigen-antibody crossed-electrophoresis technique reveals that there is a second minor component migrating at the cathodal edge of the main band (*5*). When a buffer of a lower *p*H is employed in the vertical starch-gel system (*6*), the normal α_1-at migrates as three distinct bands preceding the albumin band. Two of the bands have approximately equal staining intensity, whereas a third band, migrating more anodally, is much weaker and in some serums is barely visible (Fig. 1). An antigen-antibody crossed electrophoresis, performed with a specific antiserum, indicates that the observed bands are indeed α_1-at (Fig. 2A). Moreover, it is apparent that, at least with the antiserum used in our experiments, there is no major antigenic difference between the three components, since the precipitation lines fuse completely. Additional evidence which supports the view that these bands represent α_1-at is that the serum from an individual known to be homozygous for the deficiency gene shows, with the exception of a very faint band, a virtual absence of bands in the region anodal to the albumin (Fig. 1, −/−). Whether this faint band represents small quantities of α_1-at or a trace protein unrelated to the α-at which migrates in a similar position is unknown. The evidence from the antigen-antibody crossed-electrophoresis experiments suggests that small quantities of α_1-at are present in the region preceding the albumin band of this serum. Trypsin inhibition by normal serum can be demonstrated with the fibrin-agar technique (*7*). A broad zone of inhibition corresponding to the position of the α_1-at banding pattern was observed.

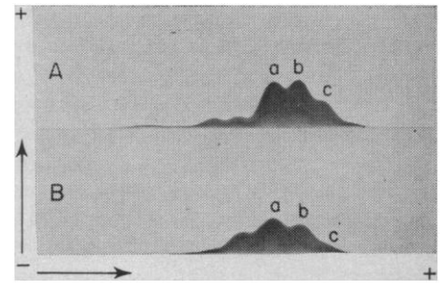

Fig. 2. Antigen-antibody crossed electrophoresis of serums. (A) Normal individual (*n/n*). (B) Heterozygous for slow variant (*n/s*). The α_1-antitrypsin peaks *a* and *b* from normal individual are of similar height; in *n/s* peak *a* is higher than peak *b*. After starch gel electrophoresis a longitudinal strip of starch was removed and placed on 1 percent agarose gel (containing 4 percent specific antiserum to α_1-antitrypsin) on a glass plate. Electrophoresis, in calcium lactate containing 0.051*M* barbital buffer, *p*H 8.65, in horizontal direction (1.5 hours, 10 volt/cm). After the second electrophoresis the starch strip was removed, and the plate was kept in 0.15*M* NaCl for 12 hours, then dried and stained.

Using the conditions described in the legend to Fig. 1, we examined a number of variations of the α_1-at. We discovered a family, some of whose members were heterozygous for a slow α_1-at variant, from an examination of the serum by antigen-antibody crossed electrophoresis with an alkaline *p*H for both electrophoretic separations. Under these conditions the results (Fig. 3) were very similar to those of Axelsson *et al.* (*2*). The two mutations are probably indistinguishable, or even identical. The electrophoretic pattern in starch gel at *p*H 4.95 of serums from those heterozygous individuals is illustrated in Fig. 1 (*n/s*). In addition to

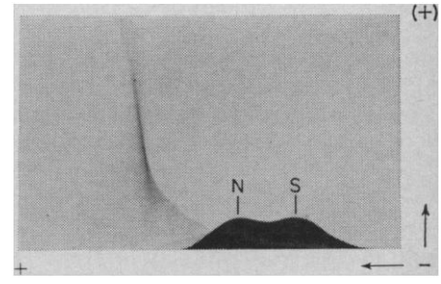

Fig. 3. Antigen-antibody crossed electrophoresis of serum from an individual heterozygous for the slow α_1-antitrypsin variant. The supporting medium was agarose. Calcium lactate containing barbital buffer, *p*H 8.65, for both electrophoretic separations. Double-peaked precipitate is due to α_1-antitrypsin. Normal antitrypsin (*N*), slow variant (*S*). The faint immune precipitate anodal of the normal α_1-antitrypsin is due to serum albumin.

the two main bands (*a* and *b*) which had a lower protein concentration than commonly observed in normal homozygous individuals, a more slowly moving band was seen. If two molecular species of α_1-at are present in these individuals, and both species can be resolved into three components, the observed pattern could be explained by partial overlapping of the bands. The slower of the two main bands of the normal α_1-at would coincide with the faster main band of the variant, thus leading to the higher protein concentration of the middle band (*a*) as seen in Fig. 1. This interpretation is consistent with the antigen-antibody crossed-electrophoresis experiments, in which the height of the peaks is a function of the antigen concentration (Fig. 2B). The weak anodal band (*c*) is unlikely to be clearly visible because, in the heterozygous state, the concentration of the protein should be approximately 50 percent of that normally present in this position. With the more sensitive antigen-antibody crossed electrophoresis, however, a small amount of material can be detected in position *c*.

The third phenotype examined was from an individual heterozygous for the α_1-at deficiency gene in whose serum half the normal trypsin inhibitory capacity was found. In this case, a normal but more weakly staining banding pattern was observed (Fig. 1, +/−). The observed phenotypic variations in the serum α_1-at appear similar to those electrophoretic patterns reported under the designation of "prealbumin" variants (*8*). Since the *p*H of the buffer in our experiments was similar to that used for the disclosure of the "prealbumin" variants, these bands probably represent variations in the serum α_1-at. If this is correct, the phenotypes not yet seen are those designated "SS" and "FS" (*8*). The "SS" pattern had one strong band approximately in position *a*, a second strong band moving cathodally of *a*, and a faint one in position *b*. The "FS" pattern showed three weak bands in positions *a*, *b*, *c*, and one moving cathodally of *a* (Fig. 1). The pattern of the "SS" phenotype would be expected to occur in an individual homozygous for the slow variant, whereas the "FS" pattern would occur in an individual who was heterozygous for the α_1-at deficiency and the slow variant. Such an observation would imply that the deficiency gene and the gene for the slow variant are probably situated at two different loci. Several authors have observed genetic variation in the electrophoretic region preceding the albumin band in species other than man (*9*). Some of these genetic variations may also represent variations in the serum α_1-at.

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10. Some of the serum samples used in the experiments were kindly supplied by Dr. C. Hames, Claxton, Georgia. Work supported in part by PHS grant GM-577 from the Institute of General Medical Sciences and by PHS grant AM01542-10 and PHS grant HEO-3341-09.

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Ribosomes from Pear Fruit

Abstract. *Ribosomes, subunits and polysomes, have been isolated from cells of ripening (senescent) fruit. Sedimentation in sucrose gradients, sedimentation constants, and electron micrographs confirm the physical resemblance of fruit ribosomes to those from other living tissues.*

Ribosomes have been isolated from pea seedlings (*1*), corn root tips (*2*), and several other actively growing plant tissues (*3*), but there are no reports of their isolation from the moribund cells of senescent (ripening) fruit. Fruit tissues have been used extensively in the study of cellular senescence (*4*) and, more recently, in that of intracellular responses to massive irradiation (*5*). Since both phenomena are underlain by protein synthesis and associated mechanisms, the isolation of ribosomes is vital to their further study.

Paucity of cytoplasm and interference from vacuolar contents, principally acids and phenolics, impede the isolation of intracellular organelles from fruit tissues. To increase cell breakage and effect good control of *p*H, we immersed 120 g of peeled and grated fleshy tissue from D'Anjou pears in liquid nitrogen in a large mortar along with 90 ml of medium containing 0.01 *M* potassium phosphate buffer, *p*H 6.4; 240 μmole of cysteine; 5 percent sucrose; 6 m*M* of $MgCl_2$ plus 12 ml of 1 percent deoxycholate and 12 ml of 10 percent polyvinylpyrrolidone (PVP); and sufficient 1 *M* KOH

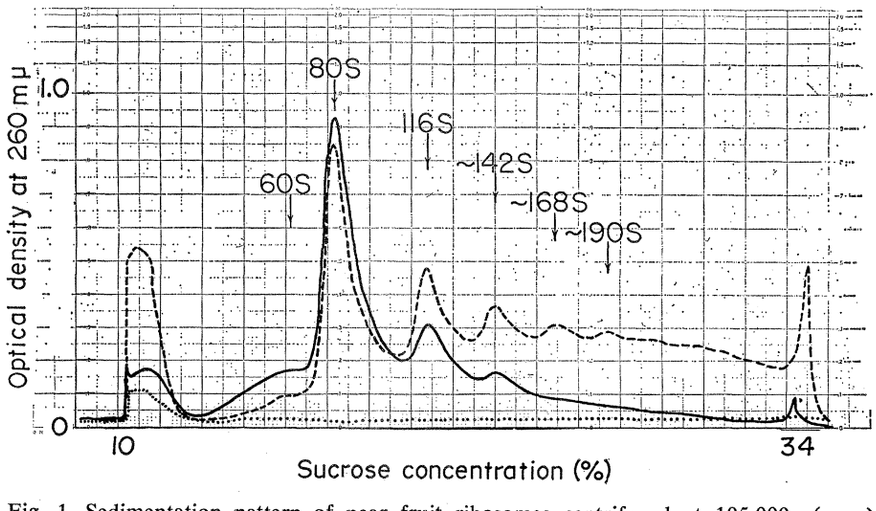

Fig. 1. Sedimentation pattern of pear fruit ribosomes centrifuged at 105,000*g* (——) in sucrose gradients after clarification and removal of the fraction sedimenting at 78,000*g* (- - - -). Omission of polyvinylpyrrolidone (PVP) from the isolation medium precludes recovery of ribosomes (. . . . .). The sucrose gradient (10 to 34 percent weight per volume) was centrifuged for 4 hours at 24,500 rev/min.