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Identical structural changes in inherited albumin variants from different populations

(bisalbuminemia/proalbumin/alloalbumin frequencies/genetic polymorphism/plasma protein variants)

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ABSTRACT **Alloalbuminemia is rare and has a cumulative frequency of only ≈1 in 3000 in Europeans and Japanese. The worldwide ethnic and geographic distribution of certain albumin genetic variants appears to be nonrandom. Moreover, we have found that structurally identical variants may occur at different frequencies in ethnically distinct populations, presumably owing to independent mutations. In this study, albumin B and two types of proalbumins, which as a group are the most common European albumin variants, have also been found in Asians. We have identified the amino acid substitution characteristic of albumin B (glutamic acid → lysine at position 570) in alloalbumins from six unrelated individuals of five different European descents and also in two Japanese and one Cambodian. The two types of proalbumins most common in Europe (Lille type, arginine → histidine at position −2; Christchurch type, arginine → glutamic acid at position −1) also occur in Japan. These results provide evidence for independent mutations at single sites in the albumin genome. The clustering of these and of several other amino acid exchanges in certain regions of the albumin molecule suggests two possibilities: that certain sites are hypermutable or that mutants involving certain sites are more subject to selection than mutants involving others.**

Alloalbuminemia is an inherited condition, usually rare and heterozygous (bisalbuminemia), in which some structural variant of common serum albumin (albumin A) is expressed without significant clinical symptoms (1–3). More than 100 alloalbumins have been identified and given geographical or ethnic names since the albumin B variant was detected (3). There are three levels of frequency of albumin variants: (*i*) Rare variants such as albumin B and two types of proalbumins, which collectively are the most common alloalbumins in people of Caucasian descent in Europe and America; these have a combined frequency of 1 in 3000 to 1 in 10,000 (1, 2). (*ii*) "Private" variants that have a polymorphic frequency (>1%); these are found in certain minimally admixed, small tribes of South American Indians such as the Yanomama and Maku (4–6). (*iii*) True polymorphic variants of which there are two (Naskapi and Mexico); these are widespread in many North American Indian tribes (3, 7). The polymorphic variants and some rare ones were discovered by geneticists who surveyed many erythrocyte and plasma proteins by using a series of electrophoretic systems and assays (3–6). Also, many apparently different, rare albumin variants have been identified by electrophoresis incidental to clinical study or blood donor screening (1, 2). Alloalbumins have been studied structurally by us (6–11) and by groups in Italy (12–13), New Zealand (14), and France (2, 15).

The structural changes in about 30 named alloalbumins have recently been identified, and at least 15 different sites of substitution have been established (6–16). Three types of alloalbumins have been identified: (*i*) single-point mutants (6, 7, 10, 11, 13), (*ii*) chain-termination mutants (12), and (*iii*) proalbumins (8, 14–16). An inherited proalbumin variant is a serum albumin molecule that retains a basic amino-terminal peptide because of a mutation in the Arg-Arg sequence required for post-transcriptional processing (8, 14–16).

This investigation consists of a structural study of 12 alloalbumins collected from diverse ethnic and geographic sources. All had similar slow mobility at pH 8.6, equivalent to an increase of +2 in unit charge compared to albumin A. Ten of these—six from five different European nationalities, one from a biracial individual, two from Japanese, and one from a Cambodian—were shown by structural study to be identical to albumin B in having the single amino acid change of glutamic acid replaced by lysine at position 570, denoted 570 Glu → Lys. The other two alloalbumins were from Japanese and represented the two types of proalbumins earlier identified in Caucasians—i.e., the Christchurch type (−1 Arg → Gln) (14) and the Lille type (−2 Arg → His) (15). The ethnic and geographic distribution of these three types of alloalbumins indicates that each of the three mutations has occurred independently several times in different populations. About half of all other reported albumin mutations are clustered nearby these two sites, one at the amino terminus, the other at the carboxyl terminus of the molecule.

MATERIALS AND METHODS

Sera from 12 unrelated individuals, each with an albumin variant discovered by clinical electrophoresis, were obtained from various sources identified later. We selected these sera on the basis of the alloalbumin mobility from >100 alloalbuminemic sera in our collection. Some specimens, such as the alloalbumin designated albumin B, had been described as long ago as 1959 (17–19); others have not previously been reported. All the sera were from heterozygotes and had a ratio of variant to normal of about 50:50. This is referred to hereafter as A/B. The following methods described previously (6–10) were used for characterization and structural study and are referred to hereafter as the standard procedure: (*i*) cellulose acetate (Microzone) electrophoresis at pH 8.6 in a Beckman model R101 apparatus (10) (Fig. 1), (*ii*) purification of the total albumin by HPLC, reduction and carboxymethylation, cleavage with CNBr (9–11), (*iii*) analytical isoelectric focusing of the CNBr digest to identify the variant fragment (6, 10, 11), (*iv*) HPLC peptide mapping of the CNBr digests of the carboxymethylated albumins (9–11), (*v*) HPLC peptide mapping of tryptic and *Staphylococcus aureus* V8

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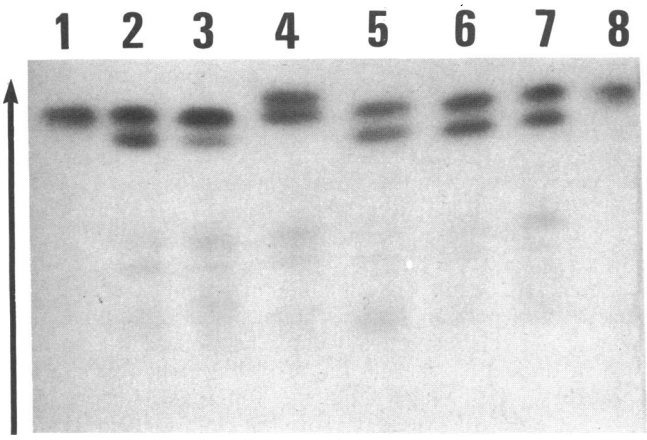

FIG. 1. Electrophoretogram at pH 8.6 of alloalbumin-containing sera: normal albumin A (lanes 1 and 8), proalbumins Fukuoka-2 (lane 2) and Gainesville (lane 3), albumin Naskapi (lane 4), and B albumins Tokyo-1 (lane 5), serum 3006 (lane 6), and Phnom Penh (lane 7).

protease digests of purified CNBr fragments (11), and (*vi*) amino acid analysis of the variant peptide(s) with the Beckman model 121M amino acid analyzer and automated sequence determination with the Beckman model 890C sequencer (9). Proalbumins were identified by their susceptibility to limited tryptic digestion and by $^{63}Ni^{2+}$ radioautography (8). For standards we used commercial human serum albumin and a series of albumin variants for which we had previously identified the sites of substitution (6–11). Purification of the total albumin (A/B) from the serum of a heterozygote was accomplished by ion-exchange chromatography followed by gel filtration chromatography (10, 11). Proalbumins were purified by HPLC (8).

For variants that appeared to be of the B type, analytical isoelectric focusing indicated the substitution was in CNBr fragment CB7 (residues 549–585), and the variant fragment was designated CB7B. The CNBr fragments were purified by reversed-phase HPLC using conditions described by Huss *et al.* (11) except that elution was for 60 min with a linear gradient from 30% to 50% buffer B. Unless otherwise stated, the variant CB7B fragment was eluted together with the normal CB4 fragment, and the two were pooled for structural study. The peptides of a tryptic digest of the pooled normal CB4 and the variant CB7B fragments were purified by reversed-phase HPLC (Fig. 2), and their amino acid composition and sequence were determined (9–11). Initially, this highly reproducible set of procedures was applied to albumin A and to all the B-type albumins except for albumin Oliphant, for which the automated tandem HPLC method (9) was used to purify the variant peptides from a tryptic digest. In later work with albumins Phnom Penh, Ann Arbor, and serum A027-001, an *S. aureus* V8 protease digest of the purified CNBr fragments (CB4 and CB7B) was prepared, and the peptides were purified by HPLC. Because albumins Fukuoka-2 and Fukuoka-3 were shown to be proalbumins by radioautography and limited tryptic digestion, the structural change was determined directly by amino acid sequence analysis of the purified carboxymethylated proteins.

RESULTS AND DISCUSSION

The Albumin B Described by Earle *et al.* (17, 18). Although bisalbuminemia had previously been described clinically by others, Earle *et al.* in 1959 (17) reported a variant they designated albumin B. It was present in 25 of 43 members of a family of Norwegian descent living in Illinois. From Karl Schmid (Boston University Medical Center, Boston, MA) we received serum from one family member and also some

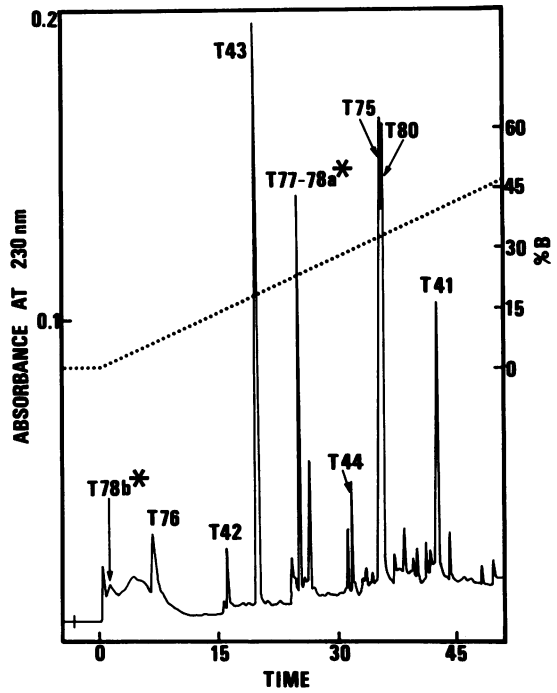

FIG. 2. HPLC elution profile on a Vydac C_{18} column of a tryptic digest of peptides CB4 and CB7B of albumin A/B (serum 3006). Time is in min. The lyophilized tryptic digest was dissolved in 0.1% trifluoroacetic acid (buffer A) and eluted at a flow rate of 1 ml/min over 60 min with a linear gradient from 0% to 60% buffer B (acetonitrile/0.1% trifluoroacetic acid). Tryptic peptides are given the prefix T and are numbered consecutively in their order in the protein sequence (9). T77-78a* and T78b* denote the two new (variant) peptides containing the amino acid substitution. T78 from albumin A is absent because the normal CB7 fragment (CB7A) was separated by HPLC.

lyophilized albumin. The albumin had been purified by the Cohn ethanol method, which did not separate A from B (17), and we repurified the albumin by HPLC, which also did not separate A from B.

When either albumin A or its CB7 fragment is digested with trypsin, a series of overlapping peptides result because of incomplete cleavage after Lys-564 and Lys-573 (Fig. 3). The three acidic residues neighboring Lys-564 inhibit cleavage, with the result that the theoretical peptides T77 and T78 remain joined and appear predominantly as T77-78. The substitution 570 Glu → Lys that is characteristic of albumin B produces a new peptide, T77-78a*, because of cleavage after Lys-570; concurrently, other new peptides, T78b*, T78b*-79, or both, are formed. In this case we isolated peptides T77-78a* and T78b*-79 and determined their se-

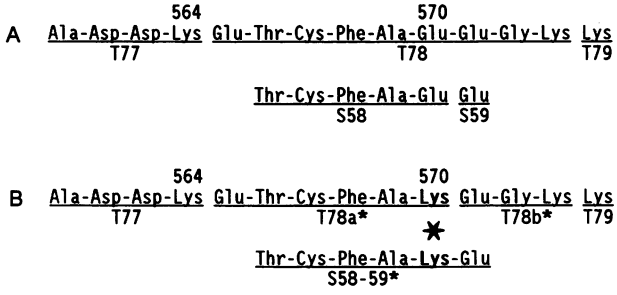

FIG. 3. Amino acid sequence analysis of albumin B variant peptides. (*Upper*) Tryptic and *S. aureus* V8 protease (prefix S) peptides of albumin A; (*Lower*) peptides of albumin B. The large asterisk and boldface type mark the substitution 570 Glu → Lys. Smaller asterisks identify new peptides generated by this substitution.

quences (Fig. 3). This established that the very albumin that was originally designated albumin B actually has the substitution 570 Glu → Lys, which was later reported by Winter *et al.* (19) for a B-type albumin designated Oliphant. This substitution increases the charge at pH 8.6 by +2 units, which accords with the mobility change. The amino acid substitution accords with a single-base change in the codon GAG (20) to AAG.

Albumins B Oliphant, B Ann Arbor, and B (Serum A027-001). Four sera containing B albumins were provided by Lowell R. Weitkamp (University of Rochester Medical Center, Rochester, NY). We studied only three of these because the fourth came from the family reported by Earle *et al.* (17). These valuable specimens had served as reference sera for electrophoretic screening of albumin variants in many surveys of population genetics, such as that of Weitkamp *et al.* (4, 21).

Albumin B Oliphant. The substitution in albumin Oliphant, from a family of German descent, had been reported by Winter *et al.* (19) to be 570 Glu → Lys on the basis of tryptic peptide mapping and manual sequence analysis of several tryptic and chymotryptic peptides, including T78b*-79 and T78b* of Fig. 3. However, we undertook to confirm their results and to extend them to the other albumin specimens. Albumin (A/B Oliphant) was purified from serum A003-002 and the substitution 570 Glu → Lys in the B albumin was confirmed by sequence analysis of peptide T77-78a*. This peptide was isolated by T. Isobe, from a tryptic digest of the carboxymethylated albumin (A/B) by means of our tandem HPLC system (9); however, the expected small peptides T78b* and T78b*-79 were not separated by this method.

Albumin Ann Arbor. This albumin variant (serum A001-026) was inherited in an American family of Danish descent and had been typed as an albumin B electrophoretically (4) and also by peptide mapping but not by sequence analysis (19). In this case we used a TSK G3000 SW column (Tosoh, Tokyo) to purify the CNBr fragments, and the normal CB7A and variant CB7B fragments eluted together. An *S. aureus* V8 protease digest of these was fractionated by HPLC, yielding the normal peptide S58-59 and the variant peptide S58-59* (Fig. 3). Peptide S58-59* had the sequence Thr-Cys-Phe-Ala-*Lys*-Glu, which represents positions 566–571 in albumin B. This new peptide results because of the substitution 570 Glu → Lys. Both the normal and the variant peptides end with Glu-571 because a Glu-Glu sequence resists cleavage by the V8 protease.

Albumin B (serum A027-001). This specimen had earlier been typed electrophoretically as an albumin B (4) and was identified as family 20 by Weitkamp *et al.* (21) in a paper that listed 14 different families with albumin variants having mobilities indistinguishable from B. The individual was of biracial (Black/White) descent. Our experimental approach and results were identical to those described above for albumin Ann Arbor; that is, the variant peptide was isolated by HPLC, and sequence analysis proved that it was peptide S58-59*.

Thus, four alloalbumins previously typed as albumin B by Weitkamp *et al.* (4, 21) and used as reference specimens by them and by other investigators did have the same amino acid substitution, 570 Glu → Lys. This finding confirms the usefulness of a combination of electrophoretic systems as a screening procedure for identifying plasma protein polymorphisms in studies of population genetics. Of course, nonidentical variants may appear to be the same even in several electrophoretic systems; therefore, structural studies are required to establish the nature and site of the structural change.

Albumin B (Serum 3006). This alloalbumin had been identified by J. M. Fine (Centre National de Transfusion Sanguine-Institut, Paris) in a systematic electrophoretic screening of 30,000 French blood donors and was judged to be the B type on the basis of its mobility in several electrophoretic surveys (2, 4). The albumin (A/B) was purified and subjected to our standard procedure, including tryptic digestion. Two tryptic peptides, T77-78a* and T78b*-79, were isolated; their amino acid compositions and sequences were determined, thereby establishing the substitution 570 Glu → Lys. Altogether, Fine *et al.* (2) identified 15 examples of the B type of variant by use of their electrophoretic methods (see later).

Albumin B London. This alloalbumin was inherited and had been the subject of an immunochemical study, but it had not been typed electrophoretically (22). The proband was born in England of Scottish parents, but resided in London, Ontario. Through C. Laschinger (National Reference Laboratory, Canadian Red Cross Society, Toronto, Ontario) we received normal and variant albumins that had been separated by the late Derek Naylor. Ethnic background was supplied by W. B. Chodirker (University Hospital, London, Ontario). We purified the variant albumin further and submitted it to our standard procedure. Peptides T77-78a* and T78b* appeared in the HPLC profile of the tryptic digest; amino acid analysis and sequence determination established the substitution 570 Glu → Lys.

Albumin B Lübeck. This serum was sent to us by K. Heyne (Medizinische Universität, Lübeck, F.R.G.) with a request for structural study because of the possibility that it might be a new variant. Inheritance had been established. We were informed that albumin Lübeck behaved as an albumin B in electrophoretic typing but it appeared to differ from albumin B in dye-binding affinity, a property proposed as an additional criterion for differentiating albumin variants (1). The total albumin (A/Lübeck) was purified, and structural study was done by our usual procedures. Two pure peptides were isolated from a tryptic digest of CB4 and CB7B. One peptide had the sequence of T77-78 and thus was derived from the albumin A in the mixture of the two albumins. The other peptide was identical in sequence to T77-78a*, and thus resulted from the substitution of 570 Glu → Lys; this showed that albumin Lübeck is a type B. A double sequence was obtained from another peptide peak. One sequence (Ala-Asp-Asp-Lys) was identical to that of T77, so this peptide could have been derived from either or both albumins A and B (Fig. 3). The other sequence (Glu-Gly-Lys) corresponded to T78b* and thus confirmed the substitution 570 Glu → Lys in albumin Lübeck. This result shows that structural study is the ultimate criterion for typing albumin variants and that even a careful combination of electrophoretic typing with other criteria may lead to ambiguous or erroneous conclusions.

Identification of Albumin B in Japanese. From various sources we received a total of 18 sera or plasmas from unrelated Japanese who are heterozygous with respect to albumin. Five of these are specimens from the Radiation Effects Research Foundation (RERF) Biochemical Genetics Study at Hiroshima and Nagasaki and are designated Hir-1, Hir-2, Nag-1, Nag-2, and Nag-3 (6). All of these differ in their sites of substitution, and none is a type B albumin or proalbumin. The other 13 specimens were identified by clinical electrophoresis in various laboratories. Two specimens from unrelated individuals that are designated here as Tokyo-1 and Shinanomachi-1 were shown by structural study to be B-type albumins. We had no information on inheritance in either case.

Serum Tokyo-1 was provided by I. Sakurabayashi (Jichi Medical School, Tochigi-ken, Japan). The total albumin was investigated by our standard procedure, including tryptic digestion. HPLC peptide mapping revealed the presence of the two peptides characteristic of albumin B—i.e., T77-78a* and T78b*-79 (Fig. 3). Amino acid analysis and sequence

determination proved that the substitution was 570 Glu → Lys, as in the Caucasian albumin B type. A similar result was obtained for albumin Shinanomachi-1 purified from serum provided by T. Ohtake and S. Kano (Keio University Hospital, Shinanomachi, Tokyo). In this case, the sequence was determined for the variant peptides T77-78a* and T78b*, which are characteristic of albumin B and prove the substitution 570 Glu → Lys. The sequence also was determined for peptide T77-78-79, which is characteristic of albumin A, and for T77, which can be derived from both albumin A and albumin B.

Albumin Phnom Penh. This serum, which was provided by C.-B. Laurell (Malmö General Hospital, University of Lund, Malmö, Sweden) was from a patient from Cambodia (Kampuchea) who was receiving treatment at Malmö. The family was Cambodian and had lived in the vicinity of Phnom Penh for several generations, but it was not feasible to ascertain inheritance of the trait. As in the case of albumin Ann Arbor, we used *S. aureus* V8 protease to digest the purified CNBr fragments (in this case normal CB4 + variant CB7B). Fractionation of the V8 protease digest by HPLC yielded the variant peptide designated S58-59*; sequence analysis of this proved the substitution 570 Glu → Lys that is characteristic of albumin B (Fig. 3). Because this procedure gives proof of the substitution by sequence analysis of a single peptide, we recommend it for use for future verification of the substitution of albumins assumed to be of the B type.

Proalbumin Fukuoka-2. Proalbumins Fukuoka-2 and Fukuoka-3 were provided by Shunsuke Migita (Cancer Research Institute, Kanazawa University, Kanazawa, Japan), who had received them from K. Ookouchi (Kyushu University, Kyushu, Japan) for our study. No family studies or other data were available. Our preliminary study suggested that Fukuoka-2 was a proalbumin as indicated by its mobility, its susceptibility to limited tryptic digestion and its reduced affinity for $^{63}Ni^{2+}$ compared to albumins A and B. Amino acid sequence analysis for 10 cycles on two different preparations established that Fukuoka-2 was a proalbumin of the Lille type with the substitution −2 Arg → His. The unfractionated albumin gave a double sequence; one sequence was that of albumin A, the other that of the proalbumin. The purified proalbumin gave a single sequence: Arg-Gly-Val-Phe-*His*-Arg-Asp-Ala-His-Lys. In this sequence the histidine residue has replaced an arginine in the normal propeptide Arg-Gly-Val-Phe-*Arg*-Arg. The substitution −2 Arg → His accords with the single-base mutation C*G*T → C*A*T.

Proalbumin Fukuoka-3. Fukuoka-3 also appeared to be a proalbumin as judged by the criteria described above. Sequence analysis for 10 cycles on two preparations showed that Fukuoka-3 was a proalbumin of the Christchurch type with the substitution −1 Arg → Gln. The unfractionated albumin gave a double sequence in approximately equal ratio. The partially purified proalbumin also gave a double sequence in which the ratio of the proalbumin sequence to that of albumin A was about 3:1. In this case, the propeptide sequence was Arg-Gly-Val-Phe-Arg-*Gln*, corresponding to the substitution −1 Arg → Gln. This substitution is attributable to a single base change in the codon, C*G*A to C*A*A.

Frequency of Albumin B and of Proalbumins in Non-European Populations. No alloalbumin corresponding electrophoretically to type B had been described in extensive genetic surveys in Japan (6, 23), and albumin B been not been reported by Japanese clinical laboratories. Nor had albumin B been reported in the North and South American Indian tribal groups that have undergone comprehensive genetic analysis (3–5). Thus, prior to this investigation, the B type of alloalbuminemia had appeared to be a Caucasian trait. However, despite our identification of albumin B in two Japanese, the frequency of the B variant in the Japanese population must be very low. Albumin B was not reported in the RERF Biochemical Genetics Study of a cohort of 15,581 unrelated children (independent samples) in which five new albumin variants were discovered (6, 23). The total number of samples examined was 23,661. Nor was a proalbumin identified in that study. We have no information on the frequency of albumin B in Cambodia. Albumin B has not been reported in tribal Amerindian and mixed-race populations in Brazil (4). Also, F. Salzano (Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil) has informed us that the only example he had seen in his surveys occurred in an individual of German descent (personal communication).

The frequency of proalbumins in non-European populations also appears to be low. Thus far, proalbumins have not been identified through the genetic screening of Amerindians (3–5) or Japanese (6, 23) and have only rarely been found in Japanese by clinical electrophoresis. Matsuda *et al.* (16) did report that albumin Tokushima was an inherited proalbumin of the Lille type (−2 Arg → His). Also, we found that albumin Taipei from a Chinese in Taiwan was a Lille type of proalbumin, and we discovered a third type in proalbumin Takefu from Japan (−1 Arg → Pro) (8).

Frequency of Albumin B and of Proalbumins in Individuals of European Descent. Estimates of the incidence of alloalbumins in individuals of European descent vary but are in the range of only 1 in 1,000 to 1 in 10,000 in sera examined in clinical electrophoresis (1, 2). Extensive data from genetic screening are not available. In a personal communication, J. M. Fine (Centre National de Transfusion Sanguine–Institut, Paris) informed us that proalbumins Christchurch and Lille and albumin B account for the majority of albumin variants detectable by clinical electrophoresis in France. In the screening of 30,000 blood donors, his laboratory detected 12 variants of 5 different allotypes for an overall frequency of 0.0004. The distribution among the allotypes was proalbumin Lille, 4; albumin B, 2; all other variants, 6. When the results for 32 sera sent for typing were included, the cumulative distribution of the 44 variants was proalbumin Christchurch, 10; proalbumin Lille, 7; albumin B, 15; all other variants (six types), 12.

In response to our inquiry Carl-Bertil Laurell reviewed 9388 serum samples (from some 7000 patients) that had been analyzed by agarose electrophoresis at the Department of Clinical Chemistry, University of Lund, Malmö, Sweden, and he recognized 18 subjects with atypical variants (personal communication). The most common variant observed clinically was the broad albumin band, for which the difference reasonably is one charge unit. In two instances the variant moved more slowly, with a mobility similar to proalbumins or albumin B. We subsequently received one of these, which we found to be an albumin of the B type, but this was from a Cambodian (albumin Phnom Penh described above).

Of course, clusters of even a rare genetic marker may occur because of consanguinity. In the most extensive study of the inheritance of albumin B, Frohring (24) identified it electrophoretically in five generations and traced the genealogy of 63 heterozygous carriers (A/B) to 117 direct ancestors over 14 generations and four centuries. Thus, albumin B must have appeared in this kindred before the 17th century. Another cluster occurs in Italy, where 36 carriers of albumin B Verona were identified (25) and the structural change was verified in one case (13). Altogether, albumin B and proalbumin types Christchurch and Lille are the most common allotypes reported in Italy, France, and Germany. These three predominant European alloalbumins might in each case be ascribed to a common ancestral gene widely dispersed, perhaps through the Roman migrations. However, the Asian examples must have arisen through independent mutations.

Evidence That 570 Glu → Lys Is the Only Structural Change in Albumin B. The electrophoretic behavior of albumin B in

a series of systems ranging from pH 5.0 to pH 8.6 (1–4, 13, 24) is fully consistent with the single substitution 570 Glu → Lys. Although the absence of other structural changes could be ruled out only by complete sequence analysis of all the specimens, much other evidence supports our conclusion. In our study, isoelectric focusing clearly identified CB7 as the variant polypeptide. HPLC peptide mapping and amino acid analysis of the tryptic and *S. aureus* V8 protease peptides of CB7 confined the substitution to 570 Glu → Lys. Furthermore, peptides representing about 80% of the total albumin sequence were identified by the automated tandem HPLC method (9), and T77-78a* was the only variant peptide found by this procedure in albumin Oliphant.

Independent Mutations at Hypermutable Sites. The findings reported here give structural evidence that a rare protein allotype has arisen through independent mutations in diverse populations. The point mutation of albumin B (570 Glu → Lys) has occurred in unrelated families of two races representing eight nationalities. In view of the relative frequency of albumin B compared to other nonpolymorphic alloalbumins and its wide geographical and ethnic dispersion, we suggest that the albumin B substitution may reflect a hypermutable site in the gene. Another possibility is that certain mutated sites in the albumin gene are more subject to selection than other sites. However, no selective advantage for any albumin variant has yet been established. In the albumin molecule the albumin B site is located in a flexible COOH-terminal segment or tail that extends from a rigid three-domain structure composed of nine loops that are yoked together by 17 disulfide bonds. The COOH-terminal tail, which contains only 18 residues and is predicted to be largely α-helical, is the site of a cluster of mutations that result in a total of four different albumin allotypes with several others nearby (Fig. 4), or about one-third of the total reported to date. As shown previously (8) and in this work, a similar cluster of mutations occurs in the propeptide and

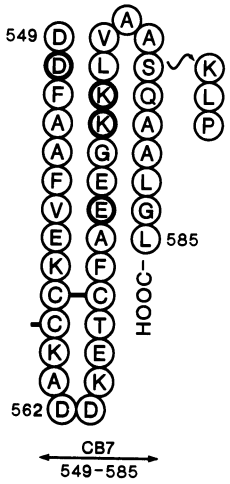

FIG. 4. Amino acid sequence in the one-letter code for CB7 (residues 549–585), the COOH-terminal CNBr fragment of human serum albumin. Boldface circles mark single-point amino acid exchanges in albumin genetic variants: 550 Asp → Gly, albumin Mexico (7); 570 Glu → Lys, albumin B (see text); 573 Lys → Glu, albumin Mi/Fg (Gent) (26); 574 Lys → Glu, albumin Vanves (13). In albumin Catania, which is a chain-termination mutant, the COOH-terminal sequence beginning at Gln-580 is replaced by the sequence Lys-Leu-Pro (12). A nearby mutation not shown is 541 Lys → Glu, albumin Maku (6).

NH_2-terminal segment of albumin. Similar clusters may be anticipated at interdomain hinge segments that are not essential for maintenance of the rigid three-domain structure of the albumin molecule.

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