Hemoglobin istanbul: substitution of glutamine for histidine in a proximal histidine (F8(92)β)

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Research Article

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Hemoglobin Istanbul: Substitution of Glutamine for Histidine in a Proximal Histidine $(F8(92)\beta)$

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A B S T R A C T A presumably spontaneous mutation has resulted in the formation of Hemoglobin (Hb) Istanbul in which glutamine is substituted for histidine in the proximal position of the β-chain (F8(92)). The anemia and other physiological effects that occur in the presence of Hb Istanbul were much ameliorated by splenectomy. Hb Istanbul is a relatively unstable molecule which produces a rather moderate case of "unstable hemoglobin hemolytic anemia."

In the determination of structure, a method of preferential cleavage of an aspartyl-proline bond at residues 99–100 of the β-chain was used.

INTRODUCTION

The work of Perutz, Muirhead, Cox, and Goaman (1) has provided a detailed model of contacts and interactions of the various parts of the hemoglobin molecule. Consequently, when an aberrant hemoglobin is identified, it is possible to examine the position of the variation in the framework of the molecule and to consider to what extent the normal functioning of the molecule may be disrupted by the substitution. In many recent detections of an abnormal hemoglobin, the very instability has indicated that identification would show substitution in a sensitive position. Substitutions of histidyl residues proximal or distal to the heme iron,

as exemplified by the hemoglobins M, have interfered with the normal functioning of hemoglobin and resulted in untoward clinical symptoms. We report on a substitution of the proximal histidine of the β-chain that produces no severe clinical symptoms in the carrier.

CASE REPORT

A 29 yr old unmarried white male (K. K.) from the vicinity of Istanbul presented at the Istanbul Medical School in 1966 with lassitude, jaundice, and anemia. Since the age of 6 yr, he had had several bouts of jaundice as well as arthralgia in his knees which increased when he walked or climbed stairs. Despite these complaints, he was not seriously incapacitated. During prior medical attention at another clinic, he had received iron and liver extract injections. A liver biopsy was normal.

Physical examination revealed a well-developed and well-nourished male with mild jaundice. The liver was enlarged two and the spleen four finger breadths below the costal margin. A bone survey revealed no abnormalities. Hematological and other laboratory data were as follows: RBC $3.60 \times 10^6/mm^3$, Hb[1] 9.1 g/100 ml, PCV 32%, MCV 88 μ^3, MCH 25 pg, MCHC 28%, platelets 290,000/mm^3, reticulocytes 4.2%, and WBC 5,000/mm^3 with 66% polymorphonuclear neutrophils, 4% band forms, 1% basophil, 3% monocytes, and 26% lymphocytes. Moderate anisocytosis, hypochromia, poikilocytosis, and occasional target cells were apparent in the peripheral blood smear, but inclusion bodies were absent. Starch-gel electrophoresis in Tris-EDTA-borate buffer of pH 9 and borate buffer at pH 8.6 showed 12–15% of an abnormal component that moved between

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[1] *Abbreviations used in this paper:* Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; WBC, white blood cells.

hemoglobins A₂ and S (2). Quantitative osmotic fragility tests with and without incubation at 37°C for 24 hr were normal. The total serum bilirubin (all indirect reacting) was 3 mg/100 ml. The serum iron was 160 μg/100 ml. Erythrocyte glucose-6-phosphate dehydrogenase activity was within normal limits. The direct Coombs's test was negative. Urobilinogen excretion was increased and fecal urobilinogen was 400 Ehrlich U/100 g feces per 2 hr. The serum cholesterol was 160 mg/100 ml. Serum electrophoresis, serum alkaline phosphatase, and liver function tests (including the Bromsulphalein [Hynson, Westcott & Dunning, Inc., Baltimore, Md.] excretion test, prothrombin time, glutamic acid oxalacetic acid transaminase, and glutamic acid pyruvic acid transaminase) were within normal limits.

After the patient had been splenectomized in 1968, microscopic examination of the spleen showed generalized passive congestion of the sinusoids with marked proliferation of the reticuloendothelial cells, but without excess of iron-staining pigment. 2 yr after splenectomy, the hematological data were as follows: RBC 4.80 × 10⁶/mm³, Hb 13 g/100 ml, PCV 48%, MCV 100 μ³, MCH 27 pg, MCHC 27%, platelets 564,000/mm³, reticulocytes between 1.2 and 4%, and WBC 6,700/mm³. Serum iron was 110 μg/100 ml. Total bilirubin was 1 mg/100 ml. Morphological abnormalities except for a mild ovalocytosis had disappeared from the blood smear, and less than 1% of the cells had Heinz bodies. His previous complaints disappeared and he is able to lead a normal active life.

Ancestors of both parents had immigrated from Caucasia to Turkey. The father of the propositus, a 70 yr old white man, with moderate iron deficiency anemia which responded to iron therapy, his mother, a 65 yr old white woman, three brothers, and one sister were all in good health. Their hematologic data were within normal limits. Starch-gel electrophoresis and DEAE-cellulose chromatography failed to reveal an abnormal hemoglobin component in any of these relatives. Parentage was supported by data from extensive blood group typing.

METHODS

Blood samples from the propositus K. K. were drawn into heparin and shipped via air mail, special delivery without refrigeration to Georgia.

Hemoglobin studies. Electrophoresis of hemolysates containing about 100 mg/ml of hemoglobin was done at 6 v/cm overnight on starch gel in Tris-EDTA-borate buffer, pH 9.0 (3). The gels were stained with *o*-dianisidine and with Buffalo black (4). Chromatography on columns of DEAE-Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) followed previously described procedures (5, 6) except that developers were 0.025 M instead of 0.05 M. Chromatography of hemoglobin on columns of Sephadex G-75 (2.5 × 150 cm) in a buffer that contained 0.12 M glycine-NaOH and 0.12 M sodium chloride at pH 10.1 followed published methods (7, 8). The technique of Betke, Marti, and Schlicht (9) was used to quantitate the alkali-resistant hemoglobin. The heat stability of the hemoglobin in hemolysates was tested by incubation at 60°C in 0.1 M sodium phosphate buffer, pH 7.4, at a concentration of 1.5 mg/ml. The percentage of heat-precipitated hemoglobin was calculated from the amount that was originally present and that which remained in solution (10, 11).

Sedimentation velocity analyses. These analyses were made with a Spinco model E analytical ultracentrifuge

(Beckman Instruments, Inc., Fullerton, Calif.) at 60,000 rpm at 25°C (3, 12). Hemoglobin concentration was 0.5 g/100 ml. Buffers were 0.1 M KH₂PO₄-NaOH at pH 7.4 or 0.12 M NaCl-glycine-NaOH at pH 10.1. Each sample was dialyzed for 24 hr at 4°C against three changes of the appropriate buffer.

Isolation of the abnormal β-chain. The abnormal hemoglobin was isolated by DEAE-Sephadex chromatography (5, 6), and heme was removed by the acid-acetone procedure of Anson and Mirsky (13). The α- and β- (and δ-) chains were separated by CM-cellulose chromatography in urea according to Clegg, Naughton, and Weatherall (14). The S-β-aminoethyl (AE) derivative was prepared with ethylenimine (15).

Preferential cleavage of the abnormal β-chain. A 213 mg sample of abnormal AE-β-chain was dissolved in 40 ml of 70% formic acid and maintained at 50°C for 16 hr. After dilution with water and lyophilization, the sample was dissolved in 50% acetic acid and chromatographed on Bio-Gel P-100 (Bio-Rad Labs, Richmond, Calif.) with the same solution as eluent. For this separation, four 2.2 × 160-cm columns were used under conditions previously described (16).

Structural analyses. Approximately 130 mg AE-β-chain and 60 mg of the amino-terminal fragment from the preferential cleavage above (residues 1 thru 99 inclusive) were hydrolyzed with trypsin (Worthington Biochemical Corp., Freehold, N. J., crystallized three times, salt-free) in a pH-stat (Radiometer) at pH 9. The hydrolysis of the AE-β-chain was made at room temperature for 4 hr, and that of the fragment at 37° C for 24 hr; trypsin equal to 0.5% of the weight of the β-chain (or fragment) was added at zero time and again 1 hr later (β-chain) or 3 hr later (fragment). Upon completion of the hydrolysis, the pH was lowered to 2.5 with N HCl, and the solvent was removed by flash evaporation.

The peptides were separated by chromatography on a 1 × 100 cm column of Dowex 50-X2 (Bio-Rad Labs, Richmond, Calif.) with volatile pyridine-acetic acid developers (17, 18). Each Dowex-50 zone was rechromatographed on a column of Dowex 1-X2 (19).

Amino acid analyses were made with a Spinco model 121 automated amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) equipped with high sensitivity cuvettes and an Infotronics model CRS-110 A integrator (Infotronics Co., River Forest, Ill.). Samples were hydrolyzed for 24 hr (and on occasion for 72 hr) at 110°C under reduced pressure with 6 N HCl. Tryptophan was identified by spot test on paper (20).

RESULTS

Properties of the abnormal hemoglobin

Chromatographic and electrophoretic behavior. The electrophoretic behavior of hemoglobin from K. K. as compared to that of Hb's A, F, S, and C is shown in Fig. 1. Chromatographic separation is depicted in Fig. 2. Although not investigated further, the first zone to emerge may contain free α-chains. The percentage of A₂ is probably too high because of incomplete separation from Hb Istanbul. The size of the second zone of Hb Istanbul increases with age of the sample. Chromatographic isolation of Hb Istanbul requires the addi-
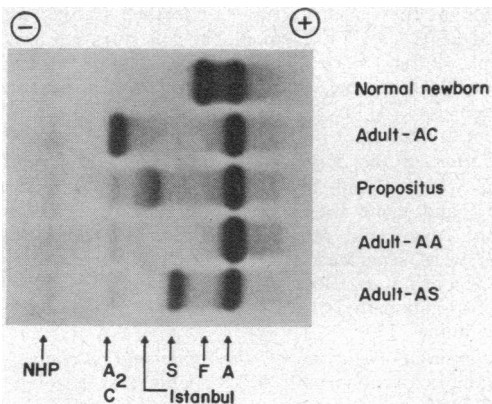
FIGURE 1 Starch-gel electrophoresis of hemoglobin from propositus K. K. in Tris-EDTA-borate buffer at pH 9.0. Amido black 10B stain. NHP designates nonhemoglobin protein fraction.

tion of potassium cyanide to the developers because otherwise the abnormal component appears in part as a broad zone in the chromatogram overlapping both Hb A_1 and Hb A, and is in part retained at the top of the column.

The approximately 15% of Hb Istanbul as determined from the chromatogram which was run in Georgia is in good agreement with the estimated 12–15% from starch-gel electrophoresis of a fresh sample in Istanbul; the sample, therefore, apparently did not deteriorate during shipment. After chromatographic isolation, Hb Istanbul has somewhat altered electrophoretic properties and is not entirely homogeneous electrophoretically. This behavior may reflect the instability and dissociation to be discussed below.

When the zone of $A_1 + F$ was examined by a recently developed method for Hb F (21), about a third of the zone of $A_1 + F$ was Hb F. Consequently, Hb F
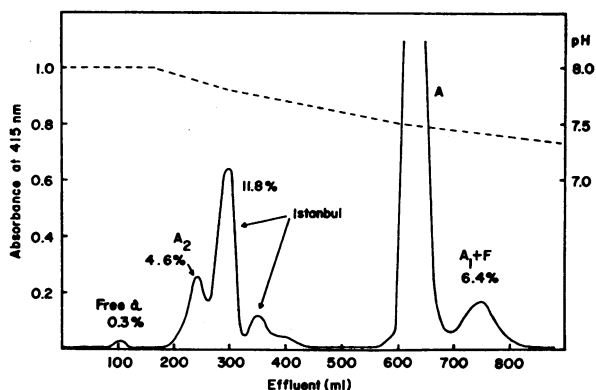

FIGURE 2 Chromatographic separation of the components from approximately 50 mg of hemoglobin from propositus K. K. on DEAE-Sephadex (0.9 × 55 cm).
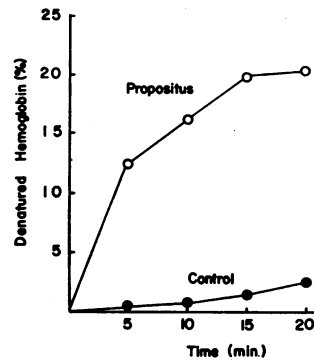

FIGURE 3 Partial instability at 60°C of the hemoglobin in the hemolysate of propositus K. K.

in K. K.'s hemolysate is about 2%. The ratio of $^G\gamma$ to $^A\gamma$ chains in this Hb F was examined by previously described methods (16); the ratio was about 2:3 as it is in Hb F of the normal adult (22).

Heat instability. During heating at 60°C there was partial precipitation of the hemoglobin in the hemolysate (Fig. 3). If the precipitate is comprised solely of Hb Istanbul, the amount (15–20%) is in approximate agreement with the chromatographically and electrophoretically estimated quantity.

Molecular weight and dissociation. Hb Istanbul dissociates to some extent at neutral pH and to a greater degree at high pH. Thus, S_{20w} from ultracentrifugal studies of Hb Istanbul was 3.39 at pH 7.4 and 2.57 at pH 10.1 in contrast to 4.12 and 4.01, respectively, for Hb A at the same pH's. Further evidence of dissociation, as detected by Sephadex filtration, may be seen in Fig. 4. Hemoglobin from a normal adult has a single peak presumably of the undissociated molecule. The
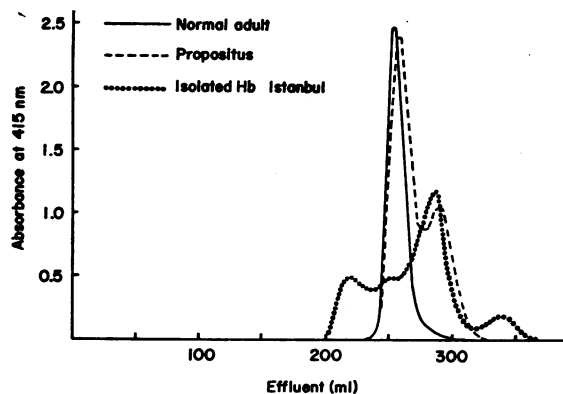

FIGURE 4 Elution profiles of hemoglobins from hemolysates of a normal adult and of propositus K. K. and of the isolated Hb Istanbul fraction on columns of Sephadex G-75. A load of 30 mg in 4 ml was applied to two 2.5 × 150-cm columns in series and developed with 0.12 M glycine-NaCl-NaOH buffer at pH 10.1.

smaller of the two peaks in the hemolysate of K. K. is mainly Hb Istanbul as shown by starch-gel electrophoresis; it also falls in the position that is occupied by the main part of a sample of isolated Hb Istanbul. However, the isolated component has a complex pattern that suggests both aggregation and dissociation and may well be responsible for the heterogeneity of chromatographically isolated Hb Istanbul in starch-gel electrophoresis.

Spectra and oxygen dissociation. Use of potassium cyanide during chromatographic isolation precluded a study of spectra and oxygen dissociation of Hb Istanbul.

Identification of the aberration

Separation of the chains. As depicted in Fig. 5, the β-chains of Hb Istanbul separate well from the α-chains as well as the δ-chains of contaminating Hb A$_2$ (Fig. 2). In the several separations of this kind that were made, the Istanbul β-chains always appeared in two peaks which had the same amino acid composition. Why two peaks appear is not known, but is presumably associated with the heterogeneity of the isolated Hb Istanbul as already described.

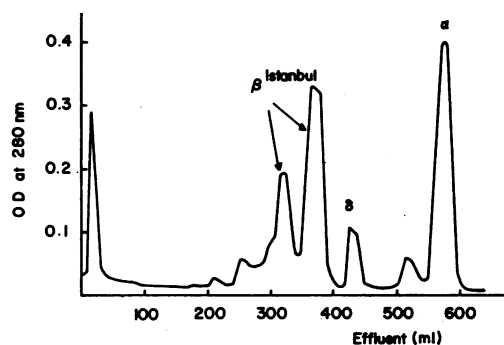*Amino acid analysis of the $\beta^{Istanbul}$ chain.* The amino

FIGURE 5 The separation of the chains from globin of 80 mg of Hb Istanbul on a 2×10-cm column of CM-cellulose in 8 M urea.

acid analysis of the AE-$\beta^{Istanbul}$ chain is compared with that of the normal β^A-chain in Table I. It is apparent that a *histidyl* residue has been replaced by a *glutamyl* or *glutaminyl* residue in Hb Istanbul. The substitution, then, should be in one of the following tryptic peptides: βT-1, βT-7, βT-9, βT-10, βT-11, βT-12, βT-14, or βT-15. The amino acid composition of the α-chain from Hb Istanbul was indistinguishable from that of the α^A-chain.

TABLE I

*Amino Acid Composition of AE-$\beta^{Istanbul}$ Chain and of Fragments from Preferential Cleavage**

	$\beta^{Istanbul}$	β^A (Theory)	Fragment residues 1–99		Fragment residues 100–146	
			Peak 2, Fig. 6	β^A (Theory)	Peak 4, Fig. 6	β^A (Theory)
Lysine	11.1	11	7.7	8	3.4	3
AE-Cysteine	1.4	2	0.8	1	0.6	1
Histidine	*7.8*	*9*	*3.9*	*5*	3.5	4
Arginine	3.0	3	1.8	2	0.8	1
Aspartic acid	12.7	13	10.1	10	3.7	3
Threonine	6.1‡	7	5.5‡	6	1.4‡	1
Serine	4.8‡	5	4.6‡	5	0.6‡	0
Glutamic acid	*12.0*	*11*	*8.3*	*7*	4.2	4
Proline	6.8	7	4.4	4	2.9	3
Glycine	12.6	13	10.3	10	3.7	3
Alanine	14.8	15	9.0	8	6.9	7
Valine	16.4‡	18	11.1‡	11	6.6‡	7
Methionine	0.6	1	0.8	1	0.2	0
Leucine	18.0	18	13.3	13	5.7	5
Tyrosine	3.2	3	1.1	1	1.7	2
Phenylalanine	7.9	8	5.3	5	3.0	3
Tryptophan	n.d.§	2	n.d.§	n.d.§	n.d.§	n.d.§

* Data are presented as residues per molecule.
‡ 24 hr hydrolysate; data uncorrected for hydrolytic loss of threonine and serine or incomplete hydrolysis of valyl bonds.
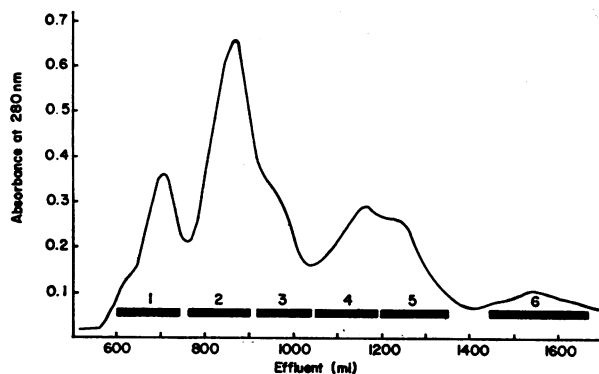§ n.d., not determined.

FIGURE 6 Separation by Bio-Gel chromatography of fragments from preferential cleavage of the AE-β^{Istanbul} chain between residues 99-100. Bars denote the portions that were pooled.
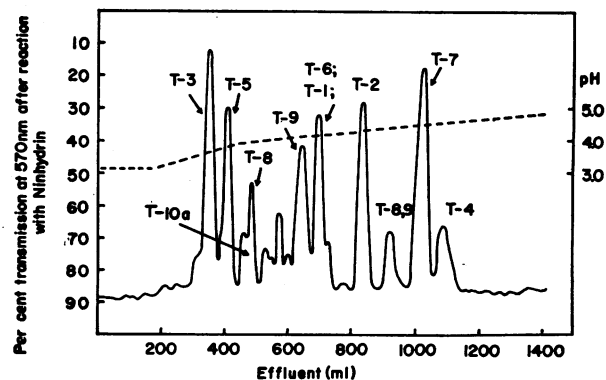

FIGURE 7 Elution profile of the 24 hr tryptic hydrolysate of the amino terminal segment (residues 1 through 99) of the AE-β^{Istanbul} chain. The pH gradient is indicated by the broken line.

Examination of the tryptic peptides. From the tryptic digest of the AE-β^{Istanbul} chain, peptides βT-1 to βT-9, βT-11, βT-14, and βT-15 were isolated and found to have the amino acid composition anticipated from the sequence of the β^A-chain. Peptides βT-10 and βT-12

could not be isolated. Both have histidine in the β^A-chain and either may contain the substituted residue. To determine this, preferential cleavage was used.

Preferential cleavage of the β^{Istanbul} chain. The rationale behind the approach to the determination of

TABLE II

Amino Acid Composition of Tryptic Peptides* of the AE-β^{Istanbul} Chain

	T-1‡	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-8,9	T-9	T-10a	T-11‡	T-13	T-14	T-15
Lysine	1.08	1.08			1.06	1.03	1.09	1.00	2.09	1.04			0.98	1.02	
AE-Cysteine												0.80			
Histidine	0.92						0.95		0.98	0.95		1.10		0.98	1.07
Arginine			1.03	0.96								1.00			
Aspartic acid			2.04		3.02				2.92	3.17		1.90		1.02	
Threonine	0.97	0.89		1.05	0.93						1.70		0.90		
Serine		0.95			1.82				1.09	1.01	1.03				
Glutamic acid	1.98		2.11	1.06	1.19						1.93	0.90	2.85		
Proline	0.88			1.02	1.89							0.74	1.89		
Glycine		1.01	2.85		1.90		1.03		2.28	1.96	1.16			1.16	
Alanine		1.85	1.00		1.00		0.92		2.05	1.97	0.90		2.00	3.89	
Valine	1.02	1.07	2.88	1.02§	1.01	0.97			0.80	1.13		0.92	0.97	2.08§	
Methionine					0.67										
Leucine	1.17	1.02	1.05	1.88	1.02				3.97	3.87	2.13	1.12		1.00	
Tyrosine				0.61									0.77		0.93
Phenylalanine					2.76				0.80	0.92	0.83	0.81	0.83		
Tryptophan		+		+											
Yield, %‖	24	30	45	12	24	48	44	20	12	16	8	20	40	30	42

* Data are presented as residues per molecule. Amino acids present in less than 0.1 residue are omitted. Tryptophan was detected by color reactions on paper and by amino acid analysis of quantities residual after hydrolysis. No correction factors have been applied to amino acids partially destroyed during acid hydrolysis.

‡ Peptides T-1 through T-10a were isolated from the digest of the N-terminal fragment (residues 1 through 99) of the chain and peptides T-11, T-13, T-14, and T-15 from the digest of the total chain. Peptide T-12 was not recovered.

§ Low recovery due to the presence of a Val-Val bond; 2.73 residues of valine were recovered from a 72 hr acid hydrolysate of T-14.

‖ Calculated on the basis of recovery after chromatography on Dowex 50 and subsequently on Dowex 1, assuming 100% hydrolysis of the appropriate cleavage points during tryptic digestion.

the substitution lies in the ready hydrolysis of bonds to aspartyl residues as reported by Partridge and Davis (23). An unusual lability of aspartyl-proline bonds was observed some years ago (J. R. Shelton, unpublished data) and is apparent also from data in the literature; the topic has been discussed by Piszkiewicz, Landon, and Smith (24). An aspartyl-proline sequence is present at residues 99-100 in tryptic peptide βT-11. Consequently, if this bond could be preferentially cleaved, the substitution of Hb Istanbul which must be either in βT-10 or βT-12 would be present in the fragment from residues 1-99 or 100-146. The sizes are such that the cleavage products should be readily separable.

After the β^{Istanbul} chain had been cleaved in formic acid as described under Methods, the fragments were isolated by Bio-Gel chromatography (Bio-Rad Labs, Richmond, Calif.) with the results that are shown in Fig. 6. That peak 2 contained the N-terminal fragment 1-99 and peak 4 the C-terminal fragment 100-146 is shown by the amino acid analyses in Table I. A decrease in histidine and an increase in glutamic acid over that of residues 1-99 of the β^A-chain is evident. On the basis of data already presented, the substitution should be present in βT-10 of Hb Istanbul.

The amino acid composition of the fragment 100-146 departs somewhat from the expected values because of some contamination with residues 53-99 which are the main component of peak 5. Technical aspects of this procedure of preferential cleavage will be discussed in the Addendum.

Examination of tryptic peptides from the N-terminal fragment. The separation of tryptic peptides from the N-terminal fragment is shown in Fig. 7, and their amino acid composition is recorded in Table II. The critical peptide is βT-10a (residues 83-93) which now has no histidine but an additional glutamic acid in comparison with T-10a of the normal β-chain. Peptide βT-10 itself, βT-10b (Asp-Lys from residues 94 and 95), as well as residues 96-99 from βT-11 were not recovered from this tryptic hydrolysate.

The substitution. The data establish that the histidyl residue at position 92 has been replaced by glutamine or glutamic acid. The electrophoretic behavior suggests the presence of glutamine as does the genetic code unless there is more than one point mutation. We conclude that in Hb Istanbul the substitution is F8 (92)β histidine $\rightarrow$ glutamine.

DISCUSSION

The substitution and the functioning of the hemoglobin. The substitution in Hb Istanbul is at the proximal histidine of the β-chain. Denaturation by heat takes place with relative ease, but quantitative comparison with other heat-denaturable variants is not possible.

The molecule is partially dissociated even at neutral pH despite the fact that the substitution involves a heme contact rather than an $\alpha_1\beta_2$ contact as, for example, in Hb Richmond (3) or Hb Kansas (25) both of which have abnormal dissociation properties. The lability of Hb Istanbul is further expressed in the heterogeneity of the chromatographically isolated material from aged hemolysates. It has been suggested (26) that instability of many hemoglobins is due to increased lability of the heme-globin bond or even heme-globin dissociation. The substitution of glutamine for histidine at the proximal β-histidine in Hb Istanbul would certainly be expected to influence the character of the heme-globin interaction: we may be seeing that effect of such alteration in the characteristics that we have been discussing.

Unfortunately, the necessity of using potassium cyanide during chromatographic isolation has made it impossible to study characteristics of Hb Istanbul that relate to heme-globin interaction. Substitutions at proximal and distal histidines by tyrosine in the Hb's M produce spectral changes and cyanosis. Although the spectrum of Hb Istanbul could not be examined, the absence of cyanosis in the propositus indicates that Hb Istanbul does not have the properties of an Hb M. This is another indication that the substitution is glutamine rather than glutamic acid because the presence of glutamic acid near to but not in the distal position produces an Hb M in Hb M-Milwaukee-1.

The substitution and its physiological effects. The absence of Hb Istanbul in the parents and siblings of K. K. suggests that a spontaneous mutation has occurred in K. K.

Hb Istanbul has distinct physiological manifestations. Although the substitution occurs at a proximal histidine, the clinical characteristics that are caused by an Hb M are not observed. Before splenectomy, however, the degree of anemia, the absence of inclusion bodies in the erythrocytes, the hepatosplenomegaly, the jaundice, and arthralgia are indicative of a moderate form of "unstable hemoglobin hemolytic anemia" (26). Splenectomy has resulted in marked improvement in well-being, disappearance of the anemia, and an essentially normal blood smear.

The percentage of Hb Istanbul in K. K. appears to be 15-20% both pre- and postsplenectomy. This low percentage, in contrast to many other β-chain-abnormal hemoglobins, may be caused by the instability of the molecule or results from its decreased rate of synthesis as has been demonstrated for other unstable hemoglobins.

ADDENDUM

Technical aspects of the preferential cleavage. The examination of the tryptic peptides of the entire AE-β^{Istanbul}

chain had narrowed the location of the substitution to a histidyl residue in βT-10 or βT-12. These peptides are normally difficult to study because of their presence in the tryptic "core." The cleavage procedure was used because it provided the opportunity to separate these two parts of the chain in fragments of the molecule. With such prior separation, each peptide should be more easily separable from a tryptic digest of each fragment.

Before its application to AE-β^{Istanbul} chains, the procedure was studied with AE-β^A-chains. The final conditions of cleavage are a compromise which gives a good cleavage of the Asp-Pro bond and yet minimizes other undesirable cleavages. If the sulfhydryl groups were not blocked (say, with β-aminoethyl groups as in this case), random disulfide bonds form during the cleavage and must be reduced before separation of the cleavage products.

Fig. 6 is typical also of results from the AE-β^A-chain. As we have seen, peaks 2 and 4 contain the desired fragments 1-99 and 100-146. The latter was isolated in about 75% yield. Peak 1 is essentially uncleaved β-chain. Although the most rapid cleavage occurs at the Asp-Pro bond, it is not negligible at other bonds that involve aspartic acid. The good yield of fragment 100-146 is no doubt due to the fact that only the Asp-Pro bond at 99-100 need be cleaved to produce it and that the fragment contains no aspartyl residues. On the other hand, fragment 1-99 has six aspartyl residues in addition to that of residue 99. Next in reaction rate to the Asp-Pro bond at 99-100 is the Asp-Ala bond at 52-53; proline in residue 51 may influence this cleavage. Peak 5 is primarily fragment 1-52. In actual amount, peak 5 contains much less than peak 4, but the presence of two tryptophyl residues in fragment 1-52 produces greater absorbance at 280 nm and gives the false impression of approximately equal amount. Although the fragments in peaks 4 and 5 are almost the same size, separation occurs probably because the tryptophyl residues retard the movement of fragment 1-52. Peak 6 contains fragments 1-21 and 80-99 whereas peak 3 is primarily fragment 1-79. Fragment 53-99 apparently is present only in small amount. It is probably present in peak 4 and is responsible for the divergence of the amino acid composition of material in peak 4 from the anticipated (Table I).

The preferential cleavage has been useful in determining the structure of Hb Istanbul despite some shortcomings. In retrospect, it may be that the isolation of βT-10a from the tryptic hydrolysate of whole AE-β^{Istanbul} chains would have been possible if the more vigorous conditions of hydrolysis that were used for the fragment had been applied to the whole chain.

ACKNOWLEDGMENTS

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