Observations on the amino acid composition of human hemoglobins

Several investigators have undertaken to ascertain whether the normal and abnormal hemoglobins that have been differentiated electrophoretically also exhibit differences in amino acid composition[1-5]. In the course of an investigation undertaken in a different connection, we recently had occasion to determine the amino acid composition of a sample of hemoglobin A prepared by zone electrophoresis. The half-cystine and isoleucine contents of this material were much lower than those reported for most of the samples of hemoglobin analyzed heretofore. In an attempt to clarify the question which this result raised concerning the homogeneity of hemoglobin preparations, seven different electrophoretically purified samples have been analyzed, with the results shown in Table I.

The hemoglobins were all isolated from the blood of single individuals (except in one instance, *cf.* Table I) by zone electrophoresis of the carbonmonoxy derivatives on starch[6]. By this procedure, hemoglobin A was freed of the minor hemoglobin components previously described[6], the part of the zone overlapping the faster moving minor component being discarded. The final preparation represented 85% of the original hemoglobin. In one case (the pooled sample of thalassemic hemoglobin, Table I), a second electrophoretic separation was carried out on a polyvinyl chloride supporting medium without causing any alteration in the ratio of ninhydrin color to cyanmethemoglobin color. Hemoglobins C and E were obtained from the blood of individuals with the heterozygous condition and after preparative electrophoresis contained no electrophoretically detectable quantities of hemoglobin A. The fetal hemoglobin was isolated similarly from umbilical cord blood. After electrophoresis, all preparations were dialyzed at 4° for 24 h against distilled water and lyophilized.

The amino acid analyses were performed by ion exchange chromatography on columns of sulfonated polystyrene resin, with the aid of automatic recording equipment[7]. Hydrolyses were carried out for 22 and 70 h at 110° in evacuated sealed tubes in the manner described by HIRS *et al.*[8]. Determinations of cysteic acid were performed according to the procedure of SCHRAM, MOORE AND BIGWOOD[9]. Amperometric titrations of -SH groups were carried out essentially by the method of BENESCH, LARDY AND BENESCH[10]. It was found important to employ solutions of 8 *M* urea that were freshly purified according to BENESCH *et al.* The presence of ammonia, which may form rapidly in concentrated solutions of urea, is deleterious. It was also found necessary to protect all portions of the silver nitrate solution from light at all times.

One prominent fact brought out by the data in Table I is the virtual absence of isoleucine in all of the preparations of hemoglobin except that of hemoglobin F, the relatively high isoleucine content of which has already been established by DUSTIN *et al.*[2] and VAN DER SCHAAF AND HUISMAN[3]. Except for hemoglobin F, none of the samples contains sufficient isoleucine to amount to one residue per molecule, which would require about 0.2 g of isoleucine per 100 g of protein. The largest amount, 0.09 g per 100 g of protein, is found in hemoglobin C, which is the most difficult to free from non-heme-containing proteins by electrophoresis. The chromatograms of the samples of hemoglobin A showed a flat baseline in the isoleucine position between methionine and leucine when quantities of hydrolysate were employed that gave a leucine peak, the ninhydrin color of which had a maximum optical density of 1.0 or higher. The effluent curves were similar in this respect to those reported by ROSSI-FANELLI, CAVALLINI AND DE MARCO[5], who are the only investigators who have heretofore described isoleucine-free hemoglobin.

Since hemoglobin can be obtained devoid of isoleucine, it may be concluded that the appreciable amounts of isoleucine (as much as 0.4 g per 100 g of protein) frequently reported to be present in hemoglobin A must have been contributed by an impurity. Whether this impurity is one of the minor hemoglobin constituents that have been removed by electrophoresis remains unanswered. This impurity was probably not fetal hemoglobin, however, about 1% of which has been reported to be present in adult hemoglobin. A 10 to 25% contamination by fetal hemoglobin would be necessary to account for the isoleucine values reported.

Both INGRAM[11] and BENESCH *et al.*[10] have reported that denatured hemoglobin A contains eight sulfhydryl groups per molecule. HOMMES, DRINKWAARD AND HUISMAN[12] found in hemoglobins A, B, and C, eight sulfhydryl groups and eight half-cystine residues per molecule, determined as cysteic acid. As can be seen from Table I, all of the samples of hemoglobin (except hemoglobin F) prepared electrophoretically contain only four to five sulfhydryl groups per molecule. The total quantity of half-cystine, determined as cysteic acid, is in most cases in acceptable agreement with the number of sulfhydryl groups determined amperometrically, indicating the absence of disulfide bonds in the samples. This was further confirmed with one sample of hemoglobin A which was titrated after treatment of the hemoglobin with Na_2SO_3, and no increase in the number of sulfhydryl groups was found. Apparently, electrophoresis removes the bulk of an impurity rich in sulfhydryl groups. The value for the sulfhydryl groups seems to vary somewhat from one hemoglobin sample to the next, and in some cases an integral molar quantity is not found. This may be a result of experimental error, or it may mean that the impurity has not

TABLE I

AMINO ACID COMPOSITION OF HYDROLYSATES OF VARIOUS HUMAN CARBONMONOXY HEMOGLOBINS

Values are given as g of amino acid per 100 g of protein (on an ash- and moisture-free basis), except for half-cystine and sulfhydryl which are expressed as groups per molecule of molecular weight 66,000.

Amino acid	*Type of hemoglobin and time of hydrolysis*													
	Hemoglobin A Normal negro		*Hemoglobin A Normal white*		*Hemoglobin A Thal. trait*		*Hemoglobin A Thal. trait**		*Hemoglobin C*		*Hemoglobin E*		*Hemoglobin F*	
	22 h	*70 h*	*22 h*	*70 h*	*22 h*	*70 h*	*22 h*	*70 h*	*22 h*	*70 h*	*22 h*	*70 h*	*22 h*	*70 h*
Aspartic acid	9.77	10.01	9.64	9.99	9.94	10.27	9.92	10.05	9.54	9.45	10.40	10.04	9.84	10.02
Glutamic acid	6.69	6.94	6.55	6.89	6.65	6.85	6.52	6.74	6.36	6.30	6.59	6.37	7.02	7.38
Glycine	4.31	4.65	4.32	4.46	4.34	4.43	4.30	4.42	4.33	4.27	4.61	4.52	4.28	4.52
Alanine	9.35	9.67	9.15	9.33	9.44	9.48	9.33	9.59	8.96	8.96	9.71	9.41	8.50	8.80
Valine	10.48	11.20	10.36	10.44	10.38	11.17	9.95	11.19	10.00	9.80	10.50	10.80	8.88	9.47
Leucine	13.44	14.33	13.94	13.49	13.98	14.37	13.86	14.10	13.58	13.20	14.10	13.55	13.57	13.75
Isoleucine	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.09	0.09	<0.04	<0.02	1.44	1.49
Serine**	4.11	3.42	4.05	2.83	3.96	2.96	4.14	3.48	4.23	3.00	4.33	3.10	5.42	4.20
Threonine**	5.16	5.03	5.13	4.44	5.12	4.66	5.33	4.79	5.20	4.39	5.38	4.72	6.22	5.69
Methionine	1.19	1.13	1.25	1.14	1.26	1.31	1.23	1.03	1.09	1.13	1.25	1.13	1.56	1.57
Proline	4.91	4.94	5.02	5.22	4.76	5.07	5.20	4.91	4.54	4.35	4.68	4.55	3.60	3.78
Phenylalanine	6.69	7.34	7.33		7.09	7.35	6.99	7.25	6.82	6.93	7.22	7.06	7.08	7.27
Tyrosine	2.71	2.88	3.05		3.06	2.85	3.19	3.03	2.98	2.71	3.01	2.84	2.54	2.41
Histidine	8.22	8.61	8.32	7.84	8.93	8.31	8.83	8.53	8.00	8.20	8.33	8.36	7.58	7.82
Lysine	9.37	9.65	9.28	9.00	9.46	9.62	9.39	9.35	9.69	9.26	9.76	9.60	10.00	9.82
Arginine	3.06	2.99	2.82	3.02	3.14	3.09	3.06	3.06	2.98	2.90	2.99	3.05	3.11	3.01
Half-cystine*** (as cysteic acid)	4.9		4.2		5.3				4.6		4.8		3.4	
Sulfhydryl groups*** (amp. titration)	4.6		5.0		5.2				4.5		4.6		3.4	
Total N (corr.) %	16.84		16.68		17.10		16.97		16.75		16.79		16.61	
Ash %	1.68		0.86		1.63		1.35		3.00		1.30		1.2	

* A pooled sample obtained from four individuals.

** Threonine corrected for decomposition on hydrolysis[8] gave the following values: Hemoglobin A (negro), 5.22; hemoglobin A (white), 5.48; hemoglobin A (thal. trait), 5.35; hemoglobin A (thal. trait, pooled sample), 5.60; hemoglobin C, 5.61; hemoglobin E, 5.72; hemoglobin F, 6.49. The corresponding values for serine were: 4.47, 4.77, 4.52, 4.48, 4.95, 5.02, 5.90.

*** Number of residues per molecule of molecular weight 66,000.

been completely removed even from the electrophoretically prepared samples. In agreement with HOMMES *et al.*[12], we find less half-cystine in fetal hemoglobin than in hemoglobin A, but the value is three to four rather than six residues per molecule. Unlike these authors, however, we have not been able to secure any evidence for the presence of a disulfide bond in fetal hemoglobin. The cysteic acid and amperometric values agree, and titration after treatment with Na_2SO_3 gave the same value for sulfhydryl groups as the one given in Table I.

Except for isoleucine and cysteine, the analytical results given in Table I are in general quite similar to other values to be found in the literature, although the present results show a tendency to be lower. It should be emphasized that the single analyses of the 22 h and 70 h hydrolysates do not provide sufficient data for the formulation of definitive tables on the complete amino acid composition of the proteins, nor do they permit decisions as to the possibility of small differences in the compositions of hemoglobins A, C, and E. This is particularly the case since there is reason to suspect from the sulfhydryl analyses that these preparations still may not be completely pure. Since further work along these lines is not contemplated, the present results are summarized simply to demonstrate that the absence of isoleucine is one criterion to be considered in the evaluation of the purity of hemoglobin, and that determinations of sulfhydryl groups and of half-cystine as cysteic acid may be useful in following the purification of this protein.

The authors are greatly indebted to Dr. JOHN LEGGETT BAILEY for preliminary work on the amperometric titrations, to Miss KERSTIN JOHANSSON and Miss JOYCE F. SCHEER for expert technical assistance, and to Mr. S. THEODORE BELLA who performed the microanalyses reported in this paper.

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Received March 22nd, 1957

Les nucléotides libres du cristallin de veau

Nous avons abordé l'étude de la répartition des nucléosides mono-, di- et triphosphates dans le cristallin comme préliminaire aux recherches sur le métabolisme nucléique de cet organe.

Nos essais ont porté sur des cristallins de veaux prélevés aux abattoirs à moins d'une minute après la mort, puis congelés dans un mélange neige carbonique–acétone. Après un broyage à l'homogénéiseur POTTER[1] dans l'acide perchlorique 0.6*N* et 0° C, la fraction acido-soluble a été séparée par centrifugation à 15,000 tours/minute pendant 20 minutes dans une centrifugeuse MSE réfrigérée 0° C. Nous avons encore effectué une extraction du culot, par l'acide perchlorique 0.2*N* et l'ensemble des fractions acido-solubles a été ensuite neutralisé par la potasse à —5°. Après séparation du perchlorate de potassium insoluble, par centrifugation, le surnageant a été soumis à une chromatographie sur colonne Dowex-1 X8, 200 à 400 mesh selon la technique de SCHMITZ[2,3,4].

Le résultat d'une des chromatographies portant sur 11 cristallins de veaux est présenté dans la Fig. 1.

La nature des composés des divers pics a été déterminée après rechromatographie, tenant compte: de l'absorption dans l'ultra-violet à deux longueurs d'onde, 2600 et 2750 A, des résultats