Distribution of Heme in Systems Containing Heme-Free and Heme-Bound Hemoglobin Chains*

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ABSTRACT: The distribution of proto-, meso-, and deuteroheme on hemoglobin apoprotein has been studied in mixtures containing heme-free and heme-bound hemoglobin chains. The difference between α and β chains in their affinity for hemin, responsible for the formation of the intermediate compound IC_{II}, is absent in the case of deuterohemin. Binding of deuterohemin by globin appears to be strongly cooperative since no intermediate can be detected in the reconstitution process. For the case of protohemin, varying the pH from 6 to 9 did not influence the difference in affinity between α and β chains. The addition of cyanide strengthens the binding of the prosthetic group to the polypeptide chains enough to prevent heme exchange in the case of proto- and mesohemin but not with deuterohemin. Globin and IC_{II} show a rapid exchange with isolated chains leading to the formation of IC_{II} and hemoglobin, respectively. α^h cyanmet chains do not exchange their prosthetic group with other heme binding proteins, whereas β_4^h do. It is concluded that two effects contribute in various degrees in the different cases, to the departure from a simple statistical distribution of the hemes on the globin: (a) greater tendency of the heme to bind to the α chains; (b) cooperativity in the binding within $\alpha\beta$ pairs.

The interaction of heme with hemoglobin apoprotein involves a number of still unsettled problems regarding the kinetic and equilibrium aspects of the reaction (Antonini and Brunori, 1971). The process under many conditions may be represented formally as

$$2\alpha\beta \text{ (globin)} + 4\text{hemes} \rightleftarrows \alpha_2\beta_2 \text{ (hemoglobin)}$$

The main sources of complexities which make the detailed investigation of the system particularly difficult are: (1) nonequivalence of α and β chains in the binding of heme; (2) homotropic and heterotropic interaction between the heme binding sites and between these and other groups in the protein; (3) inter- and intrachain structure changes associated with the reaction. These are reflected in the change of helicity even of the chains not reacted with heme in the intermediate forms (Javaherian and Beychok, 1968) and also in changes in the degree of polimerization (Winterhalter and Deranleau, 1967).

Previous studies on the reaction of heme with hemoglobin globin have dealt with some of the features just described but often with apparently conflicting conclusions. In their kinetic work, Gibson and Antonini (1966) considered, in the absence of contrary evidence, that in the combination reaction the heme binding sites behaved as if they were equivalent and independent. On the other hand, under conditions where the hemoglobin interaction can be treated as a reversible equilibrium, older experiments indicated that the binding of heme by hemoglobin globin was cooperative with a tendency of the system to contain primarily, at equilibrium, fully heme-saturated hemoglobin molecules (Banerjee, 1962; Antonini and Gibson, 1964). Later studies provided evidence for a greater affinity for heme of α than non-α chains and an intermediate carrying heme only on the α chain[1] was isolated in the reconstitution process (Winterhalter and Deranleau, 1967). Nonequivalence of α and β chains in the binding of heme is indicated by other evidence as well; the velocity constant for dissociation of hemin from the α chains of ferrihemoglobin was calculated to be about tenfold lower than that from the non-α chains (Bunn and Jandl, 1968); upon investigation of the optical activity of isolated α and β chains Beychock *et al.* (1967) found that the intensities of the heme bands at 260 and 410 nm were greater for the α chains than for the β chains. In addition, the atomic model obtained by X-ray crystallography for horse oxyhemoglobin (Perutz *et al.*, 1968) shows that the contacts between heme and α chains differ from the ones between heme and β chains.

On the basis of these observations we reinvestigated here under a wider range of conditions, the distribution of heme in systems containing heme-free and -bound hemoglobin chains. The experiments were designed especially to deal with the following questions: (a) relative role of equilibrium and kinetic factors in determining the amount of intermediates detectable under any given conditions; (b) importance of chain exchange *vs.* heme exchange in the attainment of equilibria; (c) dependence of the behavior of the system on the state of the heme iron.

Experimental Section

Materials. All chemicals used were of the highest commercially available grade. All experiments were carried out in a cold room at 4° unless otherwise specified.

Hemoglobin was purified as previously described (Winterhalter and Huehns, 1964) and globin was prepared by acetone precipitation (Winterhalter and Huehns, 1964). Hemoglobin containing heme only on the α chains $\alpha_2^h\beta_2$ was obtained according to a method reported elsewhere (Winterhalter,

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[1] $\alpha_2^h\beta_2 = IC_{II}$, where h denotes a heme molecule.

1966). Isolated α^h and β^h chains were obtained by the method of Bucci and Fronticelli (1965); the *p*-mercuribenzoate[2] reacted SH groups were regenerated with dodecanthiol (De Renzo *et al.*, 1967). Hemoglobin and the chains were always obtained in the oxy state. The deoxy forms were obtained in a tonometer at 20° by repeated evacuation and flushing with nitrogen. The cyanmet derivatives were obtained by oxidation with a 10% excess of ferricyanide over heme in the presence of excess KCN. This was followed by dialysis against at least three changes of the required buffer containing 100 mg/l. of KCN. Deoxygenation and the formation of the cyanmet derivatives were controlled spectrophotometrically.

Protohemin was prepared according to Labbe and Nishida (1957).

Mesohemin. Mesoporphyrin dimethyl ester (K & K Laboratories Inc., Plainview, N. Y.) (364 mg) was dissolved in 28 ml of 25% HCl. After 36 hr at room temperature the solution was neutralized with 25% NaOH and subsequently centrifuged after cooling to 4° for 1 hr. The precipitate was washed twice with distilled water, and then dried in the dark for 24 hr at room temperature (adapted from Grinstein, 1947). This material which consisted of mesoporphyrin was dissolved in 1 ml of pyridine and diluted with 50 ml of glacial acetic acid at room temperature. A nearly saturated aqueous solution of $FeSO_4$ (1 ml) was added. The mixture was immediately immersed in a water bath at 80° for 10 min, while a stream of dry nitrogen was passed through the flask containing the mixture. The mixture was then cooled at room temperature in contact with air. The resulting mesoheme was precipitated by the addition of ether, the precipitate removed by centrifugation, and residual porphyrin was extracted with 25% HCl (Falk, 1964).

Deuterohemin. Protohemin (500 mg) was ground with 1500 mg of resorcinol and heated under an air condenser in an oil bath to 160° for 45 min. After 3 hr at room temperature the dark brown solid was suspended in ethyl acetate and washed with ethyl acetate until the extracts were colorless. The solid residue was dried for 24 hr in a desiccator at room temperature (yield 80%) (Schumm, 1928). This deuterohemin (200 mg) was dissolved in 1 ml of pyridine and subsequently 8 ml of chloroform was added. The mixture was shaken for 15 min and then filtered. The filtrate was added to 30 ml of a saturated solution of NaCl in glacial acetic acid, containing 1 ml of concentrated HCl, which had been preheated to 105°. After cooling to room temperature and standing for 24 hr, the deuterohemin crystals were isolated by centrifugation and washed twice with 50% acetic acid, once with distilled water, twice with ethanol, and once with ethyl ether. The crystals were dried in a desiccator at room temperature (Kuester, 1903).

Radioactive Chains. Radioactive hemoglobin was obtained by incubating blood from patients with high reticulocyte contents 10–20% (pernicious anemia 5–8 days after vitamin B_{12}, subacute blood loss) with ^{14}C-labeled leucine and valine according to Lingrel and Borsook (1963) with the appropriate modifications for human hemoglobin. The radioactive hemoglobin was purified as previously described (Winterhalter and Huehns, 1964) and the chains were prepared as reported above for the nonradioactive material.

Titration of globin with the heme derivatives was carried out at 8° in a Beckman DB spectrophotometer (Antonini *et al.*, 1964). Paper chromatography of hemes was carried out, using Whatman No. 3MM paper and the modified lutidine–water system (Chu and Chu, 1955).

[2] Abbreviation used: *p*-mercuribenzoate, PMB.

TABLE I: Spectral Properties of Pyridin Hemochromogens of Hemes.[a]

	Soret	β Max.	β Min	α Max.	α/β
Protoheme	419	524	539	558	2.05
Value from literature	420	526	540	557	2.00
Mesoheme	407	516	531	547	1.65
Value from literature	407	518	531	547	1.68
Deuteroheme	405	512	529	544	1.46
Value from literature	406	515	530.5	545	1.44

[a] Literature values from Antonini *et al.* (1964).

Starch gel electrophoresis was carried out according to Poulik (1957). Chromatographic separation of the reaction products resulting from the incubation of globin or IC_{II} with ^{14}C-labeled α^h or β^h chains was carried out by the method described for the isolation of IC_{II} (Winterhalter, 1966).

Reduced pyridine hemochromogens were prepared by adding solid dithionite to a solution of hemin in alkaline pyridine (De Duve, 1948) (1 ml of heme solution in 0.5 M phosphate buffer (pH 7.0), 3 ml of a solution containing 50 ml of pyridine, and 15 ml of 1 N NaOH diluted to 150 ml with water).

Results

Properties of the Hemes. SPECTRA. The absorption maxima and minima obtained from the pyridine hemochromogens of the hemes are summarized in Table I and compared to values from the literature. The unnatural hemes combined with stoichiometric amounts of globin giving hemoglobins which had absorption characteristics closely similar to the ones reported before (Antonini *et al.*, 1964). These values and the ones obtained previously are reported in Table II.

PAPER CHROMATOGRAPHY. All three heme derivatives were chromatographically pure giving R_F values identical with the ones reported in the literature (Chu and Chu, 1955).

FORMATION OF IC_{II}. It was mentioned in the introduction that the main intermediate in the reconstitution of hemoglobin from globin and heme is the compound, IC_{II}, containing heme only on the α chains. The effect of several variables on the amount of IC_{II} which may be detected by gel electrophoresis was therefore investigated in experiments in which less than the stoichiometric amount of heme was added to globin, or in which hemoglobin was incubated with globin.

Effect of pH. In the pH range from 6 to 9, on addition of protohemin cyanide in half-stoichiometric amounts to globin, there was no apparent difference in the amount of IC_{II} formed. In this pH range stoichiometric addition of protohemin cyanide to globin always resulted in the formation of hemoglobin (Figure 1). Equally, no difference in the formation of IC_{II} from mixtures of methemoglobin and globin could be observed as a function of pH; the rate of formation of IC_{II} was the same for all four pH values. No formation of IC_{II} takes place on addition of globin to ferric cyanide hemoglobin.

Effect of Changes in the Structure of Heme, in the Absence and Presence of CN. In experiments in which globin was mixed with half-stoichiometric amounts of protohemin, it became evident that the presence of CN enhanced the formation of IC_{II} considerably (Figure 2). A similar influence of the

TABLE II: Spectral Properties of Reconstituted Hemoglobins.[a]

		Max. Soret	Max. 2	Max. 1	OD Max. 1/OD Max. 2
CyanmetHb	Proto	419	540		
	Meso	410	532		
	Deutero	407	522		
DeoxyHb	Proto	430 (430)	554 (555)		
	Meso	420 (421)	545 (550)		
	Deutero	420 (421)	543 (544)		
OxyHb	Proto	415 (414.5)	540 (541)	576 (577)	1.0 (1.04)
	Meso	405	543 (543)	567 (568)	0.84 (0.83)
	Deutero	403 (403)	530 (532)	563 (565)	0.73 (0.75)
CarboxyHb	Proto	419 (419)	537 (539)	567 (569)	0.98 (0.99)
	Meso	409 (410)	530 (532)	558 (560)	0.85 (0.90)
	Deutero	407 (408)	527 (528)	556 (556)	0.75 (0.76)

[a] The values in brackets in each column represent values from Antonini *et al.* (1964). The values of OD max. 1/OD max. 2 from the literature were calculated from the relative values of ϵ M $\times 10^{-3}$.

presence of CN on the quantity of IC_{II} formation was noted with mesohemin. With deuterohemin, however, this difference was much more marked in as much as there was no formation of IC_{II} at all when deuterohemin was mixed in half-stoichiometric amounts with globin in the absence of cyanide ions, whereas a considerable amount is formed when CN ions are present (Figure 3). A collateral observation is borne out by Figure 4. In this experiment reconstituted hemoglobins from proto-, meso-, and deuterohemin were incubated in the presence or absence of cyanide ions with globin. In the absence of cyanide, some IC_{II} formed from protohemoglobin, a considerable amount formed from mesohemoglobin but none at all from deuterohemoglobin (Figure 4a). In the presence of cyanide no IC_{II} formed from protohemoglobin, a small amount from deuterohemoglobin (Figure 4b).

Incubation of Isolated Chains with Globins. When globin was incubated with equimolar amounts of protoheme α chains either in the oxy, deoxy, or cyanmet form, a rapid formation of IC_{II} took place to the same extent in all three cases (Figure 5). In all three cases a minimal band of Hb A also became apparent in the benzidine stain. Upon incubation of globin with equimolar amounts of β chains, the formation of IC_{II} could also be observed in all three forms, but was less marked than with α chains. The formation of IC_{II} decreased in the order oxy, deoxy, and cyanmet form (Figure 5). Here faint but clearly detectable band of Hb appeared upon staining with benzidine.

Incubation of Globin and IC_{II} in the Cyanmet Form with ^{14}C-Labeled Cyanmet α^h and β^h Chains. In analogous experiments ^{14}C-labeled α^h and β^h chains in the cyanmet form were incubated with equimolar amounts of IC_{II} in the cyanmet form. As evident from Figure 6, even after prolonged incubation very little Hb A formed from $IC_{II} + \alpha^h$ chains, whereas a considerable amount of Hb A formed from the mixture containing β chains. The reaction products of these mixtures and of the ones resulting from similar incubations of radioactive α^h and β^h chains in the cyanmet form with globin were isolated by column chromatography in order to assess the relative importance of transfer of entire subunits *vs.* transfer of heme. The fractions thus obtained were identified by gel electrophoresis and absorption spectroscopy. The results of this experimental series are summarized in Table III.

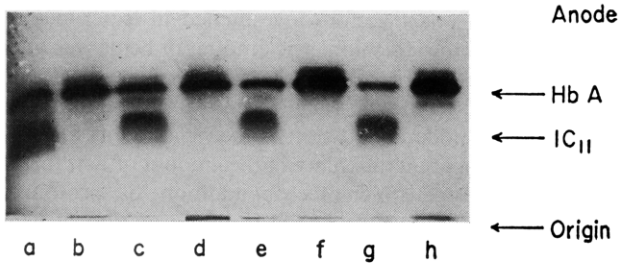

FIGURE 1: Starch gel electrophoresis of mixtures of protohemin cyanide with globin after 48-hr incubation at 4°. pH range from 6 to 9. Benzidine stain. (a) pH 9.0 borate buffer 2%; globin + half-stoichiometric amount of protohemin cyanide; (b) as part a with twice the amount protohemincyanide; (c) pH 8.0 phosphate buffer (0.1 M), globin + half-stoichiometric amount of protohemin cyanide; (d) as part c with twice the amount protohemincyanide; (e) as part c but pH 7; (f) as part e but twice the amount protohemincyanide; (g) as part c but pH 6; (h) as part g but twice the amount protohemincyanide.

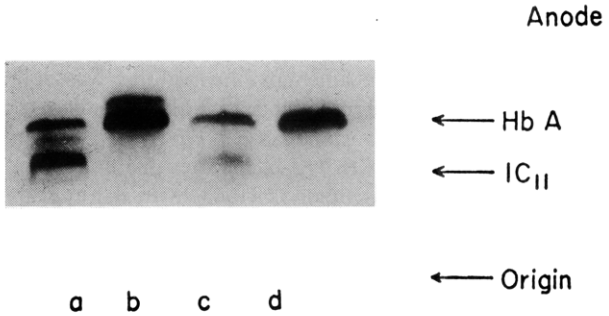

FIGURE 2: Starch gel electrophoresis of mixtures of protohemin with globin in phosphate buffer (0.1 M, pH 7.0). Incubation of solution for 48 hr in 0.1 M phosphate buffer (pH 7.0). Benzidine stain. (a) Globin + half-stoichiometric amount of protohemincyanide; (b) globin + stoichiometric amount of protohemincyanide; (c) globin + half-stoichiometric amount of protohemin without cyanide; (d) globin + stoichiometric amount of protohemin without cyanide.

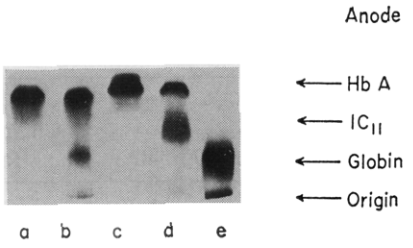

FIGURE 3: Starch gel electrophoresis of mixtures of deuterohemin with globin. Incubation of solutions for 48 hr at 4° in 0.1 M phosphate pH 7. Amido-Schwarz stain. (a) Globin + stoichiometric amount of deuterohemin, no cyanide; (b) globin with half-stoichiometric amounts of deuterohemin, no cyanide; (c) globin: stoichiometric amounts of deuterohemin cyanide; (d) globin: half-stoichiometric amounts of deuterohemincyanide; (e) globin marker.

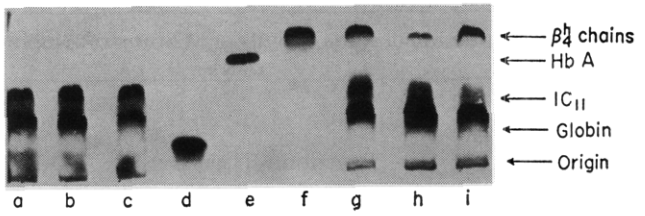

FIGURE 5: Starch gel electrophoresis of hemoglobin chains in oxy, deoxy, cyan met form after 48-hr incubation with globin. Phosphate buffer (0.1 M, pH 7.0), 4°. Amido-Schwarz stain. (a) αO_2 + globin, (b) α-deoxy + globin; (c) α^{CN} + globin; (d) αO_2 chains; (e) Hb A marker; (f) βO_2 chains; (g) βO_2 + globin; (h) β-deoxy + globin; i) β + N + globin.

Discussion

Most of the information reported here is obtained by starch gel analysis of the components which are present in mixtures of incompletely reconstituted hemoglobin. Particularly relevant in this respect is the amount of IC_{II}, containing heme only on the α chains, which represents the main intermediate in the reconstitution of hemoglobin.

In evaluating the results, it is important to discriminate between equilibrium and kinetic effects in the reaction system studied. Therefore the following two basic types of experiments were carried out: (a) mixing of hemin and apoprotohemin, which should primarily reflect the kinetic aspects of the recombination reactions (particularly in the presence of cyanide ions); (b) incubation of partially and fully heme saturated hemoglobins with globin in order to gain insight into the equilibrium aspects of the system. Both exchange of heme and of chains may contribute to the attainment of equilibrium in a system containing heme-bound and heme-free hemoglobin chains. The experiments with the radioactively labeled hemoglobin chains were performed in order to discriminate between these two mechanisms. From the results reported in Figures 1, 2, and 4 it becomes evident that the difference in binding of heme by α and β chains is not solely due to differences in the kinetics of the reaction since also incubation of methemoglobin with globin leads to the formation of IC_{II}; in this case a transfer of heme from the β chains of methemoglobin to α chains of globin takes place. However, the amount of IC_{II} is greater when the reconstitution is carried out with protohemin cyanide than with protohemin, indicating that kinetic effects contribute significantly to its formation, since binding of protoheme cyanide to globin may be considered an irreversible process. Accordingly, there is no appreciable formation of IC_{II} on incubation of globin with cyanide methemoglobin.

As evidenced by the results illustrated in Figure 1, pH in the range from 6 to 9 did not influence significantly the relative amount of IC_{II} formed.

Different results were observed with mesohemin and deuterohemin. In the absence of cyanide, no formation of IC_{II} could be observed in the case of deuterohemin, neither when the heme was mixed with globin nor when deuteromethemoglobin was incubated with globin. The binding of heme is therefore in this case clearly cooperative, leading exclusively to the formation of fully reconstituted deuterohemoglobin.

This observation also suggests that an important role in the difference of affinity for heme between α and β chains is played by the contacts between the vinyl groups in position 2 and 4 and the protein. In fact, a number of differences between α and β chains have been found in this region in the atomic model of horse oxyhemoglobin by Perutz *et al.* (1968). In the incubation experiments (globin + heme cyanide hemoglobin), formation of IC_{II} was minimal from mesohemoglobin, but pronounced from deuterohemoglobin (Figure 4). Apparently, the CN ion is able to stabilize the heme–polypeptide bond in the case of proto- and mesohemin but not in the case of deuterohemin. This is consistent with the lower affinity of deuteroheme for globin in comparison to protoheme (Banerjee, 1962; Gibson and Antonini, 1966). Thus, it appears that the presence of CN ions brings out the difference in affinity for heme between α and β chains. There is of course the possibility that the observed slower band labeled IC_{II} in fact consists of

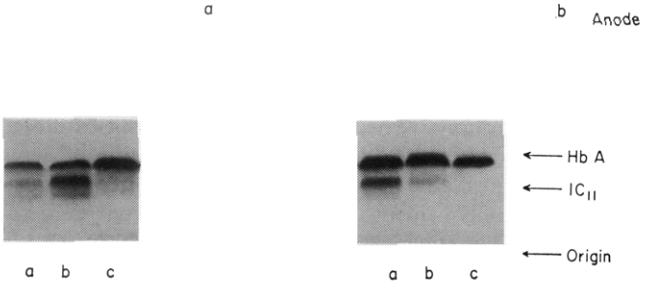

FIGURE 4: Starch gel electrophoresis of reconstituted hemoglobins from proto-, meso-, and deuterohemin after 48-hr incubation with globin. Phosphate buffer (0.01 M, pH 7), 4°. Benzidine stain. Part a is without cyanide: (a) protoHb + globin; (b) mesoHb + globin; (c) deuteroHb + globin. Part b is with cyanide: (a) deuteroHb + globin; (b) mesoHb + globin; (c) protoHb + globin.

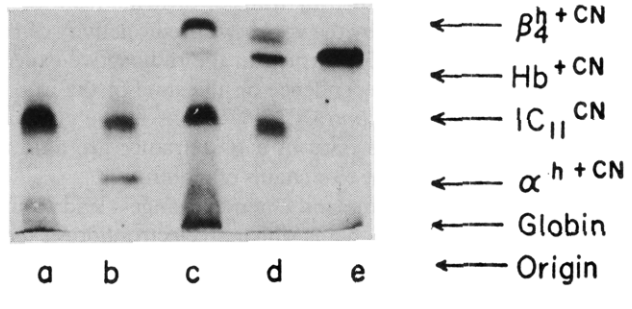

FIGURE 6: Starch gel electrophoresis of hemoglobin chains in cyanmet form after 96-hr incubation with IC_{II} in cyanmet form; 0.1 M phosphate buffer (pH 7.0), 4°. Amido-Schwarz stain. (a) IC_{II} marker; (b) α + IC_{II}; (c) β + globin; (d) β + IC_{II}; (e) HbA marker. Note: Hb A formation from IC_{II} + α^h chains is minimal (channel 1b) but marked for IC_{II} + β^h chains (channel d).

TABLE III: Distribution of Radioactivity in Mixtures of Globin and IC_{II} with Hemoglobin Chains.[a]

No.	Reaction Mixtures	Reaction Products	cpm/mg	275/540[b]	Sp Act. of Chains in Final Product
1	Globin + 14C α^h chains	HbA[c]	~38	2.68	0.27
		IC_{II}	135	3.50	0.96
		Globin	8		0.05
		α^h chains	280		1.0
2	IC_{II} + 14C α^h chains	IC_{II}	153	3.54	1.03
		α^h chains	297		1.0
3	Globin + 14C β^h chains	HbA	128	2.66	0.86
		IC_{II}[d]	34	3.46	0.23
		Globin	28		0.19
		β^n chains	294		1.0
4	IC_{II} + 14C β^h chains	HbA	313	2.70	1.09
		C_{II}	14	3.48	0.05
		β^h chains	570		1.0

[a] Conditions: 96 hr at 4° in 0.1 M phosphate pH 7 prosthetic groups in the cyanmet form. [b] Values for Hb = 2.6 ± 0.1 for IC_{II} = 3.6 ± 0.2 (Winterhalter and Deranleau, 1967). [c] Too little material was obtained to get accurate data. [d] A small second IC_{II} peak with an electrophoretic mobility slightly slower than IC_{II} showed a higher specific activity (~410). Its 275/540 ratio was 4.7.

two different molecules, namely $\alpha_2{}^D\beta_2 + \alpha_2\beta_2{}^D$. This explanation is however unlikely, since at least $\alpha_2\beta^h$ from protohemin has a slower electrophoretic mobility than IC_{II} (Cassoly, 1968).

The incubation of globin with heme containing isolated α and β chains led to the formation of IC_{II} in both cases, although by a different mechanism. As shown in Figure 5, IC_{II} formed rapidly from mixtures of globin with α^h chains either in the oxy, deoxy, or cyanmet form. No dependency of the state of the iron or the absence of ligand could be demonstrated. This is consistent with the idea that IC_{II} is formed in in this case by exchange of α^h chains with the α chains of globin, a concept that is supported by the finding that the α chains in IC_{II} obtained in the experiments reported in Table III have the same specific activity as the ones originally added to the mixture.

In the second set of experiments, also shown in Figure 5 dealing with the incubation of β^h chains with globin, the state of the iron and the presence of the ligand influences greatly the formation of IC_{II}. IC_{II} having its prosthetic group on the α chains can only be formed by transfer of heme from the β chains. This is borne out by the very low specific activity of the β chains isolated from IC_{II} formed in the radioactive experiments (Table III). The dependence on the state of the iron is thus to be expected. In contrast to β^h chains in the cyanmet form in hemoglobin, the ones in a β^h tetramer are able to rapidly transfer their heme to α chains of globin.

Both mechanisms—heme and chain exchange—lead to unstable heme-free-isolated chains which on electrophoresis tend to precipitate at the origin (Figure 5).

Lastly, a rapid exchange of free α chains with the α^h chains of IC_{II} seems to take place, as shown by the equal specific activity of α chains in IC_{II} after 24-hr incubation (Table III). Globin and IC_{II} rapidly exchange their component chains with isolated chains; they must therefore have the capability to dissociate significantly into monomers in contrast to hemoglobin which in this pH, concentration, and temperature range does not dissociate into monomers (Glatthaar and Winterhalter, 1970; Antonini and Brunori, 1971). Although IC_{II} is suspected to have a helical content similar to the one of hemoglobin (Javaherian and Beychok, 1968), there must be important differences in the interchain contacts and probably in the spatial arrangement of the chains not linked to heme. This fact is borne out by the difference in the reaction rate of the SH groups in position 93 (Jacob and Winterhalter, 1970) and the fluorescence of IC_{II}, which would be quenched if the β chains of IC_{II} were closely packed as in hemoglobin.

In summary, taking into consideration all the results it may be concluded that binding of the heme to globin is dominated by two kinds of effects which make the system depart from a simple situation corresponding to a statistical distribution of the hemes on the protein: (1) higher rates of combination and higher affinity of α chains in comparison to β chains. These effects are responsible for the appearance of the compound IC_{II}, carrying heme only on the α chains, as an intermediate in the reconstitution of hemoglobin. (2) Cooperativity in the binding of heme within $\alpha\beta$ pairs by which the form containing full heme complement (hemoglobin) tends to be favored in comparison to intermediates, thus leading to a greater relative proportion of heme-free and fully heme-saturated protein than expected from a statistical distribution of heme molecules on available binding sites. The case where cooperativity is especially evident is in the binding of ferric deuteroheme where no intermediate of any type is detectable.

The electrophoretic pattern which is observed in systems containing heme-free and -bound hemoglobin chains, varies in the different cases due to different contributions of the two effects just mentioned and different tendency to reach an equilibrium through heme transfer or exchange of chains. These conclusions appear to reconcile some of the apparently conflicting results which have been reported in the past.

Acknowledgment

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Complexes of Deoxyribonucleic Acid with Fragments of Lysine-Rich Histone (f-1). Circular Dichroism Studies*

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ABSTRACT: Lysine-rich (f-1) histone from calf thymus was cleaved by *N*-bromosuccinimide, to yield a large carboxyl-terminal polypeptide of high lysine content and a smaller amino-terminal fragment with relatively low lysine content. Reconstituted complexes of calf thymus DNA with these two fragments were examined by means of circular dichroism. Although the N-terminal fragment (6000 molecular weight) failed to cause significant change in DNA circular dichroism upon complexation, the C-terminal fragment (15,000 molecular weight) effected even larger changes than did equivalent molar amounts of intact f-1. These observations were not simply related to differences in lysine content of the fragments, or to the reduced binding affinity of the N-terminal fragment for DNA. The two f-1 fragments showed no tendency to interact with each other in the absence of DNA. However, complexes formed with DNA and a mixture of the two polypeptides (in such amounts as to reconstitute f-1) exhibited augmented circular dichroism changes. This finding suggests that, although binding of the highly cationic C-terminal end of f-1 histone is the primary cause of conformational change in DNA, the N-terminal fraction of the molecule may modify this interaction. In the intact f-1 molecule, the N segment appears to moderate the conformational effect of the C segment upon DNA, while when cleaved from the C fragment it enhances this effect. This finding might suggest that in the intact f-1 molecule the N-terminal segment folds back on the C-terminal segment, thus acting as a modifier.

In the cell nucleus of higher organisms DNA is found complexed to basic proteins, the histones, as well as to other constituents. The interaction between histones and DNA is dominated by electrostatic forces; the histones in chromatin contain sufficient basic amino acid residues to neutralize nearly all the negative charges on the ionized phosphates of the DNA. However if any specificity between DNA and histones exists, other interactions must play a controlling role.

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