aniline as determined by thin layer chromatography on silica gel G, buffered with acetic acid–sodium acetate, and with ethanol–0.22 M ammonia (89:11) as eluent, R_f 0.77. The second yellow band was eluted from the column with ether–methanol–acetic acid (49:49:2.5), and on thin layer chromatography as above had R_f 0.12. This is the value found for DNP-aspartic acid. There were no traces of DNP-serine (R_f 0.36), nor of DNP-glycine (R_f 0.55) in any fraction.

The DNP-aspartic acid was taken up in 25 ml of a sodium borate buffer and absorbance read at 361 mμ. When the above procedure starting with the hydrochloric acid hydrolysis was carried out on pure DNP-aspartic acid, it was found that recovery was only 48%. Losses have been noted before during the hydrolysis steps.

The calculated number-average molecular weight, correcting for the loss, was 7200.

Number-Average Molecular Weights by Dinitrophenylation. An appropriate weight of sample (1–6 mg) was dissolved in 2 ml of 2% sodium bicarbonate, warming if necessary to effect solution. To this was added 2 ml of a freshly prepared 1–3% solution of 2,4-dinitrofluorobenzene in ethanol. After 2 hr at room temperature the solution was transferred quantitatively to a separatory funnel using 6 ml of 3 N hydrochloric acid for rinsing. The solution was extracted with two 10-ml portions of carbon tetrachloride and the aqueous layer filtered through wet filter paper into a 25-ml volumetric flask and diluted to volume with 3 N hydrochloric acid. Absorbance was read at 353 mμ. A blank run was used to correct for absorbance owing to dissolved carbon tetrachloride or other substances. Sample size was chosen to give an absorbance of about 0.8, and an extinction coefficient of 1.59×10^4 l. mole^{-1} cm^{-1} was used. The rationale is that the extinction coefficient of the DNP derivatives may be expected to be relatively insensitive to the amino acid or the peptide to which the DNP is attached. In support of this it was found that five samples of DNP-Asp(OH)-OH in 2% sodium bicarbonate and in hydrochloric acid ranging from 1.2 to 6 N all had an extinction coefficient at 353 mμ of 1.59×10^4 with a standard deviation of 0.022 per run or 0.01 for the average. The value for DNP-Gly-Gly-Gly-OH was 1.60. Values reported at 350 mμ for DNP-Gly-OH (1.55), DNP-Phe-OH (1.57), DNP-Gly-Gly-OH (1.58), DNP-Phe-Val-OH (1.55), and for H-Lys-(DNP)-OH (1.49) are close.[25] (All values are to be multiplied by 10^4.) Properties of the polymers are summarized in Table IV.

(25) F. Sanger, *Biochem. J.*, **39**, 507 (1945); R. R. Porter, *Methods Enzymol.*, **4**, 221 (1957).

$$\bar{M}_n = \text{mg of sample} \times 1.59 \times 10^4/(A_{samp} - A_{blank})(\text{volume in milliliters})$$

Weight-Average Molecular Weights from Ultracentrifuge Measurements. The data in this paper are based on the Archibald method using the Beckman-Spinco Model E ultracentrifuge.[26] Runs were made in aqueous solution (mostly in 0.01 M KCl, but sometimes in water alone with comparable results) and at a concentration of 0.5–1%, usually using a Schlieren angle of 70° (50–85°) and a speed of 12,590, 20,410, or 24,630 rpm with photographs at intervals ranging from 10 to 100 min. In most cases only the meniscus values were used.

The principal source of error is the estimation of the intercept at the meniscus, and the error arises both from the problem of accurately identifying the meniscus and from the fading of the pattern. In the present series the meniscus was taken as the boundary as it appeared on the photographic plate, and extrapolation procedures were used to get the $(dc/dx)_0$ value at this point. Plots of several sets of data showed that the arbitrary function log (dc/dx) gave good agreement with the visual estimated of $(dc/dx)_0$ and that parabolic extrapolation was less satisfactory. Errors arising from $(dc/dx)_0$ estimates are the major source of the roughly 10% standard deviation per exposure. Data were processed by the computer program ARCHBD.

The calculated molecular weight is sensitive to the assumed density of the polymer, or of its reciprocal, the partial specific volume $\bar{V}$. Estimates of solution densities at concentrations of 1% with a precision of 1 part in 5000 lead to a 25% error in $\bar{V}$. We therefore used a calculated value for $\bar{V}$ of 0.61 for poly Asp(OCH$_3$)-Ser(H)-Gly.[27] The weight-average molecular weights in this paper have an estimated accuracy of about 20%. All data have been processed in a uniform fashion so that comparisons between samples involve only the standard deviation of the scatter which is 10%. Control runs on ribonuclease gave much sharper curves and satisfactory agreement with published results.

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Halide Ions as Probes for Nuclear Magnetic Resonance Studies of Proteins. The Sulfhydryl Groups of Hemoglobin

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Contribution from the Department of Chemistry, Stanford University, Stanford, California. *Received January 21, 1967*

Abstract: Chlorine nmr spectroscopy is used to verify that the unreactive sulfhydryl groups of hemoglobin are incapable of forming structures of the type protein–S–HgCl even when the protein is dissociated in media of high ionic strength or low pH. However, dissociation of hemoglobin into fragments appears to be accompanied by conformational changes that restrict the motion of chlorine ions bound to mercury atoms complexed at the sites of the reactive sulfhydryl groups. The changes in structure and chemical reactivity of hemoglobin in urea solution are complex.

In recent years there has been a great deal of interest in the application of nmr techniques to systems of biological importance.[2] Since the direct application of nmr to proteins in solution is seldom fruitful, several indirect methods involving relaxation effects have been developed.[3,4] Recently Stengle and Baldeschwieler[5] reported a novel technique which involves the use of a halide ion (usually chloride) as a probe for the study of mercury complexes of proteins. In this paper, this

(1) On leave from the Department of Chemistry, University of Massachusetts, Amherst, Mass.

(2) (a) A. Kowalsky and M. Cohn, *Ann. Rev. Biochem.*, **33**, 481 (1964); (b) O. Jardetzky, *Advan. Chem. Phys.*, **7**, 499 (1964).

(3) A. S. Mildvan and M. Cohn, *Biochemistry*, **2**, 910 (1963).

(4) O. Jardetzky, N. G. Wade, and J. J. Fisher, *Nature*, **197**, 183 (1963).

(5) T. R. Stengle and J. D. Baldeschwieler, *Proc. Natl. Acad. Sci. U. S.*, **55**, 1020 (1966).

method is applied to a specific protein, hemoglobin, with the aim of developing some information on the behavior of the sulfhydryl groups.

An interesting property of the hemoglobin molecule is its ability to dissociate into halves when dissolved in certain media. This phenomenon has been extensively investigated since it serves as a model for a variety of protein reactions.[6] From the nature of the media capable of causing dissociation it seems that the forces holding the halves together are primarily electrostatic, whereas further dissociation or unfolding, often leading to denaturation, must also involve the rupture of hydrophobic bonds.[7,8] The problem of the function of the unreactive sulfhydryl groups has been intimately connected with this aspect of the tertiary structure. There is some evidence to indicate that the unreactive sulfhydryl groups are directly involved in the interchain linkage,[9] although other work has not substantiated this contention.[8] This question can be resolved by a study of the SH groups of the dissociated molecule. If dissociation exposes originally unreactive SH groups to the solvent, hence activating them, the inference is that they are involved in the interchain binding. However, if the unreactive SH groups remain unreactive when the protein is cleaved, they must not be involved in interchain binding. Cecil and Thomas[8] studied the unreactive SH groups in a mixed water–alcohol solvent which was designed to rupture hydrophobic bonds without causing dissociation of the hemoglobin molecule. They found that the addition of alcohol to the solvent did cause an enhancement of the SH reactivity, indicating that the SH groups were involved in hydrophobic bonding and, hence, in intrachain structure.

Since the halide ion nmr technique can be specific for SH groups, it can be used to verify and extend the earlier results. The nmr method is particularly appropriate since it examines reactive SH groups, whereas Cecil and Thomas[8] worked with the unreactive ones.

Theory

For a nucleus of spin greater than $^1/_2$ (*e.g.*, ^{35}Cl, ^{79}Br, ^{127}I) the interaction of the nuclear electric quadrupole moment, Q, with the fluctuating field gradient, q, at the nucleus can provide a simple and dominant relaxation mechanism. In the extreme narrowing approximation, the contribution to the nuclear resonance line width from quadrupole relaxation is

$$\Delta\nu = K(e^2qQ)^2\tau_c \qquad (1)$$

where $\Delta\nu$ is the full line width at half-height in cycles per second, e^2qQ is the quadrupole coupling constant in cycles per second, τ_c is the correlation time for molecular rotation in seconds, and K equals $2\pi/5$ for a nucleus of spin $^3/_2$ if the asymmetry parameter is neglected. A large range of line widths is possible depending on the values of these quantities. For example, for a chloride ion in dilute aqueous solution, the solvation of the ion is essentially symmetric, and the electric field gradient at the nucleus is nearly zero. This results in a line width of 15–20 cps for the ^{35}Cl signal in aqueous solutions of NaCl. However, when the chlorine atom is involved in covalent binding, the value of e^2qQ is quite large; the line width for CCl_4 is 14.5 kc/sec. Even greater line widths are expected for molecules larger than CCl_4 with longer τ_c.

If a quadrupolar nucleus can be located at different kinds of sites in solution, the resulting line shape depends on the relative concentrations of the various sites, the values of e^2qQ and τ_c at each site, and the rate of exchange of chlorine among the various sites. If there are two possible sites, and the exchange of chlorine between them is very rapid, the spectrum will be a single composite signal of line width

$$\Delta\nu = \Delta\nu_a P_a + \Delta\nu_b P_b \qquad (2)$$

where $\Delta\nu_a$ and $\Delta\nu_b$ are the line widths at sites a and b, and P_a and P_b are the probabilities that the nucleus is at sites a and b. It is apparent from eq 2 that if $\Delta\nu_b$ is very large, a very small value of P_b may produce an observable effect on the line width $\Delta\nu$. Hence the chloride ion can be used as a probe for interesting sites in low concentrations, and the exchange process functions as a chemical amplifier. For small values of $P_b, P_a \cong 1$ and

$$\Delta\nu - \Delta\nu_a \cong \Delta\nu_b P_b \qquad (3)$$

The conditions for the binding and exchange process are reasonably restrictive. The chloride ion must be able to enter the first coordination sphere of the site and form a sufficiently strong bond to give a large value of q, the electric field gradient at the nucleus. Furthermore, the ion must remain bound for a time long compared with τ_c, while exchange with ions in the bulk solvent must occur in a time short compared with $1/\pi\Delta\nu_b$. The binding of chloride to mercuric ion appears to satisfy these requirements.[5]

It is possible to label specific sites in proteins with mercury, and thus use the chloride ion as a probe to examine these sites. Reaction of $HgCl_2$ directly with SH groups on protein molecules can produce sites of the type P–S–Hg–Cl. In a typical experiment the chlorine nmr line width of a 0.5 M NaCl solution is 16 cps. Addition of 1.4×10^{-5} M hemoglobin causes the line to broaden slightly to 17 cps. When the solution is made 2.8×10^{-5} M in $HgCl_2$, the line width changes to 55 cps.

The effect of mercury on the chlorine line width in the presence of hemoglobin is very large. This is due to the long effective correlation time of ^{35}Cl bound to the protein–mercury complex. If it is assumed that the mercuric ions react to completion with the two accessible SH groups, and that q has about the same value in P–S–Hg–Cl as in $HgCl_4^{-2}$, then the effective τ_c for the Hg–hemoglobin site must be about 100 times as long as that for the small $HgCl_4^{2-}$ complex. The correlation times observed in this work are frequently one or two orders of magnitude shorter than the time of rotation of the protein molecule as a whole.[10] In eq 1, τ_c refers to the correlation time for the change in angle between the electric field gradient at the chlorine nucleus, q, and the external magnetic field, H_0. This angle is affected by motions within the protein–mercury complex as well as by rotations of the entire molecule. Hence the effective correlation times will be a com-

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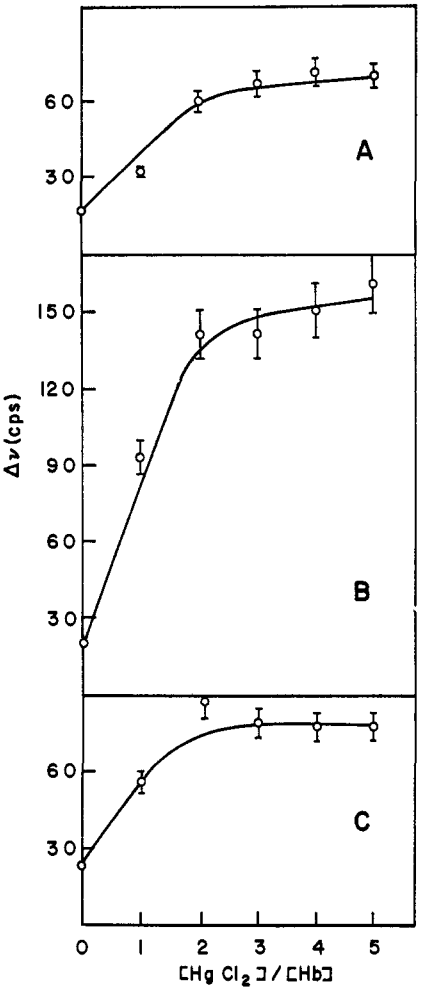

Figure 1. Titration of equine methemoglobin *vs.* $HgCl_2$ at various NaCl concentrations. All solutions were adjusted to pH 7 with 0.05 *M* phosphate buffer: A, NaCl = 0.5 *M*, Hb(E) = 1.4×10^{-5} *M*; B, NaCl = 2.0 *M*, Hb(E) = 7.0×10^{-5} *M*; C, NaCl = 4.0 *M*, Hb(E) = 7.0×10^{-5} *M*.

posite of these two factors. Thus τ_c provides a measure of the rigidity of the structure at the cysteine site, as well as the rotational correlation time of the entire protein, and should be quite sensitive to any unfolding of the molecule.

Experimental Section

Materials. Twice-recrystallized horse methemoglobin, Hb(E), was obtained from Pentex, Inc. The human hemoglobin, Hb(A), supplied by the Department of Biochemistry, Stanford Medical School, was freshly prepared from normal adult human blood and stored in the cold. It was used in the oxy form, and was never kept for more than 1 week. All other chemicals were analytical reagent grade. When hemoglobin was dissolved in dissociating media, the spectral measurements were made on the same day. Some of the solutions were quite unstable; in these cases spectra were obtained within a few minutes after the solutions were prepared.

Nmr Measurements. The ^{35}Cl spectra were obtained at 4.33 Mc/sec using a Varian V-4311 fixed-frequency radiofrequency unit. Base-line stabilization was achieved by field modulation and phase-sensitive detection using a PAR Model JB-4 lock-in amplifier. Calibration of the spectra was effected by the usual sideband technique. All spectra were obtained at room temperature. The reported line widths are the average of at least five spectra, except in the case of very unstable samples where only the first few runs could be trusted.

Results

The experimental measurements are most conveniently carried out in the form of a titration. Hemo-

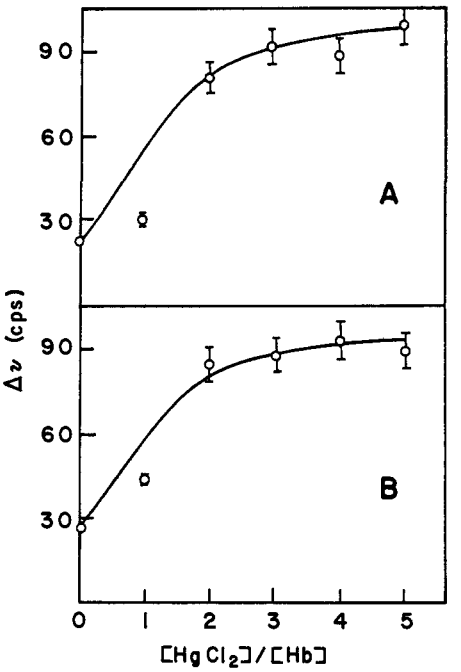

Figure 2. Titration of human oxyhemoglobin *vs.* $HgCl_2$ at various NaCl concentrations. All solutions were adjusted to pH 7 with 0.05 *M* phosphate buffer: A, NaCl = 1.0 *M*, Hb(A) = 2.6×10^{-5} *M*; B, NaCl = 4.0 *M*, Hb(A) = 5.1×10^{-5} *M*.

globin was dissolved in the selected medium containing 0.5 *M* NaCl, enough NaCl so that the ^{35}Cl resonance is readily observable. The nmr line width was followed as increasing amounts of $HgCl_2$ were added. In the early stage of the titration, when reactive SH groups are present, each addition of $HgCl_2$ causes the formation of P–S–Hg–Cl groups which results in a large increase in the ^{35}Cl line width. When all the available SH groups have reacted, further addition of $HgCl_2$ has little effect on the spectrum. A typical curve for horse methemoglobin is shown in Figure 1A. In this solution the dissociation of the protein is minimized by the low ionic strength and neutral pH. The end point at $[HgCl_2]/[Hg] = 2$ is well defined showing that there are only two reactive SH groups per molecule. From eq 2 it is apparent that

$$\Delta\nu - \Delta\nu_a = K(M[\mathrm{Hb}]/[\mathrm{Cl}^-])(e^2qQ)^2\tau_c \qquad (4)$$

where *M* is the number of Cl^- binding sites per labeled hemoglobin molecule, [Hb] is the concentration of labeled hemoglobin molecules, and $[Cl^-]$ is the chloride ion concentration. If e^2qQ is assumed to be the same for all these solutions, then for given hemoglobin and chloride ion concentrations the slopes of the titration curves are simply proportional to τ_c. It is known that hemoglobin will dissociate in solutions of high ionic strength.[11] At an NaCl concentration of 4 *M* the dissociation should be essentially complete. The titration curves in 2.0 and 4.0 *M* NaCl are presented in Figures 1B and C, respectively. These curves clearly show that only two SH groups are reactive. The unreactive SH groups are not exposed to the solvent, even when the molecule is completely dissociated into halves. Similar curves for human oxyhemoglobin in 1.0 and 4.0 *M* NaCl are shown in Figures 2A and B. The re-

(11) A. Rossi-Fanelli, E. Antonini, and A. Caputo, *J. Biol. Chem.*, **236**, 391 (1961).

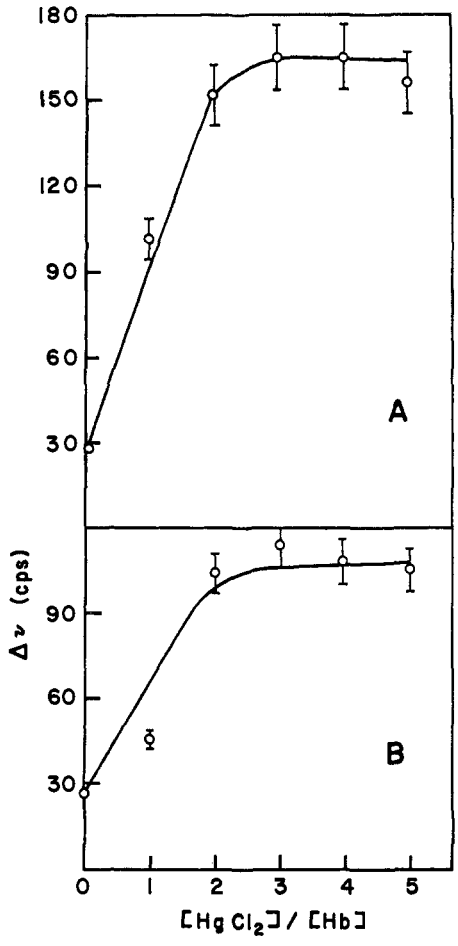

Figure 3. Titration of equine and human hemoglobin *vs.* $HgCl_2$ under acid conditions. All solutions were adjusted to pH 5.1 with 0.1 *M* acetate buffer: A, NaCl = 1.0 *M*, Hb(E) = 2.8×10^{-5} *M*; B, NaCl = 2.0 *M*, Hb(A) = 2.6×10^{-5} *M*.

sults demonstrate that all but two of the SH groups are masked even in 4 *M* NaCl. Several of the titration curves show an anomaly at the point corresponding to $[HgCl_2]/[Hb] = 1$; this solution shows less line broadening than expected. This is probably due to incomplete reaction between the $HgCl_2$ and the active SH groups; the high chloride ion concentration may tend to hold a small fraction of the metal in the form of $HgCl_4^{2-}$.

The relative values of the effective correlation times calculated for these solutions are given in Table I. The correlation times measured by this method appear to *increase* under dissociating conditions. This behavior indicates that in the dissociated protein the motions of the ^{35}Cl nucleus are more restricted than in the whole molecule. For a molecule of the size of hemoglobin, one would expect a rotational correlation time on the order of 100 nsec from the model of a sphere turning in a viscous liquid. Using eq 1 to calculate a value of τ_c from this work we obtain an order of magnitude of 1 nsec, under the assumption that e^2qQ for the protein–Hg–Cl complex is the same as for solid $HgCl_2$. Hence τ_c must be determined by motions faster than the rotation of the molecule as a whole, probably motions within the protein chains. Since τ_c increases in dissociating media, it appears that dissociation into fragments may be accompanied by a change in conformation of each fragment so as to restrict the motion of the bound chloride ions.

Table I. Relative Values of τ_c for Hemoglobin

Figure	$[Cl^-]$	[Hb] $\times 10^5$	Type of hemoglobin	$\Delta\nu - \Delta\nu_a$, cps at $[HgCl_2]/[Hb] = 2.0$	Rel τ_c	Remarks
1A	0.5	1.4	E	43	1.0	
B	2.0	7.0	E	128	2.4	
C	4.0	7.0	E	55	2.0	
2A	1.0	2.6	A	58	1.5	
B	4.0	2.6	A	58	5.8	
3A	1.0	2.8	E	125	3.1	pH 5.1
B	2.0	2.6	A	80	4.0	pH 5.1
4A	1.0	2.8	E	30	0.7	4.0 *M* urea
B	1.0	2.8	E	20	0.5	6.0 *M* urea
5A	1.0	2.6	A	50	1.3	4.0 *M* urea
B	1.0	2.6	A	25	0.6	6.0 *M* urea

Since hemoglobin is known to dissociate in an acid medium,[6] a series of measurements was attempted on both the equine and human samples at a pH of 4.5. In this medium, protein denaturation occurred immediately after addition of the $HgCl_2$, and no meaningful data could be obtained. It is interesting however that the precipitation of the protein could be followed by a fall in the ^{35}Cl line width with time. At a pH of 5.1 the denaturation was slowed to the point where the solutions were stable for a few minutes. By taking the spectrum immediately after the addition of $HgCl_2$, the curves shown in Figures 3A and B were obtained for equine and human hemoglobin at 1 and 2 *M* NaCl. Here again, only two active SH groups were found. Thus there is no difference in dissociation brought about by high ionic strength and that caused by a low pH insofar as events about the SH groups are concerned. Again the effective values of τ_c appear to increase as shown in Table I.

The effect of urea on hemoglobin has been the subject of some confusion in the past. Steinhardt[12] showed that urea causes dissociation of equine CO-hemoglobin, whereas Gutter, *et al.*,[13] reported that human CO-hemoglobin is unfolded by urea without appreciable dissociation. Recently Kawahara, *et al.*,[7] showed that human CO-hemoglobin is indeed dissociated by urea, but they also reported a series of experiments indicating that simultaneous unfolding does not occur. Chlorine nmr titrations on both human and equine hemoglobin in 4 and 6 *M* urea solutions are shown in Figures 4 and 5. These results are quite different from the other titration curves. Although vestiges of the end points are evident for the titrations in 4 *M* urea, the results for 6 *M* urea show no sign of a break at $[HgCl_2]/[Hb] = 2$. Furthermore, for a given concentration of hemoglobin and $HgCl_2$, the line-broadening effect in the presence of urea and hence the effective τ_c is less than is otherwise produced, as shown in Table I. This behavior cannot be due to an interaction of $HgCl_2$ with urea; when the experiment is performed in the absence of hemoglobin, such effects are not observed. Urea seems to be unique in other respects as well. For ex-

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(13) F. J. Gutter, H. A. Sober, and E. A. Peterson, *Arch. Biochem. Biophys.*, 62, 427 (1956).

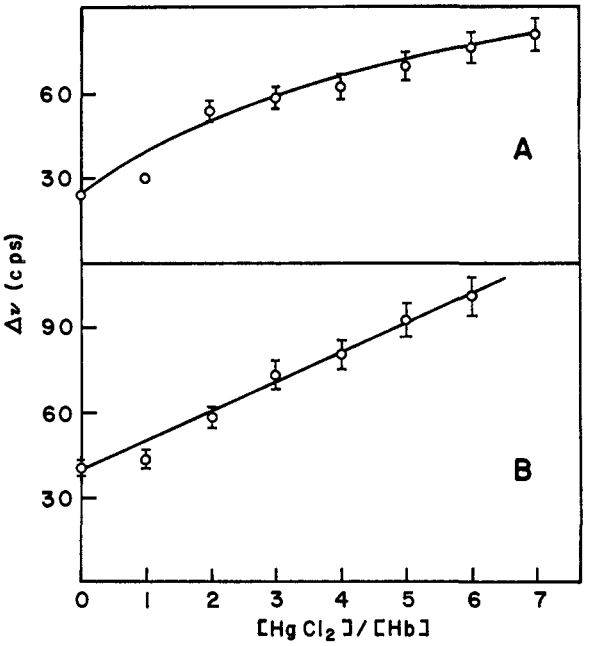

Figure 4. Titration of equine methemoglobin *vs.* $HgCl_2$ at various urea concentrations. All solutions were adjusted to pH 7 with 0.05 *M* phosphate buffer: A, NaCl = 1.0 *M*, Hb(E) = 2.8×10^{-5} *M*, urea = 4.0 *M*; B, NaCl = 1.0 *M*, Hb(E) = 2.8×10^{-5} *M*, urea = 6.0 *M*.

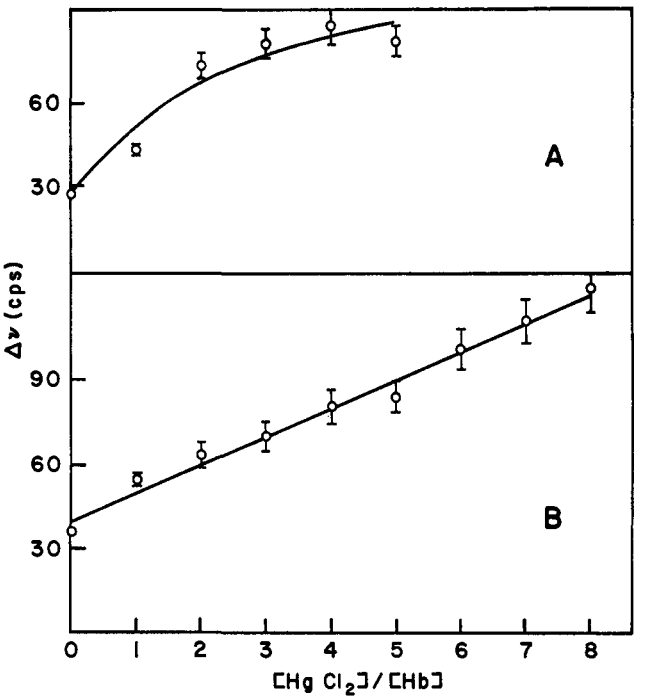

Figure 5. Titration of human oxyhemoglobin *vs.* $HgCl_2$ at various urea concentrations. All solutions were adjusted to pH 7 with 0.05 *M* phosphate buffer: A, NaCl = 1.0 *M*, Hb(A) = 2.6×10^{-5} *M*, urea = 4.0 *M*; B, NaCl = 1.0 *M*, Hb(A) = 2.6×10^{-5} *M*, urea = 6.0 *M*.

ample, the protein solutions were extremely stable in the presence of urea; no precipitation was ever observed in this medium. Unfortunately it was not possible to study solutions more concentrated in urea than 6 *M*, since at higher molarity of amide the solubility of NaCl was too low to obtain a good nmr signal.

Discussion

The most interesting result of this work is the lack of activity of the unreactive SH groups even when the hemoglobin molecule is dissociated into halves. This indicates that the unreactive SH groups are incapable of forming structures of the type P–S–Hg–Cl which are exposed to the solvent. Such behavior could be due to any one of several causes. The mercuric chloride could react with the SH groups producing P–S–Hg–S–P linkages. These groups would probably produce no effect on the ^{35}Cl nmr line width as shown by an earlier study of $Hg(SCH_2COOH)_2$.[5] Such a result is unlikely. Since the reactive SH groups do not form such bridges, it is improbable that the unreactive ones would. Another process consistent with the experimental data is the formation of P–S–Hg–Cl groups at the sites of the unreactive sulfhydryls, with the subsequent screening of these from the solvent by virtue of their location in unaccessible regions of the protein. A third possibility is that there is simply no reaction between $HgCl_2$ and the unreactive SH groups. In either of the latter two cases, the inference is the same; the behavior of the unreactive SH groups is essentially unaffected by dissociation of the molecule. This result is consistent with the conclusion of Cecil and co-workers[8,14] that the unreactive SH groups are involved in intrachain binding and would be affected by an unfolding of the individual chains, but not by a simple dissociation of the hemoglobin molecule. This result is also consistent with the observation that the unreactive SH groups fail to react with iodoacetamide in 2 *M* NaCl.[15]

There appears to be no simple explanation for the behavior of the urea–hemoglobin systems. This is not surprising since these solutions contain two substances capable of affecting the protein. Urea solubilizes amide, peptide, and hydrophobic groups, while sodium chloride decreases the free energy of ionic groups in contact with the solvent. It is not clear what effect a combination of the two will have, but one would expect the mixture to be exceptionally active by analogy with guanidine hydrochloride which also contains an amide-like and an ionic component.

In complex systems it is not always possible to derive an unequivocal explanation of the experimental results from the nmr data alone. The line width depends on the product of two variables, P_b and τ_c; there is no simple way of separating the effect of each one. It is highly probable that the flexibility of the hemoglobin molecule is enhanced in urea solution, thus lowering τ_c. The absence of an end point in the titrations in 6 *M* urea shows that P_b has also been affected. The absence of an end point even at mole ratios of $[HgCl_2]/[Hb]$ in excess of six indicates that sites in addition to the sulfhydryl group for the formation of mercury complexes with the protein may be available in concentrated urea solutions or the equilibria involving the mercury atom are more complex. Speculation on the changes in gross structure, conformation, and chemical reactivity of hemoglobin in urea solution requires information such as molecular weights in addition to these nmr results.

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(15) R. E. Benesch and R. Benesch, *Biochemistry*, **1**, 735 (1962).

Acknowledgments. Helpful discussions with Professors L. Stryer and H. Taube are gratefully acknowledged. This research was supported by the National Science Foundation under Grant GP-4924, and by the National Institutes of Health under Grant GM-13545-01. T. R. S. was also supported in part by the Center for Materials Research, Stanford University.

Communications to the Editor

Rearrangement of the Allylcarbinyl Radical[1]

Sir:

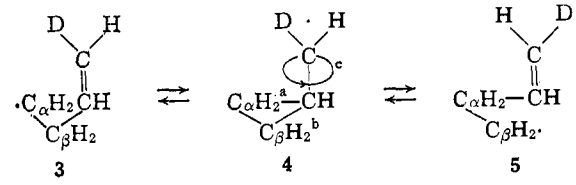
Extensive carbon skeletal rearrangement accompanies the solvolyses of allylcarbinyl derivatives. These fascinating rearrangements have been the subject of a number of studies.[2] Vicinal vinyl group migrations occur with facility in allylcarbinylmagnesium halides and diphenylallylcarbinyl radicals.[3] In this communication we wish to report two types of intramolecular structural transformations that allylcarbinyl radicals undergo, as well as several observations which are germane to the detailed mechanism of these changes.

In preliminary experiments it was determined that 4-pentenal decarbonylates by way of a radical chain process (di-*t*-butyl peroxide, chlorobenzene, 130°), yielding 1-butene as the virtually exclusive hydrocarbon product. In order to seek out conceivable carbon skeletal rearrangements that might take place during this reaction, the decarbonylation products of 4-pentenal-2,2-d_2 were examined. A 1.0 *M* solution of 4-pentenal-2,2-d_2 afforded 1-butene-4,4-d_2 and 1-butene-3,3-d_2 in roughly equal quantities (mass spectral analysis). Recovered, unreacted 4-pentenal-2,2-d_2 was not rearranged. Following a procedure developed previously in studies of the decarbonylations of 2- and 3-methyl-4-pentenals and 2- and 3-methyl-*trans*-4-hexenals[4] which facilitates quantitative interpretation of the rearrangement data, a series of 4-pentenal-2,2-d_2 solutions, varying in concentration from 0.50 to 6.0 *M*, was prepared and allowed to react to only a few per cent conversion. 1-Butene-4,4-d_2:1-butene-3,3-d_2 ratios were determined for each solution. Ratios for the 0.50–1.5 *M* solutions were all close to 1.0:1.0 There was, however, a small but clearly discernible monotonic increase in the ratios (10–20%) in going from the solutions in the concentration range 0.50–1.5 *M* to the 6.0 *M* solution.[5] The magnitude of this increase is in reasonable agreement with that which would be predicted from the decarbonylation studies on 2-methyl- and 3-methyl-4-pentenals.[4a] The variation in ratios demonstrates that *a minimum* of two radical intermediates gives rise to the observed olefinic products. Classical[4a,6] radicals **1** and **2**, which are interconvertible by vicinal vinyl group migration, suffice nicely as the required intermediates. Homoallylic radicals **1** and

$$\underset{\mathbf{1}}{CH_2{=}CHCH_2CD_2\cdot} \underset{\leftarrow}{\overset{\sim CH{=}CH_2}{\rightarrow}} \underset{\mathbf{2}}{\cdot CH_2CD_2CH{=}CH_2}$$

2 must interconvert moderately fast to be compatible with the data. In terms of the proposed mechanistic scheme, the decrease in the extent of rearrangement with increasing aldehyde concentration results from increased trapping of **1**, which is formed initially from 4-pentenal-2,2-d_2.

If the interconversion of **1** and **2** is intramolecular, a reasonable assumption, a half-migrated entity of cyclopropylcarbinyl structure must be implicated in the very least as a transition state in the rearrangement sequence. The question arises as to whether a cyclopropylcarbinyl intermediate is also involved. In an effort to probe this point, *cis*-4-pentenal-5-d_1 (95% *cis* as inferred by proton nmr analysis) was synthesized and its decarbonylation products were examined. Radical chain decomposition of *cis*-4-pentenal-5-d_1 should generate homoallylic radical **3** initially. If half-migrated structure **4** is merely a transition state for carbon skeletal rearrangement, bond a should be formed



3 **4** **5**

and bond b broken without a loss of geometrical identity about bond c, for transition-state lifetimes are generally considered to be short[7] relative to internal rotational lifetimes.[6] Decarbonylation of a 1.0 *M* solution of *cis*-4-pentenal-5-d_1 gave *cis*-1-butene-1-d_1 and *trans*-1-butene-1-d_1 in a 1.03:1.00 ratio (nmr). Recovered starting material had not rearranged. A straightforward explanation of these results is that a cyclopropylcarbinyl radical like **4** is a reaction intermediate and that the double bond geometry of the starting aldehyde is lost in intermediate **4** through rotation

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(2) K. L. Servis and J. D. Roberts, *J. Am. Chem. Soc.*, 87, 1331 (1965), and pertinent references cited therein.

(3) (a) M. S. Silver, P. R. Shafer, J. E. Nordlander, C. Rüchardt, and J. D. Roberts, *ibid.*, 82, 2646 (1960); (b) D. J. Patel, C. L. Hamilton, and J. D. Roberts, *ibid.*, 87, 5144 (1965); (c) T. A. Halgren, M. E. H. Howden, M. E. Medof, and J. D. Roberts, *ibid.*, 89, 3051 (1967).

(4) (a) L. K. Montgomery, J. W. Matt, and J. R. Webster, *ibid.*, 89, 923 (1967); (b) L. K. Montgomery and J. W. Matt, *ibid.*, 89, 934 (1967).

(5) The presence of minor contaminants prohibited precise measurement of the 1-butene-4,4-d_2:1-butene-2,2-d_2 ratios. The ratios are presently being redetermined using chromatographed (glpc) 1-butene-x,x-d_2 samples. Preliminary experiments using this technique support the qualitative trend cited here.

(6) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, 39, 2147 (1963).

(7) S. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p 189.