Acknowledgment. We are grateful to Messrs. Chung Kuang Chen, Toshio Nakao, Hiroyuki Kino, and Toshio Miyoshi for their assistance in the preliminary experiments of this work.

Supplementary Material Available: Spectral and elementary analysis of the derivatives (5 pages). Ordering information is given on any current masthead page.

References and Notes

(1) During the course of study on the preparation of tetracyanoethylene, Cairns and co-workers[2] obtained 7-cyano-7-ethoxycarbonylbicyclo[4.1.0]heptane in 10% yield by the reaction of ethyl dibromocyanoacetate with copper in the presence of cyclohexene. The reaction of dibromomalononitrile with copper in the presence of cyclohexene was also suggested to afford 7,7-dicyanobicyclo[4.1.0]heptane, although the structure of the product was not finally elucidated. These experiments are previous examples of reaction 1, but details are not available in the literature.
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(3) Bromoiodomethane,[4] chlorodiiodomethane,[5] dichloroiodomethane,[5] and methyl dibromoacetate[6] were prepared by minor modification of literature methods. Diiodomethane, olefins, and solvents were purified by distillation. The ordinary commercial grade of copper powder (particle size was 5–15 μ) provided by Nakarai Chemicals, Ltd., Kyoto, was used without further purification. Copper powder was allowed to react with a small amount of iodine in solvent at room temperature. After the brown color of iodine disappeared, olefin and organic *gem*-dihalide were added, and the mixture was heated at the prescribed temperature with stirring. After the reaction, the inorganic products were separated by filtration, and the organic layer was analyzed. Yields were determined by VPC analysis of the reaction mixture.
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(12) VPC analysis of the reaction mixture showed the formation of an unknown material in the neighborhood of 7-methoxycarbonylbicyclo[4.1.0]heptanes in estimated yield of 5%. ^{1}H NMR spectrum of this material appeared consistent with methyl 1-cyclohexen-1-ylacetate.
(13) These reactions were carried out with the aid of Mr. Ichiro Kameura.

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Received September 30, 1975

Oligopeptides as Potential Antiaggregation Agent for Proteins: Hemoglobin S Gel and Insulin Dimer

Sir:

The ordered aggregation of certain proteins requires specific contact areas between associating protein molecules. Oligopeptides mimicking a portion of the amino acid sequence at the contact region has been proposed as potential antiaggregation agents.[1] This is based on a working hypothesis that such oligopeptides (A) might compete for the binding sites on the protein (P) molecules if they are energetically favorable or if their concentrations are sufficiently high, thereby shifting the equilibria:

$$P + P \overset{K_P}{\rightleftarrows} P_2 \ldots \rightleftarrows P_n; \quad P + A \overset{K_A}{\rightleftarrows} PA \qquad (1)$$

toward the monomeric complex PA which is incapable of association. Reaction 1 does not rule out the possibility that P aggregates to form a nucleus P_m ($m \ll n$),[2] but stops at P_m·PA instead of polymeric P_n. To test this idea, we present some preliminary studies of the influence of antiaggregation agents on two proteins: deoxygenated hemoglobin (Hb) S which gels and insulin which dimerizes.

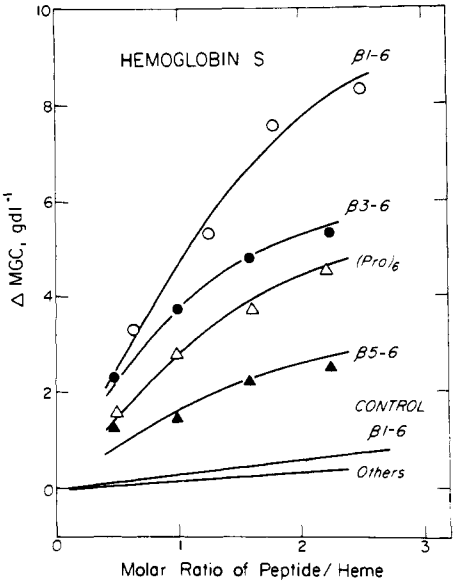

Figure 1. Increase in the minimum gelling concentration of deoxyhemoglobin S in the presence of various oligopeptides in phosphate buffer (pH 6.8; ionic strength 0.1) at 37 °C. The MGC of deoxyHb S alone is 9.5 g dl^{-1}. The baselines refer to the correction of additional ionic strength due to positively charged oligopeptide amides. See text.

Hb S: Out of 574 amino acid residues Hb S differs from normal Hb A in only two mutation sites, that is, two β6 valine residues instead of glutamic acid. It is highly suggestive that the β1–6 region:

1 6
Val His Leu Thr Pro Val . . .

might constitute one of the contact areas between neighboring molecules when deoxyHb S gels. Thus, we have synthesized a series of oligopeptide amides containing β1–6, β3–6, β5–6, and also a hexa-L-prolineamide (the latter is to test the specificity of these peptides). The effectiveness of these oligopeptides is determined by comparing the minimum gelling concentration (MGC)[3] of deoxyHb S in phosphate buffer (pH 6.8; I = 0.1) at 37 °C. For control, the MGC of Hb S alone was found to be 9.5 g/dl at I = 0.1; addition of NaCl to increase its ionic strength raised the MGC to 10.3 g/dl at I = 0.16. Figure 1 shows the increase in MGC of deoxyHb S in the presence of various oligopeptides. (The imidazole·HCl in the hexapeptide was first neutralized with concentrated NaOH, which produced additional NaCl. This accounts for the difference in the baselines.) The most prominent feature is that the MGC increases almost linearly with the molar ratio of the hexapeptide β1–6 amide to Hb S. At a molar ratio of 2.5 peptide per heme, the increment in MGC amounts to 75%. Shorter peptides such as tetrapeptide β3–6 and dipeptide β5–6 amides are less effective than the hexapeptide amide β1–6. These results are consistent with the view that the oligopeptides compete for the binding site at the contact area. Hexa-L-prolineamide also raises the MGC of deoxyHb S, although it is not as effective as β1–6 amide. It is possible that $(Pro)_6$ might interfere with some other contact area, thus making gelation difficult. (We also attempted to use $(Gly)_6$ and $(Ser)_6$, but their low solubilities at neutral pH made meaningful measurements of ΔMGC difficult.) Hemoglobin which is much larger than our other example, insulin, may provide locations other than those involved in intersubunit contacts which can associate less specifically with oligopeptides but with subsequent modification of aggregation tendencies. Recently, small quantities (3.8 mM) of L-homoserine, L-glutamine, and L-asparagine, but no other amino acids, have been reported to inhibit and reverse the sickling of erythrocytes.[4] Our preliminary studies

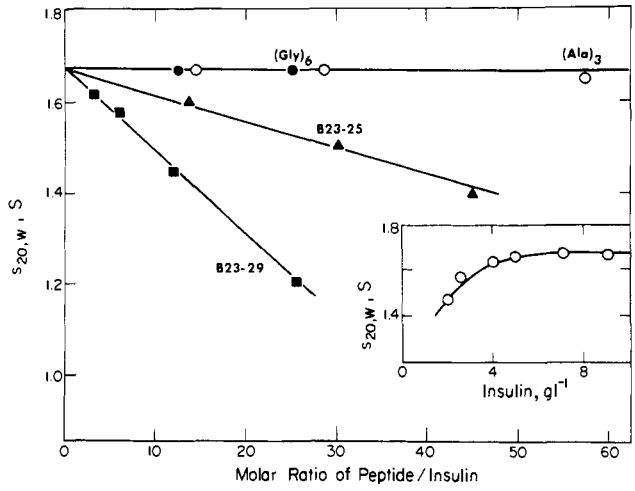

Figure 2. Effect of oligopeptides on the sedimentation coefficient of 0.38% insulin in glycine-HCl–NaCl buffer (pH 2; ionic strength 0.1) at 20 °C. Inset: concentration dependence of the s value of insulin in the same buffer.

indicated that these three compounds had little or a small effect on the MGC of deoxyHb S.[5] It seems that the morphology of red cells can be altered by agents which are without effect on in vitro gelation.

Insulin: For insulin dimerization, the extended C-terminal residues of the B chains:

$$\begin{matrix} 23 & & & & & & & & 29 \\ \cdots & \text{Gly} & \text{Phe} & \text{Phe} & \text{Tyr} & \text{Thr} & \text{Pro} & \text{Lys} & \text{Ala} \end{matrix}$$

run antiparallel to each other. This makes possible the antiparallel β-form between B24 and B29 which contains four hydrogen bonds between two monomers (B24 of one molecule to B26 of the other).[6,7] Figure 2 shows the effect of several oligopeptides on the dissociation of insulin dimer in glycine-HCl–NaCl buffer (pH 2; $I = 0.1$). The concentration of the protein was kept at 0.38% (6.6×10^{-4} M (monomer)), since the sedimentation coefficient, s, of insulin began to drop below this concentration (see inset; also ref 8). Addition of a heptapeptide B23–29 (prepared by trypsin digestion of insulin)[9] reduces the s value linearly from about 1.7 S (mostly due to the dimer)[8] toward the monomer (about 1.2 S at infinite dilution of the protein solution).[10,11] Even a tripeptide B23–25 appears to shift the dimer–monomer equilibrium, although it is less effective than the heptapeptide B23–29. On the other hand, hexaglycine and $(Ala)_3$ have no effect on the sedimentation coefficient. These results again suggest that oligopeptides having the same sequence as a portion of the C-terminal residues of the B chain might interfere with the dimerization of insulin.

Segments of the peptide chain in a protein molecule are fixed in a right conformation, but isolated fragments such as the oligopeptides are largely random in solution. The binding, if any, of these compounds to proteins would result in a loss of configurational entropy, which must be compensated by a decrease in enthalpy through hydrogen bonding or hydrophobic interaction or both. Therefore, in general, the equilibrium association constant K_p is expected to be much larger than the equilibrium binding constant K_A in reaction 1. The standard free energy change, $\Delta G°$, for the equilibrium involving PA would determine the effectiveness of these antiaggregation agents. Raising their concentrations could help shift the equilibrium toward the monomeric PA. In this respect, the work of Eisinger et al. on $tRNA^{Phe}$ may have some bearing on our findings.[12] A codon trinucleotide does not bind with the complementary anticodon trinucleotide. However, a weak, but detectable binding does occur when the anticodon triplet is fixed in the $tRNA^{Phe}$ molecule. The binding is the strongest when the triplets are parts of tRNA and mRNA molecules, respectively. Our protein–oligopeptide interaction may represent a similar intermediate case.

Our initial objective is to seek an antisickling agent for deoxyHb S gel without introducing any chemical modification of the intact native proteins (an effective antisickling compound is simply one that raises the MGC so high that deoxyHb S would not gel under physiological conditions).[1] The idea that is implicit in reaction 1 can equally well be applied to other biological aggregates if the binding of the antiaggregation agent to a biopolymer is energetically favorable. This leads us to study the insulin dimerization. Although the evidence is not yet conclusive, the proposed working hypothesis merits further investigations of other biological systems.

Acknowledgment. This work was aided by USPHS Grants HL-18818, HL-06285, and GM-10880. We thank Drs. W. Gratzer and L. Peller for their valuable comments and Drs. S. B. Shohet and B. Lubin for their encouragement and supply of blood samples.

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Interpretation of Mass Spectroscopic Fragmentation by State Correlation Diagrams

Sir:

State correlation diagrams are useful in the interpretation of photochemical mechanisms.[1] Here we apply such diagrams to a fundamental mass spectroscopic reaction, the fragmentation (or α-cleavage) of ketone positive radical-ions.[2,3] The departure of the alkyl fragment is assumed to occur in a coplanar fashion. It is then possible to correlate all the low-lying states of reactant ion with the states of primary products. The coplanar path is not necessarily the best pathway for a given state (specific geometry reorganization occurs in each state), but it reveals correlations which control the dynamical behavior of even noncoplanar pathways.[1] We illustrate the process with the α-cleavage of the acetaldehyde radical-cation, whose mass spectrum[3b] shows peaks for HCO^+ (abundance 100) and CH_3^+ (abundance 32)—corresponding to CC bond cleavage—as well as a peak for CH_3CO^+ (abundance 42), corre-