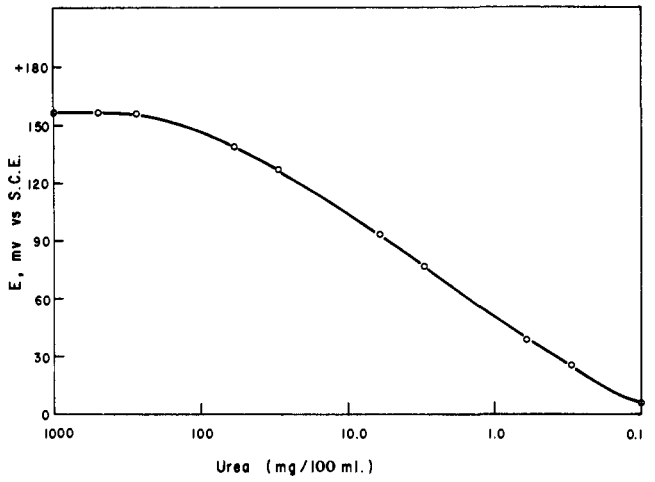

Figure 1. Response of enzyme electrode with 175 mg of urease/100 ml of gel.

it is made by polymerizing a gelatinous membrane of immobilized enzyme over a Beckman cationic glass electrode which is responsive to ammonium ions. Specificity for urea is obtained by immobilizing the enzyme urease in a layer of acrylamide gel 60–350 μ thick on the surface of the glass electrode. When the urease electrode is placed in contact with a solution containing urea, the substrate diffuses into the gel layer of immobilized enzyme. The enzyme catalyzes the decomposition of urea to ammonium ion as shown in the following equation.

$$\text{urea} + H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_2$$

The ammonium ion produced at the surface of the electrode is sensed by the specially formulated glass which measures the activity of this monovalent cation in a manner analogous to pH determination with a glass electrode.

The potential of this electrode is measured after allowing sufficient time for the diffusion process to reach the steady state. This interval varies from about 25 to 60 sec for 98% of the steady-state response, depending on the thickness of the gel membrane.

When the urea concentration is below the apparent K_m for the immobilized enzyme, but above 0.6 mg of urea/100 ml of solution, the potential of the electrode varies linearly with the logarithm of the urea concentration. Also, the response curve goes from first order at low urea concentrations to zero order at high substrate concentrations. A typical calibration curve is shown in Figure 1.

A detailed report on the parameters (enzyme concentration in gel, per cent gel, etc.) that effect the response of this electrode, the stability of the immobilized enzyme electrode on prolonged storage in aqueous buffer at 25°, and application to determination of urea in blood serum and urine will be published in the near future.

George G. Guilbault, Joseph G. Montalvo, Jr.
Department of Chemistry,
Louisiana State University in New Orleans
New Orleans, Louisiana
Received February 7, 1969

Side Chain Effect on the Helix Stability of Poly-α-amino Acids

Sir:

In the course of our investigations[1] in the correlation of structures of poly-L-lysine–metal complexes as enzyme models with their catalytic behaviors, it seemed to be of interest to study the complexes of poly-α-amino acids consisting of the lower homologs of L-lysine. As the first step of these studies, this communication presents preliminary results obtained on the relative stability of the helical structures of poly-α-amino acids derived from L-lysine and its lower homologs. Though there have been many studies[2] on the helix–coil transitions of poly-ε-N-carbobenzoxy-L-lysine $(Cbz\text{-}Lys)_n$ and poly-L-lysine $(Lys)_n$ and some studies[3-5] on poly-L-ornithine $(Orn)_n$, no conformational study of poly-δ-N-carbobenzoxy-L-ornithine $(Cbz\text{-}Orn)_n$, poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid), and poly(L-α,γ-diaminobutyric acid) has been reported.

The samples of $(Cbz\text{-}Lys)_n$, $(Cbz\text{-}Orn)_n$, and poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) used here have degrees of polymerization (DP's) of 300, 190, and 33, respectively, estimated from viscosity measurements.[6] The helix–coil transitions of these polymers were studied in a chloroform–dichloroacetic acid solvent system by measuring optical rotatory dispersion. The ORD measurements were carried out over the wavelength range of 290–500 mμ at 25° with a JASCO Model ORD/UV-5 optical rotatory dispersion recorder. The concentration range of the polymers was 0.2–1.0 g/dl. The normalized ORD curves were independent of the solute concentration in the range studied. The curves obtained were analyzed with the Moffitt equation,[7] assuming a λ_0 of 212 mμ.

Figure 1 shows variations of Moffitt's b_0 values with the compositions of the mixed solvent. Of the three polymers, as shown in Figure 1, the helix content of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) decreases most slowly with increasing content of dichloroacetic acid in the mixed solvent. Even at 40 vol % of dichloroacetic acid, poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) exists in a helical form to a considerable extent in spite of its relatively low DP, while the others are virtually random coils at the same ratio of the solvent components. Thus, the helical structure of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) seems to be the most stable of those of the three polymers. It seems due to the relatively low DP of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) that the absolute value of b_0 of poly(γ-N-carbobenzoxy-L-α,γ-diamino-

(1) In the oxidation reaction of 3,4-dihydroxyphenylalanine catalized by poly-L-lysine–copper(II) complex, a relationship between the helix content of the catalyst and the asymmetric selectivity of the substrate by the catalyst was observed: M. Hatano, T. Nozawa, S. Ikeda, and T. Yamamoto, to be submitted.

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(3) G. Blauer and Z. B. Alfassi, *Biochim. Biophys. Acta*, 133, 206 (1967).

(4) M. J. Grourke and J. H. Gibbs, *Biopolymers*, 5, 586 (1967).

(5) S. R. Chaudhuri and J. T. Yang, *Biochemistry*, 7, 1379 (1968).

(6) The equation $\log DP = 1.47 \log [\eta]^{25}_{DCA} + 2.99$ was used, where $[\eta]^{25}_{DCA}$ is an intrinsic viscosity measured in DCA at 25.0°.

(7) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, 42, 596 (1956).

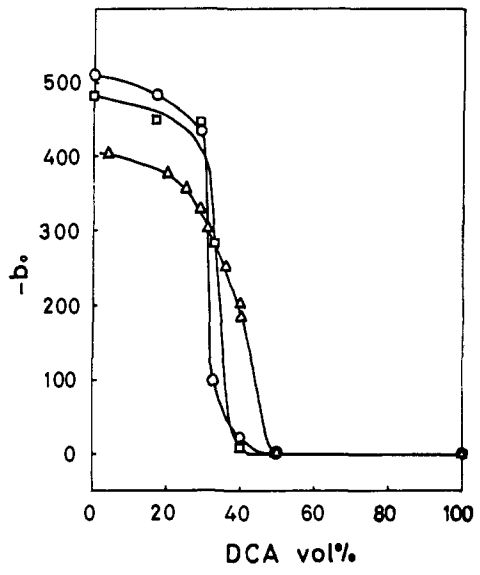

Figure 1. Moffitt's b_0 *vs.* solvent composition in a chloroform–dichloroacetic acid system: ○, $(Cbz\text{-}Lys)_n$; □, $(Cbz\text{-}Orn)_n$; Δ, poly(γ-*N*-carbobenzoxy-L-α,γ-diaminobutyric acid).

butyric acid) extrapolated at 100 vol % of chloroform is rather low. Though the difference between the curves of $(Cbz\text{-}Lys)_n$ and $(Cbz\text{-}Orn)_n$ is small, the helix content of $(Cbz\text{-}Orn)_n$ is, for example, about three times that of $(Cbz\text{-}Lys)_n$ at 33 vol % of dichloroacetic acid in spite of the DP of $(Cbz\text{-}Orn)_n$ being lower than that of $(Cbz\text{-}Lys)_n$. This seems, in turn, to show that the helical structure of $(Cbz\text{-}Orn)_n$ is a little more stable than that of $(Cbz\text{-}Lys)_n$. It can be considered that, in the case of the polymers studied here, the urethan linkages in the side chains, which can combine with one another through hydrogen bonds, may serve to stabilize the helical structures of the polymers in the helix solvent, and that in the helical structure of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid), which has shorter side chains, interaction between the urethan linkages in the side chains may be relatively large.

The samples of $(Orn)_n$ and poly(L-α,γ-diaminobutyric acid) were obtained by the decarbobenzoxylation of $(Cbz\text{-}Orn)_n$ (DP = 190) and poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) (DP = 110), respectively. The ORD and circular dichroism measurements were carried out in aqueous solution at various pH values at 20° with the same recorder as mentioned above. The concentration of the polymers was about 0.2 g/dl. From the spectra, Moffitt's b_0, reduced mean residue rotation at 233 mμ, $[m']_{233}$, in deg cm²/dmol, and residue ellipticity at 222 mμ, $[\theta]_{222}$, in deg cm²/dmol, were calculated. The estimation of fraction of helix, f_H, was made with the following empirical equations:[5,8] $f_H = -b_0/630$, $f_H = -([m']_{233} + 2000)/13{,}000$, and $f_H = (4000 - [\theta]_{222}))/42{,}000$.

The helix content of $(Orn)_n$ was estimated to be about 60% at pH 12 using any of these three methods. Our estimate of the helix content of $(Orn)_n$ agrees well with that obtained from the CD measurement by Grourke and Gibbs.[4] However, the estimate of the helix content of $(Orn)_n$ by Blauer and Alfassi[3] based on b_0 was about 20% at pH 11.4 and that by Chaudhuri and Yang[5] based on b_0 and $[m']_{233}$ was about 25% at pH 12. These two estimates do not agree with ours.

The helix content of poly(L-α,γ-diaminobutyric acid) was found to be practically zero even at pH 12 from any of the three methods mentioned above. This extraordinary instability of the helical structure of poly(L-α,γ-diaminobutyric acid) may be due to the stronger interaction between the amino groups in the side chains and the carbonyl groups in the main chain than in the cases of $(Orn)_n$ and $(Lys)_n$, though the instability may be partly due to the rather low DP of the sample of poly(L-α,γ-diaminobutyric acid) used here. It seems of interest to study the oxidation reaction of 3,4-dihydroxyphenylalanine using poly(L-α,γ-diaminobutyric acid)–copper(II) complex as a catalyst in order to elucidate whether the asymmetric selectivity by $(Lys)_n$–Cu(II) complex in the reaction is due to the helical structure of the polymer.[1]

Acknowledgment. The authors wish to thank Professor Junzo Noguchi for supplying samples of $(Cbz\text{-}Orn)_n$ and also Ajinomoto Co., Inc., and Kyowa Hakko Kogyo Co., Ltd., for supplying amino acids.

Masahiro Hatano, Michio Yoneyama, Ichi Ito
Tsunenori Nozawa, Michio Nakai
The Chemical Research Institute of Non-Aqueous Solutions
Tohoku University, Sendai, Japan
Received December 9, 1968

Reactions of Nitric Oxide with Methemoglobin[1]

Sir:

We wish to report that the reaction of nitric oxide and methemoglobin[1b] is accompanied by the reduction of methemoglobin.

Hemoglobin is known to react with molecular NO to form nitrosylhemoglobin (HbNO) having a paramagnetic susceptibility[2] of 3.07 BM. The electron paramagnetic resonance spectra (epr) of this hemoprotein has been briefly studied.[3,4] More recently, Sancier, *et al.*,[5] compared the reaction products of Hb and of Hi with NO. The authors reported that the long-wavelength absorption (α band) of HiNO was shifted slightly toward the blue and reduced in intensity as compared to the same transition in HbNO. Both hemoproteins were found to have the same spin concentrations as determined by epr; they also have the same isotropic *g* value. This similarity in paramagnetism of the two hemoproteins is surprising and

(1) (a) Much of the experimental work was carried out in the Department of Chemistry at Stanford University and was supported by the Office of Naval Research under Contract 228(88); research also benefited from facilities made available by the Advanced Research Projects Agency through the Center for Materials Research at Stanford University. The hospitality of Professor H. M. McConnell is gratefully acknowledged. (b) Hemoglobin and methemoglobin are represented by Hb and Hi, respectively.

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