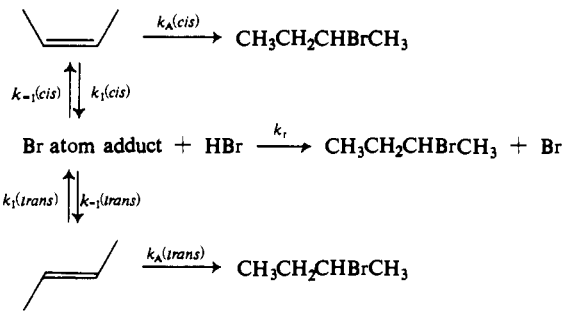
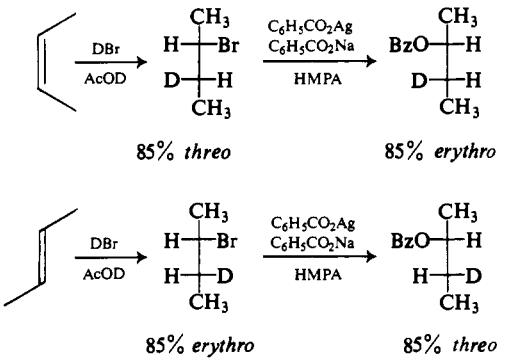
the reaction mixture, or substantial quantities of benzoyl peroxide are added, is any substantial amount of 1-bromobutane formed.

All of the foregoing observations are readily rationalized by Scheme I in which the rates of bromine atom isomerization are substantially greater than the rate of addition to *cis*-2-butene which in turn is greater than the rate of addition to *trans*-2-butene. Although the specific rate constants for the chain propagation steps for the radical chain addition of hydrogen bromide to the *cis*- and *trans*-2-butenes would appear to be much larger than the specific rate constants for the ionic addition reactions, the low concentration of radical intermediates, except in the presence of large quantities of oxygen or benzoyl peroxide, preclude the formation of substantial amounts of product *via* a radical chain mechanism. The interference of these radical-induced reactions would not be apparent in the additions to the unconjugated cyclic olefins because of the inability to observe olefin isomerization.[17]



The addition of *cis*- or *trans*-2-butene to a freeze-degassed acetic acid-O-*d* solution of deuterium bromide[18] maintained under a helium atmosphere in the dark produces a highly stereoselective *trans* addition (85 ± 1%) of deuterium bromide[19] as determined by the direct conversion of the bromides to the benzoates with silver benzoate–sodium benzoate in hexamethylphosphoramide.



The kinetics of the addition under various conditions is under study in an attempt to determine the mechanism of formation of the *cis* and *trans* addition products (Ad_E2 *vs.* Ad_E3 transition states).

7.88 Hz, $J_{CHDCHBr}$ = 0.8 Hz; *threo*, δ_{CHBr} 4.075, $J_{CHDCHBr}$ = 5.16 Hz, $J_{CHDCHBr}$ = 1.2 Hz. The nmr spectra indicate stereochemical purities of > 80%.

(20) Alfred P. Sloan Fellow, 1967–1969.

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A Urea-Specific Enzyme Electrode

Sir:

A prime objection to the use of enzymes as analytical reagents lies in the high cost of using large quantities of these materials. The immobilization or insolubilization of the enzyme eliminates this problem, because the enzyme could be used over and over again. The immobilization of several enzymes by various techniques has been recently reported. A review on the preparation of insoluble enzymes has been prepared by Chibata and Tosa.[1] Guilbault[2] has presented a more recent review of immobilized enzymes.

Very little work has been reported on immobilization of urease. In 1925, Sumner and Graham[3,4] obtained an enzymatically active water-insoluble urease preparation on adding small amounts of sodium chloride to neutral 30% alcohol urease. The water-insoluble product, which probably consists of intermolecularly disulfide cross-linked urease molecules, was only partially characterized. Its possible use as a heterogeneous catalyst was not investigated. Recently Riesel and Katchalski[5] reported the preparation and properties of water-insoluble derivatives of urease prepared by chemically binding urease with the diazotized copolymer of *p*-amino-DL-phenylalanine and L-leucine. The stability of the various urease preparations on prolonged storage at 4° varied from loss of 80% of the initial activity within a day to 35% of its initial activity within 5 months.

Bernfeld and Wan[6] and Hicks and Updike[7,8] have demonstrated the immobilization of enzyme activity in polyacrylamide gel. Our investigations have shown that urease immobilized in this gel possesses high activity. We wish now to report the preliminary results of our studies.

A urea transducer, suitable for rapid, continuous determination of urea in body fluids, has been developed. The urea transducer is called a urease electrode because

(17) A similar mechanistic scheme has been proposed by C. Walling and W. Helmreich (*J. Amer. Chem. Soc.*, **81**, 1144 (1959)) to account for the thiyl radical catalyzed isomerization of *cis*- and *trans*-2-butene.

(18) The acetic acid-O-*d* solution of deuterium bromide is prepared by the addition of deuterium oxide to phosphorus tribromide and bubbling the deuterium bromide into acetic acid-O-*d* prepared by the addition of deuterium oxide to distilled acetic anhydride, in a stream of nitrogen.

(19) The hydrogen nmr spectra of the *erythro*- and *threo*-3-deuterio-2-butanols are distinctly different: *erythro*, δ_{CHBr} 4.028, $J_{CHDCHBr}$ =

(1) I. Chibata and T. Tosa, *Tampakushitu Kakusan Koso*, **11**, 23 (1966).
(2) G. G. Guilbault, *Anal. Chem.*, **40**, 459R (1968).
(3) J. B. Sumner and V. A. Graham, *Proc. Soc. Exptl. Biol. Med.*, **22**, 504 (1925).
(4) J. B. Sumner, *Science*, **108**, 410 (1948).
(5) E. Riesel and E. Katchalski, *J. Biol. Chem.*, **239**, 1521 (1964).
(6) P. Bernfeld and J. Wan, *Science*, **142**, 678 (1963).
(7) G. P. Hicks and S. J. Updike, *Anal. Chem.*, **38**, 726 (1966).
(8) S. J. Updike and G. P. Hicks, *Nature*, **214**, 986 (1967).

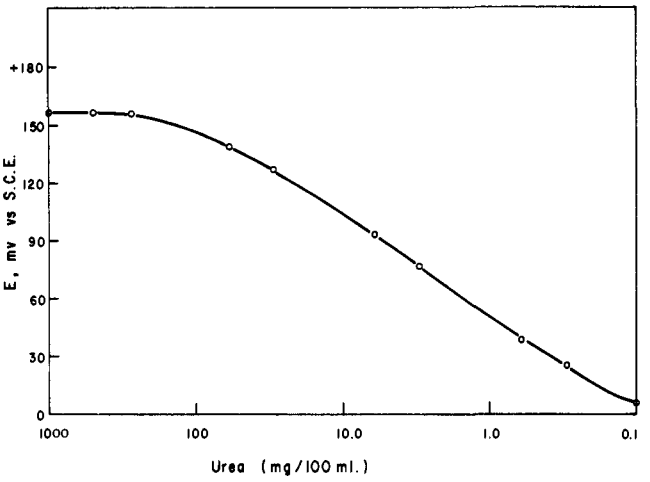

Figure 1. Response of enzyme electrode with 175 mg of urease/100 ml of gel.

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.

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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.

(2) G. D. Fasman, "Poly-α-amino Acids," G. D. Fasman, Ed., Marcel Dekker, Inc., New York, N. Y., 1967, p 499.

(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).