Ultrasonification of Ethylene Glycol[1]

As far as we know, all known synthetic sonochemical reactions require the presence of water[2]. We wish to report that ultrasonic waves[3] may be used to oxidize pure ethylene glycol in the presence of oxygen at 0 °C to peroxides[4] (of undetermined composition) and glycolaldehyde and/or glyoxal. In the presence of pure argon this reaction does not occur. Dilution with water (up to 75% H_2O) lowers the peroxide yield. Under identical reaction conditions pure ethylene glycol affords more peroxide than does pure water.

The kinetics of peroxide formation (up to 24 h) appear to be zero order with $k = 0.23 \pm 0.06$ mM/l/h. Aldehyde formation, evidenced by the osazone obtained with 2,4-dinitrophenylhydrazine[5], stops at ca. 10 mM/l[6] after 12 h of ultrasonification, although after 24 h both the yellow colour and distinctive odour of glyoxal were detected. Thus, the apparent cessation of total aldehyde formation appears to be due to further oxidation of glycolaldehyde to glyoxal.

Our observations indicate that the mechanism of oxidation of ethylene glycol may be different from that which occurs in aqueous solution, since the glycol reaction requires the presence of oxygen, whereas the water reaction does not[2]. This procedure does not constitute a practical method for the synthesis of the aldehydes obtained.

Zusammenfassung. In Gegenwart von Sauerstoff wird Äthylenglycol durch Ultraschall zu Peroxiden, Glycolaldehyd und/oder Glyoxal oxidiert.

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Department of Chemistry, Israel Institute of Technology, Haifa (Israel), August 10, 1966.

[1] This work was supported in part by Grant No. F2-MH-10,498 of the Department of Health, Education and Welfare (USA).

[2] P. RENAUD and H. GILLIER, Bull. Soc. Chim. Fr. *2381* (1964) and references therein.

[3] A model T200 (Lehfeldt-GmbH) ultrasonic generator operating at 800 kc/sec with an energy input of 3.8 W/cm² was used. The glycol samples were 6 ml.

[4] Peroxide was determined by the spectrophotometric method of A. O. ALLEN, C. J. HOCHANADEL, J. A. GHORMLEY, and T. W. DAVIS, J. phys. Chem., Ithaca *56*, 575 (1952).

[5] T. BANKS, C. VAUGHN, and L. M. MARSHALL, Analyt. Chem. *27*, 1348 (1955); C. NEUBERG and E. STRAUSS, Archs Biochem. *7*, 211 (1945).

[6] The aldehyde concentration was determined after all the peroxides had been destroyed by allowing the reaction mixture to stand overnight and heating it at ca. 100 °C for 1 h. The yield therefore consists of aldehyde formed both directly and via thermal decomposition of the peroxides.

Stereoselectivity in Peptide Synthesis under Simple Conditions

Previous studies have indicated that a suitable system for studying peptide synthesis in aqueous solution under simple conditions employs the cyanamides, with best results coming with sodium dicyanamide[1]. It was the objective of the present study to determine if stereoselectivity was active in oligopeptide synthesis which would depend on the nature of primary (neighbor) interactions rather than secondary ones (such as α-helix, which first appears around the octapeptide level[2]).

To carry out this study, L-amino acids were bound (carboxylic ester) to a chloromethylated polystyrene resin according to the method of MERRIFIELD[3]. This procedure permitted easier separation of products and restriction of the variety of mixed peptides that could be produced than if free monomer-monomer interactions had been used.

Preliminary results using free leucine (^{14}C-labeled) and bound tyrosine suggested that stereoselectivity might exist at the dipeptide level[4]. If this were so, one would expect to see the selectivity increase as the size of the interacting sidechains got bigger.

To test this possibility, the following experiments were carried out: in each case, a separate aqueous mixture was prepared containing 0.0125 M resin-bound L-amino acid, 0.00125 M ^{3}H-labeled D,L-leucine, and 0.1 N HCl. While the mixture (sample A) was being stirred, four 10 λ aliquots of sodium dicyanamide (DCA) were added 5 min apart so as to bring the total concentration of the condensing agent to 0.1 M. 5 min later, the resin was thoroughly washed with 100 ml of 0.01 N HCl, then water, and finally methanol. The dried resin was placed in 1 ml of *Tris* buffer (pH 8.0) containing leucine aminopeptidase (LAP). After the incubation period, the resin was again washed with 100 ml of 0.01 N HCl, then water, and finally acetone. Another sample (B) was treated in exactly the same fashion except that the enzyme step was eliminated. Both samples were measured by scintillation counting. Leucine aminopeptidase hydrolyzes off N-terminal L-amino acids exclusively[5]; the reading for sample A would therefore indicate the amount of D-leucyl-L-amino acid dipeptide produced because the radioactivity is due to leucine and the enzyme had removed (hydrolyzed) L-leucine from the resin. On the other hand, the reading for sample B would indicate the sum of the D-leucyl-L-amino acid and L-leucyl-L-amino acid dipeptides formed. Thus,

$$B = [D] + [L];\ A = [D];\ [L]/[D] = \frac{B - A}{A} = \frac{[D + L] - [D]}{[D]}.$$

According to the hypothesis set up for this work, if stereoselectivity does in fact occur at the dipeptide level, the L/D value should increase (L/D ≧ 1) as the sidechains (R) involved get bigger (i.e. increased sidechain interaction). The L-amino acids selected to bind to the resin were members of the straight chain homologous aliphatic series of alanine (R = CH_3), α-aminobutyric acid (R = CH_2CH_3), norvaline (R = $CH_2CH_2CH_3$), and norleucine (R = $CH_2CH_2CH_2CH_3$). Glycine (R = H), although it does not contain an asymmetric carbon, is the parent amino acid of this series and its hypothetical 'L/D' value can be taken as 1.0. The branched chain series of valine

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[2] G. WALD, Ann. N.Y. Acad. Sci. *69*, 352 (1957).

[3] R. B. MERRIFIELD, Biochemistry *3*, 1385 (1964).

[4] G. STEINMAN and C. ESHELMAN, unpublished results.

[5] E. L. SMITH, in *Methods in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press Inc., New York 1957), vol. II.

(R = $CH(CH_3)CH_3$), leucine (R = $CH_2CH(CH_3)CH_3$) and isoleucine (R = $CH(CH_3)CH_2CH_3$) was also studied.

The results failed to demonstrate a definite pattern of increasing L/D values as the size of the sidechain of the resin-bound L-amino acid increased. (In each case, D,L-leucine was the free amino acid used.) A mean value of 1.0 with an average deviation of $\pm$ 0.2 was observed. There was no apparent correlation of resultant values with the size of the sidechain involved. (Appropriate control experiments for the experimental method used have already been reported[6].) The positive results noted with the leucyltyrosine system may involve the additional factor of the polar nature of the sidechain of tyrosine, although this remains to be examined more thoroughly.

In order to be certain that the results noted with the resin-bound procedure were also applicable to unbound systems, the combination yielding the least number of different dipeptides (leucyl-leucine) was studied. With the use of D,L-leucine-4,5-H^3 and L-leucine-1-C^{14}, as well as leucine aminopeptidase (LAP), the following analytical scheme, outlined in terms of the labeled compounds involved, was devised. (The word 'leucine' has been omitted.):

(^{3}H): L* + D* $\xrightarrow{\text{DCA}}$ L*-L* + L*-D* + D*-L* + D*-D* (1)

L* + D*, L* + D*-L* + D*-D* (2) — LAP

(^{14}C): L* + D $\xrightarrow{\text{DCA}}$ L*-L* + L*-D + D-L* + D-D (3)

L* + L*+D + D-L* + D-D (4) — LAP

In this scheme, labeled substances are indicated with an asterisk (*). Since in the ^{14}C experiments (Nos. 3 and 4) D-leucine is unlabeled, D-leucyl-D-leucine (D-D) is not observed by radiotracer techniques. Scintillation spectrometry can readily differentiate between tritium and carbon-14 in the same sample.

An aqueous solution was prepared containing D,L-leucine, HCl, and sodium dicyanamide (DCA) as before. The only exceptions were that the HCl concentration was 0.12*N*, the D,L-leucine concentration (all unbound) was 0.02*M*, and both ^{14}C-L-leucine and ^{3}H-D,L-leucine were included together in the same reaction mixture. The enzyme step (LAP) was carried out by first neutralizing the product solution with $NaHCO_3$ and then placing it in *Tris* buffer (pH 8.0) containing the leucine aminopeptidase. Aliquots of the products, both after reaction with DCA (Nos. 1 and 3) and after subsequent treatment with LAP (Nos. 2 and 4), were resolved by paper electrophoresis in borate buffer at pH 9.2. The dimer bands, detected by autoradiography, were cut out and analyzed by scintillation spectrometry. By the electrophoretic method used, all the diastereomeric dipeptide products appeared together as one band and the monomers as another.

This method led to the following calculations (P = dimer product; example, 1P = dimer band from experiment No. 1):

$$\left.\begin{aligned}(1P) &= [L\text{-}L] + [L\text{-}D] + [D\text{-}L] + [D\text{-}D]\\ (2P) &= [D\text{-}L] + [D\text{-}D]\end{aligned}\right\}\ {}^3H$$

$$\left.\begin{aligned}(3P) &= [L\text{-}L] + [L\text{-}D] + [D\text{-}L]\\ (4P) &= [D\text{-}L]\end{aligned}\right\}\ {}^{14}C$$

$$(2P)\text{-}(4P) = [D\text{-}D] \qquad \text{Nos. 2, 4 = LAP-treated}$$

$$L/D = \frac{4P}{2P\text{-}4P} = \frac{[D\text{-}L]}{[D\text{-}D]}$$

Thus, appropriate analysis of the LAP-treated dimer product plus knowledge of the total activity employed would provide the desired data. The results gave an L/D value of 1.2, which compares quite well with the 1.1 figure found with the resin-bound system. This would further corroborate the conclusion that little, if any, stereoselectivity is evident at the oligopeptide level on the basis of preferential interactions.

In the context of possible events which may have occurred on the primitive Earth, these results suggest that the synthesis of stereohomogeneous polypeptides would have had to depend on chance associations at the simple peptide level and then on stabilization of homopolymers by the α-helix at higher degrees of polymerization[7]. (See reference [2] for a more detailed discussion of the problem of prebiological stereoselectivity.)

Zusammenfassung. Die Umsetzung von D,L-Leucin mit verschiedenen L-Aminosäuren und Dicyanamid als Kondensationsmittel zu Oligopeptiden verläuft nicht stereoselektiv. Die Bedeutung dieser Beobachtung in bezug auf den Ursprung des Lebens wird diskutiert.

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[7] The author is indebted to the College of Science and the Department of Biochemistry of the Pennsylvania State University for providing funds with which this investigation was carried out. The skillful technical assistance of Mr. W. DRESSELHAUS, Mrs. CAROL ESHELMAN and Mr. A. TISCHLER is gratefully acknowledged. Mr. R. COTNER graciously helped with the scintillation counting.

Dietary Cholesterol and Activity of Enzymes Hydrolyzing and Synthesizing Cholesterol Ester in Pancreatic Juice of Rats

Dietary cholesterol esters are hydrolyzed, presumably in the lumin of the small intestine, before absorption into the intestinal mucosa[1]. Free cholesterol is esterified prior to the transfer of cholesterol into the intestinal lymphatics, maintaining a ratio of esterified to free cholesterol of approximately 2–3:1 in intestinal lymph[1]. Enzymes derived from the pancreas are believed to be involved in both the hydrolysis of the esters and the esterification of free cholesterol[1]. Feeding rats a high cholesterol diet does not increase the activity of these enzymes in the pancreas[2]. The present study was undertaken to determine

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