COMMUNICATIONS TO THE EDITOR

POLYMORPHISM AND RADIATION DECOMPOSITION OF CHOLINE CHLORIDE[1]

Sir:

Solid choline chloride at room temperature is extremely sensitive to ionizing radiation and the choline ion breaks down into trimethylamine and acetaldehyde.[2,3] Recently Serlin[4] reported that for γ-radiation the decomposition per 100 e.v. absorbed was higher at 50° than at 20° but at 150° the solid choline chloride was much more stable than at room temperature. X-Ray diffraction experiments on both powders and single crystals of choline chloride have been carried out in this laboratory and a phase transition has been found in the range 73–78°. This transition has been verified also with simple cooling curves.

At room temperature after only a half hour exposure to a collimated beam from a molybdenum target X-ray tube the crystals become quite cloudy while exposures of 20 hours length at 80° cause only a very faint yellow color to develop in the crystal. This lends added support to Serlin's more convincing experiments.

The crystal structure of the room temperature phase has been worked out by Senko[5] and is based on an orthorhombic unit cell with axial lengths a = 11.21, b = 11.59, c = 5.87Å., space group $P2_12_12_1$, and containing four molecules. A single crystal slowly heated from room temperature transforms at about 73° into another single crystal with a few smaller satellites randomly oriented with respect to it. The high temperature phase is face-centered cubic with four molecules in a unit cell of axial length 9.5Å. The [110] axis of the main crystal in the high temperature phase coincides with the [001] direction of the room temperature crystal. If the cubic phase is now cooled back down to room temperature it fractures into a large number of small crystals when the transition to the orthorhombic phase occurs.

The 4-fold positions in the face-centered cubic space groups have at least 23 or m3 point symmetry[6] and since the choline ion can have at most a mirror plane (the room temperature molecular structure as described by Senko has no symmetry) the cubic phase must be disordered. There appears to be sufficient room in the unit cell for spherical rotation of the choline ion but symmetry and space requirements do not preclude a disordered structure involving motion of the ion about a number of different equilibrium positions or structures involving internal disorder of the choline ion.

The exact mechanism for the room temperature decomposition of solid choline chloride upon irradiation is still not clear. However, the transition to the disordered phase and the subsequent increased stability furnish further evidence that the decomposition is highly stereospecific.

Studies are now under way to investigate further the nature of the disorder in the cubic phase, the structural details of the transition and the structural changes occurring in the orthorhombic room temperature phase when exposed to ionizing radiation.

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(1) This work was done under U. S. Atomic Energy Commission Contract AT(30-1)-901 with the New England Deaconess Hospital.

(2) B. M. Tolbert, *et al.*, This Journal, **75**, 1867 (1953).
(3) R. M. Lemmon, M. A. Parsons and D. M. Chin, *ibid.*, **77**, 4139 (1955).
(4) I. Serlin, *Science*, **126**, 261 (1957).
(5) M. E. Senko, U. S. Atomic Energy Comm. UCRL-3521 (1956).
(6) "International Tables for X-Ray Crystallography," Vol. 1, The Kynock Press, Birmingham, England, 1952, pp. 306–346.

ACTION OF THROMBIN ON LYSINE SUBSTRATES[1]

Sir:

Sherry and Troll[2] have shown that thrombin catalyzes the hydrolysis of arginine esters. We wish to report that thrombin is also active in catalyzing the hydrolysis of ester and peptide bonds involving lysyl residues.

Using the analytical procedure previously described[3] for following the thrombin-catalyzed hydrolysis of p-toluenesulfonyl-L-arginine methyl ester (TAMe), it was found that 0.02 M lysine ethyl ester (LEe) at pH 6.5 in 0.15 M KCl at 25° was hydrolyzed to the extent of 10% in 30 min. by 100 TAMe units/ml. of Parke, Davis thrombin (having an activity of 10 TAMe units mg.) The definition of the TAMe unit has been given elsewhere.[3] From similar experiments, the reaction was found to be zero order in LEe and first order in thrombin in the limited range employed (0.02-0.04 M LEe, and 80–280 TAMe units/ml. thrombin). Seegers' purified citrate thrombin (having an activity of approximately 1000 TAMe units/mg.) gave essentially the same result under the same conditions.

The addition of large amounts (about 1 mg./ml.) of soybean trypsin inhibitor (STI) failed to alter the rate of hydrolysis of LEe significantly. Since thrombin itself, unlike plasmin[4] and trypsin, is not inhibited by STI,[5,6,7] this result suggests that the hydrolysis of LEe is not catalyzed by plasmin or by trypsin impurities in the thrombin. When thrombin activity toward TAMe was eliminated by acid treatment of the enzyme,[3] the activity toward LEe was also eliminated, suggesting that the origin of the LEe esterase activity may be the same as the TAMe esterase activity, which in turn has been shown to be the same as the fibrinogen-clotting

(1) This investigation was supported by research grant No. H-1662 from the National Heart Institute, Public Health Service.

(2) S. Sherry and W. Troll, *J. Biol. Chem.*, **208**, 95 (1954).
(3) S. Ehrenpreis and H. A. Scheraga, *ibid.*, **227**, 1043 (1957).
(4) W. Troll, S. Sherry and J. Wachman, *ibid.*, **208**, 85 (1954).
(5) M. M. Guest and A. G. Ware, *Science*, **112**, 21 (1950).
(6) E. Mihalyi, *J. Gen. Physiol.*, **37**, 139 (1953).
(7) S. Sherry, W. Troll and H. Glueck, *Physiol. Revs.*, **34**, 736 (1954).

activity.[2] However, it is worth noting that much higher concentrations of thrombin are required to hydrolyze LEe than are needed to hydrolyze TAMe[3] or to activate fibrinogen[8] at the same *p*H. It is interesting to speculate that, while the primary action of thrombin on fibrinogen may be toward arginyl bonds, a slower secondary action may take place at lysyl bonds. If so, the latter activity may possibly be the origin of the phenomenon reported by Guest and Ware[5] wherein purified thrombin at very high concentration caused the lysis of fibrin clots, an action which was not prevented by STI.

In support of this additional specificity for thrombin we may cite some preliminary experiments involving Seegers' citrate thrombin and the oxidized B chain of insulin, prepared as described elsewhere.[9] Thrombin (50–100 TAMe units ml.) was incubated with oxidized B-chain (4–8 mg./ml.) at *p*H 8 in 0.1 *M* ammonium acetate for 18–24 hr. at 25°. In some experiments STI was added (0.8 mg./ml.) Parallel experiments were carried out using trypsin (0.1–0.2 mg./ml) in place of thrombin. The hydrolysis of the lysyl-alanine bond of the B-chain was assessed by detection of the alanine fragment by paper chromatography of the free amino acid and also of its DNP-derivative. With either method the intensity of the spot was undiminished by the presence of STI when thrombin was used rather than trypsin. No quantitative data are yet available for the degree of hydrolysis of the lysyl-alanine bond. Qualitatively, it appeared that thrombin was 10–20% as effective as trypsin under the conditions stated.

The activity of thrombin toward the arginylglycine bond of the B-chain is still under investigation. Further studies are also being carried out on the activity of thrombin toward synthetic lysyl substrates. We are indebted to Dr. W. H. Seegers for his generous gifts of purified thrombin.

(8) E. Mihalyi, quoted by J. A. Gladner and K. Laki, *Arch. Biochem. and Biophys.*, **62**, 501 (1956).

(9) S. J. Leach and H. A. Scheraga, *Compt. rend. Lab. Carlsberg*, in press.

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THE AZEOTROPE OF MONOCHLORODIFLUOROMETHANE AND DICHLORODIFLUOROMETHANE

Sir:

There is an azeotrope of monochlorodifluoromethane and dichlorodifluoromethane. That these two common refrigerants form an azeotrope has not been generally recognized, since the feasibility of their separation by simple distillation has been tacitly assumed in both the technical and patent literature in various instances. Furthermore, the azeotrope may occur in practical refrigeration systems, since dichlorodifluoromethane is sometimes added to monochlorodifluoromethane, when the latter is used as a refrigerant, in order to improve the low-temperature solubility of lubricating oil.

The existence of the azeotrope was demonstrated in two ways.

Reflux boiling points were measured, Table I, showing a minimum at about −41.4°, only 0.6° below the boiling point of monochlorodifluoromethane, at a composition of about 25% dichlorodifluoromethane by weight. There is little change in boiling point between 10 and 50% dichlorodifluoromethane by weight, the values lying between about −41.0 and −41.4°.

The existence of the azeotrope was confirmed by fractionating a mixture of 58.2% dichlorodifluoromethane and 41.8% monochlorodifluoromethane at high reflux in a low-temperature Podbielniak still (Cat. No. 407). Portions of the constant-boiling distillate analyzed 24.5 to 26.7% dichlorodifluoromethane by weight on the basis of the density of the gas and 25 to 29% dichlorodifluoromethane by weight based on infrared absorption. These results are in accord with expectation from the boiling-point data.

TABLE I

NORMAL BOILING POINTS OF MIXTURES OF MONOCHLORODIFLUOROMETHANE AND DICHLORODIFLUOROMETHANE

Weight % dichlorodifluoromethane in mixture	Boiling point °C.	Weight % dichlorodifluoromethane in mixture	Boiling Point °C.
0.0	−40.80	51.6	−40.73
1.4	−40.76	53.9	−40.63
2.9	−40.80	57.5	−40.54
5.2	−40.94	58.4	−40.54
6.9	−40.89	64.2	−39.84
9.0	−40.99	69.9	−38.93
15.0	−41.31	73.1	−38.33
21.9	−41.41	78.4	−37.14
27.8	−41.41	83.9	−35.87
32.5	−41.39	89.4	−34.26
38.8	−41.39	100.0	−29.80
44.4	−40.93		

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STUDIES ON POLYPEPTIDES. XI. PREPARATION OF AN OCTAPEPTIDE POSSESSING MELANOCYTE-STIMULATING ACTIVITY[1]

Sir:

Structural studies of the corticotropins[2–5] and of the melanocyte-stimulating hormones (α- and β-M.S.H.)[6–8] have shown that the molecules of these substances contain a common amino acid sequence ("core") possessing the structure met-glu-his-phe-arg-try-gly. Since all these hormones stimulate

(1) Supported by grants from the U. S. Public Health Service, the National Science Foundation, Armour and Company, and Eli Lilly and Company.

(2) P. H. Bell, THIS JOURNAL, **76**, 5565 (1954).

(3) W. F. White and W. A. Landmann, *ibid.*, **77**, 1711 (1955).

(4) R. G. Shepherd, S. D. Willson, K. S. Howard, P. H. Bell, D. S. Davies, S. B. Davis, E. A. Eigner and N. E. Shakespeare, *ibid.*, **78**, 5067 (1956).

(5) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 687 (1955).

(6) J. I. Harris and A. B. Lerner, *ibid.*, **179**, 1346 (1957).

(7) J. I. Harris and P. Roose, *ibid.*, **178**, 90 (1956).

(8) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **78**, 4494 (1956).