COMMUNICATIONS TO THE EDITOR

TRANSFORMATION OF POLYMERIC RUTHENIUM(IV) TO THE MONOMERIC SPECIES ON ION EXCHANGE RESIN

Sir:

Although the existence of polymeric forms of ruthenium(IV) had been suspected, Gortsema and Cobble[1] were able to prepare both the monomeric and polymeric species and study them extensively in perchloric and nitric acid solutions. Ion exchange provided a simple method for transforming the polymeric form to the monomeric. This was done by equilibrating a quantity of low cross-linked Dowex 50W resin (2 to 4%) with Ru(IV) solution known to contain extensive amounts of polymer and then eluting the resin with 0.1 *M* cerium(III) perchlorate or 1 *M* perchloric acid. They make no statement as to whether the ion exchange is a separation of RuO^{++} (the monomer) from solutions of high polymer concentration, or whether there is a transformation of the polymer to the monomer on the resin. From direct observation of the spectrum of Ru(IV) on the resin it can be concluded that an actual transformation of polymer to monomer occurs as it passes through the ion exchange resin. This is also significant in that it shows that the polymeric Ru(IV) structure is not extremely stable and that it can be broken down into monomer by use of rather mild conditions.

The spectra were scanned with a Cary Model 14 recording spectrophotometer. One cm. Beckman fused silica cells were fitted with cell spacers to reduce the optical path to 2 mm. By increasing the dynode voltage on the phototube it was possible to record data down to 250 mμ or less. The ruthenium(IV) perchlorate solutions were prepared using a procedure described by Yaffe and Voight[2] and by Niedrach and Tevebaugh.[3] Gortsema and Cobble[1] determined the absorbancy index, *A* (1 cm., moles/liter), to be about 700 for the monomeric Ru(IV) and 800 to 1600 for the polymer at 480 mμ. The 0.0025 *M* ruthenium perchlorate solutions with A_{480} ranging from 730 to 1000 were stirred with Dowex 50WX2, 200 to 400 mesh, ion exchange resin. After a short period of time the resin containing Ru(IV) was washed repeatedly with distilled water to remove any ruthenium ion not in the resin phase. A portion of the loaded resin then was tapped and settled in one of the 2-mm. cells and the spectrum scanned *versus* a blank of the unloaded resin in the other 2 mm. cell. After equilibration with the resin, no ruthenium could be found in the residual solution. This enabled calculation of the concentration of the ruthenium in the resin phase.

Data were were obtained for several determinations on three different preparations, both of monomeric Ru(IV), A_{480} = 733, and polymeric Ru(IV), A_{480} = 1003. The results, given in Table I, indicate that the absorbancy index in the resin phase is a constant, independent of the value for the original solutions and approximates the value for the monomeric Ru(IV).

TABLE I

ABSORBANCY INDEX OF Ru(IV) ON RESIN

Expt.	Sample no. 1	2	3	Average
	A. Monomeric Ru(IV)			
1	800	863	785	
2	806	816	764	806
	B. Polymeric Ru(IV)			
1	686	895	882	
2	817	842	755	
3	..	808	782	801

(1) F. P. Gortsema, Doctoral thesis, Purdue University, January, 1960; *Dissert. Abstracts*, **21**, 48 (1960), L. C. Card No. Mic 60-2206.

(2) R. P. Yaffe and A. F. Voight, *J. Am. Chem. Soc.*, **74**, 2500 (1952).

(3) L. W. Niedrach and A. D. Tevebaugh, *ibid.*, **73**, 2835 (1951).

(4) From the Ph.D. thesis of D. K. A. entitled "A Study of Ru(III), and Ru(IV) Species in Aqueous Perchloric Acid Solutions," Purdue University, August, 1960.

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SPECIFIC CLEAVAGE OF METHIONYL PEPTIDES

Sir:

Sulfonium derivatives of methionine have been known to undergo easy elimination of the sulfur function with concomitant formation of homoserine lactone.[1,2,3] It has now been demonstrated that in peptides of methionine this intramolecular displacement occurs with participation and cleavage of the C-peptide bond (I—IV) in high yields. The data presented in Table I show that the extent of

TABLE I

INFLUENCE OF ALKYL GROUP ON THE CLEAVAGE OF SULFONIUM SALTS DERIVED FROM ETHYL N-ACETYLMETHIONYLGLYCINATE

Alkylating agent	Molar concn. of peptide in reaction mixture[a]	Equivalents of alkylating agents	Per cent. of peptide cleavage[b]
Iodoacetic acid[c]	0.005	3	6.0
Methyl iodide[c,d]	.01	4	3.6
Ethyl bromoacetate[c,d]	.01	4	43
Iodoacetamide[e]	.01	3	53
2,4-Dinitrofluorobenzene[e]	.01	4	~2
Diethyl bromomalonate[e]	.01	3	5

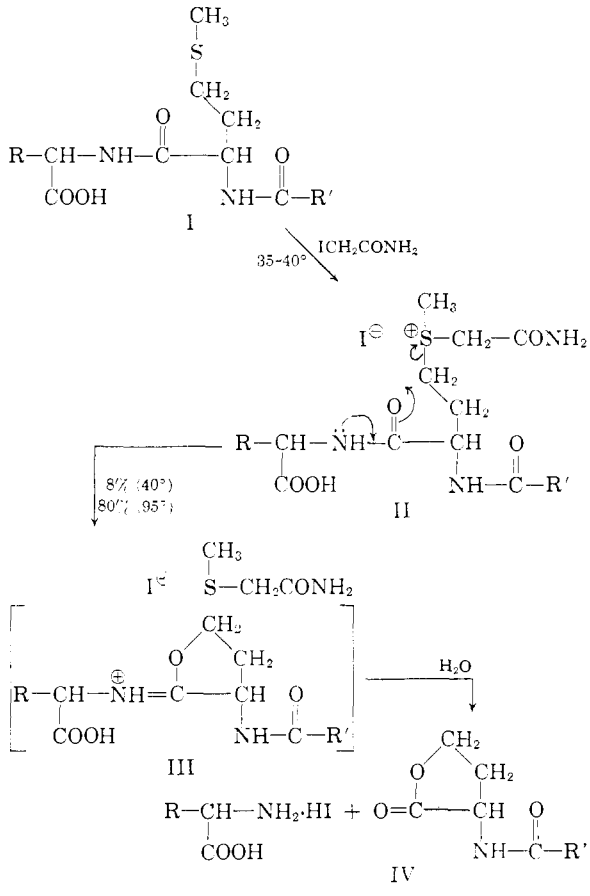
[a] Reactions were allowed to proceed at room temperature for 24 hr. [b] After removal of excess of alkylating agent by ether extraction the reaction mixture was heated for one hour at 100°. The liberated amino acid was determined by ninhydrin assay.[4] [c] Reaction medium was 0.1 *M* *p*H 3 citrate buffer. [d] Alkylation was conducted in a sealed tube. [e] Reaction medium was 1:1 mixture of EtOH and 0.1 *M* *p*H 3 citrate buffer.

(1) G. Toennies and J. J. Kolb, THIS JOURNAL, **67**, 1141 (1945).

(2) R. A. McRorie, G. L. Sutherland, M. I. Lewis, A. D. Barton, M. R. Glazener and W. Shive, *ibid.*, **76**, 115 (1954).

(3) H. G. Gundlach, W. H. Stein and S. Moore, *J. Biol. Chem.*, **234**, 1754, 1761 (1959).

(4) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).



cleavage of ethyl N-acetylmethionylglycinate depends on the nature of the alkylating agent. Carbamylmethylsulfonium salts gave the highest yield (~50%).

TABLE II

ALKYLATION AND CLEAVAGE OF METHIONYL PEPTIDES

Peptide	Time of alkylation, hr.	Degree of alkylation by titration, %	Per cent. of peptide cleavage
Carbobenzoxy-L-methionyl-L-glutamic acid[a,b]	68[c]	92	8.1[d]
	135[e]	91	80[f]
	135[e]	91	85[g]
	135[e]	91	81[h]
Benzoyl-DL-methionylglycine[a,b]	68[c]	99	8.6[d]
Benzoyl-DL-methionylglycine ethyl ester[a,b]	135[e]	93	54[f]
	135[e]	93	65[g]
	135[e]	93	62[h]
Carbobenzoxy-L-methionyl-L-tyrosine[a,b]	68[c]	92	7.9[d]
	65[c]	84	84[g]

[a] Concentration in ethanol-water (1:1) was 2×10^{-2} *M*. [b] Three equivalents of alkylating agent were used. [c] Temperature of alkylation was 40°. [d] Mixture was fractionated on columns of Amberlite IR 120 and the cleaved amino acid determined with automatic recording equipment.[6] [e] Temperature of alkylation was 35–40°. [f] Reaction mixture was analyzed directly by ninhydrin method.[4] [g] Reaction mixture was heated for 1 hr. at 95° before analysis.[4] [h] Reaction mixture was extracted with ether, heated for 1 hr. at 95° and analyzed.[4]

The procedure was applied to additional dipeptides and the course of the alkylation at 35–40° followed by argentometric titration.[5] With over 90% formation of carbamylmethylsulfonium salts only 8% of peptide cleavage occurred at 40°; however, on brief heating at 95° the yields of cleaved amino acid increased to 54–85% (Table II). Paper chromatography and paper electrophoresis of such reaction mixtures showed that in each case the cleaved amino acid was the only ninhydrin-positive substance present.

Since in peptides and proteins alkyl halides at *p*H 2.8 react only with the sulfur of methionine,[3,7] this procedure permits specific chemical cleavage of methionyl peptide bonds. The application of an improved methionine peptide cleavage to the selective splitting of ribonuclease is described in a following communication.

(5) G. Toennies and J. J. Kolb, THIS JOURNAL, **67**, 849 (1945).

(6) D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(7) P. T. Vithayathil and F. M. Richards, *J. Biol. Chem.*, **235**, 2343 (1960).

(8) Max-Planck Institut für Biochemie, München, Germany.

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SELECTIVE CLEAVAGE OF THE METHIONYL PEPTIDE BONDS IN RIBONUCLEASE WITH CYANOGEN BROMIDE[1]

Sir:

Cyanogen bromide reacts with ethyl N-benzoyl-DL-methionylglycinate in alcohol–water *at room temperature* to yield, *via* the unstable cyanosulfonium salt, (i) 70% N-benzoyl-DL-homoserine lactone (m.p. 143°, rep. 142°),[2a] (ii) methyl thiocyanate, assayed by gas chromatography and infrared absorption, and (iii) 75–90% of ethyl glycinate. By contrast, the von Braun cyanogen bromide cleavage of dialkyl thioethers in which there is no intramolecular assistance by functional groups requires *elevated temperatures* for the formation of alkylthiocyanate and alkyl bromide.[2b]

The usefulness and selectivity of this improved method for the cleavage of methionyl peptide bonds[3] was demonstrated with ribonuclease, which in a chain of 124 amino acids contains four methionines.[4] Bovine pancreatic ribonuclease[5] in 0.1–0.3 *N* HCl solution reacted with up to 30 equivalents of cyanogen bromide at 20° for 24 hours. After removal of solvent, excess reagent and methyl thiocyanate by lyophilization the residue in aliquots of 1.5–2.0 mg. was subjected to paper electrophoresis for 4 hours at 1100 v., 60 m A. and *p*H 6.5 in a pyridine–acetate buffer system. In order

(1) Presented in part at the Annual Meeting of the Chemical Society of Japan (NIHON KAGAKU KAI NENKAI), April 1–4, 1961, in Tokyo.

(2) (a) E. Fischer and H. Blumenthal, *Ber.*, **40**, 106 (1910); (b) J. von Braun *et al.*, *Ber.*, **56**, 1573 (1923); **59**, 1202 (1926); *Ann.*, **490**, 189 (1931).

(3) *Cf.* W. B. Lawson, E. Gross, C. M. Foltz and B. Witkop, *J. Am. Chem. Soc.*, **83**, 1509 (1961).

(4) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **235**, 633 (1960); C. H. W. Hirs, *Ann. N. Y. Acad. Sci.*, **88**, 611 (1960).

(5) Sigma Chemical Company, St. Louis, Lot R60B-069.