THE REACTION OF IODATE WITH CYSTINE AND WITH INSULIN

George Gorin and Walter E. Godwin
Department of Chemistry, Oklahoma State University
Stillwater, Oklahoma

Received September 14, 1966

This communication reports that cystine can be converted rapidly and quantitatively to cysteic acid by iodate in 0.1-1M HCl. It also reports on the reaction with insulin in 0.5M HCl; the amount of iodate consumed is nearly that required to oxidize two disulfide groups, and an insoluble product is formed, which can be separated by electrophoresis into two components. The molecule of insulin consists of two polypeptide chains, A and B, linked by two disulfide bonds, and the A-chain contains a third disulfide bond. It appears that the principal action of iodate is to cleave the interchain disulfide bonds.

EXPERIMENTAL

Oxidation of Cystine.-- Cystine, 10^{-3} M, and KIO_3, 2.4×10^{-3} M, in 0.1 or 1M HCl were mixed. To determine the stoichiometry, 3-ml aliquots were treated with 3 ml of 1M HCl + 5 ml of 1M KI + 1 ml of 1% starch and then titrated with 0.01M thiosulfate. To identify the product, the reaction was conducted with ten times more concentrated reagents, a drop of the reaction mixture was placed on a 2x13-cm strip of Whatman No. 1 paper, chromatographed with 88% phenol, and developed with ninhydrin (0.4% + 1.5% 2,4,6-trimethylpyridine in 95% ethanol).

Oxidation of Insulin.-- Insulin, 2.5 μmoles, was dissolved in 10 ml of 0.01M HCl and mixed with an equal volume of 1M HCl;

to this was added 100 µl of 5×10^{-3} M KI and 5 ml of 4×10^{-3} M KIO_3. A precipitate began to form after 15-30 min. In some cases it was removed 30 min after the onset of precipitation, in other cases the precipitate was not removed and aliquot portions of the reaction mixture were withdrawn at intervals, centrifuged and analyzed as described above.

Characterization of Oxidized Insulin.-- The precipitate was soluble in 0.05M $NaHCO_3$ and the spectrum was determined in this medium. Electrophoresis was conducted in a Gelman Rapid Electrophoresis Chamber; about 0.7 mg of product in the chosen solvent was placed in the middle of a 2x7-cm Whatman No. 1 paper strip and then 200 v was applied for 1 hr. After drying, the strip was stained with 0.5% ninhydrin in 95% ethanol.

RESULTS AND DISCUSSION

The reaction of iodate with cystine in 0.1M HCl was complete in less than 1 hr; 2.00 ± 0.02 moles of iodate was consumed per mole of cystine. Chromatography of the product gave a single ninhydrin-positive spot, with the same R_f (0.12) as an authentic sample of cysteic acid. The following equation represents the reaction: $cySScy + 2HIO_3 \longrightarrow 2cySO_3H + I_2$ (other iodine-containing products, e.g. HIO, might be formed without changing the overall stoichiometry, inasmuch as the final product, iodide, is finally formed after quenching and titration with thiosulfate). In 0.1M HCl the reaction rate was slower but the stoichiometry the same.

In the reaction of iodate with insulin, an induction period was observed, illustrated by curve A, Fig. 1. Added iodide catalyzed the reaction; with 2×10^{-5} M iodide (curve B), precipitation commenced in 15-30 min and was substantially

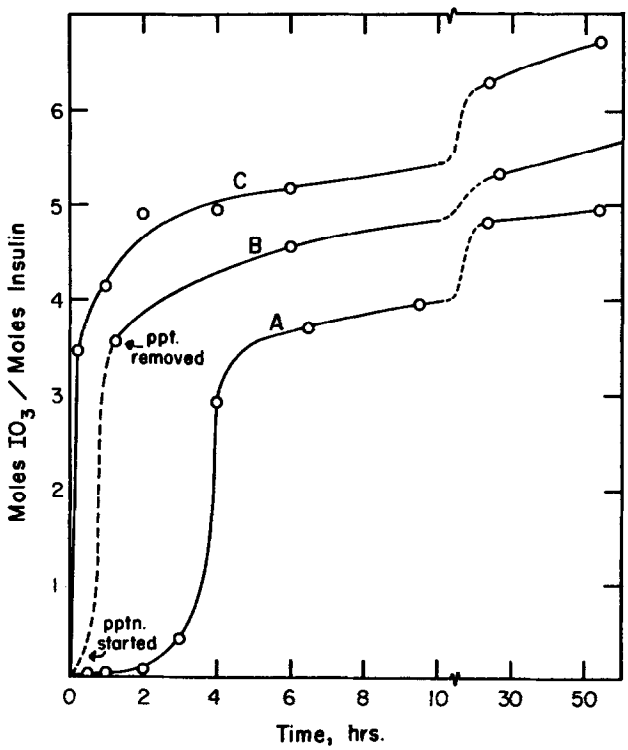

Fig. 1. Reaction of insulin with iodate; (A) no I^-; (B) 2×10^{-5} M I^-, see text; (C) 10^{-3} M I^-.

complete 30 min later; the amount of iodate consumed at this point was 3.8 ± 0.1 moles (4 moles are required to oxidize 2 disulfides) and the weight of precipitate was 98% of that of the insulin taken (95% of the theoretical, assuming oxidation of 2 disulfides).

If the precipitate was not removed, consumption of iodate continued. A sample exposed to iodate for 4 days consumed as much as 5-6 moles. Since the tyrosine residues of insulin react with iodine to give iodo and diiodo derivatives (Gruen et al., 1959; Brunfeldt, 1965), it may be inferred that this was the process responsible for the continued reaction. The other amino acids present in insulin do not react with iodate

in the conditions. The spectrum of the 4-day product indeed showed the maximum at 310 mμ that is characteristic of the iodinated derivatives (Fig. 2, curve A). Untreated insulin does not absorb in this region, and the 30-min precipitate shows little absorption; this confirms the hypothesis that the initial, rapid reaction with iodate is oxidation of two disulfide groups.

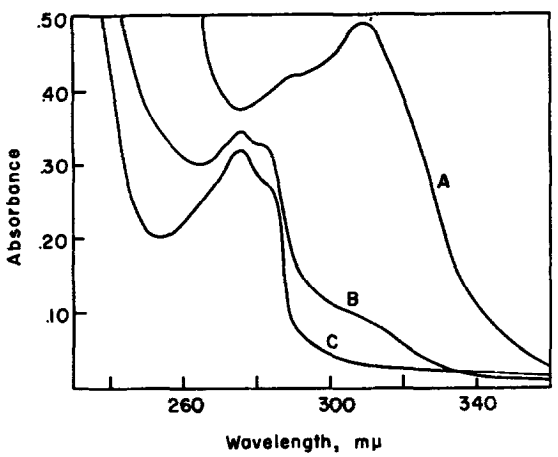

Fig. 2. Spectra of: (A) 7.6 mg of 4-day precipitate; (B) 7.6 mg of 30-min precipitate; (C) 7.3 mg of untreated insulin; all in 25 ml of 0.05M $NaHCO_3$.

Treatment of insulin with performic acid oxidizes all three disulfide groups (Sanger, 1947) and the product can be separated into two components by paper electrophoresis. In 20% formic acid, the B-chain derivative, that contains two sulfonate residues, moves toward the anode, while the A-chain derivative, with four sulfonate residues, moves toward the cathode (van Doesburg and Havinga, 1964); in barbiturate buffer both components move toward the cathode and the A-chain deriv-

ative, that has the higher negative charge, moves faster (Voelker et al., 1962). The 30-min iodate-oxidized product was subjected to electrophoresis in the aforementioned solvents and compared with untreated insulin and performic acid-oxidized insulin. Table 1 summarizes the results of the present work:

TABLE I. PAPER ELECTROPHORESIS OF INSULIN AND OXIDATION PRODUCTS

Position of band edges in cm from center of strip toward: (+) cathode (-) anode

	Untreated	Performic Oxidized	Iodate Oxidized	Performic +Iodate
20% formic acid	1.6-2.2(+)	0.9-1.4(+)(B) 0.8-1.3(-)(A)	0.9-1.4(+) 1.6-2.1(+)	0.9-1.4(+) 1.0-1.5(-)
barbiturate buffer	2.0-3.1(-)	0.6-1.5(-)(B) 4.2-5.2(-)(A)	0.9-1.5(-) 2.6-3.3(-)	- - - -

It can be seen that one component of the iodate-oxidized product behaves the same as the B-chain derivative from performic-acid oxidation, while the properties of the other component are consistent with what might be expected from an A-chain derivative with only two (or fewer) sulfonate residues. Since this component has about the same mobility as untreated insulin, it could not be an insulin molecule in which one interchain and the intrachain disulfide bonds had been oxidized without cleaving the molecule. If the iodate-oxidized insulin was isolated, treated with performic acid and then chromatographed, the bands obtained were essentially the same as for the product of simple performic acid oxidation; this is consistent with the view that iodate cleaves the two interchain disulfides and performic acid the third, intrachain disulfide.

Other investigators have found that the intrachain disul-

fide is more resistant to reduction (Cecil and Loening, 1960; Cecil and Weitzmann, 1964). That result and the present one suggest that the intrachain disulfide may be located in a relatively inaccessible position within the insulin molecule.

Acknowledgement.-- This investigation was aided by National Science Foundation Cooperative Fellowships to W.E.G. and by a Career Development Award from the National Institutes of Health, Department of Health, Education and Welfare to G.G.

REFERENCES

Brunfeldt, K., Science Tools, 12, 5 (1965).
Cecil, R., and Loening, U.E., Biochem. J., 76, 146 (1960).
Cecil, R., and Weitzmann, P.D.J., Biochem.J., 93, 1 (1964).
Gruen, L., Laskowski, M., and Scheraga, H.A., J. Biol. Chem., 234, 2050 (1959).
Sanger, F., Nature, 160, 295 (1947).
van Doesburg, J.Th.S. deV., and Havinga, E., Biochim. Biophys. Acta, 82, 96 (1964).
Voelker, I., Schuman, E., and Holt, C.v., Biochem. Z., 335, 382 (1962).