antibody (anti-m^{7}G) and a corresponding hapten-containing nucleic acid (chorion mRNA) should provide interesting and relevant data regarding the biological function (translation) of the hapten component.

Studies are now in progress to assess the in vitro translation of m^{6}A-containing mRNA in the presence of anti-m^{6}A antibodies. It is anticipated that these results may provide some insight into the location of m^{6}A within these mRNAs.

References

Both, G. W., Banerjee, A. K., & Shatkin, A. J. (1975a) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1189–1193.
Both, G. W., Furuichi, Y., Muthukrishnan, S., & Shatkin, A. J. (1975b) *Cell 6*, 185–195.
Chu, L., & Rhoads, R. E. (1978) *Biochemistry 17*, 2450–2454.
Erlanger, B. F., & Beiser, S. M. (1964) *Proc. Natl. Acad. Sci. U.S.A. 52*, 68–74.
Gelinas, R. E., & Kafatos, F. C. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3764–3768.
Grace, T. D. C. (1962) *Nature (London) 195*, 788–790.
Hickey, E. D., Weber, L. A., & Baglioni, C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 19–23.
Kemper, B. (1976) *Nature (London) 262*, 321–323.
Levine, L., Van Vunakis, H., & Gallo, R. C. (1971) *Biochemistry 10*, 2009–2013.
Lodish, H. F., & Rose, J. K. (1977) *J. Biol. Chem. 252*, 1181–1188.
Marcu, K., & Dudock, B. (1974) *Nucleic Acid Res. 1*, 1385–1397.
Munns, T. W., Podratz, K. C., & Katzman, P. A. (1973) *J. Chromatogr. 76*, 401–406.
Munns, T. W., Podratz, K. C., & Katzman, P. A. (1974) *Biochemistry 13*, 4409–4416.
Munns, T. W., Liszewski, M. K., & Sims, H. F. (1977a) *Biochemistry 16*, 2163–2168.
Munns, T. W., Sims, H. F., & Liszewski, M. K. (1977b) *J. Biol. Chem. 252*, 3102–3104.
Muthukrishnan, S., Both, G. W., Furuichi, Y., & Shatkin, A. J. (1975) *Nature (London) 255*, 33–37.
Palacios, R., Palmiter, R. D., & Schimke, R. T. (1972) *J. Biol. Chem. 247*, 2316–2321.
Paul, M., Goldsmith, M. R., Hunsley, J. R., & Kafatos, F. C. (1972) *J. Cell Biol. 55*, 653–680.
Revel, M., & Groner, Y. (1978) *Annu. Rev. Biochem. 47*, 1079–1126.
Shatkin, A. J. (1976) *Cell 9*, 645–653.
Shimotohno, K., Kodama, Y., Hashimoto, J., & Miura, K. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 2734–2738.
Weber, L. A., Hickey, E. D., & Baglioni, C. (1978) *J. Biol. Chem. 253*, 178–183.

High-Yield Cleavage of Tryptophanyl Peptide Bonds by *o*-Iodosobenzoic Acid†

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ABSTRACT: A new procedure to cleave tryptophanyl peptide bonds in high yield is reported. The method involves treatment of the S-alkylated protein with *o*-iodosobenzoic acid. The procedure is highly selective for tryptophan and does not modify tyrosine or histidine, but may convert methionine to its sulfoxide derivative. The yields in the cleavage are 70–100%. Tryptophanyl bonds to alanine, glycine, serine, threonine, glutamine, arginine, and *S*-(pyridylethyl)cysteine are split in nearly quantitative yield, while those preceding isoleucine or valine are split in ~70% yield in the proteins examined in this work. The chemical mechanism for tryptophanyl bond cleavage has not been defined, but it is likely that oxidation of the indole ring occurs during the reaction with *o*-iodosobenzoic acid. Some problems with the quality of commercial preparations of the reagent are discussed.

Automatic amino acid sequence analysis depends upon efficient procedures for the selective chemical or enzymatic cleavage of polypeptide chains in order to generate a limited number of large fragments. Many procedures have been devised to effect protein cleavage at various amino acid residues [see review by Spande et al. (1970)]. However, the number of such procedures that are useful in protein structure work is quite limited since many procedures suffer from lack of specificity, low yields of cleavage, or incompatibility of the reaction conditions with polypeptide solubility. Frequently, results obtained on model compounds and small peptides do not extend to proteins.

Residues that are infrequent in most proteins (e.g., Trp, Met, Arg) are the best candidates for cleavage sites. The digestion of methionyl bonds with cyanogen bromide provides the best example of a near quantitative and highly selective procedure. Several methods have been proposed to cleave polypeptide chains at tryptophanyl bonds (Konigsberg & Steinman, 1977; Shechter et al., 1976; Ozols & Gerard, 1977; Sakiyama, 1977; Savige & Fontana, 1977b). Most of these procedures have involved either oxidative bromination or oxidative chlorination. However, nonselective oxidation of many residues and low yields of cleavage have caused these methods to be of limited utility.

The best available method and the one most widely used for cleaving at tryptophan in polypeptides utilizes BNPS-skatole[1] (Omenn et al., 1970). After cysteine is reduced and

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S-alkylated, side reactions are limited to producing methionine sulfoxide (Omenn et al., 1970). The bromination of tyrosine and histidine can be prevented by using less than the optimum amount of BNPS-skatole required to effect cleavage. Typically, the cleavage yield is in the range of 15 to 60%.

In the present study a new procedure is described for the cleavage of tryptophanyl bonds with *o*-iodosobenzoic acid which largely overcomes the problems encountered with the previous methods.

Materials

Human serum albumin, bovine serum albumin, chicken lysozyme, and bovine trypsin were purchased from Sigma Chemical Co. Dithiothreitol, 2-mercaptoethanol, acrylamide (product no. 501), and urea were from Eastman. (Carboxymethyl)cellulose (CM23, Advanced Fibrous) came from Whatman, mixed bed resin AG-501-X8 from Bio-Rad, and Sephadex and Sephacryl gels from Pharmacia. 4-Vinylpyridine was purchased from Aldrich and *o*-iodosobenzoic acid from Chemical Dynamics. Guanidine hydrochloride (Sequenal grade) was obtained from Pierce.

Adult human hemoglobin α and β chains were isolated on columns of (carboxymethyl)cellulose according to the method of Clegg et al. (1968), with the exception that the 8 M urea solution was made and deionized on a column of AG-501-X8 resin just before use.

The major γ chain of *Macaca nemestrina* (pig-tailed macaque) fetal hemoglobin was isolated as described by Nute & Mahoney (1979).

Following S-pyridylethylation (Hermodson et al., 1973), both albumins and trypsin were further purified. Reduced and S-alkylated human and bovine serum albumins were gel-filtered through a column of Sephacryl S-200 (2.5 × 90 cm) in 50 mM imidazole, pH 6.9, to eliminate high molecular weight aggregates. *S*-(Pyridylethyl)-β-trypsin was isolated by gel filtration on a column of Sephadex G-75 superfine (2.5 × 90 cm) equilibrated and developed with 9% formic acid. Chicken lysozyme was used without further purification. The globin chains and lysozyme were S-pyridylethylated before use.

Methods

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. Proteins fragmented by treatment with *o*-iodosobenzoic acid were analyzed by $NaDodSO_4$ slab gel electrophoresis (Laemmli, 1970) using a discontinuous buffer system (Neville, 1971). The separation of globin chain fragments was achieved by using a separating gel containing 20% acrylamide. A 10% acrylamide separating gel was used to separate albumin fragments. The acrylamide, bis(acrylamide), and $NaDodSO_4$ were recrystallized before use (Weber & Osborn, 1975). Staining and destaining were done with Coomassie blue R-250 as described by Weber & Osborn (1969), except that the staining time was increased to 15 h and the dye concentration was reduced to 0.007%. The destained gels were analyzed with a scanning densitometer.

Amino Acid Analysis. Analyses were performed on a Durrum D-500 amino acid analyzer according to the manufacturer's instructions.

Sequence Analysis. Total digests or isolated fragments (2–5 mg) were analyzed in a Beckman sequencer Model 890C (Hermodson et al., 1972) using a "peptide program" (Hermodson et al., 1977). Direct identification of the resulting Pth-amino acids was accomplished by gas–liquid chromatography (Hermodson et al., 1972; Nute & Mahoney, 1979) and by high-performance liquid chromatography. LC analyses were performed by using a Bioanalytical Systems (W. Lafayette, IN) liquid chromatograph and a slight modification of the method of Zimmerman et al. (1977). Stepwise yields for the degradations ranged from 94 to 96%.

Quantitation of Cleavage Yields. The calculated yield of cleavage was determined by one of several ways depending upon the protein. Globin chains, β-trypsin, lysozyme, and both albumins were cleaved, and the unfractionated mixtures of fragments were degraded in the sequencer. The yields of newly generated peptides were determined by using the amino-terminal sequence of the protein as an internal standard. Only amino acid residues that can be quantitated easily by gas–liquid chromatography or by LC were utilized. In addition, only residues appearing within the same cycle were compared so that an internal control for all possible losses was always present. The yield of cleavage was also estimated by examination of the chromatographic profiles obtained during peptide purification and by scanning densitometry of $NaDodSO_4$ gels of the unfractionated digests.

Cleavage of Tryptophanyl Peptide Bonds. Various protein samples (1–10 mg/mL) were dissolved in 80% acetic acid and 4 M guanidine hydrochloride. To this solution was added *o*-iodosobenzoic acid after being completely dissolved in the same solvent (see Results for details). The reaction was allowed to proceed at room temperature in a foil-covered vessel for 1–24 h. The mixture was then dialyzed in Spectrapor no. 3 membrane tubing against 10% acetic acid and lyophilized. When small peptides (less than 30 residues) were anticipated in the digest, the solvent was evaporated under reduced pressure without preliminary dialysis. The mixture was redissolved in 9% formic acid and desalted on a column of Sephadex G-25 superfine (2.5 × 40 cm) equilibrated and developed with 9% formic acid.

The cleavage of the globin γ chain with BNPS-skatole was accomplished according to Nute & Mahoney (1979).

Results

Tryptophan Polypeptide Bond Cleavage. That tryptophanyl bonds can be efficiently cleaved by *o*-iodosobenzoic acid was shown by the digest of globin β chain, which has two tryptophans. The chain (20 mg) was disolved in 2.0 mL of 12 mM potassium phosphate and 4 M guanidine hydrochloride, pH 6.0. After 30 min of exposure to 50 mg of *o*-iodosobenzoic acid in this buffer, the solution was made 80% in acetic acid and allowed to react for 24 h. When the digest was subjected to G-50 filtration, two prominent peptide peaks were resolved (Figure 1). Amino acid sequence analysis demonstrated that peak I contained only one peptide with an amino acid sequence beginning at residue 38 of the β chain. Peak II contained an equimolar mixture of two peptides with sequences corresponding to residues 1–15 and 16–37. The amino acid sequence data and the absence of observable overlap fragments on gel filtration chromatography indicated greater than 95% cleavage of the β chain at tryptophanyl residues 15 and 37. Subsequently, it was found that the digestion was identical when the first step of the reaction procedure was omitted and both protein and reagent were placed directly into 80% acetic acid and 4 M guanidine hydrochloride. When a desalted but unfractionated digest of β chain was degraded in the sequencer,

[1] Abbreviations used: BNPS-skatole, the compound formed by the reaction of *N*-bromosuccinimide with 2-(2-nitrophenylsulfenyl)-3-methylindole, the most likely product being 2-(2-nitrophenylsulfenyl)-3-methyl-3-bromoindolenine; $NaDodSO_4$, sodium dodecyl sulfate; Pth, phenylthiohydantoin; LC, high-performance liquid chromatography; PE, pyridylethyl.

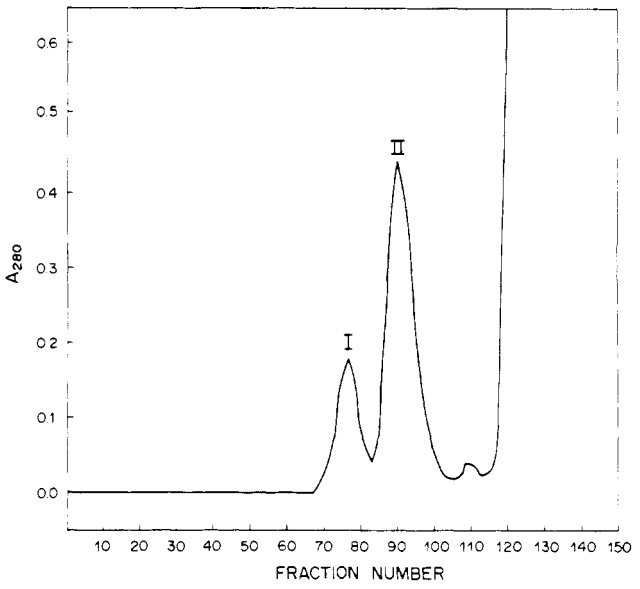

FIGURE 1: Gel filtration of fragments produced by cleavage of *S*-pyridylethyl β chain of hemoglobin at tryptophanyl residues. The reaction products were chromatographed on a column of Sephadex G-50 superfine (2.5 × 95 cm) eluted with 9% formic acid. The large peak at the end of the column is due to reagents.

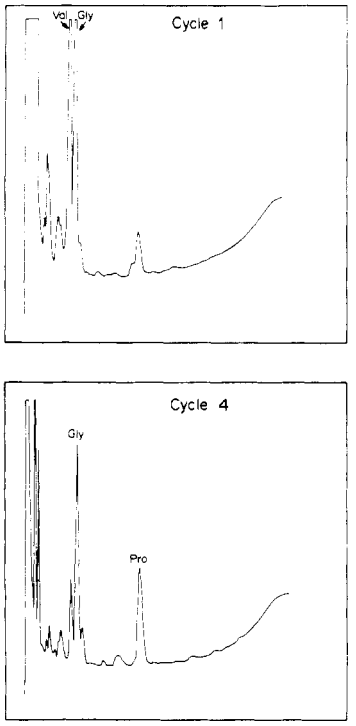

FIGURE 2: Gas–liquid chromatographic analyses of the silylated Pth-amino acids from cycles 1 and 4 of cleaved hemoglobin α chain. The α-chain digest was accomplished by digesting 20 mg of protein with 50 mg of *o*-iodosobenzoic acid in 80% acetic acid and 4 M guanidine hydrochloride for 24 h at room temperature in the dark. The peaks were integrated with a Hewlett-Packard 3380S integrator.

only the three expected fragments were observed, and the Val_1/Gly_{16} ratio in the first cycle and the Val_{20}/Phe_{42} and Val_{23}/Phe_{45} ratios in the fifth and seventh cycles were all 1:1 (0.9–1.1), again indicating quantitative cleavage at the tryptophanyl residues.

Hemoglobin α chain, which contains only one tryptophan at position 14, yielded similar results to those of the β chain. Figure 2 shows the first and fourth cycles of the Edman degradation performed on the desalted fragment mixture. The N-terminal valine and glycine-15 were found in equimolar

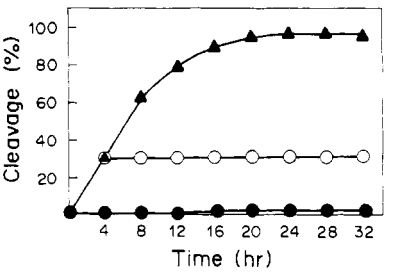

FIGURE 3: Formation of the large peptide (residues 15–141) of globin α chain with time (▲). Cleavage at tryptophan residue 14 was effected under the conditions described for Figure 2. The effect of dithiothreitol added at 0 time (●) and after 4 h (○) is presented. Cleavage yields were determined by scanning densitometry of $NaDodSO_4$ gels.

Table I: Amino Acid Compositions of Hemoglobin β and γ Chains[a]

amino acid	β chain		50×	γ chain	
	untreated	treated[b]	treated[c]	untreated	treated[b]
Asx	13.4 (13)	13.5	13.1	14.1 (14)	14.0
Thr	6.4 (7)	6.4	6.3	9.0 (9)	8.9
Ser	4.1 (5)	4.2	4.1	10.6 (11)	11.1
Glx	11.1 (11)	11.3	11.2	12.4 (12)	12.3
Pro	6.7 (7)	7.0	6.6	3.8 (4)	3.9
Gly	13.2 (13)	12.8	13.0	12.8 (13)	13.1
Ala	14.9 (15)	15.4	15.2	11.7 (12)	12.3
Val[f]	17.5 (18)	18.0	18.0	12.7 (13)	13.1
Met	0.94 (1)	0.90	0.64[d]	1.7 (2)	1.8
Ile[f]	0.0 (0)	0.0	0.0	3.9 (4)	3.9
Leu	18.3 (18)	17.8	17.9	16.9 (17)	17.1
Tyr	3.0 (3)	2.8	0.3	1.9 (2)	2.0
Phe	7.7 (8)	8.4	8.1	7.8 (8)	7.9
Lys	11.0 (11)	10.9	10.9	10.7 (11)	11.1
His	8.8 (9)	9.0	5.9	4.8 (5)	4.8
Arg	2.9 (3)	3.2	3.1	4.6 (5)	5.1
Cys[e]	1.78 (2)	1.71	1.69	0.7 (1)	0.6
Trp[g]	ND (2)	ND	ND	ND (3)	ND

[a] Determined after hydrolysis in 6 N HCl at 110 °C for 24, 48, 72, and 96 h. Values in the parentheses show compositions as determined by amino acid sequence (Dayhoff, 1972; Nute & Mahoney, 1979). Ser and Thr were extrapolated to zero time. All other values were averages of the data except as noted. [b] Amino acid compositions after digestion with *o*-iodosobenzoic acid under optimal conditions. [c] Amino acid compositions after digestion of β chain with *o*-iodosobenzoic acid using 50 times the usual amount of reagent. [d] Detected as methionine sulfone. [e] Detected as *S*-(pyridylethyl)cysteine. [f] Determined from 96-h hydrolyses. [g] Not determined.

yield, as were proline-4 and glycine-18.

Conditions for Tryptophanyl Bond Cleavage. Hemoglobin α chain was used to determine the optimal conditions for the cleavage of tryptophanyl bonds. The cleavage reaction was found to be slow (Figure 3), taking nearly 24 h at room temperature. When sufficient amounts of mercaptoethanol or dithiothreitol were added to reduce the remaining *o*-iodosobenzoic acid at various times during the reaction, further cleavage was prevented (Figure 3). This indicated that the modification reaction was a slow process, continuing throughout the digest. Therefore, the reagent must be present throughout the reaction.

A reagent to protein concentration of 2 mg of *o*-iodosobenzoic acid to 1 mg of protein brought about optimal cleavage of both human serum albumin and globin α chain (Figure 4). Amino acid analysis of the globin β and γ chains cleaved under optimal conditions showed no modification of histidine or tyrosine (Table I). When the hemoglobin α chain was cleaved, comparable results were obtained. However, when the α-chain peptide containing residues 15–141 was isolated and treated with cyanogen bromide in 70% formic acid for 24

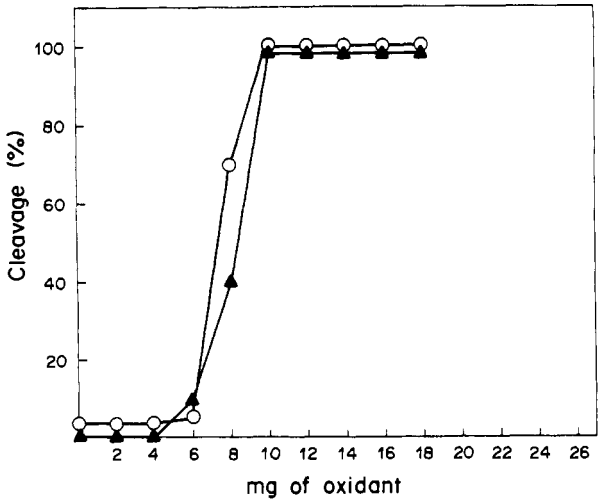

FIGURE 4: Cleavage of human serum albumin (▲) and globin α chain (○) as a function of the amount of *o*-iodosobenzoic acid. The proteins (5 mg) were dissolved in 1.0 mL of 80% acetic acid and 4 M guanidine hydrochloride and treated with *o*-iodosobenzoic acid for 24 h. The reaction was stopped by destroying excess reagent with mercaptoethanol; yields were determined by scanning densitometry of $NaDodSO_4$ gels.

h, no additional peptides were generated, suggesting modification of methionine to its sulfoxide derivative during the digestion at tryptophanyl residues (data not shown). At high concentrations of *o*-iodosobenzoic acid (50× excess over optimum), methionine is oxidized to the sulfone, tyrosine is destroyed, and the histidine yield is reduced (Table I). The destruction of these residues under these conditions may be due to the *o*-iodosobenzoic acid or could conceivably be due to impurities in the reagent.

Concentration Dependence of the Reaction. Protein–reagent concentrations were investigated with the albumins and the globin α chain. Maintaining a fixed amount of reagent (3 mg) and protein (1.5 mg), we varied the reaction volume between 0.1 and 2.9 mL. Analysis by $NaDodSO_4$ gels indicated that the cleavage reaction was concentration independent. We found a protein concentration of 10 mg/mL convenient, although protein and reagent solubility could dictate the concentration used.

Comparison of BNPS-skatole and o-Iodosobenzoic Acid Cleavage of Tryptophanyl Bonds. Hemoglobin γ chain was cleaved by BNPS-skatole and *o*-iodosobenzoic acid and chromatographed on Sephadex G-50 superfine (Figure 5). The peak centering on tube 68 from the iodosobenzoic acid digest was pure peptide containing residues 38–100 of the γ chain. The corresponding peak in the BNPS-skatole digest was a mixture of that fragment plus several "overlap" fragments due to incomplete digestion. The peaks centering on tubes 98, 118, and 150 were peptides 16–37, a mixture of peptides 1–15 and 131–146, and reagent, respectively. The yield of the large peptide starting at residue 38 was ~42% in the BNPS-skatole digest, while the same peptide was produced in quantitative yield after fragmentation with *o*-iodosobenzoic acid.

Yield of Tryptophanyl Peptide Bond Cleavage in Various Proteins. The yields of cleavage of hemoglobin α, β, and γ chains, human serum albumin, bovine serum albumin, lysozyme, and β-trypsin are shown in Table II. Tryptophanyl bonds preceding Ala, Gly, Ser, Thr, or Gln are cleaved in nearly quantitative yield, while those preceding Ile or Val are split in ~70% yield. Those preceding the cationic residues Arg and *S*-(pyridylethyl)cysteine also cleaved in good yield in the two cases examined.

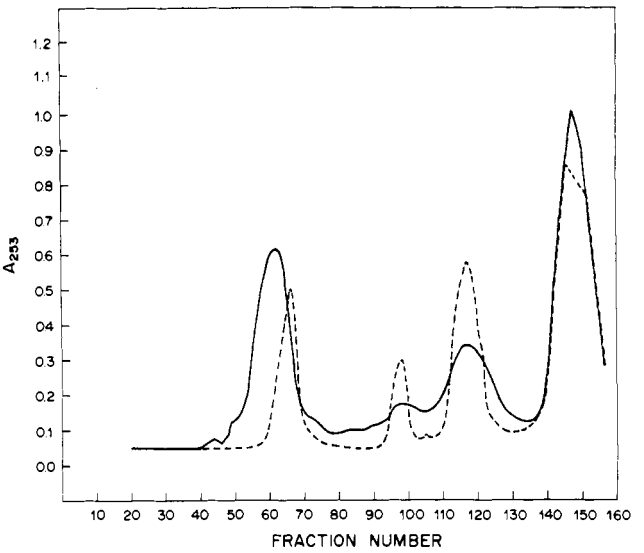

FIGURE 5: Gel filtration of fragments produced by cleavage of 30 mg of *S*-pyridylethyl γ chain of hemoglobin at tryptophanyl residues. Cleavage with BNPS-skatole (solid line) was accomplished according to Nute & Mahoney (1979). Cleavage with *o*-iodosobenzoic acid (broken line) was accomplished by using 60 mg of oxidant in 80% acetic acid and 4 M guanidine hydrochloride for 24 h. The reaction was stopped by addition of mercaptoethanol. The reaction products were chromatographed on a column of Sephadex G-50 superfine (2.5 × 95 cm) eluted with 9% formic acid.

Table II: Cleavage of Tryptophanyl Peptide Bonds in Proteins

protein	bond cleaved	yield (%)
hemoglobin α chain	Trp^{14}-Gly Lys Val	>95
hemoglobin β chain	Trp^{15}-Gly Lys Val	>95
	Trp^{37}-Thr Gln Arg	>95
hemoglobin γ chain	Trp^{15}-Gly Lys Val	>95
	Trp^{37}-Thr Gln Arg	>95
	Trp^{130}-Gln Lys Met	>95
bovine serum albumin	Trp^{134}-Gly Lys Tyr	>95
	Trp^{211}-Ser Val Ala	90
human serum albumin	Trp^{213}-Ala Val Ala	90
bovine β-trypsin	Trp^{40}-Val Val Ser	65
	Trp^{121}-Gly Asn Thr	90
	Trp^{199}-Gly Ser Gly	90
	Trp^{221}-Ile Lys Gln	70
chicken lysozyme	Trp^{28}-Val Cys Ala	70
	Trp^{62}-Trp Cys Asn	ND[a]
	Trp^{63}-PE-Cys Asn Asp	80
	Trp^{108}-Val Ala Trp	ND
	Trp^{111}-Arg Asn Arg	90
	Trp^{123}-Ile Arg Gly	70

[a] Not determined.

Discussion

The results of these studies indicate that the mild oxidant *o*-iodosobenzoic acid is the most practical reagent available for the cleavage of tryptophanyl peptide bonds. The yields of cleavage exceed that of any method previously described. Tryptophanyl bonds preceding certain residues (Table II) are split with yields which compare favorably to the yields obtained for cleavage of methionyl bonds with cyanogen bromide. After protection of the sulfhydryl groups by reversible modifications (e.g., disulfide formation) or S-alkylation, the oxidation of Cys by *o*-iodosobenzoic acid to either the sulfenic acid or unwanted disulfide species can be prevented. Methionine sulfoxide, if it occurs, can be reduced easily by thiols (Savige & Fontana, 1977a), and thus protein fragments can be obtained in which only the tryptophan residues are modified. One of the major obstacles to automatic protein sequence analysis is the limited number of methods available to specifically cleave peptide

bonds in high yield. Many of the available methods have the complications of low selectivity and yield. Use of these procedures on even the smallest of proteins makes purification of fragments difficult. This procedure is highly specific, nearly quantitative, and directed at a residue that is relatively rare in most proteins. Thus, excellent fragments for sequence analysis are generated.

The mechanism of this cleavage reaction is not understood. It is unlikely that the iodine atom is transferred from the reagent to the tryptophan since it is covalently bonded to the benzene ring. Thus, in situ production of HOI analogous to the postulated HOBr intermediate in the BNPS-skatole reaction does not seem probable. Iodoso compounds are generally used only as oxidants. Iodosobenzene diacetate oxidizes thiaxanthone only to the sulfoxide (Castrillon & Szmant, 1967) and reacts with pyrocatechols to give *o*-quinones and iodobenzene. When iodosobenzene diacetate reacts with cycloalkenes (good halogen acceptors) in the presence of trimethylsilyl azide, α-azido ketones are produced (Ehrenfreund & Zbiral, 1973). In no case has halogenation been detected.

The use of *o*-iodosobenzoic acid with proteins also demonstrates its mild oxidative property. First introduced for the determination of protein sulhydryl groups, the reagent has been used to inactivate the catalytically active sulfhydryl group of glucose-6-phosphate dehydrogenase (Parker & Allison, 1969) and papain (Allison, 1976). In both cases critical sulfhydryl residues are modified to the sulfenic acid. When [^{125}I]-*o*-iodosobenzoic acid is used, halogenation of the enzyme is not observed (Allison, 1976).

The postulated mechanisms of previously described methods to cleave tryptophanyl peptide bonds (e.g., BNPS-skatole and *N*-chlorosuccinimide) have included halogenated intermediates in the reaction sequence. Since halogenation probably does not occur using *o*-iodosobenzoic acid, the mechanism of tryptophanyl bond cleavage with the other halogenated oxidants must be questioned.

Iodoso compounds can be oxidized to iodoxy compounds and for this reason are not stable for long periods of time in the presence of air. They are also easily reduced by various reducing agents. One lot of *o*-iodosobenzoic acid which we received from Sigma Chemical Co. requires 1.5 times as much reagent to effect polypeptide cleavage as the material procured from Chemical Dynamics. We do not presently have a routine method for assessing the quality of the reagent short of testing it on a protein. Once an effective lot of reagent is obtained, we are able to store it in dry form in the freezer for more than 8 months without an observable decrease in cleavage yield. Pierce Chemical Co. will have quality-controlled reagent on the market by early summer, 1979 (Robert Vigna, personal communication).

References

Allison, W. S. (1976) *Acc. Chem. Res. 9*, 293.

Castrillon, J. P. H., & Szmant, H. H. (1967) *J. Org. Chem. 32*, 976.

Clegg, J. B., Naughton, M. A., & Weatherall, D. J. (1968) *Nature (London) 219*, 69.

Dayhoff, M. O. (1972) *Atlas of Protein Sequence and Structure*, Vol. 5, National Biomedical Research Foundation, Silver Spring, MD.

Ehrenfreund, J., & Zbiral, E. (1973) *Justus Liebigs Ann. Chem.*, 290.

Hermodson, M. A., Ericsson, L. H., Titani, K., Neurath, H., & Walsh, K. A. (1972) *Biochemistry 11*, 4493.

Hermodson, M. A., Ericsson, L. H., Neurath, H., & Walsh, K. A. (1973) *Biochemistry 12*, 3146.

Hermodson, M. A., Schmer, G., & Kuruchi, K. (1977) *J. Biol. Chem. 252*, 6276.

Konigsberg, W. H., & Steinman, H. M. (1977) *Proteins, 3rd Ed. 3*, 1.

Laemmli, U. K. (1970) *Nature (London) 227*, 680.

Neville, D. M., Jr. (1971) *J. Biol. Chem. 246*, 6328.

Nute, P. E., & Mahoney, W. C. (1979) *Biochemistry 18*, 467.

Omenn, G. S., Fontana, A., & Anfinsen, C. B. (1970) *J. Biol. Chem. 245*, 1895.

Ozols, J., & Gerard, C. (1977) *J. Biol. Chem. 252*, 5986.

Parker, D. J., & Allison, W. S. (1969) *J. Biol. Chem. 244*, 180.

Sakiyama, F. (1977) *J. Biochem. (Tokyo) 82*, 365.

Savige, W. E., & Fontana, A. (1977a) *Methods Enzymol. 47*, 453.

Savige, W. E., & Fontana, A. (1977b) *Methods Enzymol. 47*, 459.

Shechter, Y., Patchornik, A., & Burstein, Y. (1976) *Biochemistry 15*, 5071.

Spande, T. F., Witkop, B., Degani, Y., & Patchornik, A. (1970) *Adv. Protein Chem. 24*, 97.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406.

Weber, K., & Osborn, M. (1975) *Proteins, 3rd Ed. 1*, 179.

Zimmerman, C. L., Appella, E., & Pisano, J. J. (1977) *Anal. Biochem. 77*, 569.