Vol. 94, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1980 Pages 697-703

o-IODOSOBENZOIC ACID: PEPTIDE BOND CLEAVAGE AT TYROSINE IN ADDITION TO TRYPTOPHAN RESIDUES

Peter Johnson and Verna B. Stockmal

Department of Chemistry and College of Osteopathic Medicine
Ohio University, Athens, Ohio 45701, USA

Received April 21,1980

SUMMARY. Treatment of carboxymethylated actin with o-iodosobenzoic acid (Mahoney, W.C., and Hermodson, M.A. (1979) Biochemistry, 18, 3810-3814) did not produce the peptide pattern expected on the basis of specific peptide bond cleavage at tryptophanyl bonds. Isolation and amino acid sequence characterization of peptides from the digest indicated that in addition to cleavage at Trp residues, cleavages occurred at Tyr-53, Tyr-198, Tyr-218, Tyr-239 and probably at Tyr-91. These results indicate that the specificity of o-iodosobenzoic acid as a reagent for peptide bond cleavage is wider than previously reported. A simple explanation for the different susceptibilities of tyrosyl-containing peptide bonds to cleavage was not apparent from inspection of the sequences adjacent to these residues.

The technique of efficient cleavage of selected peptide bonds in polypeptides is an important tool in both amino acid sequence determination and structure-function studies of the parent polypeptide. In addition to the use of various proteolytic enzymes for this purpose, non-enzymic methods of cleavage have also been used, particularly for cleavage at methionine, cysteine and tryptophan as these residues are found relatively infrequently in most protein polypeptides. In the case of cleavages directed at tryptophan residues, several reagents have been used, including N-bromosuccinimide (1), N-chlorosuccinimide (2), BNPS-skatole (3), cyanogen bromide (4) and o-iodosobenzoic acid (5). The latter reagent, which is the most recently studied, was found to be superior to the other reagents as it effected peptide bond cleavage in higher yields and the reagent appeared to be completely specific under the selected conditions.

In this study, which describes the results of cleavage of carboxymethylated actin with BNPS-skatole and o-iodosobenzoic acid, we report that o-iodosobenzoic acid effects cleavage at certain tyrosyl-containing peptide bonds in addition

0006-291X/80/100697-07$01.00/0
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to cleavages at tryptophanyl-containing peptide bonds. Comparisons of the amino acid sequences around the susceptible tyrosyl bonds with those around tyrosyl bonds which are not susceptible to cleavage did not suggest an obvious reason for selective susceptibility to cleavage by o-iodosobenzoic acid.

METHODS

Rabbit skeletal muscle actin was prepared as in (6) and carboxymethylated according to (7). BNPS-skatole and o-iodosobenzoic acid were purchased from Pierce Chemical Company, Illinois, and S. aureus V8 protease was obtained from Miles Laboratories, Indiana.

Carboxymethylated actin was cleaved using BNPS-skatole and o-iodosobenzoic acid under the recommended reaction conditions for each reagent (8,5). The large molecular weight fragment (approximately 30,000 daltons) produced by BNPS-skatole treatment was separated from uncleaved actin and smaller polypeptides by chromatography on a 2 x 100 cm Sephadex G-200 column equilibrated in 8M urea - 10% acetic acid, and the large (> 3500 daltons) molecular weight fractions produced by BNPS-skatole and o-iodosobenzoic acid treatment were separated from smaller polypeptides by dialysis using Spectrapor-3 dialysis membrane. The large molecular weight polypeptides from the BNPS-skatole digest were also redigested with o-iodosobenzoic acid under the recommended conditions (5). The initial digests and isolated fragments were characterized by SDS-poly acrylamide gel electrophoresis in 12% acrylamide gels containing 6M urea (9), and by identification of free N-terminal amino acids (10,11). Peptides on SDS-gels were located by staining with Coomassie Brilliant Blue R250 dye and photographic negatives of stained gels were scanned with a Joyce Loebl Microdensitometer.

For isolation of N-terminal peptides from the large molecular weight fractions, the general procedure of (12) was used in which the maleylated polypeptides were initially digested with S. aureus V8 protease in 50mM ammonium bicarbonate, pH 8.0 (13). After digestion, samples were applied to a 2 x 15 cm Dowex 50 x 2 column equilibrated in 0.1M acetic acid, and 100 ml of eluant was collected and lyophilized. This fraction was then de-maleylated, and ninhydrin-reactive peptides were purified by consecutive paper electrophoresis at pH 6.5 and pH 1.8, after which their mobilities, amino acid compositions and N-terminal residues were identified as described elsewhere (14,15), thereby establishing their location in the actin amino acid sequence (16).

Amino acid analyses of peptides were performed on a Beckman 119 CL amino acid analyzer equipped with a Spectraphysics System 1 computing integrator.

RESULTS

As shown in Fig. 1A, cleavage of carboxymethylated actin by BNPS-skatole resulted in the production of a 30,000 dalton polypeptide in addition to uncleaved actin (43,000 daltons) and lower molecular weight material. After isolation of the 30,000 dalton polypeptide by chromatography using Sephadex G-200, amino acid analysis (not shown) and the identification of histidine as

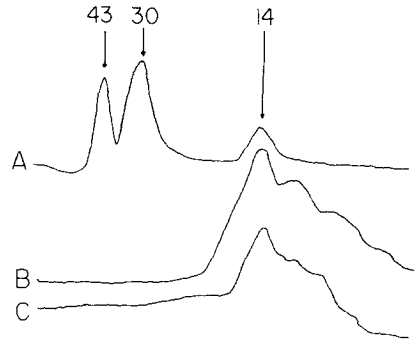

Fig. 1. Gel electrophoretic analysis of carboxymethylated actin treated with BNPS-skatole and o-iodosobenzoic acid. Conditions for cleavage, gel electrophoresis and scanning are given in the Methods. Approximately 40 µg of each sample was applied to the gel. Arrows indicate the approximate molecular weights (in kilodaltons) of polypeptides. A: BNPS-skatole cleavage, B: o-iodosobenzoic acid cleavage, C: o-iodosobenzoic acid cleavage of the large molecular weight fraction from the BNPS-skatole cleavage.

the N-terminal residue indicated that the polypeptide represented residues 87-339 in actin (16). Analysis of the digest by paper electrophoresis at pH 6.5 (not shown) also confirmed the presence of peptides comprising residues 75-86, 340-355 and 356-374, indicating that cleavages had occurred at each of the tryptophan residues in actin (Trp-74, Trp-86, Trp-339 and Trp-355).

In contrast to the BNPS-skatole cleavage results, neither intact actin nor the 30,000 dalton fragment were seen on gel analysis of the o-iodosobenzoic acid treated actin, and the digest appeared to contain only smaller molecular weight peptides in the 8,000-14,000 dalton range (Fig. 1B). That some of these smaller polypeptides were derived from the 30,000 dalton polypeptide by cleavage at peptide bonds not involving tryptophan residues was verified by o-iodosobenzoic acid treatment of the BNPS-skatole large molecular weight fraction. As shown in Fig. 1C, the redigested material contained only the smaller polypeptides.

Control experiments (not shown) demonstrated that carboxymethylated actin was not cleaved in the solvent used (4M guanidine-HCl in 80% acetic acid), thereby indicating that the additional cleavages observed were a consequence of o-iodosobenzoic acid treatment.

TABLE I

N-terminal peptides isolated from the large molecular weight fraction of the o-iodosobenzoic acid digest of actin.

Peptide	Composition and Terminal Residues [a]	Yield [b] (μmoles)	Electrophoretic mobility [c] at pH 6.5	Residues in the actin sequence [d]
1	Ser(Phe,Val,Thr,Thr,Ala)Glu ⟶ 1.0 0.8 1.0 0.9 0.9 0.9 1.0	0.08	-0.33	199-205
2	Val(Ala,Leu,Asp,Phe)Glu ⟶ 0.9 1.0 0.9 1.1 0.9 1.1	0.07	-0.62	219-224
3	Glu(Leu,Pro,Asp,Gly,Gln,Val,Ile,Thr,Ile,Gly,Asn)Glu ⟶ 1.0 1.0 1.2 1.1 1.1 1.0 0.7 0.8 1.0 0.8 1.1 1.1 1.0	0.05	-0.62	240-252
4	Asn-Glu ⟶ 1.0 1.0	0.10	-0.65	92-93 or 225-226 or 251-252
5	Val(Gly,Asp)Glu ⟶ 0.9 1.0 1.0 1.0	0.02	-0.83	54-57

[a] N-terminal residue identified after dansylation (10,11), C-terminal residue deduced from the specificity of S. aureus V8 protease (13).

[b] From 1.6 μmole carboxymethylated actin treated with o-iodosobenzoic acid.

[c] Calculated according to (17).

[d] Sequence data from (16).

In addition to the polypeptides identified by gel analysis, other smaller peptides were also produced in the cleavage by o-iodosobenzoic acid, and these were identified by pH 6.5 paper electrophoresis (results not shown) as residues 75-86, 340-355 and 356-374 of the actin sequence. This result indicates that cleavage had occurred at each of the four tryptophan residues in actin, as with BNPS-skatole.

Selective purification of N-terminal peptides from the large molecular weight fractions of the BNPS-skatole and o-iodosobenzoic acid cleavages was attempted as described in the Methods section. No N-terminal peptides were located from the BNPS-skatole fragments by this method. Table 1 shows the properties of peptides identified in the o-iodosobenzoic acid cleaved

material and these results indicate that additional cleavages have occurred in actin at the C-terminal side of Tyr-53, Tyr-198, Tyr-218, Tyr-239 and probably Tyr-91. The N-terminal peptide corresponding to residues 87-93 (His-His-Thr-Phe-Tyr-Asn-Glu) was not detected in either digest, despite the identification of histidine as the N-terminal residue in the 30,000 dalton fragment. This result probably occurred because the protonated imidazoles on the maleylated N-terminal peptides caused binding to the Dowex 50 resin, whereas the other maleylated N-terminal peptides were not positively charged and were washed directly through the column (18).

DISCUSSION

Although a previous study (5) indicated that o-iodosobenzoic acid effects peptide bond cleavage only at tryptophanyl-containing peptide bonds, this report clearly shows that cleavages at tyrosyl-containing bonds may also occur under identical reaction conditions.

Out of a total of 16 tyrosines in actin, peptide bonds at four of these (Tyr-53, Tyr-198, Tyr-218 and Tyr-239) were definitely cleaved in addition to cleavage at the four tryptophan residues in the molecule (Trp-74, Trp-86, Trp-339, and Trp-355). In addition to these definite cleavages, the isolation of the dipeptide Asn-Glu (see Table 1) is strongly suggestive of cleavage at Tyr-91. However, as this dipeptide occurs at two other regions of the sequence (after Glu-224 and Gly-250), the assignment of a cleavage at this position is tentative although it corresponds to the specificity of cleavage seen in the other isolated peptides.

Although cleavage at the tryptophan residues was complete, cleavage at the susceptible tyrosines appeared to be incomplete as five different N-terminal peptides were identified in the large molecular weight polypeptide fraction isolated from the digest. The yields of the N-terminal peptides isolated (see Table 1) also suggest that different extents of cleavage occurred at each susceptible tyrosine.

A comparison of the sequences around the susceptible tyrosines with those of non-susceptible tyrosines in actin and the proteins studied earlier (5) does not reveal any exclusive and obvious feature related to susceptibility. Each of the susceptible bonds has a basic residue (which would be protonated under the reaction conditions) within three residue positions on the N-terminal side, but this is not unique as twenty out of 49 non-susceptible sequences examined also show this feature.

Although we have not examined the mechanism of cleavage at tyrosine residues, it has been reported that an unspecified modification of tyrosine (possibly caused by impurities in the reagent) may occur at higher than recommended excesses of o-iodosobenzoic acid (5). In the present study however, we have observed cleavage at tyrosine residues under the recommended cleavage conditions (reagent: protein weight ratio of 2:1) and with four different batches of the quality-controlled commercial reagent (Pierce Chemical Co.). In the light of these findings, we therefore suggest that the possibility of a somewhat broader specificity of peptide bond cleavage for o-iodosobenzoic acid should be taken into account in future fragmentation studies.

ACKNOWLEDGEMENT

This research was carried out under a grant to P.J. from Muscular Dystrophy Association.

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