# Protein Splicing: Evidence for an N−O Acyl Rearrangement as the Initial Step in the Splicing Process[†]

Yang Shao,[‡,§] Ming-Qun Xu,[∥] and Henry Paulus*[,‡,⊥]

*Boston Biomedical Research Institute, 20 Staniford Street, Boston, Massachusetts 02114, New England Biolabs Inc., 32 Tozer Road, Beverly, Massachusetts 01915, Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138, and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115*

*Received November 1, 1995; Revised Manuscript Received January 31, 1996*[⊗]

ABSTRACT: Protein splicing involves the self-catalyzed formation of a branched intermediate, which then resolves into the excised intervening sequence and the spliced protein. A possible mechanism for branched intermediate formation is an N−O rearrangement of the peptide bond involving the amino group of the conserved serine/cysteine residue at the upstream splice junction to yield a linear peptide ester intermediate. This possibility was examined using an *in vitro* splicing system involving the intervening sequence from the DNA polymerase of the extremely thermophilic archeon, *Pyrococcus* sp. GB-D. Because thioesters react much more rapidly with nitrogen nucleophiles at neutral pH than do oxygen esters, protein-splicing precursors in which the serine residue of interest was replaced by cysteine were constructed and purified. In the presence of 0.25 M hydroxylamine or 0.1 M ethylene diamine at pH 6 or higher, these constructs underwent rapid cleavage at the upstream splice junction, consistent with the aminolysis of a thioester. The site of hydroxylaminolysis was identified by analysis of the C-terminus of the polypeptide cleavage products. Comparison of the C-terminal peptide hydroxamate with the synthetic peptide hydroxamates with respect to chromatographic mobility, colorimetric assay, amino acid composition, and high-resolution mass spectrometry showed that the hydroxylamine-sensitive site in the splicing precursor was the peptide bond adjacent to the serine residue at the upstream splice junction. These results provide evidence that the peptide bond at the upstream splice junction can undergo a self-catalyzed N−O or N−S acyl rearrangement to yield a linear polypeptide ester intermediate and suggest that this kind of rearrangement constitutes the first step in protein splicing.

In[1] the last two years, much progress has been made in elucidating the mechanism of protein splicing, a process in which an internal polypeptide segment (the intein; Perler et al., 1994) is precisely excised from a precursor protein and the flanking N- and C-terminal segments (the exteins) are ligated together to yield a new protein (Xu et al., 1993, 1994; Shao et al., 1995). A major breakthrough was the development of an *in vitro* protein-splicing system in which the intein from the DNA polymerase of the extremely thermophilic *Pyrococcus* sp. GB-D was inserted into a foreign context. The unspliced precursor protein could thus be purified at low temperatures and be used to study the splicing reaction that occurred as the temperature was raised (Xu et al., 1993). A key advance was the identification of a branched intermediate with two N-terminal polypeptide chains, one of which is linked to an amino acid side chain by an alkali-labile bond (Xu et al., 1993, 1994).

The most likely candidate for the branch point in the splicing intermediate is Ser 538, the residue just downstream of the C-terminal splice junction. Two pathways by which such an intermediate could arise are illustrated in Figure 1 (Xu et al., 1994). In pathway A, the peptide bond involving the amino group of the serine residue at the upstream splice junction (Ser 1) undergoes an N−O acyl rearrangement to yield a peptide ester linked to the hydroxyl side chain of Ser 1, followed by transesterification through nucleophilic displacement by the side chain of Ser 538. Pathway B involves direct nucleophilic attack by the hydroxyl side chain of Ser 538 on the peptide bond at the upstream splice junction, displacing the amino group of Ser 1 and leading to the formation of a peptide ester. There are no *a priori* reasons to favor one or the other of these mechanisms since both involve reactions for which there is biochemical precedent. Self-catalyzed N−O acyl rearrangements have been observed in other proteins (van Poelje & Snell, 1990), and nucleophilic attack on a peptide bond by a serine hydroxyl group is seen in the formation of the acyl−enzyme intermediates by serine proteases.

In this paper, we present experimental evidence that supports a mechanism for the formation of the branched intermediate in which an N−O acyl rearrangement occurs at the upstream splice junction as the first step in protein splicing.

[†] Supported by a New England Biolabs predoctoral fellowship to Y.S.

* To whom correspondence should be addressed. Tel: (617) 742-2010. FAX: (617) 523-6649. E-mail: paulus@bbri.harvard.edu.

[‡] Boston Biomedical Research Institute.

[§] Department of Chemistry, Harvard University.

[∥] New England Biolabs.

[⊥] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School.

[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1996.

[1] Abbreviations: Dnp, 2,4-dinitrophenyl; HPLC, high-performance liquid chromatography; ILVA, L-isoleucyl-L-leucyl-L-valyl-L-alanine; Bis-Tris-Propane, 1,3-bis-[tris(hydroxymethyl)methylamino]propane; SDS−PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

0006-2960/96/0435-3810$12.00/0 

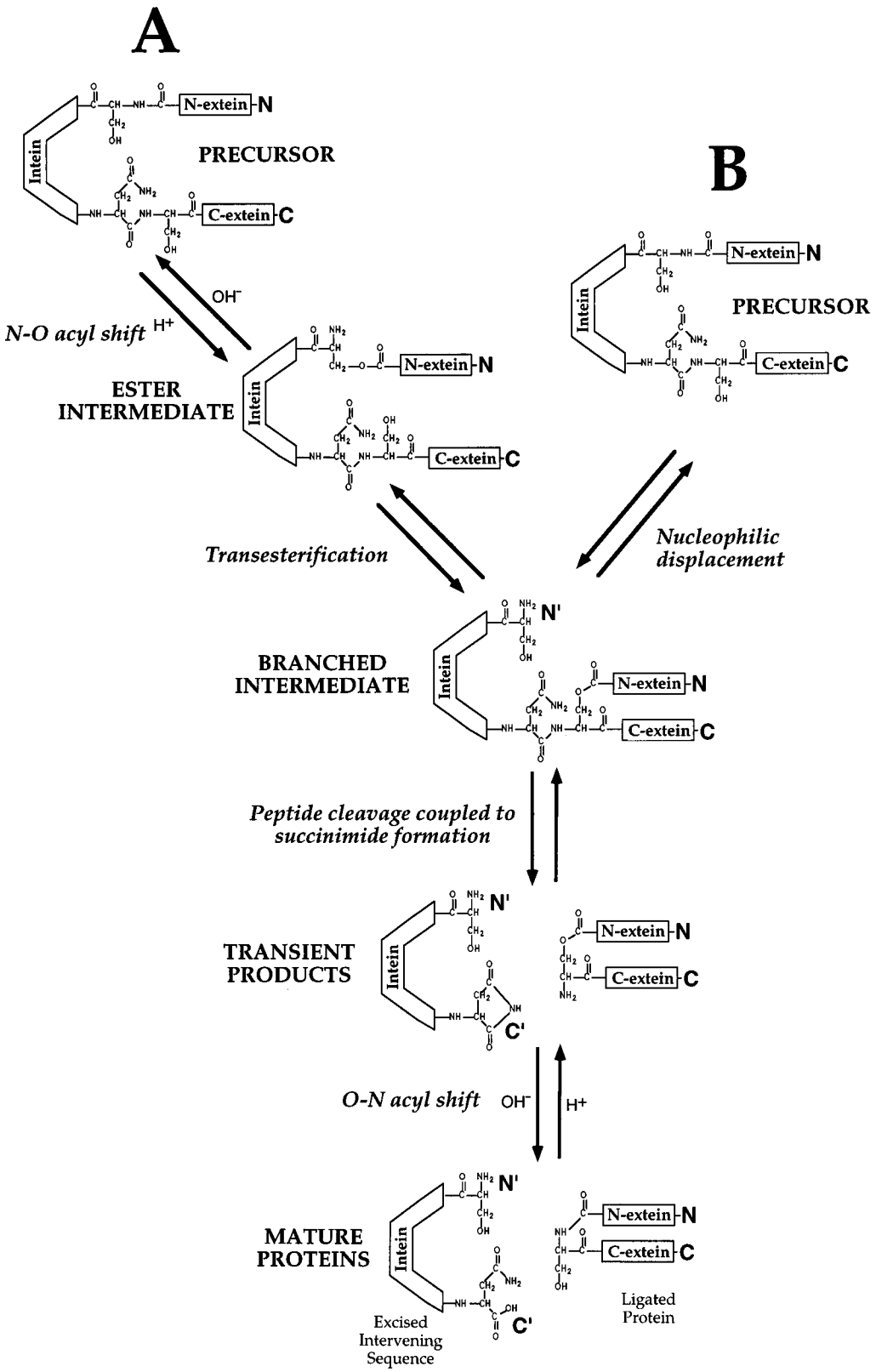


FIGURE 1: Two possible mechanisms for protein splicing. Pathway A (on the left) involves an initial N−O acyl rearrangement followed by transesterification to yield a branched intermediate. Pathway B (on the right) involves nucleophilic attack by a serine hydroxyl group on a peptide bond to yield the identical branched intermediate [After Xu et al. (1994)].

## EXPERIMENTAL PROCEDURES

*Materials.* Protected amino acids and other reagents for peptide synthesis were obtained from Applied Biosystems, except for Dnp-L-leucine, which was obtained from Sigma. $\gamma$-Butyrolactone (4-hydroxybutyric acid $\gamma$-lactone) and $\gamma$-thiobutyrolactone (4-thiobutyric acid $\gamma$-thiolactone) were from Aldrich. Plasmids pMIP-59, pMIP-47/59, pMI-94, pMIP-52, and pMIP-98 were derived from pMIP-21 by cassette replacement of splice junction sequences along the lines described earlier (Xu et al., 1994). The amino acid sequences at the splice junctions of the proteins encoded by the various plasmids are shown in Figure 2.

*Peptide Synthesis.* Peptides were synthesized on an Applied Biosystems model 431 peptide synthesizer as

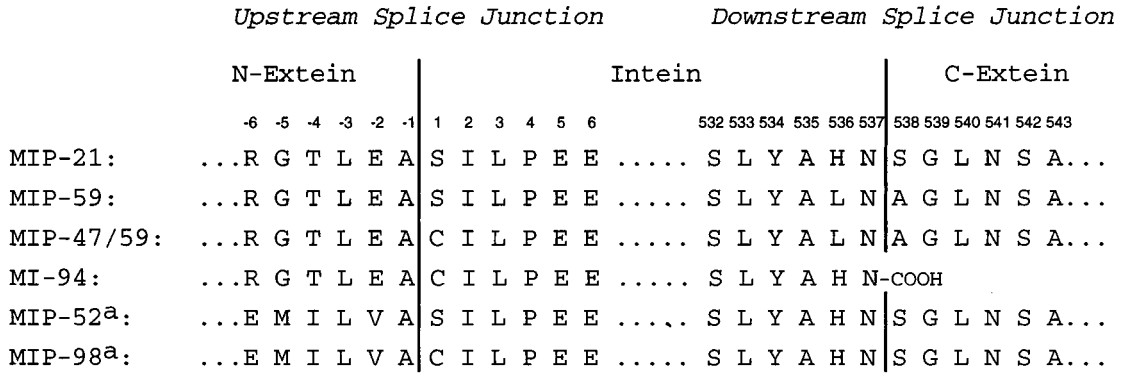


FIGURE 2: Amino acid sequences at the splice junctions of the precursor proteins encoded by the mutant pMIP plasmids used in this work. The amino acid residues are numbered as in Xu et al. (1994). [a] MILV is inserted between residues E and A of MIP-21.

described earlier (Xu et al., 1994). Peptide methyl esters were prepared as described previously (Shao et al., 1995). The tetrapeptide derivative ILVA-OMe was obtained by CNBr cleavage of Dnp-LMILVA-OMe as described below. ILVA hydroxamic acid was prepared from ILVA-OMe by heating with 2 M $NH_2OH$, pH 9.0, for 30 min at 65 °C, essentially as described by Kwong and Harris (1994). The reaction products were then repeatedly purified by HPLC to remove traces of $NH_2OH$ that would interfere with the colorimetric assay for hydroxamic acids described below.

*Cyanogen Bromide Cleavage of Methionine Peptides or Proteins.* Polypeptides (1 mg or less) were dissolved in 1 mL of 70% formic acid and treated with 20 mg of CNBr under nitrogen at 25 °C in the dark for 15−20 h, followed by evaporation in a vacuum (Gross, 1967). The residue was redissolved in a small volume of water and purified by reverse phase HPLC.

*Analytical Methods.* The separation of peptides by HPLC employed a Rainin system with an analytical C-18 reverse phase column (Vydac; 5 $\mu$m pores, 4.6 × 250 mm) at room temperature and a flow rate of 1 mL/min, employing linear gradients of solvent A (0.1% aqueous trifluoroacetic acid) and solvent B (0.1% trifluoroacetic acid in acetonitrile). The following elution program was used for the separation of small peptides: sample injection; 30 min, 0%−60% B; 5 min, 60%−100% B; 5 min, 100% B; 5 min, 100%−0% B; 5 min, 0% B.

Amino acid analysis employed a Beckman model 7300 high-performance analyzer with a System Gold data analysis module after vapor phase hydrolysis with HCl using a Waters PicoTag work station.

High-resolution mass spectra were recorded on a Jeol JMS-SX102 spectrometer at the Department of Chemistry, Harvard University.

The colorimetric determination of hydroxamic acids was done by the method of Seifter et al. (1960) as described earlier (Shao et al., 1995). A standard curve with ILVA-hydroxamate showed a linear relationship between hydroxamic acid concentration and absorbance at 520 nm from 5 to 50 nmol of ILVA-hydroxamate, with 50 nmol yielding $A_{520}$ of 1.4.

The kinetics of hydroxylaminolysis of esters and thioesters were followed by the method of Jencks et al. (1960). $\gamma$-Butyrolactone (10 mM) or $\gamma$-thiobutyrolactone (2 mM) was incubated at 37 °C with 0.05 M $NH_2OH$ in 0.1 M Bis-Tris-Propane and 15 mM $NaP_i$, pH 6.0. At various times, 0.3 mL samples were diluted with 0.4 mL of 0.05 M $NH_2OH$ in 0.1 M Bis-Tris-Propane, pH 6.0, and 0.3 mL of 20% $FeCl_3 \cdot 6H_2O$ in 2.5 M HCl, and the absorbance at 540 nm was measured.

Protein determination was done by the method of Bradford (1976).

*Purification of MIP Fusion Proteins.* The precursor protein MIP-98 (Figure 2) was purified from *Escherichia coli* ER2426/pMIP-98 essentially as described earlier (Shao et al., 1995), except that the final elution step from the amylose column was done at pH 6.0. Analysis of the purified protein (about 8 mg) by SDS−PAGE showed about 95% intact precursor (MIP) and 5% N-terminal cleavage product (M + IP), presumably produced by cleavage of MIP-98 during the last stage of purification or subsequent storage. MIP-52 , MIP-59, MIP-47/59, and MI-94 were purified in a similar manner from *E. coli* ER2426 transformed with the correponding plasmids.

*In Vitro Hydrolysis and Aminolysis of MIP Fusion Proteins.* For large-scale hydroxylaminolysis, a portion of purified MIP-98 (1.5 mg) in 5 mL of 0.5 M NaCl in 20 mM sodium phosphate, pH 6.0, was mixed with 1.7 mL of 1 M $NH_2OH$ in 0.4 M Bis-Tris-Propane, pH 6.0, and incubated at 50 °C for 4 h, conditions adequate for complete hydroxylaminolysis. The mixture was then cooled to 4 °C and adjusted to 0.6 M with cold trichloroacetic acid. The precipitate was collected by centrifugation, washed twice with 1 mL of cold 0.6 M trichloroacetic acid and twice with 1 mL of ethanol, and then dried in a vacuum for storage at −20 °C.

The kinetics of hydrolysis and aminolysis of MIP fusion proteins were studied by incubation at 37°C either in aqueous buffers, 0.25 M $NH_2OH$, or 0.1 M ethylene diamine, adjusted to the desired pH with 0.1 M Bis-Tris-Propane. Samples were removed at various times, subjected to SDS−PAGE, and analyzed for the relative amounts of precursor and cleavage products by scanning with a CCD camera and analyzing the scans using the NIH Image 1.58b program. A pseudo-first-order rate constant for protein cleavage was estimated from the slope of a first-order plot of the logarithm of precursor concentration against time.

## RESULTS AND DISCUSSION

*Strategy.* A unique feature of one of the proposed mechanisms for the formation of the branched intermediate in protein splicing (pathway A in Figure 1) is the occurrence of a linear ester intermediate in which the N-extein domain is esterified with the side chain hydroxyl of Ser 1 as a result of an N−O acyl rearrangement at the upstream splice junction. Unfortunately, experimental evidence for peptide

esters produced by an N−O acyl rearrangement is difficult to obtain because the nucleophilic reagents which are ordinarily used for identifying esters are effective only at alkaline pH, where the amide−ester equilibrium is far toward the amide form (Iwai & Ando, 1967). Self catalyzed N−O acyl rearrangements of the type postulated here have been observed in the biosynthesis of pyruvoyl-dependent enzymes such as histidine decarboxylase from *Lactobacillus*, in which the amino-terminal pyruvoyl moiety is generated by an N−O acyl rearrangement at an internal serine residue followed by a $\beta$-elimination step (van Poelje & Snell, 1990). Although experiments to trap the postulated ester intermediate as the hydroxamate at first seemed to succeed (Huynh & Snell, 1986), their interpretation was later questioned (van Poelje, 1988). However, hydroxylaminolysis of histidine decarboxylase at the predicted site could be achieved after replacing the relevant serine residue with cysteine (van Poelje, 1988; Vanderslice et al., 1988), owing to the much higher susceptibility of thioesters than oxygen esters to attack by nitrogen nucleophiles (Jencks et al., 1960).

We used an analogous strategy to explore the involvement of an N−O acyl rearrangement in protein splicing by replacing Ser 1 at the upstream splice junction with cysteine. If an ester intermediate involving the side chain of Ser 1 were involved in the splicing process, replacement of the serine hydroxyl by a cysteine thiol group should significantly enhance the rate of protein cleavage at the upstream splice junction by neutral hydroxylamine. To obtain an estimate of the relative reactivities of thio- and oxygen esters with hydroxylamine, we compared the rates of hydroxylaminolysis of $\gamma$-thiobutyrolactone and $\gamma$-butyrolactone. At pH 6.0, 7.0, and 8.0, the relative rates of aminolysis by 50 mM $NH_2OH$ of $\gamma$-thiobutyrolactone in comparison with $\gamma$-butyrolactone were found to be 130, 40, and 20, respectively, verifying the assumption that thioesters are cleaved much more rapidly by neutral hydroxylamine than are oxygen esters.

*Comparison of the Rates of Hydrolysis and Aminolysis of MIP-59, MIP-47/59, and MI-94.* The rate of hydrolysis and hydroxylamine-dependent cleavage at the upstream splice junction was compared in three fusion proteins containing intein-1 of *Pyrococcus* sp. GB-D DNA polymerase, MIP-59, MIP-47/59, and MI-94 (Figure 2). In all three proteins, the serine residue at the downstream splice junction was either replaced by alanine or deleted in order to prevent formation of the branched intermediate (Xu et al., 1994). Cleavage of the fusion proteins at the upstream splice junction was measured by densitometry after SDS−PAGE, following incubation at 37 °C in buffers containing no amine, 0.25 M hydroxylamine, or 0.1 M ethylene diamine, which has been reported to be an effective nucleophile for the cleavage of thioesters (Bruice & Benkovic, 1966). The rate constants for protein cleavage were deduced from first-order plots, an example of which (for the cleavage of MIP-47/59 at pH 7) is shown in Figure 3. In the absence of amines, the fusion proteins, being unable to undergo protein splicing, underwent slow cleavage at the upstream splice junction to yield M and IP. The pH optimum of this hydrolytic reaction was pH 6 (Figure 4A,B), which is also the optimum pH for protein splicing (Xu et al., 1993), suggesting that hydrolysis and splicing are interrelated processes. Whereas neither hydroxylamine nor ethylene diamine had a significant effect on the cleavage of MIP-59 (Figure 4A), the amines greatly enhanced the cleavage of MIP-47/59, the corresponding protein in which Ser 1 was replaced by cysteine (Figures 3 and 4B). Unlike hydrolysis, which, like protein splicing, was favored at low pH, aminolysis proceeded more rapidly as the pH increased from 6 to 8, as expected for nucleophilic attack on a thioester (Bruice & Benkovic, 1966). An interesting observation was that MI-94, a fusion protein in which Ser 1 was replaced by cysteine and which in addition was truncated by the introduction of a nonsense mutation at the downstream splice junction (Figure 2), underwent aminolysis at about twice the rate as MIP-47/59 (Figure 4C), suggesting that steric interactions between the two splice junctions may interfere with the attack of amines at the upstream junction.

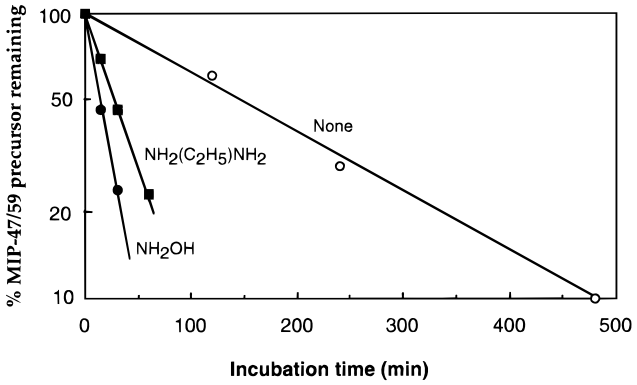


FIGURE 3: Kinetics of hydrolysis and aminolysis of fusion protein MIP-47/59. Purified MIP-47/59 (0.7 mg/mL; about 5 $\mu$M) was incubated at 37 °C and pH 7.0 without added amine (○), with 0.25 M hydroxylamine (●), or with 0.1 M ethylene diamine (■). At the times indicated, samples were removed, subjected to SDS−PAGE, stained with Coomassie Blue, and analyzed as described under Experimental Procedures. The graph plots the logarithm of the percent of intact MIP-47/59 precursor protein remaining against time. A correction was made for 9% of the 132 kDa material that co-migrated with MIP-47/59 precursor but was not cleaved even after prolonged incubation, probably owing to misfolding. Assuming first-order kinetics, the slopes of the lines obtained yielded the following rate constants: no amine, $k = 0.002$ min$^{-1}$; hydroxylamine, $k = 0.019$ min$^{-1}$; ethylene diamine, $k = 0.011$ min$^{-1}$.

The observation that cleavage of the unspliced precursor protein could be effected by nitrogen nucleophiles, provided that the N-terminal amino acid of the intein was cysteine instead of serine, suggests that the peptide bond at the upstream splice junction can undergo an N−O or N−S acyl rearrangement to yield either an ester or a thioester. An alternative interpretation, that the aminolysis observed in these experiments occured at the ester bond of the branched splicing intermediate (Figure 1), which involves the side chain hydroxyl of Ser 538 (Xu et al., 1993; Xu et al., 1994), is excluded by two observations: (a) aminolysis occurred only when cysteine was substituted for Ser 1 at the upstream splice junction, a residue which is presumably not involved in branched intermediate formation (Xu et al., 1994); and (b) the experiments involved fusion proteins in which Ser 538 was replaced by alanine or deleted so as to prevent the formation of the branched intermediate. The fact that cleavage of the thio-substituted precursor protein occurred under very mild conditions (pH 6.0, 37 °C) not only with hydroxylamine but also with ethylene diamine argues against the possibility of cleavage at a uniquely hydroxylamine-sensitive peptide bond such as asn-gly (Bornstein & Balian, 1977).

*Identification of the C-Terminal Peptides of the Hydroxylaminolyzed MIP-98.* In order to identify precisely the site of precursor cleavage by hydroxylamine, the splicing-

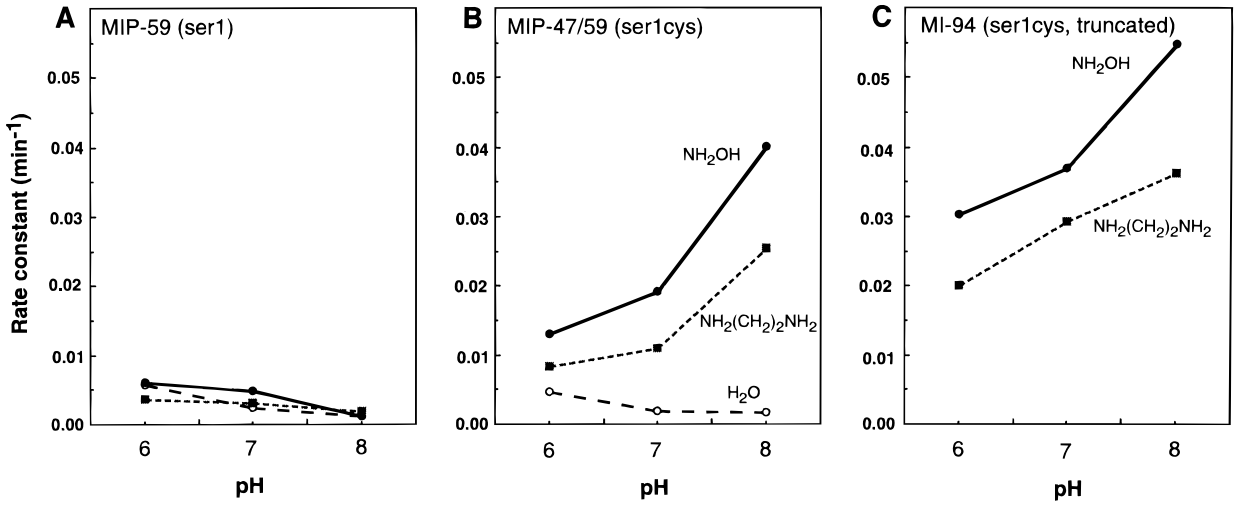


FIGURE 4: Effect of pH and various amines on the rates of hydrolysis or aminolysis. The kinetics of cleavage at the upstream splice junction of MIP-59, MIP-47/59, and MI-94 were studied in the absence of added amines (○), with 0.1 M ethylene diamine (■), or with 0.25 M hydroxylamine (●) in Bis-Tris-Propane buffers at the pH values indicated. The values plotted on the ordinate are pseudo-first-order rate constants derived from the slopes of first-order plots of precursor disappearance as described in Figure 3.

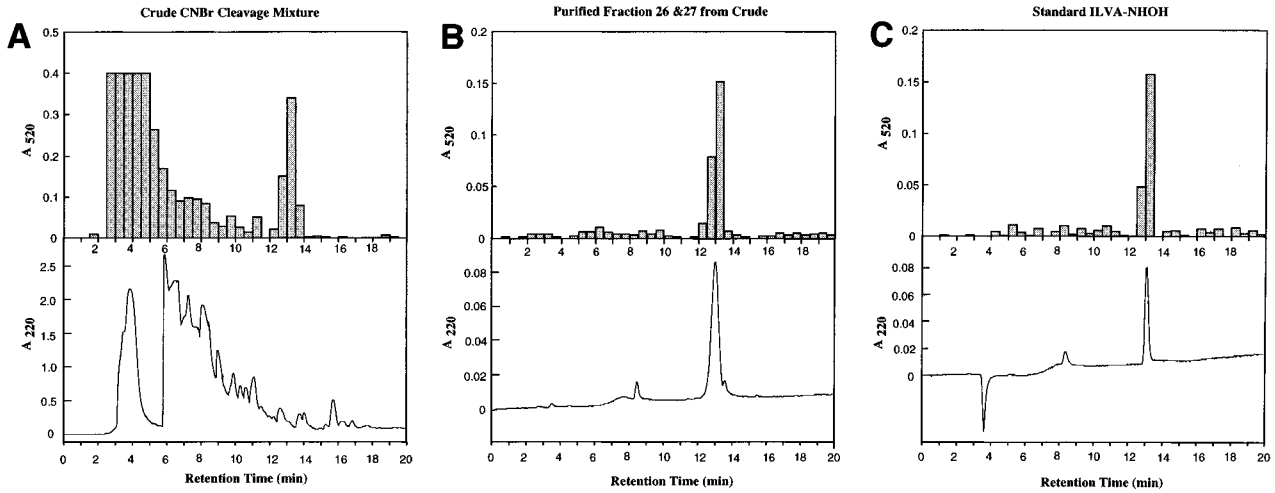


FIGURE 5: HPLC elution profiles and colorimetric assay of hydroxamic acids obtained after CNBr cleavage of the hydroxylaminolysis products of MIP-98. Samples were subjected to HPLC, and the eluate was monitored by its ultraviolet absorbance (bottom panels), with fractions being collected every 0.5 min for the colorimetric determination of hydroxamic acids as described under Experimental Procedures: A, crude CNBr cleavage mixture; B, fractions 26 and 27 from A after two additional cycles of purification by HPLC; C, synthetic ILVA-hydroxamate.

competent fusion protein MIP-21 (Xu et al., 1993) was modified at the C-terminus of the N-extein by inserting the tetrapeptide met-ile-leu-val just before the terminal alanine residue to yield MIP-52; further substitution of Ser 1 by cysteine yielded MIP-98 (Figure 2). The introduction of a methionine residue at position 5 of the N-extein facilitated the analysis of the C-terminus of the product of hydroxylaminolysis so as to define the cleavage site. At 37 °C and pH 6.0, MIP-52 underwent splicing to MP and I at about 25% the rate of MIP-21, perhaps owing to the cluster of hydrophobic amino acid residues at the upstream splice junction, and also underwent slow hydrolysis at the upstream splice junction into M and IP (data not shown). Replacement of Ser 1 with cysteine in MIP-98 further reduced the rate of protein splicing but had little effect on upstream cleavage, which proceeded with a pseudo first-order rate constant of 0.0022 $min^{-1}$ at 37 °C and pH 6.0. In the presence of 0.25 M hydroxylamine under the same conditions, cleavage at the upstream splice junction of MIP-98 was significantly enhanced, occurring with a rate constant of 0.0089 $min^{-1}$, indicating that hydroxylamine affected cleavage of MIP-98 in a similar manner as that of MIP-47/59 (Figure 4B). In contrast, hydroxylamine had no effect on upstream cleavage of MIP-52, the Ser-containing analog of MIP-98 (data not shown). To analyze the products of hydroxylaminolysis, MIP-98 underwent reaction with 0.25 M hydroxylamine at pH 6 for four half-lives and the products were subjected to CNBr cleavage, followed by HPLC on a reverse phase column to separate the resulting peptides. The fractions near the elution position of synthetic ILVA-hydroxamate (13 min) were collected and rechromatographed until a single peak was obtained, both by absorbance at 220 nm and by the colorimetric assay for hydroxamic acids of Seifter et al. (1960), as shown in Figure 5. Table 1 summarizes the properties of the peptide hydroxamate derived from MIP-98 and synthetic ILVA-hydroxamate, which are in close agreement with respect to chromatographic properties, color yield at 520 nm in the colorimetric assay, amino acid composition, and high-resolution fast atom bombardment mass spectrometry, which yielded ($M + H^+$) of 430.3020 and 430.3018, respectively, compared to a calculated value for ILVA-hydroxamate of 430.3029.

The isolation of a tetrapeptide hydroxamate corresponding to the C-terminus of the N-extein clearly establishes that the

Table 1: Comparison of Synthetic Ile-Leu-Val-Ala-NHOH with the Peptide Hydroxamate Obtained after Hydroxylaminolysis of MIP-98

| peptide | amino acid composition (A; I; L; V) | $M + H^+$ found (predicted) | HPLC retention time (min) | colorimetric assay ($A_{520}$ per $\mu$mol) |
|---|---|---|---|---|
| ILVA-NHOH[a] (synthetic) | 1.00; 0.82; 1.08; 0.91 | 430.3018 (430.3029) | 13.1 | 28 |
| 13-min peak from MIP-98 | 1.00; 1.05; 1.03; 1.00 | 430.3020 | 13.1 | 25 |

[a] ILVA-NHOH, ILVA-hydroxamate.

hydroxylamine-dependent cleavage of MIP-98 occurred precisely at the upstream splice junction and not at some other labile bond. It thereby confirms our earlier conclusion that hydroxylaminolysis of proteins containing the intervening sequence derived from *Pyrococcus* sp. GB-D in which cysteine replaces the N-terminal serine residue involves nucleophilic attack on a thioester bond to produce the corresponding peptide hydroxamate.

## PERSPECTIVE

The characterization of the linear ester intermediate described here, together with the earlier characterization of a branched intermediate (Xu et al., 1993, 1994) and of a succinimide intermediate produced as the primary cleavage product (Xu et al., 1994; Shao et al., 1995), establishes the identity of the major intermediates in protein splicing and thereby defines the basic chemical steps in the splicing process. The conclusions of these relatively chemical studies are supported by complementary experiments in which the protein-splicing pathway was dissected by mutagenesis of the conserved regions near the splice junctions (M. Q. Xu and F. B. Perler, personal communication) and which also throw light on questions such as whether Asn 537 participates in the N−O acyl rearrangement as proposed by Clarke (1994). Indeed, the analysis of intein function by mutation will be an important first step in the identification of the amino acid side chains and structural elements that participate in the catalysis of the reactions outlined in Figure 1A so as to define the mechanism of protein splicing in molecular detail.

Protein splicing is one of a class of reactions in which the peptide backbone of a protein undergoes a self-catalyzed rearrangement. Until recently, it was thought that polypeptide rearrangements, such as zymogen activation, polyprotein processing, and excision of signal sequences, are always intermolecular processes requiring the intervention of accessory enzymes such as processing proteases. In the case of protein splicing, it has been clearly established that the excision of the intervening sequence and the ligation of the flanking polypeptides is self-catalyzed (Xu et al., 1993). Similarly, polypeptide cleavage and generation of the N-terminal pyruvoyl moiety of various bacterial amino acid decarboxylases is an intramolecular process catalyzed by the proenzyme itself (van Poelje & Snell, 1990). Very recently, it was found that the cleavage of the inactive protein precursors of hedgehog protein (Lee et al., 1994, Porter et al., 1995) and of glycosylasparaginase (Guan et al., 1996) involves autoproteolysis. In each of these cases, evidence has been presented that the first step in the self-catalyzed activation of the precursor protein involves an N−O or N−S acyl rearrangement. N−O acyl rearrangements in polypeptides had been thought to occur only under extreme, nonphysiological conditions (Iwai & Ando, 1967), but this new information shows that they play an important biological role. The formation of peptide esters involving the side chain of serine or cysteine of the type described in this paper may be the hallmark of a diverse group of self-catalyzed protein rearrangements.

## ACKNOWLEDGMENT

We thank Dr. Donald Comb for his encouragment and for valuable discussions and comments on the manuscript and New England Biolabs for the generous support of this work.

## REFERENCES

Bornstein, P., & Balian, G. (1977) *Methods Enzymol. 11*, 132−145.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.
Bruice, T. C., & Benkovic, S. J. (1966) *Bioorganic Mechanisms*, Vol. 1, pp 268−294, W. A. Benjamin, New York.
Clarke, N. D. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 11084−11088.
Gross, E. (1967) *Methods Enzymol. 11*, 238−255.
Guan, C., Cui, T., Rao, V., Liao, W., Benner, J., Lin, C. L., & Comb, D. (1996) *J. Biol. Chem. 271*, 1732−1737.
Huynh, Q. K., & Snell, E. E. (1986) *J. Biol. Chem. 261*, 1521−1524.
Iwai, K., & Ando, T. (1967) *Methods Enzymol. 11*, 262−282.
Jencks, W. P., Cordes, S., & Carriulo, J. (1960) *J. Biol. Chem. 235*, 3608−3614.
Kwong, M. K., & Harris, R. J. (1994) *Protein Sci. 3*, 147−149.
Lee, J. J., Ekker, S. C., Kessler, D. P., Porter, J. A., Sun, B. I., & Beachy, P. A. (1994) *Science 266*, 1528−1537.
Perler, F. B., Davis, E. O., Dean, G. E., Gimble, F. S., Jack, W. E., Neff, N., Noren, C. J., Thorner, J., & Belfort, M. (1994) *Nucleic Acids Res. 22*, 1125−1127.
Porter, J. A., Kessler, D. P. v., Ekker, S. C., Young, K. E., Lee, J. J., Moses, K., & Beachy, P. A. (1995) *Nature 374*, 363−366.
Seifter, S., Gallop, P. M., Michaels, S., & Meilman, E. (1960) *J. Biol. Chem. 235*, 2613−2618.
Shao, Y., Xu, M.-Q., & Paulus, H. (1995) *Biochemistry 34*, 10844−10850.
van Poelje, P. D. (1988) Kinetics and mechanism of activation of prohistidine decarboxylase, Ph.D. Dissertation, University of Texas, Austin.
van Poelje, P. D., & Snell, E. E. (1990) *Annu. Rev. Biochem. 59*, 29−59.
Vanderslice, P., Copeland, W. C., & Robertus, J. D. (1988) *J. Biol. Chem. 263*, 10583−10586.
Xu, M.-Q., Comb, D. G., Paulus, H., Noren, C. J., Shao, Y., & Perler, F. B. (1994) *EMBO J. 13*, 5517−5522.
Xu, M.-Q., Southworth, M. W., Mersha, F. B., Hornstra, L. J., & Perler, F. B. (1993) *Cell 75*, 1371−1377.

BI952592H