Tetrapeptide Inhibitors of the IgA1 Proteinases from Type I *Neisseria gonorrhoeae*

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The six series of unique tetrapeptides and their blocked *N*-acetyl, *C*-amide, and *N*-acetyl-*C*-amide analogues which comprise the hinge region of human IgA1 (Ser^{224} to Ser^{240}) have been synthesized and tested as inhibitors of the type 1 IgA1 proteinase elaborated by *Neisseria gonorrhoeae* (EC 3.4.24.13). Most series had at least one member with an IC_{50} value <1 mM. The most effective inhibitors came from the series Ser-Thr-Pro-Pro (P_4-P_1) and Pro-Thr-Pro-Ser (P_1-P_3'). One member from each series had an IC_{50} value in the low μM range. Magnetic resonance studies (Siemion, I. Z.; et al. *Biophys. Chem.* **1988**, *31*, 35) indicate that the various tetrapeptide series appear to have different preferred solution conformations. However, these do not appear to be correlated with affinity for the neisserial proteinase. The most effective inhibitors tend to have a threonine residue adjacent to the N-terminus and the P_1 or P_1' residues at either the N- or the C-terminus. These relationships are not exclusive however, as other inhibitors, which do not meet these criteria, bind reasonably well. The most effective substrate analogues outlined here are about one-half the size and bind to the neisserial proteinase 2 orders of magnitude more tightly than previously reported inhibitors.

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About 70% of human bacterial infections in the developed world are caused by organisms which elaborate a high molecular weight proteinase that selectively inactivates human secretory IgA1.[1] Presumably the IgA1 proteinases (EC 3.4.24.13) inactivate mucosal IgA1 and allow the invading microorganism to more readily attach to—and ultimately breach—the mucosal barrier which protects the host. Examples of pathogens which secrete IgA1 proteinases are given in Table I.[2]

Inhibitors of bacterial IgA1 proteinases could perhaps enhance the activity of naturally occurring IgA1, which at an early stage of the pathogenic process would be diminished by the proteinases secreted by the bacteria. Drugs which specifically inhibit IgA1 proteinases may thus find application in states of relative immune deficiency which occur in various types of cancer and also in HIV infections.

Octapeptide analogues of the amino acid sequence of the hinge region of human IgA1 (Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser) have been reported to inhibit both the type 1 and type 2 IgA proteinases produced by *N. gonorrhoeae* with IC_{50} values in the 0.05–10 mM range.[3] All unique tetrapeptides and their *N*-acetyl, *C*-amide, and *N*-acetyl-*C*-amide analogues from the hinge region (Ser^{224}–Ser^{240}) have been prepared, characterized, and tested for inhibition of the type 1 neisserial IgA1 proteinase. Limited structure–activity relationships for the inhibitors, several of which have IC_{50} values 50-fold better than for previously reported inhibitors, are presented.

Results

The unique peptides which span the hinge region of human IgA1 were prepared by solid-phase synthesis, purified by gel filtration on Sephadex G-15, and characterized. Physical properties of the synthetic peptides are given in Table II.

The hinge-region tetrapeptides were tested for their inhibitory properties against the type 1 IgA1 proteinases secreted by *N. gonorrhoeae*. Peptides based on the sequence Ser-Thr-Pro-Pro (P_4-P_1) are reasonable inhibitors of type I IgA1 proteinases (Table III, Figure 1).

Table I. Bacteria Which Secrete IgA1 Proteases

Hemophilus influenzae
Hemophilus aegyptius
N. gonorrhoeae
Neisseria meningitidis
Stretococcus pneumoniae
Streptococcus sanguis
Streptococcus mitior
Bacteriodes melaninogenicus
Bacteroides assacharolyticus
Capnocytophaga ochracea
Capnocytophaga gingivalis

From the sequence Thr-Pro-Pro-Thr (P_3-P_1') only one peptide shows significant inhibition. Hinge-region peptide HRP-7, Thr-Pro-Pro-Thr-NH_2, has an IC_{50} of 419 μM. Other members of the series do not inhibit the enzyme.

The sequence Pro-Pro-Thr-Pro (P_2-P_2') spans the cleavage site. The free peptide (HRP-42) is a fair inhibitor of the type 1 proteinase (40.5 μM). Peptides in this series with a C-terminal amide do not inhibit the IgA1 proteinases.

The sequence Pro-Thr-Pro-Ser contains the P_1-P_3' side chains. The most effective tetrapeptide inhibitors come from this series of peptides. Pro-Thr-Pro-Ser-NH_2 (HRP-48) has an IC_{50} of 5.3 μM while Ac-Pro-Thr-Pro-Ser (HRP-47) has an IC_{50} value of 24 μM.

The sequence Thr-Pro-Ser-Pro (P_1'-P_4') provides two inhibitors. HRP-54 (Thr-Pro-Ser-Pro) and HRP-55 (Ac-Thr-Pro-Ser-Pro). HRP-54 has nearly the same inhibitory properties as HRP-47 (33.1 M vs 24 M). Other members of the series are less effective than corresponding members of the series based on Pro-Thr-Pro-Ser (P_1-P_3').

The series of peptides based on the sequence Pro-Ser-Pro-Ser (P_2'-P_5') has one compound. HRP-77 (Ac-Pro-Ser-Pro-Ser), which shows slight inhibition of the neisserial proteinases (675 μM). Other members of the series inhibit the IgA1 proteinase poorly if at all (Figure 1).

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Table II. Physical Properties of the Tetrapeptide IgA1 Proteinase Inhibitors

HRP no.	amino acid analysis			thin-layer chromatography R_f			ϵ_{220}	sp act., Ci/mol	HPLC t_r, min
	Ser	Thr	Pro	T_2	T_3	T_4			
7		2.05	1.95	0.23	0.47	0.00	7.870	0.055	10.4
8		2.05	1.95	0.34	0.52	0.00	6.910	0.048	13.6
9	1.83		2.17	0.18	0.57	0.03	6.280	0.039	12.3
10	2.09		1.91	0.17	0.52	0.13	5.623	0.073	12.8
42		0.93	3.07	0.11	0.58	0.03	6.850	0.030	14.9
43		0.97	3.03	0.17	0.44	0.00	5.565	0.052	15.2
44		0.89	3.11	0.13	0.52	0.03	6.480	0.034	15.7
45		0.97	3.03	0.13	0.65	0.13	7.960	0.040	16.7
46	1.02	0.96	2.01	0.05	0.40	0.14	4.360	0.034	13.1
47	0.95	0.99	2.06	0.15	0.38	0.05	5.682	0.067	13.6
48	0.91	0.98	2.11	0.10	0.48	0.00	5.431	0.078	12.5
49	0.94	0.98	2.08	0.21	0.47	0.00	6.950	0.062	13.9
54	0.91	1.01	2.09	0.02	0.45	0.07	7.910	0.073	13.2
55	0.93	1.00	2.08	0.07	0.54	0.08	6.670	0.099	14.6
56	0.99	0.95	2.05	0.20	0.54	0.05	5.489	0.036	13.7
57	0.98	0.97	2.05	0.25	0.66	0.17	6.134	0.045	13.9
65		2.04	1.96	0.13	0.43	0.05	6.515	0.036	13.3
66		2.02	1.98	0.10	0.45	0.03	6.250	0.062	12.2
76	1.90		2.10	0.18	0.45	0.00	8.290	0.054	13.3
77	1.87		2.13	0.22	0.44	0.10	4.790	0.078	13.8
81	0.88	1.01	2.11	0.19	0.37	0.00	7.530	0.010	12.3
82	0.93	1.02	2.06	0.26	0.41	0.05	7.650	0.011	12.5
131	0.96	1.09	1.95	0.17	0.45	0.00	7.867	0.030	12.8
132	0.82	1.03	2.16	0.23	0.39	0.03	7.923	0.014	13.1

Table III. Inhibitory and Conformational Properties of the Tetrapeptide IgA1 Proteinase Inhibitors

HRP no.	sequence	IC_{50},[a] μM	polv-Pro	β-turn	unordered	ref
131	STPP	73.0				
132	AcSTPP	9.7				
81	$STPPNH_2$	*				
82	$AcSTPPNH_2$	*				
65	TPPT	*	1		X	5
66	AcTPPT	*	1		X	5
7	$TPPTNH_2$	419.0	1		X	5
8	$AcTPPTNH_2$	*	1		X	5
42	PPTP	40.5		X		5
43	AcPPTP	*			X	5
44	$PPTPNH_2$	*		X		5
45	$AcPPTPNH_2$	*				
46	PTPS	*				
47	AcPTPS	23.8			X	6
48	$PTPSNH_2$	5.3			X	6
49	$AcPTPSNH_2$	*			X	6
54	TPSP	33.1				
55	AcTPSP	183.0		X		6
56	$TPSPNH_2$	*		X		6
57	$AcTPSPNH_2$	*		X		6
76	PSPS	*				
77	AcPSPS	675.0				
9	$PSPSNH_2$	*				
10	$AcPSPSNH_2$	*				

[a] An asterisk signifies an $IC_{50} > 1$ mM.

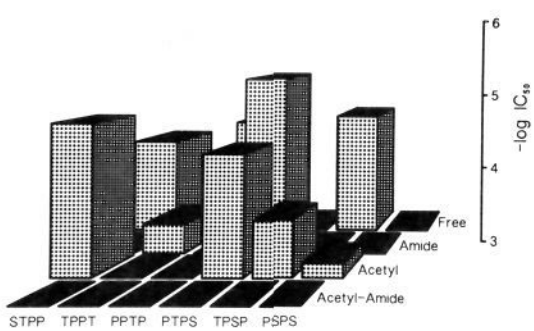

Figure 1. $-\log IC_{50}$ values of the hinge region spanning peptides and their analogues.

Discussion

The inhibitory properties of the substrate analogue peptides are difficult to predict. The best inhibitors (IC_{50} < 10 μM) are Ac-Ser-Thr-Pro-Pro (HRP-132) and Pro-Thr-Pro-Ser-NH_2 (HRP-48). The charge on the two peptides is different at the pH at which the assays were performed and would not appear to influence binding to the IgA1 proteinase. There is a tendency for the inhibitors to be negatively charged at the assay pH (Table IV).

Table IV. IgA1 Tetrapeptide Substrate Analogues Arranged in Order of Affinity (<100 μM)[a]

	IC_{50}, μM		IC_{50}, μM
Pro↓ThrProSerNH_2	5	↓ThrProSerPro	33
AcSerThrProPro↓	10	ProPro↓ThrPro	41
AcPro↓ThrProSer	23	SerThrProPro↓	73

[a] An arrow indicates a cleavage site.

Many of the better inhibitors have a threonine residue adjacent to the N-terminus. Other substrate analogue peptides containing a threonine residue at this position are poor inhibitors, however. In addition, HRP-54 (Thr-

Pro-Ser-Pro) and HRP-42 (Pro-Pro-Thr-Pro) are moderate inhibitors of the neisserial enzyme (33 and 41 μM) but lack a threonyl residue at the second position. The inhibitors would thus appear to bind to different subsites on the enzyme.

If binding is evaluated by the subsite occupancy[7] rather than homology, most of the effective inhibitors appear to have a residue which can fill either the S_1 or the S_1' subsites of the IgA1 proteinase at either the N- or the C-terminus of the inhibitors (Table IV). As with other structure–activity relationships, these conditions are not sufficient to ensure good binding. Substrate analogues such as HRP-81 and HRP-57 fulfill this criterion, but they bind to the IgA1 proteinase only poorly (>1 mM).

All tetrapeptide substrate analogues blocked at both the N- and C-termini are uniformly poor inhibitors (Figure 1). Reasons for this are unclear, but similar results were obtained for the octapeptide substrate analogues.[3]

Results from NMR conformational analysis (Table III)[5-7] indicate that the best inhibitors have an unordered conformation in solution. No obvious relationship between solution conformation and affinity has been identified.

Molecular modeling studies of the series using CHARMm-21[8] and a dynamics approach have been started in an effort to identify subtle conformational details which can be correlated with binding to the IgA1 proteinase.

Structure–activity relationships reported here may permit the design of research drugs that will help define the role of the IgA1 proteinases in the pathogenesis of gonorrhoeal infections.

Experimental Section

Determination of IC_{50}. Purification of the type 1 IgA1 proteinase from *N. gonorrhoeae* has been previously reported.[3] Human IgA1 was purified to homogeneity from serum by using the methods outlined by Plaut et al.[9] IgA1 was then iodinated by the method of Hunter and Greenwood[9] to a specific activity of 8 Ci/g. Methods for measurement of IgA1 are outlined in the first publication of this series.[3]

Measurement of Peptide Conformation. Measurement of the solution conformation of the tetrapeptide IgA1 hinge-region fragments is presented in detail elsewhere.[5-7] Conformational preferences of the various tetrapeptides are given as part of Table III to indicate that a readily apparent relationship between conformation and inhibitory conformation does not appear to exist.

Peptide Synthesis. (*tert*-Butyloxycarbonyl)amino acids were purchased from Peninsula Laboratories (San Mateo, CA). Side chain protecting group was benzyl for serine and threonine. Tritiated proline was prepared by reaction of labeled proline (Amersham, Arlington Heights, IL), diluted to approximately 0.05 Ci/mol with unlabeled proline (Eastman Kodak, Rochester, NY), with di-*tert*-butyl dicarbonate (Fluka, Ronkonkoma, NY)). Both Et_3N and CF_3COOH were purchased from Pierce Chemical Co. (Rockford, IL). Dicyclohexylcarbodiimide (DCC) was obtained from Fluka Chemical Corp. Dichloromethane (Dow, Midland, MI) was redistilled before used. The support used for solid-phase synthesis was either LS-601 Merrifield resin containing 0.74 mM Cl^-/g (Laboratory Systems, San Mateo, CA), or *p*-methylbenzhydrylamine-resin containing 0.46 mmol of NH_2/g (USB, Cleveland, OH). Dioxane (Eastman Kodak) was redistilled from sodium metal and gave a negative KI test for peroxides. HCl (6 N) in dioxane was prepared by bubbling semiconductor-grade HCl gas (Medtech, Medford, MA) through dioxane cooled in an ice bath. After addition of an aliquot of the reagent to excess 1 N NaOH and back-titration with 1 N H_2SO_4 to determine concentration, the reagent was diluted to 6 N with redistilled dioxane and stored in a closed container. Other reagents were analytical grade.

Synthetic reactions were performed in 60-mL polypropylene syringes fitted with a high-density polyethylene frit (70-μm porosity, Bel Art, Pequannock, NJ) with apparatus and techniques previously described.[11] Completeness of coupling was judged by viewing beads which had been reacted with the ninhydrin reagent[11] on filter paper at 40× under a dissecting microscope.

Peptides were hydrolyzed at 110 °C for 24 h under vacuum in 6 N HCl. Amino acid analyses were performed with a Beckman D-6000 analyzer. Yields are calculated from amino acid analysis data.

Thin-layer chromatography was done on 250 silica gel H plates (Analtech. Newark, DE) in solvents previously described.[11] Peptide was detected by sequential application of ninhydrin spray and chlorine–starch/KI.

Tritium was quantitated by counting aliquots of peptide solution with Hydroflor (National Diagnostics, Manville, NJ) in a Beckman LS-100 scintillation counter until at least 1000 counts above background were obtained. Tritiated water standards (New England Nuclear, Boston, MA) diluted to 10000 dpm/50 μL were counted in series with most samples to determine the efficiency, which was typically about 17%.

UV spectra were obtained on an HP8450A spectrophotometer and stored in memory until quantitative data became available to allow calculation of ϵ vs λ.

HPLC was done with a 410 system (Beckman Instruments, Palo Alto, CA), eluted through a 178.32 flow cell (Helma, Jamaica, NY). Data were collected and converted into an elution profile with an HP-216 computer. HPLC purifications were done on a Beckman ODS column (1 × 25 cm) using a linear gradient of CH_3CN–0.2% CF_3COOH in 0.2% CF_3COOH over 20 min at 2 mL/min. When different elution conditions were used, these are identified.

Serylthreonylprolylproline (HRP-131). The C-terminal proline residue was attached by refluxing a 2-fold excess of Boc-Pro and a 1.8-fold excess of Et_3N in ethanol (10 mL/g) with the copolymer for 24 h. On completion of the esterification, the peptidyl-resin was washed well with ethanol and dried to constant weight over P_2O_5. The copolymer contained 0.23 mmol of proline/g. For the synthesis of HRP-131, 3.00 g of the protected aminoacyl-resin was used. Amino acid residues were added to the peptide with the standard cycle with a 5-fold excess of the Boc-protected amino acid and DCC. HCl (6 N)-dioxane was used for deprotection. On completion of synthesis, the peptidyl-resin was deprotected with HCl–dioxane, washed well with dioxane, CH_2Cl_2, and ethanol, and dried to constant weight over P_2O_5. Peptidyl-resin, (3.00 g) was then treated with 30 mL of HF–anisole (9:1) for 1 h at 0 °C. On completion of cleavage, HF and anisole were removed at high vacuum. The cleaved product was then transferred to a Hirsch funnel (porosity A, Ace Glass, Vineland, NJ) with 50 mL of cold EtOAc. After removal of EtOAc by suction, the peptidyl-resin was sequentially extracted with 100 mL of 1, 5, 10, and 25% (v/v) acetic acid solution. Aliquots (100 mL) of each extract were counted and the 1% extract, which contained 96% of the radioactive label, was lyophilized (42%, 117 mg). The peptide was dissolved in 2 mL of 5% AcOH solution and gel filtered on a Sephadex G-15 column (1.1 × 110 cm) at a flow rate of 12 mL/h. Fractions (2.6 mL) were collected, aliquots of each were counted, and the UV absorption at 245 nM was measured. Both physical data were plotted and fractions in which the ratio of counts/absorption were constant (52–63 mL) were

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pooled and lyophilized (102 mg, 87%, overall yield 37%). Peptide (1 mg) was dissolved in 1 mL of H_2O and subjected to HPLC. A single peak was observed. Physical properties of the peptide are given in Table II.

Acetylserylthreonylprolylproline (HRP-132). HRP-132 was synthesized in parallel with HRP-131. After deprotection, the tetrapeptidyl-resin was neutralized and acetylated with a 5-fold excess of acetic anhydride and Et_3N in CH_2Cl_2 over a 1-h period. The peptidyl resin gave a negative ninhydrin test after acetylation. After drying to constant weight, 3.10 g of peptidyl-resin were cleaved with HF–anisole by using conditions reported for HRP-131. After extraction (90% radioactivity in 1% AcOH solution) and lyophilization (139 mg, 46%), the crude peptide was gel filtered as described. Fractions eluting between 50 and 65 mL were lyophilized to yield 118 mg (85%, overall yield 39%) of homogeneous peptide by HPLC.

Serylthreonylprolylprolinamide (HRP-81). *p*-Methylbenzhydrylamine resin containing 1.41 mmol (3 g) of attachment sites was neutralized, washed well with CH_2Cl_2, and reacted with a 5-fold excess of Boc-Pro and DCC. After coupling and washing, the protected aminoacyl polymer gave a negative test with ninhydrin. The remaining amino acids were then added, and one-half of the peptidyl polymer (1.50 g) was cleaved with HF–anisole, and the 1% AcOH solution (radioactivity 93%) was lyophillized (140 mg, 49%). Fractions from gel filtration eluting between 61 and 68 mL were pooled and lyophilized to yield 129 mg (92%, 45% overall yield) of homogeneous peptide by HPLC.

Acetylserylthreonylprolylprolinamide (HRP-82). One-half of the protected tetrapeptidyl-resin synthesized during the preparation of HRP-81 (1.50 g) was neutralized and acetylated as described for HRP-132. Crude peptide obtained after HF cleavage (130 mg, 41%) was gel filtered, and fractions eluting between 52 and 59 mL were pooled and lyophilized to yield 116 mg (89%, overall yield 37%) of homogeneous product by HPLC.

Threonylprolylprolylthreonine (HRP-65). HRP-65 was prepared with chloromethylated resin containing 0.25 mmol of threonine per gram of resin. The tetrapeptidyl-resin (3.0 g) was subjected to HF cleavage and 84% of the extracted label was found in the 1% AcOH extract. This was lyophilized and the product (284 mg, 91%) was gel filtered on Sephadex G-15. Material eluting between 37 and 50 mL was pooled to yield 253 mg (89%, overally yield 71%) of homogeneous peptide.

Acetylthreonylprolylprolylthreonine (HRP-66). HRP-66 was prepared with the protected aminoacyl-resin used for HRP-65. After deprotection and neutralization, the peptidyl resin was acetylated as for HRP-82. Following HF–anisole cleavage, 84% of the radioactive label was extracted into 1% acetic acid solution. After lyophilization, the peptide (309 mg, 87%) was gel filtered, and fractions eluting between 34 and 46 mL were pooled to yield 281 mg (91%, overall yield 79%) of homogeneous peptide.

Threonylprolylprolylthreoninamide (HRP-7). The synthesis of HRP-7 was begun with 3 g of *p*-methylbenzhydrylamine-resin containing 1.41 mmol of attachment sites. The protected tetrapeptidyl-resin (1.63 g) was subjected to HF cleavage. Radioactivity was found in the 1% acetic acid extract (77%) and in the 5% extract (24%). These solutions were combined and lyophilized. Crude peptide (309 mg, 97%) was gel filtered on Sephadex G-15 (1.1 × 100 cm). Peptide eluting between 34 and 46 mL was pooled and lyophilized to yield 294 mg (95%, overall yield 92%) of homogeneous HRP-7.

Acetylthreonylprolylprolylthreoninamide (HRP-8). One-half of the α-amino-deprotected tetrapeptidyl-resin from the synthesis of HRP-7 was acetylated as described for HRP-66. The tetrapeptidyl-resin (1.84 g) was cleaved with HF–anisole and after extraction 72% of the radioactivity was found in the 1% acetic acid extract, which was lyophilized (285 mg, 72%). The EtOAc extract contained radioactivity (20%) but was discarded. The crude peptide was gel filtered, and fractions eluting between 32 and 43 mL were pooled to yield 213 mg (75%, overall yield 54%) of homogeneous HRP-8.

Prolylprolylthreonylproline (HRP-42). Boc-Pro-resin prepared for the synthesis of HRP-131 was used for the preparation of HRP-42. On completion of synthesis, one-half (1.77 g) of the peptidyl polymer was cleaved with HF–anisole and 87.1% of the label was found in the 1% AcOH extract. After lyophilization (167 mg, 92%), this was gel filtered, and material eluting between 41 and 54 mL was pooled and lyophilized to yield 137 mg (82%, overall yield 75%) of homogeneous HRP-42.

Acetylprolylprolylthreonylproline (HRP-43). One-half of the α-amino-deprotected tetrapeptidyl polymer prepared in the synthesis of HRP-42 was acetylated by using standard techniques. The peptidyl-resin (1.71 g) was cleaved with HF–anisole and 70% of the radioactive label was found in the 1% AcOH extract, which was lyophilized (166 mg, 86%) and gel filtered. Material eluting between 43 and 60 mL was lyophilized to yield 113 mg (68%, overall yield 59%) of homogeneous HRP-43.

Prolylprolylthreonylprolinamide (HRP-44). HRP-44 was synthesized with 3.00 g of *p*-methylbenzhydrylamine-resin containing 0.47 mmol of attachment sites per gram of resin. On completion of synthesis, 1.71 g of peptidyl-resin was cleaved with HF. Most of the radioactivity (89%) was found in the 1% AcOH extract, which was lyophilized (308 mg, 93%) and gel filtered. Material eluting between 36 and 48 mL was lyophilized to yield 262 mg (85%, overall yield 79%) of homogeneous HRP-44.

Acetylprolylprolylthreonylprolinamide (HRP-45). One-half of the α-amino-deprotected tetrapeptidyl resin synthesized for the preparation of HRP-44 was neutralized and acetylated as described previously. The peptidyl-resin (1.47 g) was treated with HF–anisole. About 54% of the radioactivity was found in the AcOEt extract and 44% of the label was in the 1% AcOH extract. The AcOEt extract was evaporated and the residue was dissolved in a 10% AcOH solution, filtered, and lyophilized. This material was then added to the 1% AcOH extract (286 mg, 91%) and gel filtered. Material eluting between 42 and 51 mL was pooled and lyophilized to yield 249 mg (87%, overall yield 79%) of homogeneous HRP-45.

Prolylthreonylprolylserine (HRP-46). HRP-46 was synthesized with 3.00 g of resin containing 0.69 mmol of serine. After addition of the amino acid residues, the peptidyl-resin (3.37 g) was treated with HF–anisole and extracted as described. Most of the radioactivity (91%) was found in the 1% acetic acid extract, which was lyophilized (292 mg, 94%). The crude material was then gel filtered, and fractions eluting between 54 and 69 mL were pooled and lyophilized to yield 254 mg (87%, overall yield 82%), of homogeneous HRP-46.

Acetylprolylthreonylprolylserine (HRP-47). HRP-47 was synthesized with 3.00 g of resin containing 0.69 mmol of serine. After addition of all the amino acid residues, the peptide was acetylated with acetic anhydride by using standard techniques. After HF cleavage of 3.27 g of peptidyl-resin, 40% of the extracted radioactivity was found in the AcOEt wash and 58% was in the 1% acetic acid extract. The AcOEt extract was evaporated and the residue was dissolved in 1% acetic acid solution. The solution was filtered and added to the original 1% extract, which was then lyophilized (320 mg, 96%). Crude peptide was then gel filtered on Sephadex G-15 and material eluting between 54 and 63 mL was pooled and lyophilized to yield 288 mg (90%, overall yield 86%) of homogeneous HRP-47.

Proylthreonylprolylserinamide (HRP-48). HRP-48 was synthesized with 3.00 g of resin containing 1.41 mmol of attachment sites. After addition of the residues and deprotection of the N-terminal prolyl residue, 1.77 g of peptidyl-resin was cleaved with HF–anisole. On extraction, 95% of the radioactivity was found in the 1% AcOH solution, which was lyophilized (308 mg, 92%) and gel filtered on Sephadex G-15. Material eluting between 41 and 53 mL was pooled and lyophilized to yield 280 mg (91%, overall yield 84%) of homogeneous HRP-48.

Acetylprolylthreonylprolylserinamide (HRP-49). The tetrapeptidyl-resin (1.76 g) obtained from the synthesis of HRP-48 was acetylated by using standard conditions and cleaved with HF–anisole. Most of the radioactivity (96%) was found in the 1% AcOH extract, which was lyophilized (360 mg, 98%) and gel filtered on Sephadex G-15. Fractions eluting between 53 and 75 mL were pooled and lyophilized to yield 335 mg (93%, overall yield 91%) of homogeneous HRP-49.

Threonylprolylserylproline (HRP-54). Resin containing 0.69 mmol of serine was used for the synthesis of HRP-54. After addition of the amino acid residues, 1.68 g of the α-amino-deprotected tetrapeptidyl-resin was cleaved with HF–anisole. On extraction, most (84%) of the radioactivity was found in the 1% acetic acid extract, which was lyophilized to yield 143 mg of material (92%). This was gel filtered on Sephadex G-15 in 1%

acetic acid solution and material eluting between 37 and 50 mL was pooled and lyophilized to yield 132 mg (92%, overall yield 85%) of homogeneous HRP-54.

Acetylthreonylprolylserylproline (HRP-55). One-half of the α-amino-deprotected tetrapeptidyl-resin (1.53 g prepared in the synthesis of HRP-54 was acetylated by using standard procedures. After HF cleavage and extraction, 84% of the radioactive label was found in the 1% AcOH extract, which was lyophilized to yield 168 mg (108%) of crude peptide. This was gel filtered and material eluting between 37 and 50 mL was pooled and lyophilized to yield 147 mg (88%, overall yield 94%) of homogeneous HRP-55.

Threonylprolylserylprolinamide (HRP-56). *p*-Methylbenzhydrylamine-resin (3 g) containing 1.41 mmol of attachment sites was used for the synthesis of HRP-56. After addition of the amino acid residues, 1.02 g of α-amino-deprotected tetrapeptidyl-resin was cleaved with HF. The 1% AcOH extract was lyophilized to yield 176 mg (91%) of crude peptide. This was gel filtered (G-15), and fractions eluting between 40 and 45 mL were pooled and lyophilized to yield 132 mg (75%, overall yield 68%) of homogeneous HRP-56.

Acetylthreonylprolylserylprolinamide (HRP-57). *p*-Methylbenzhydrylamine-resin (3 g) containing 1.41 mmol of attachment sites was used for the synthesis of HRP-57. After addition of the amino acid residues, the peptidyl-resin was acetylated as before. After HF cleavage of 3.46 g of the peptidyl resin, the 1% acetic acid extract (70% radioactivity) and the 5% acetic acid extract (23% radioactivity) were pooled and lyophilized to yield 659 mg (91%) of crude peptide. This was gel filtered (G-15) in 10% AcOH solution, and fractions eluting between 60 and 76 mL were pooled and lyophilized to yield 574 mg (87%, overall yield 79%) of homogeneous HRP-57.

Prolylserylprolylserine (HRP-77). Resin containing 0.69 mmol of serine was used for the synthesis of HRP-77. After addition of the amino acid residues, 2.97 g of tetrapeptidyl-resin was treated with HF–anisole. After evaporation and extraction, 91% of the label was found in the 1% AcOH extract, which was lyophilized to yield 248 mg (94%) of crude HRP-77. This was gel filtered on Sephadex G-15 and material eluting between 42 and 54 mL was pooled and lyophilized to yield 208 mg (84%, overall yield 79%) of homogeneous HRP-77.

Acetylprolylserylprolylserine (HRP-76). Resin containing 0.69 mmol of serine was used for the synthesis of HRP-76. After addition of the remaining amino acid residues, 3.11 g of tetrapeptidyl-resin was cleaved with HF–anisole. After evaporation and extraction, 97% of the label was found in the 1% AcOH extract, which was lyophilized to yield 303 mg (99%) of crude peptide. This was gel filtered on Sephadex G-15 and material eluting between 53 and 68 mL was pooled and lyophilized to yield 254 mg (84%, overall yield 83%) of homogeneous HRP-76.

Prolylserylprolylserinamide (HRP-9). *p*-Methylbenzhydrylamine-resin containing 1.41 mmol of attachment sites was used for the synthesis of HRP-9. After addition of the amino acid residues, 3.11 g of peptidyl polymer was cleaved with HF–anisole. After evaporation and extraction, 95% of the radioactivity was found in the 1% AcOH extract, which was lyophilized to yield 471 mg (83%) of crude HRP-9. This was gel filtered on Sephadex G-15 and material eluting between 54 and 59 mL was pooled and lyophilized to yield 419 mg (89%, overall yield 74%) of homogeneous HRP-9.

Acetylprolylserylprolylserinamide (HRP-10). *p*-Methylbenzhydrylamine-resin containing 1.41 mmol of attachment sites was used for the synthesis of HRP-10. After addition of the amino acid residues, 3.00 g of tetrapeptidyl-resin was treated with HF–anisole. After evaporation and extraction, 92% of the radioactivity was found in the 1% acetic acid wash, which was lyophilized to yield 522 mg (86%) crude HRP-10. This was gel filtered on Sephadex G-15 and material eluting between 59 and 82 mL was pooled and lyophilized to yield 470 mg (90%, overall yield 77%) of homogeneous HRP-10.

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