a molecular weight of about 92 000, which consists of two subunits of identical molecular weight.

In addition, data presented here confirm that the molecular weight of the testicular enzyme is smaller than those reported for the membrane-bound cyclases solubilized with detergents. The latter ranged from about 114×10^3 to 270×10^3 (Neer, 1974, 1978; Haga et al., 1977; Asbury et al., 1978; Stellwagen & Baker, 1976).

The testicular enzyme is the only nonsedimentable adenylate cyclase found in mammalian tissues, but little is known about its regulation and analogies with membrane-bound cyclases. The role of this enzyme in testicular germ cells is an important point to be elucidated.

Acknowledgments

We express our gratitude to Dr. Luis F. Leloir for his advice and support and to our colleagues at the Instituto de Investigaciones Bioquímicas for helpful discussions and criticism.

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Preparation and Activation of a Spin-Labeled Pepsinogen†

Sally S. Twining, Roger C. Sealy, and David M. Glick*

ABSTRACT: A porcine pepsinogen derivative has been prepared, using 3-[[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl]-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrolyl-1-oxy with 3 spin-labels/protein molecule. Two are located in the peptide that is released first (1–16) and the other is in the secondarily removed sequence (17–44). Activation of the labeled zymogen is a faithful model of native pepsinogen activation because the two processes are closely related in rate, pH dependence of rate, and sites of peptide bond cleavage. The ESR signal associated with the bound label changes during activation due to release of the activation peptide. The rate of release (0.5 min^{-1} at pH 2, 22 °C) is an order of magnitude slower than is the rate of activation, i.e., cleavage of the peptide bond that holds the peptide to the enzyme. Activation in the presence of pepstatin, however, results in peptide release (2 min^{-1} at pH 2, 22 °C) nearly as fast as activation occurs. At pH values between 2.5 and 3, there is a lag in the change of the ESR signal following acidification. This indicates accumulation of an intermediate with pH 8.5 labile activity that still has its activation peptide attached. The strong temperature dependence of the rate of activation (26 kcal/mol) is reflected neither in reported characteristics of pepsin catalysis nor in the measured rate of release of the activation peptide from the enzyme (13 kcal/mol).

Activation of porcine pepsinogen occurs when the zymogen is brought to pH values below 4. The first well-described activation event is cleavage of the Leu_{16}–Ile_{17} peptide bond, generating pseudopepsin (abbreviated as ϕ-pepsin), which is enzymatically active (Dykes & Kay, 1976; Christensen et al., 1977). This step is unimolecular. This and subsequent self-processing of ϕ-pepsin by bimolecular processes cleaves a total of 44 amino acid residues from pepsinogen (Al-Janabi et al., 1972). Both the rate of activation of pepsinogen and the rate of proteolysis by pepsin are pH dependent, apparently controlled by a group with a pK of 2.1 that must be protonated for activity (Bull & Currie, 1949). Although pepsinogen is quite acidic, having a pI of 3.7 (Herriott, 1938), pepsin with

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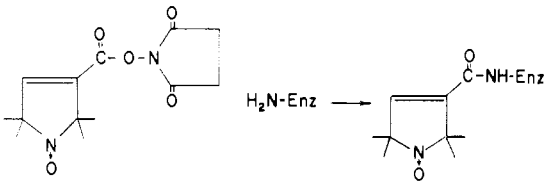
a p*I* below 1 (Perlmann, 1958) is considerably more acidic because the activation sequence has two of the four arginine and nine of the ten lysine residues (Foltmann & Pedersen, 1977). How acidification results in the first bond cleavage is unknown, but it is assumed to be closely related to pepsin catalysis.

Electron spin resonance (ESR) is useful in monitoring changes in spin-labeled macromolecules when these changes result in an altered mobility of the label. This approach seemed appropriate for study of the activation of pepsinogen, provided that a suitable labeling procedure could be devised. By specifically labeling the activation sequence, we hoped to be able to follow removal of peptides from pepsinogen or ϕ-pepsin. A spin-labeling reagent directed toward the amino groups concentrated in the activation sequence seemed to offer the best chance of success. 3-[[(2,5-Dioxo-1-pyrrolidinyl)-oxy]carbonyl]-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrolyl-1-oxy (CA Registry No. 37558-29-5), which reacts with amino groups according to reaction 1, was therefore selected as a



reagent. The activation sequence of the zymogen has been successfully and specifically labeled, and the resulting spin-labeled pepsinogen and its activation have been studied.

Experimental Procedure

Materials. Pepsin, pepsinogen, and substrate hemoglobin were purchased from Sigma Chemical Co., and pepstatin was purchased from Bristol Laboratories. 3-[[(2,5-Dioxo-1-pyrrolidinyl)oxy]carbonyl]-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrolyl-1-oxy was purchased from Molecular Probes. TPCK-treated trypsin was a Worthington product.

General Methods. Pepsin was assayed according to Chow & Kassell (1968) or Al-Janabi et al. (1972), and activation of pepsinogen was followed by the method of Al-Janabi et al. (1972). Inhibition of pepsin was assayed by the method of McPhie (1976). ESR spectra were measured in 50-μL capillary tubes on a Varian E-109 spectrometer with 100-kHz modulation. High-pressure liquid chromatography was performed on Laboratory Data Control equipment: Constametric I and II pumps, Gradient Master, and SpectroMonitor III. Amino acid analyses of 24-h HCl hydrolysates were performed on a Beckman 120B instrument modified for a single-column operation or on a Dionex analyzer kit.

Spin-Labeling of Pepsinogen. A solution of 2.5–8 mg of 3[[(2,5-dioxo-1-pyrrolidinyl)oxyl]carbonyl]-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrolyl-1-oxy in 0.09 mL of dimethylformamide was added to 10–100 mg of porcine pepsinogen in 3 mL of 0.1 M sodium phosphate, pH 7.0. The mixture was gently agitated at 22 °C for varying times and then dialyzed at 4 °C against several changes of a suspension of activated powdered charcoal in 0.02 M sodium phosphate, pH 7.0. Preparations were used within 5 days.

A standard aqueous solution (8×10^{-5} M) of the spin-labeling reagent was used to calculate the concentration of bound nitroxide. For nitroxide bound to released peptides, the concentration relative to that of the standard was obtained from measurements of peak amplitude and line width, while for nitroxide bound to pepsinogen, double integrations of the overlapping peaks were initially performed. As expected, this showed that the total calculated concentration of nitroxide did not change following activation. Consequently, the concentration of nitroxide bound to pepsinogen was routinely estimated from the concentration of spin-labeled peptide released upon activation. Pepsinogen concentration was calculated from $\epsilon_{280} = 5.13 \times 10^4$ L·mol^{-1}·cm^{-1} (Arnon & Perlmann, 1963). The remaining free amino groups on spin-labeled pepsinogen were estimated by fluorescence yield with fluorescamine (Udenfriend et al., 1972). Native pepsinogen with 11 amino groups (1 α- and 10 ϵ-amino groups) was used as a standard.

Rate of Activation of Spin-Labeled Pepsinogen. At 0 °C and 22 °C, 1.0-mL solutions of 0.38 mg of spin-labeled pepsinogen were brought to pH values between 1.0 and 3.0 with 0.1 M HCl. At intervals between 5 s and 6 min, 100-μL aliquots were removed and added to 300 μL of 0.1 M Tris-HCl, pH 8.5, to terminate activation and to denature any active forms of pepsin. These quenched solutions were assayed for remaining potential proteolytic activity. Semilog plots of remaining activity vs. time yielded first-order rate constants for activation.

Peptide Separations. Spin-labeled activation peptides were separated from pepsin and spin-labeled ϕ-pepsin by chromatography on polylysine–Sepharose at 4 °C (Nevaldine & Kassell, 1971). The sample was introduced onto a 2.3 × 14 cm column in 0.05 M ammonium formate, pH 5.5, and the peptides were eluted with the same buffer. The column was monitored at 230 nm. Larger proteins were subsequently eluted when the eluting buffer was supplemented with 1 M NaCl.

Individual peptides were separated on a Whatman Partisil PXS 5/ODS column (4.6 × 250 mm), monitored at 230 or 280 nm, eluted with 0.1% H_3PO_4, and delivered at 1 mL/min.

Tryptic Digestion of Spin-Labeled Pepsinogen. Spin-labeled pepsinogen, 8.45 mg, was digested with 80 μg of TPCK-treated trypsin in 5 mL of 10 mM Tris-HCl, pH 7.8, and 2 mM $CaCl_2$ at 37 °C. Another 80 μg of trypsin was added after 3 h, and the digestion was allowed to continue 16 h more. The product was then lyophilized and separated on a PXS 5/ODS column.

Edman Degradation of Activated Pepsinogens. Lyophilized native and spin-labeled pepsinogen were taken up in 1 mL of water that had been brought to pH 2.0 with 97% formic acid. After 70 s at 22 °C, the reaction was terminated with 100 μL of concentrated NH_3 (final pH 8.5). The samples were lyophilized and submitted to Edman degradation (Edman, 1950). The products were separated on a Partisil PXS 10/ODS column (4.6 × 250 mm), eluted with a 15%–50% linear acetonitrile gradient in water (over 30 min), and monitored at 269 nm. The flow rate was 2 mL/min.

Kinetics of ESR Spectral Changes. Spin-labeled pepsinogen (200 μL) that had been dialyzed against 0.02 M sodium phosphate, pH 7.0, was acidified with varying amounts of 0.5 M HCl and immediately placed in sealed capillaries, and the downfield ESR peak was repeatedly scanned to detect changes in peak height. At the pH values used, no significant decrease in nitroxide radical concentration was observed over the course of the experiment.

Results

Porcine pepsinogen was reacted with the spin-labeling reagent for varying periods of time. The level of labeling rose to a plateau of 3 nitroxides/mol (Figure 1). This degree of labeling was confirmed by fluorescamine titration, which generates a fluorescent product for each reacting amino group. Over 7 h, 2.8 groups became nontitrable. The 7-h reacted material was used for all subsequent studies.

Spin-labeled pepsinogen possessed full potential proteolytic activity. The rate of activation of spin-labeled pepsinogen

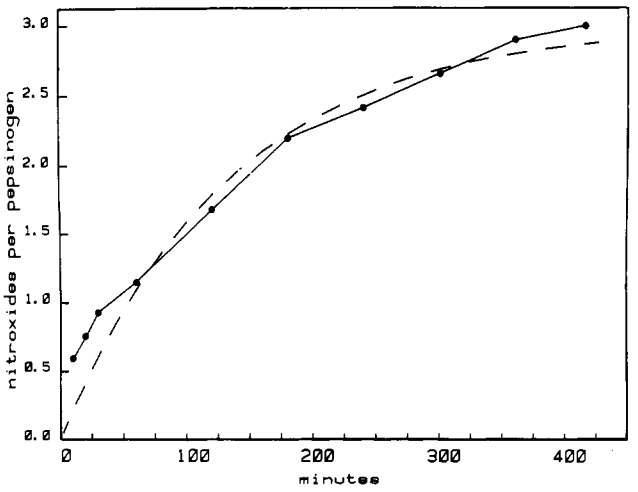


FIGURE 1: Time course of spin-labeling of pepsinogen. Pepsinogen was reacted with 9.2 mM spin-labeling reagent for varying times at 22 °C. The broken line is a calculated curve for three groups reacting, each at 0.00756 min^{-1}. The degree of labeling was determined by ESR measurements on the protein products.

Table I: Effect of pH and Temperature on Rate of Activation of Spin-Labeled Pepsinogen

	first-order rate constant (min^{-1})	
pH	0 °C	22 °C
1.9		5.4
2.0	0.16	
2.3		3.5
2.4	0.07	
3.0	0.04	1.9

(Table I) is about twice the rate for that of the native zymogen, and the pH dependence of these rates is the same (Al-Janabi et al., 1972).

For determination of the site of nitroxide attachment to the zymogen, spin-labeled pepsinogen was activated for 45 min at pH 2 and then separated on a polylysine–Sepharose column. ESR measurements showed that the pepsin fraction had only 0.11 nitroxide/mol of enzyme, and this residuum corresponded to the level of unactivated pepsinogen detected by enzyme assay (9%). The three nitroxides were, therefore, in the activation sequence, 1–44. For further localization of the nitroxides, spin-labeled pepsinogen was activated in the same way, but in the presence of a 2:1 mole ratio of pepstatin, and then separated on a polylysine–Sepharose column. Pepstatin binds to ϕ-pepsin and prevents bimolecular proteolysis (Dykes & Kay, 1976). This time the ϕ-pepsin fraction contained 0.86 nitroxides/mol. Therefore two nitroxides were in the 1–16 sequence and one in the 17–44 sequence.

To determine the three spin-labeled sites, we digested the spin-labeled pepsinogen with trypsin to cleave at arginyl and nonmodified lysyl residues. Amino acid analysis of the separated peptides representing 60% of the spin-label indicated the sites of labeling. One spin-labeled tripeptide corresponds in composition to the N-terminal sequence H_2N-Leu_1-Val_2-Lys_3. Further evidence for a tryptic peptide with one nitroxide per two lysines suggests that the ϵ-amino group of Lys-9 of the Lys_9-Lys_{10} sequence is labeled. In the 17–44 sequence, Lys-21 is labeled, as a peptide with the composition of Asp_{19}-Gly_{20}-Lys_{21}-Leu_{22}-Lys_{23} was found.

The ESR spectrum of spin-labeled pepsinogen was measured before and after activation (Figure 2). The spectral changes observed demonstrate a marked increase in motion of the label and an associated decrease in the rotational correlation time

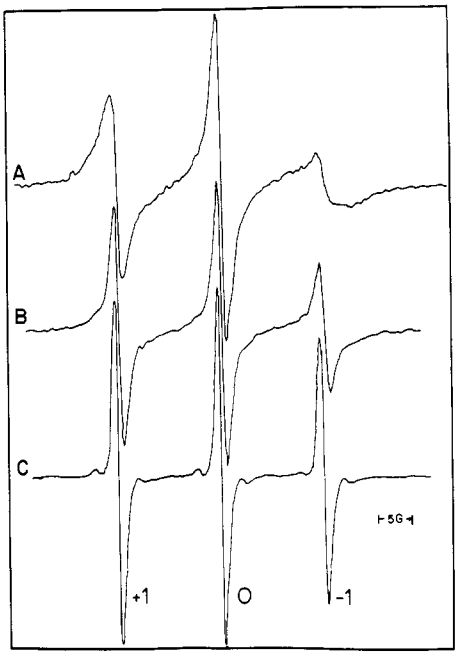


FIGURE 2: ESR spectra of spin-labeled pepsinogen and two activated forms. (A) Spin-labeled pepsinogen (32.3 μM) in 0.02 M sodium phosphate, pH 7.0. (B) Same solution as (A) with 2.0:1 molar ratio of pepstatin brought to pH 1.5 with HCl. (C) Spin-labeled pepsinogen brought to pH 1.3 for 300 min with HCl. Conditions: 100-kHz modulation, 20-mW microwave power, 9.51 GHz, modulation amplitude 0.4 G, scan range 100 G, time constant 1 s (A, B) or 0.25 s (C).

Table II: Characteristics of Electron Spin Resonance Spectra[a]

	peak height ratios		
sample	h_{+1}/h_0	h_0/h_{-1}	τ (ns)
spin-labeled pepsinogen[b] (pH 7.0)	0.56	5.74	1.55
spin-labeled pepsinogen (pH 1.3)	0.95	1.37	0.13
spin-labeled pepsinogen (with pepstatin, pH 1.5)	0.84	2.20	0.44
spin-labeled peptide 1-16	0.96	1.35	0.19
spin-labeling reagent	0.99	1.09	0.03

[a] The signal peak heights were measured in the ESR spectra from Figure 2 and from other spectra which are not shown. τ was calculated according to Kivelson (1960), taking the mean of values obtained from linear and quadratic terms. [b] Identical values were observed in the presence of pepstatin at this pH.

following activation. The qualitative changes evident from the spectra can be quantitated from ratios of peak heights of the $M_I = +1$, 0, and −1 resonances, h_{+1}, h_0, and h_{-1}, respectively (Table II), in the usual manner (Kivelson, 1960). The spin-label bound to pepsinogen is the most highly immobilized, with a calculated correlation time, τ, of 1.6 ns. However, the correlation time calculated is likely to be approximate in this case since some heterogeneity is in the ESR spectrum. Evidently the three bound nitroxides are not immobilized to the same extent. When spin-labeled pepsinogen is activated for 45 min at pH 2, the h_{+1}/h_0 ratio rises and the h_0/h_{-1} ratio falls, reflecting a mobilization of the nitroxides. In the presence of pepstatin, acidification releases only peptides 1–16, and the peak height ratios change only about 70% compared with complete activation, which is consistent with the finding that peptide 1–16 contains two of the three nitroxides.

Activation of spin-labeled pepsinogen results in the same initial peptide bond cleavages as activation of native pepsinogen because the same N-terminal groups are exposed. Edman degradation of 70-s-activated spin-labeled pepsinogen produced 1 leucine to 1.70 isoleucine phenylthiohydantoin derivatives,

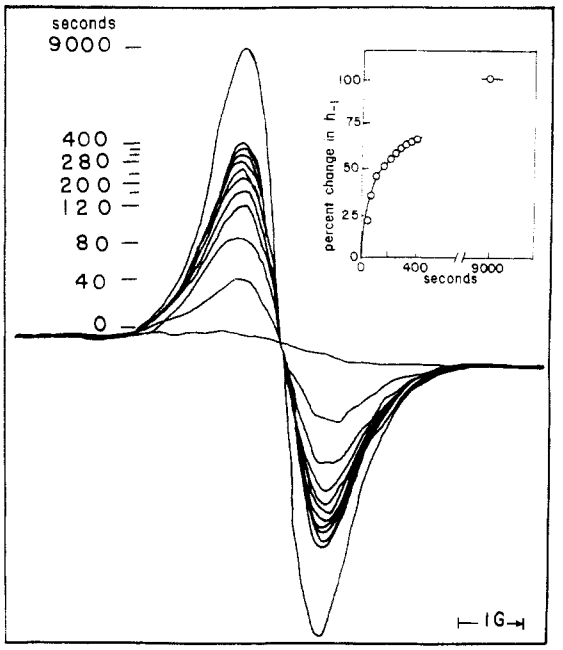


FIGURE 3: Repeated scans of the downfield peak following activation. The ESR spectrum of the peak was scanned at 40-s intervals following acidification of 200 μL of a 32.3 μM solution of spin-labeled pepsinogen with 10 μL of 0.5 M HCl (final pH 2.38). Insert shows peak height vs. time.

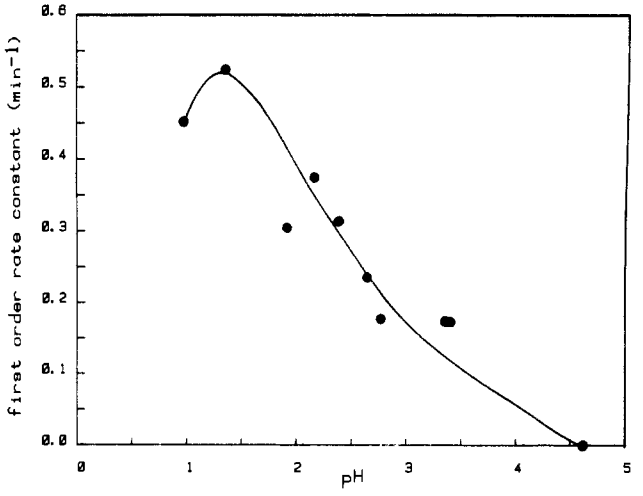


FIGURE 4: Rate of ESR spectral changes vs. pH. Acidified spin-labeled pepsinogen solutions were scanned as shown in Figure 3. First-order rate constants were extracted from the initial 20% of the total change.

the same as native pepsinogen, due to the N-terminal leucine and cleavage of the Leu_{16}–Ile_{17} and Leu_{44}–Ile_{45} peptide bonds.

Because the signal peak heights change during the activation process, it is possible to follow activation continually scanning the downfield $M_I = -1$ peak, which shows the greatest change in signal intensity (Figure 3). The initial signal change is due to release of peptide 1–16. Subsequent changes are due to further processing of spin-labeled ϕ-pepsin, which is a second-order process, and to enzymatic hydrolysis of the labeled peptides. Extraction of first-order rate constants from initial rates of change of h_{-1} at various pH values reveals that the pH dependence of this phenomenon parallels that of zymogen activation (Figure 4 and Table II). When the change in the ESR signal at intermediate pH values was monitored (2.5–3), a lag was invariably present (e.g., Figure 5).

The spin-labeled activation peptide fraction was recovered from the separation on polylysine–Sepharose of a 1-min activation of 24.8 mg of spin-labeled pepsinogen in 16 mL of

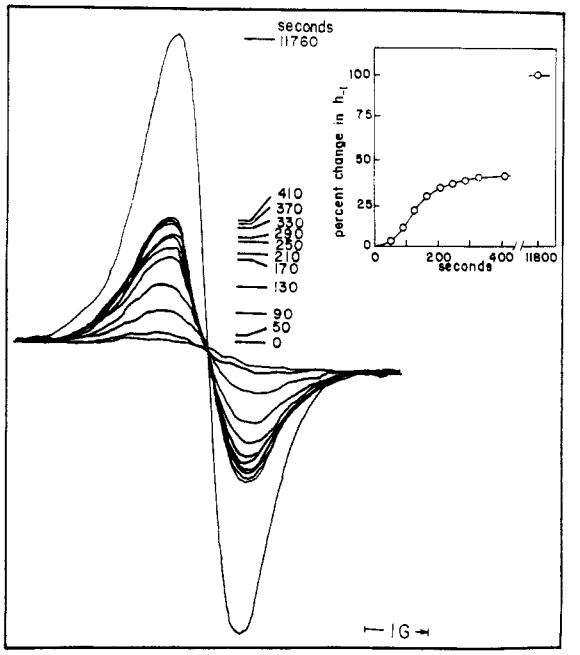


FIGURE 5: Repeated scans of the down–field peak following activation. The ESR spectrum of the peak was scanned at 40-s intervals following acidification of 200 μL of a 32.3 μM solution of spin-labeled pepsinogen with 8 μL of 0.5 M HCl (final pH 2.77). Insert shows peak height vs. time.

Table III: Temperature Dependence of Constants for Spin-Labeled Derivatives[a]

	temp (°C)				Arrhenius activation energy (kcal/mol)
	0	17	22	31	
pepsinogen to ϕ-pepsin first-order rate constant (min^{-1})	0.16		5.0		26.0
release of peptides 1–16 from ϕ-pepsin (min^{-1})	0.04	0.09	0.50		13.0
rotational correlation time (ns) of pepsinogen, pH 7.2	2.24	1.95	1.55	1.45	2.3

[a] Constants are given for processes at pH values between 1.9 and 2.1.

0.05 M ammonium formate, pH 7, with 0.16 mL 97% formic acid. The concentration of peptide 1–16 was determined by amino acid analysis to be 7.75 μM. This solution was used in a milk clotting assay to determine its inhibition constant with 0.52 nM native porcine pepsin. Plotting the ratio of free to bound peptide vs. peptide concentration (Munck, 1976) yields an inhibition constant of 63 ± 18 nM, which is close to the value for the unmodified peptide, 70 nM (Harish Kumar & Kassell, 1977). Having this equilibrium constant and the rate for the release of the peptide from ϕ-pepsin allows estimation of the rate for reassociation of the peptide with the enzyme.

The rates are indicated in the kinetic model for spin-labeled pepsinogen activation (Figure 6). Finally, Table III shows the effect of temperature on the rate of activation, on the rate of change of the ESR signal, and on the rotational correlation time of the spin-label estimated from the ESR signal.

Discussion

Activation of the derivative is a faithful model for activation of native porcine pepsinogen: (a) the product is fully active; (b) the product contains <0.1 nitroxide/mol; (c) activation

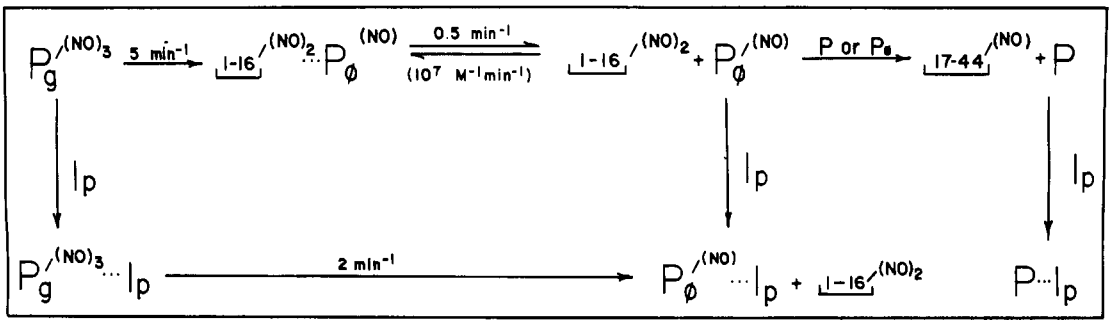


FIGURE 6: Kinetic model of spin-labeled pepsinogen activation. Pg, pepsinogen (includes residues 1–44); P_ϕ, ϕ-pepsin (includes residues 17–44); P, pepsin, Ip, pepstatin; (NO), spin-label; 1–16, peptide 1–16; 17–44, peptide 17–44; ···, noncovalent binding. Each transformation shown may, in fact, occur in more than one stage; this kinetic model is presented only to summarize the findings of this work.

results in the same peptide bond cleavages; and (d) the pH dependence of the process is similar to that for native pepsinogen.

The increase in the height of the downfield peak, which we monitor, reflects the release of the peptide from the macromolecule rather than a conformational change in the zymogen because (a) the increase occurs much slower than activation measured in chemical quench experiments, and (b) the increases are accompanied by a drop in the rotational correlation time, τ, from 1.6 ns at pH 7.2 to 0.13 ns at pH 1.3. That τ is already low for a nitroxide rigidly attached to a globular protein of 370 amino acid residues is consistent with the spin-label in the zymogen being attached to a segment that is somewhat loosely held to the bulk of the molecule when one considers that labeled chymotrypsin with 242 residues has τ = 12 ns (Shimshick & McConnell, 1972) and human oxyhemoglobin with 574 residues has τ = 20 ns (McCalley et al., 1972).[1] The absence of any evidence for spin–spin interaction between nitroxides supports this. The further drop in τ that takes place during activation undoubtedly reflects dissociation of the activation peptide from the protein. When the rates of dissociation were calculated only the initial 20% of the change in h_{-1} was used, to avoid confusion with the bimolecular processes that occur subsequently.

The calculated rate at which the spin-labeled peptide binds to pepsin is $\leq 10^7$ $M^{-1}\cdot min^{-1}$. As this is considerably slower than the diffusion-controlled rate of 4×10^9 $M^{-1}\cdot min^{-1}$, arbitrarily assuming 3% of the surfaces to be involved in association (North, 1964), serious conformational constraints may be involved in binding the peptide to the enzyme.[2] Indeed, it seems that the binding occurs in at least two stages (B. Dunn, personal communication).

The activation of spin-labeled pepsinogen shows an activation energy of 26 kcal/mol, much higher than the 18–20 kcal/mol reported for pepsin catalysis of small substrates (Casey & Laidler, 1950). The rotational correlation time τ, however, has only a weak temperature dependence, which probably reflects the small activation energy for viscious flow of water (Weast, 1975). This suggests that there is no extensive change in the mobility of the activation peptide due to unfolding of the zymogen between 0 and 31 °C. There is, therefore, a strongly temperature-dependent event associated with conversion of pepsinogen to ϕ-pepsin.

Pepstatin accelerated the initial rate of change of h_{-1}, indicating that pepstatin binds to the protein before the release of the activation peptide. This does not necessarily mean that pepstatin binds to pepsinogen before any exposure to a low pH—and we found no evidence for any change in spin-label motion in the presence of pepstatin at pH 7—as the activation rate is at least as fast as the rate of peptide release from the macromolecule. Pepstatin may bind as fast as bond 16–17 is cleaved, and once bound, speed the release of the activation peptide, as observed, by displacing it from its site in the zymogen. Because of the manner in which measurements of activation are conventionally made, "activation" means only conversion of pepsinogen to a form that is labile at pH 8.5, being either denatured itself at that pH or being converted to ϕ-pepsin, which is denatured. The phenomenon of the lag after acidification until h_{-1} begins to change, which we always see in the pH range where both activation and peptide release are slowed, may bear on this issue. The material that accumulates during this lag is (a) like pepsin but unlike pepsinogen in being pH 8.5 labile but is (b) unlike pepsin and like pepsinogen in having the activation peptide attached to it. It is possible that this is a covalent intermediate formed by transpeptidation, analogous to the acyl-enzyme proposed for pepsin catalysis (Fruton, 1976; Kaiser & Nakagawa, 1977), or it may be the noncovalent complex of ϕ-pepsin and peptide that may accumulate at higher pH values.[2]

Concerning these early events in pepsinogen activation, we have evidence for a change in fluorescence of pepsinogen that is complete 50 ms following acidification, but we do not yet have quantitative data to report.

Acknowledgments

We thank Marifran Cataldi for her dedicated, expert technical assistance.

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[1] The mobility of the spin-labels bound to pepsinogen may also reflect the flexibility of the lysine side chains by which two of them are attached.

[2] This binding constant value indicates that reassociation of peptide with ϕ-pepsin could be important at least at the higher pH value employed for measurement of K_i (pH 5.3). Actually, the reassociation rate constant is only an estimate, because the measured equilibrium constant is for the spin-labeled peptide, leaving spin-labeled ϕ-pepsin at considerably lower pH values. The binding constant to pepsin is quite insensitive to a variety of modifications, so the difference in species being compared is unlikely to invalidate the comparison with the diffusion-controlled rate.

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Purification and Characterization of Human Pancreatic Ribonuclease†

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ABSTRACT: A ribonuclease (RNase) has been isolated from normal human pancreas obtained upon autopsy. About 5 mg of RNase is normally recovered per kilogram of pancreas, equivalent to ca. 70% of the total activity and a 700-fold purification from the initial acidified extract. The specific activity of the purified enzyme is identical with that of bovine pancreatic ribonuclease, and a single component is found in sodium dodecyl sulfate–polyacrylamide gel electrophoresis, gel filtration, and reversed-phase high-pressure liquid chromatography. Aggregation of the protein is found upon ultracentrifugation under native and denaturing conditions, and several bands of equal specific activity are seen in polyacrylamide gel electrophoresis of the native protein. At least two components are glycoproteins. A molecular weight of 15 000 is estimated from sodium dodecyl sulfate gel electrophoresis, gel filtration, and amino acid and peptide analyses. The enzyme is related to bovine pancreatic RNase, but distinguishable by amino acid analysis, tryptic peptide maps, and low cross-reactivity of antibodies with the heterologous enzymes. The human enzyme is also inactivated by treatment with iodoacetic acid at pH 5.5 and is essentially identical with bovine RNase in its far-ultraviolet circular dichroism spectrum. The human RNase is like bovine pancreatic RNase catalytically; RNA is cleaved at pyrimidine residues, and activity against poly(cytidylic acid) is high.

Bovine pancreatic ribonuclease is a very well-characterized enzyme. In part because of its easy availability and unusual stability, it frequently serves as a model in biochemical investigations; as a result, much is known about both the protein and its actions [reviewed in Richards & Wyckoff (1971) and Sierakowska & Shugar (1977)]. Investigations of pancreatic ribonucleases from other species have been of interest to workers studying protein structure and function as well as biochemical evolution [e.g., Beintema (1980) and references cited therein, Lenstra et al. (1977), and Welling et al. (1975)].

Human pancreatic ribonuclease has not played a role in these studies. Human tissues are frequently difficult to obtain in large quantities, and the physiological function of the human enzyme is questionable. Bernard (1969) suggests that pancreatic ribonuclease is vestigial in nonruminant vertebrates where it is not needed for salvage of phosphorus from microbial RNA. The enzyme is thus present only at a very low level [less than 10 μg/g of tissue, relative to 1200 μg/g of bovine pancreas (Barnard, 1969)]. Nevertheless, some attempts to purify the human RNase[1] have been reported (Delaney, 1963; Ukita et al., 1964; Bardon et al., 1976; Neuwelt et al., 1977). Our interest in this enzyme was stimulated by the suggestion of Reddi & Holland (1976) that serum ribonuclease, presumably of pancreatic origin, could serve as a specific marker for pancreatic carcinoma. Peterson (1979) has since questioned the diagnostic value of measurement of serum RNase. This disagreement may result from measurements which were limited to enzymatic activity, since several RNases are known to be present in serum (Akagi et al., 1976; Blank & Dekker, 1977). In part to help clarify this question, we have undertaken the biochemical and immunological characterization of some human ribonucleases. Here, we describe the purification and properties of human pancreatic ribonuclease.

Materials and Methods

Assays. Ribonuclease activity was measured by the formation of perchloric acid soluble nucleotides from wheat germ ribosomal RNA (Glitz & Dekker, 1963) or poly(cytidylic acid)

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[1] Abbreviations used: RNase, ribonuclease; $NaDodSO_4$, sodium dodecyl sulfate; poly(C), poly(cytidylic acid); CM-cysteine, carboxymethylcysteine; BSA, bovine serum albumin; Mes, 2-(*N*-morpholino)-ethanesulfonic acid.

0006-2960/81/0420-1272$01.25/0