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Human Serum Procarboxypeptidase A†

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ABSTRACT: Zymogen activation is an important biochemical control process and has important physiological and pathological implications. We have simultaneously measured both procarboxypeptidase A, the enzyme precursor, and carboxypeptidase A, its active product, in serum by using an affinity resin and the synthetic peptide substrate *N*-(2-furanacryloyl)-L-phenylalanyl-L-phenylalanine. Serum procarboxypeptidase A is activated by trypsin, chymotrypsin, plasmin, subtilisin, or urokinase but not by thrombin or enteropeptidase. The molecular weight of the precursor is approximately 5000–10 000 greater than that of the active product. Both enzyme and precursor increase in serum in the course of pancreatic inflammation, but the degree of activation can vary up to 2000-fold, independent of the amount of precursor present. The existence of this pancreatic proteolytic precursor in serum opens new avenues for the investigation of zymogen activation and its regulation.

Conversion of inactive enzyme precursors to their physiologically active forms by limited proteolysis is a major biological control mechanism (Neurath & Walsh, 1976). Several of the digestive enzymes are stored in the pancreas as precursors or zymogens that are activated in the duodenum by the limited proteolytic removal of a peptide from the proenzyme (Maroux et al., 1971). The precursors in the pancreas are actually synthesized as pre-proenzymes that contain an amino-terminal peptide which is removed prior to release of the proenzyme from the acinar cell (Blobel & Dobberstein, 1975). Both limited proteolytic processes are irreversible under physiological conditions; as a result, the enzymatic activity measured is a reflection of the concentration of the proenzyme. Most important, generation of activity by an activating proteolytic enzyme is strong evidence for the existence of the precursor.

It has long been known that the presence of amylase in serum serves as a diagnostic guide for the detection of various disorders of the pancreas. The utility of serum amylase is limited, however, since the pancreas is not the sole tissue of origin for this enzyme, but the search for other pancreatic enzymes in serum that have greater diagnostic significance has been largely futile. The presence of protease inhibitors in serum and the lack of tissue specificity for most of the pancreatic proteases have contributed to this problem. Our efforts in this regard have focused on the procarboxypeptidase–carboxypeptidase A system and are based on the properties and characteristics of zymogens and their constituent enzymes outlined above.

We have synthesized a new substrate and developed a new method for measuring low concentrations of carboxypeptidase A, thereby overcoming technical problems in detecting the activity of this enzyme in serum (Peterson et al., 1982). Yet, despite the sensitivity of the assay and the detection of high activities in some patients with pancreatitis, carboxypeptidase A activity was not detectable in the serum of normal individuals. We have found, however, that their sera do contain the precursor, procarboxypeptidase A, which can be detected following proteolytic activation.

This report details the evidence for the existence of procarboxypeptidase A in human serum and describes conditions for its activation. The demonstration of such a precursor and the capacity to measure it in serum constitute a new approach for the investigation of zymogen activation, in general, and for the evaluation and diagnosis of human pancreatic disease. Furthermore, simultaneous measurement of both the active and inactive forms of this enzyme allows assessment of its state of activation as well as concentration. The ability to determine the degree of activation of such biological molecules introduces

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an additional means for understanding the biochemical basis of conditions which depend on limited proteolysis for their control.

Materials and Methods

Bovine trypsin (EC 3.4.4.4), trypsinogen, and chymotrypsin (EC 3.4.4.5) were purchased from Worthington Biochemical Co., porcine plasmin (EC 3.4.4.14), porcine enteropeptidase (EC 3.4.4.8), and bovine thrombin (EC 3.4.4.13) were from Sigma, and human urokinase (EC 3.4.99.26) was from Calbiochem. Subtilisin Carlsberg (EC 3.4.4.16) was a gift from the Chemical Division of the Carlsberg Laboratory. All enzymes except enteropeptidase were free of carboxypeptidase A (EC 3.4.2.1) activity when tested at their highest concentration in the assay and were used without further purification. Tosyl-L-arginine methyl ester (TosArgOMe)[1] was purchased from Sigma and the trypsin assay performed according to Walsh (1970).

Human trypsin was isolated from pancreatic juice by using a lima bean trypsin inhibitor affinity resin (Feinstein et al., 1974). Contaminating carboxypeptidase A activity was removed from human trypsin and porcine enteropeptidase by passage over the CABS–Sepharose affinity resin (vide infra). Passage of these enzymes over this column did not affect their activity as assayed by using TosArgOMe for trypsin and trypsinogen for enteropeptidase.

The carboxypeptidase A affinity resin, *p*-caproylamino-DL-benzylsuccinic acid–Sepharose 4B (CABS–Sepharose), and the peptide substrate, *N*-(2-furanacryloyl)-L-phenylalanyl-L-phenylalanine (FAPP), were prepared as described (Peterson et al., 1976, 1982). Activity was measured at 35 °C in 50 mM Tris, pH 7.5, and 0.45 M NaCl with 2×10^{-4} M substrate. Activities were recorded as nanomoles of substrate hydrolyzed per minute per milliliter of serum.

Gel filtration was performed on a 2.5 × 50 cm Sephadex G-100 column equilibrated with 20 mM Tris, pH 7.5, and 0.1 M NaCl at 4 °C. One milliliter of serum was applied, and the column was eluted with equilibrating buffer.

Blood was collected and allowed to clot and serum removed. Sera were stored at −20 °C. Storage for up to 6 months did not affect the total carboxypeptidase A content, but storage for more than 1 month did alter the ratio of zymogen to enzyme in sera with a high procarboxypeptidase A content. In the present study, procarboxypeptidase A content and carboxypeptidase A activity in pancreatitis patients were determined within 48 h of blood collection.

Activation of procarboxypeptidase A was carried out at room temperature, 22 °C, in 0.5-mL aliquots of serum. One hundred microliters of a 10 mg/mL stock solution of bovine trypsin in 1 mM HCl was added to 0.5 mL of serum for routine activation; a second aliquot was treated in parallel without trypsin. Addition of up to 20 mM $CaCl_2$ did not alter the rate or extent of procarboxypeptidase A activation.

Following activation, serum was brought to pH 6 by adding 50 μL of 0.2 M Mes and adjusting the pH with 0.1 M HCl. Activated and unactivated sera were chromatographed on separate 0.25-mL columns of CABS–Sepharose, and the pH 8 eluate was assayed as described (Peterson et al., 1982). One serum, chosen as a standard, was measured repeatedly with each group of assays; the coefficient of variation for this standard analyzed 22 times in the course of 6 weeks was 12.5%.

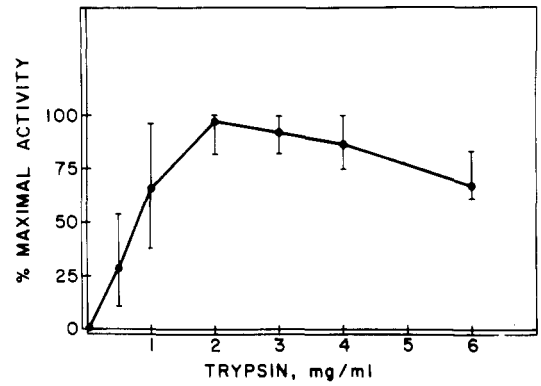

FIGURE 1: Carboxypeptidase A activity of sera following treatment with bovine trypsin. Trypsin was added to six sera at the concentrations indicated. Incubation was for 30 min before affinity chromatography followed by FAPP activity measurement. Points are mean values, and vertical lines indicate the range.

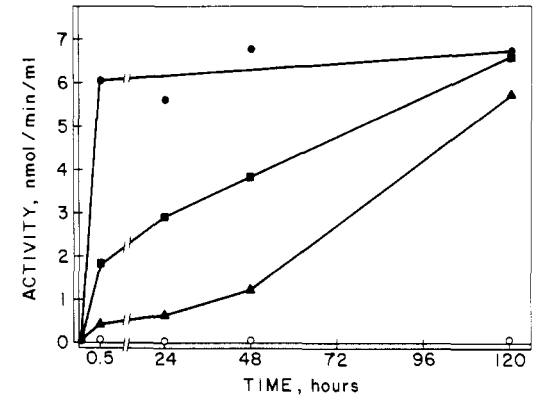

FIGURE 2: Time course of trypsin activation of serum procarboxypeptidase A. Bovine trypsin concentrations: (●) 2 mg/mL; (■) 1 mg/mL; (▲) 0.5 mg/mL; (○) no added trypsin.

Results

Addition of trypsin to a control serum, i.e., one without inherent carboxypeptidase A activity, generates carboxypeptidase activity after a suitable period of incubation. Examination of the effect of trypsin concentration on procarboxypeptidase A activation revealed that the addition of 2 mg/mL serum is optimal (Figure 1). Activity toward TosArgOMe (a trypsin substrate) was detectable in sera prior to trypsin addition. However, no increase in this activity could be detected until the added bovine trypsin concentration reached approximately 0.5 mg/mL serum, i.e., the minimal concentration of this enzyme required to initiate activation of procarboxypeptidase A (Figure 1).

The extent of procarboxypeptidase A activation was dependent jointly on the duration of incubation and on trypsin concentration (Figure 2). With 2 mg/mL trypsin, full activation occurred in 30 min while with 1 mg/mL trypsin 120 h were required. With 0.5 mg/mL trypsin, activation was incomplete after 120 h. There was no loss of carboxypeptidase A activity after 120 h at 22 °C, nor was there any evidence of activation without added trypsin.

The effects of other enzymes on the activation of serum procarboxypeptidase A were compared with those of trypsin (Figure 3). For subtilisin, almost complete activation was achieved at the lowest concentration examined (0.25 mg/mL); at higher concentrations, carboxypeptidase A activity was actually diminished. Urokinase, a plasminogen activator, also

[1] Abbreviations: FAPP, *N*-(2-furanacryloyl)-L-phenylalanyl-L-phenylalanine; TosArgOMe, tosyl-L-arginine methyl ester; CABS, *p*-caproylamino-DL-benzylsuccinic acid; Tris, tris(hydroxymethyl)aminomethane; Mes, 2-(*N*-morpholino)ethanesulfonic acid; BzGlyGlyOPhe, benzoylglycylglycyl-L-α-hydroxy-β-phenyllactate; BzGlyArg, benzoylglycyl-L-arginine.

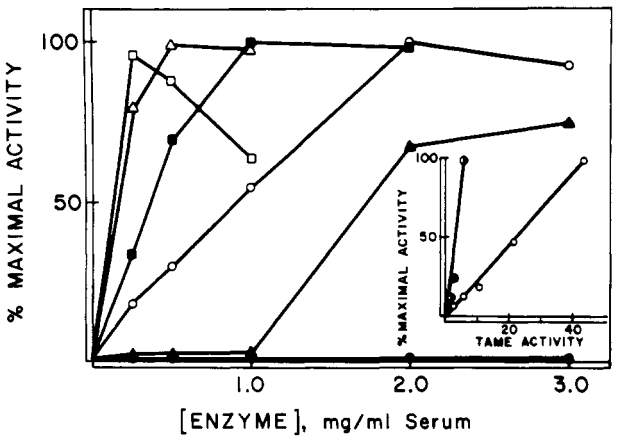

FIGURE 3: Effect of various proteolytic enzyme concentrations on the extent of serum procarboxypeptidase A activation. Each activating enzyme was added to serum aliquots in triplicate and compared to 2 mg/mL bovine trypsin added to the same serum. FAPP activity produced by 2 mg/mL bovine trypsin equals 100%. Incubation was for 30 min prior to assay. Inset: TosArgOMe esterase activity in arbitrary units added to 0.5 mL of serum vs. carboxypeptidase A activity produced. Enzymes used were as follows: (□) subtilisin; (△) urokinase; (■) plasmin; (○) bovine trypsin; (▲) chymotrypsin; (●) thrombin; (◑) human trypsin. Enteropeptidase (not shown) had no effect.

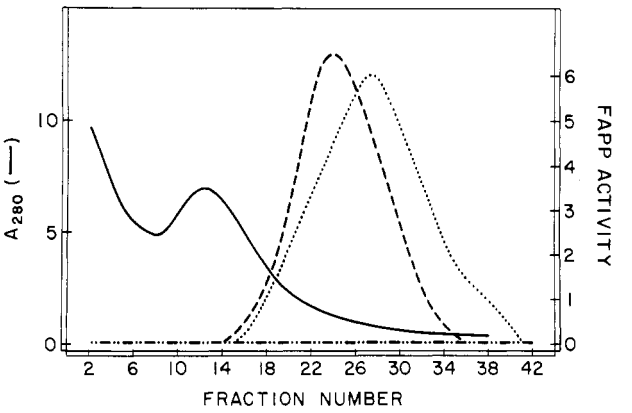

FIGURE 4: Gel filtration of human serum on Sephadex G-100. Fractions are 1 mL. (—) A_{280}; FAPP activity: (-···-) unactivated serum, no trypsin; (- - -) unactivated serum + trypsin; (···) activated serum.

activated fully at relatively low concentrations while plasmin was almost twice as effective as trypsin. Chymotrypsin was less effective than trypsin while thrombin and enteropeptidase (not shown) had no activating effect at the concentrations employed. Since there were no striking differences among the enzymes which activate procarboxypeptidase A, bovine trypsin was employed routinely, since it is readily available in a state of high purity.

The activating efficiency of human trypsin was compared with that of the corresponding bovine enzyme (Figure 3 inset). Neither the human nor the bovine trypsin employed had been subjected to extensive, rigorous purification; hence, their capacity to activate procarboxypeptidase A was based on their activity toward TosArgOMe. While the specific activity of bovine trypsin is almost twice that of human trypsin (1.03×10^4 vs. 6.13×10^3 mol min^{-1} mol^{-1}) with TosArgOMe as substrate (Brodrick et al., 1978), the human enzyme was 6 times more active than the bovine enzyme with procarboxypeptidase A as the substrate.

Procarboxypeptidase undergoes a change in molecular weight on activation. A serum sample lacking carboxypeptidase A activity was gel filtered on Sephadex G-100 (Figure 4). The second peak appearing at fraction 13 in the A_{280} profile is largely serum albumin. Each of the column fractions was examined and found to be devoid of FAPP activity. However, after addition of trypsin (0.25 mg of bovine trypsin/mL) to each of the column fractions, a single peak of carboxypeptidase A activity could be detected, indicating the presence of procarboxypeptidase A and defining its elution volume. A second sample of the same serum which had been activated with trypsin prior to chromatography was then passed over the same column under identical conditions; in this case, carboxypeptidase A activity appeared as a single peak but at an elution volume greater than that of serum albumin or of the procarboxypeptidase A from the same serum, thus indicating a size change. Although activity can be detected, it should be noted that the enzyme protein concentration is too low to allow detection of the 280-nm absorbance in these positions.

Procarboxypeptidase A and carboxypeptidase A activities were determined in three groups of individuals: (1) a control group of 33 hospital personnel with no history of pancreatic disease and with a normal serum amylase; (2) 4 patients who had undergone total pancreatectomy, 2 for pancreatitis and 2 for cancer; and (3) 13 patients with acute pancreatitis. The control group had no activity before activation but after activation had a mean and standard deviation of 3.77 ± 1.22 nmol min^{-1} mL^{-1}. The apancreatic group had no activity before or after activation. All 13 patients with pancreatitis had increased procarboxypeptidase A activities, ranging from 9 to 926 nmol min^{-1} mL^{-1} (all greater than 2 standard deviations above the control mean); 11 of the 13 pancreatitis patients had intrinsic carboxypeptidase A activity before addition of trypsin, and the ratio of enzyme to proenzyme varied from 0.004 to 8.6 independent of the amount of proenzyme present.

Discussion

By using a sensitive and specific assay for measuring carboxypeptidase A activity, we have demonstrated the presence of a zymogen or an activatable precursor of this pancreatic proteolytic enzyme in human serum. Previously we could not detect free carboxypeptidase A activity in serum samples taken from a healthy population, although such activity was present in the sera of many individuals with pancreatitis (Peterson et al., 1982). The explanation for this discrepancy was unclear. Now, however, after addition of trypsin to serum, carboxypeptidase A activity is detected even in normal individuals, suggesting the presence of a precursor form of this enzyme.

The following approaches have been used to confirm that this activity is due to the presence of a zymogen or precursor of carboxypeptidase A: (1) varying the trypsin concentration and duration of tryptic activation; (2) using enzymes other than trypsin which would be expected to have an effect on procarboxypeptidase A activation; and (3) gel chromatography of serum before and after activation.

Addition of bovine trypsin in varying amounts to different sera produced maximal carboxypeptidase A activity in 30 min when the trypsin concentration reached 2 mg/mL. Thus, even though the amount of carboxypeptidase A activity generated varied considerably among sera, the amount of trypsin required to reach the peak effect for each serum was the same. The carboxypeptidase A activity is dependent on a unique property of each serum and independent of the trypsin added. Smaller amounts of trypsin produced the same carboxypeptidase A activity if the time of trypsin exposure is increased, but again the maximum carboxypeptidase A activity found is characteristic of the particular serum studied. Furthermore, in the

absence of trypsin, no carboxypeptidase A activity is generated even after 5 days. The dependence of this process on the activator, the concentration of the activator, and the duration of activation is consistent with activation of a zymogen.

Freisheim et al. (1967a) found that proteolytic enzymes other than trypsin can activate purified bovine procarboxypeptidase A. We added these activators to serum and measured the resultant activity. The bacterial endoprotease subtilisin produced the greatest degree of activation on a weight basis, in accord with its relative potency in activating purified bovine procarboxypeptidase A. The decrease in serum carboxypeptidase A at higher subtilisin concentrations was more striking than with the other enzyme activators. Chymotrypsin activates bovine procarboxypeptidase A and also activates human procarboxypeptidase A in serum; it differed from the other activators in that it failed to activate at low concentrations but suddenly became effective at 1 mg/mL (Figure 3). Chymotrypsin interacts with α_1-antitrypsin, α_2-macroglobulin, and possibly a specific serum antichymotryspin (Heimburger, 1974), and it might well be that the different pattern for chymotryptic activation of procarboxypeptidase A in serum is due to differences in inhibition as well as a reduced capacity of chymotrypsin for activating human procarboxypeptidase A.

Four other enzymes, not studied previously, were examined because of their potential importance in understanding the biological implications of the presence of enzyme precursors in serum. Plasmin was studied because of its presence in serum and its ability to hydrolyze tryptic substrates and because it can be produced by tryptic action on plasminogen (Robbins & Summaria, 1970). Indeed, plasmin was more effective than trypsin on a weight basis. Differences between plasmin and trypsin could be due to specific effects on the procarboxypeptidase A molecule as well as differences in the levels of serum inhibitors or inactivators for each. Urokinase, a plasminogen activator, also activated procarboxypeptidase A in serum, perhaps due to plasminogen activation but possibly by a direct effect on procarboxypeptidase A. Thrombin had no effect on procarboxypeptidase A activation despite its tryptic-like activity; this is not unexpected since blood coagulation with concomitant conversion of prothrombin into thrombin had no effect on activation of this zymogen. Enteropeptidase also had no effect on procarboxypeptidase A, suggesting that this intestinal enzyme is not capable of generating carboxypeptidase A either by acting directly or by activating any trypsinogen, its specific substrate, that might be present in serum.

Assay of serum to which known amounts of human carboxypeptidase A had been added produced virutally complete recovery of the activity added (Peterson et al., 1982). Therefore, it would seem unlikely that there is a major serum inhibitor interfering with carboxypeptidase measurement and also unlikely that trypsin activates serum by displacement or removal of such an inhibitor. This is further indirect but important evidence for zymogen activation as the mechanism for inducing the appearance of carboxypeptidase in serum by trypsin and other enzyme activators.

Human trypsin was studied and compared to bovine trypsin to detect species differences, if any, in the capacity to activate human procarboxypeptidase A in serum. Brodrick et al. (1978) have demonstrated a striking species difference in the ability of enteropeptidase to activate trypsinogen: human enteropeptidase was 150 times more effective than porcine enteropeptidase in activating human trypsinogen. The present study suggests that a species difference also exists for human procarboxypeptidase A in serum since human trypsin was 6 times more potent than bovine trypsin. However, other differences between these two trypsins (human and bovine) could also account for the difference in procarboxypeptidase A activation: e.g., human trypsin binds less tightly to α_1-antitrypsin and has greater activity when complexed with α_2-macroglobulin than the bovine enzyme (Bieth et al., 1974).

Gel chromatography demonstrates a trypsin-activatable procarboxypeptidase A in human serum with a molecular weight less than that of serum albumin. Following tryptic activation of serum, the carboxypeptidase A activity appears at a larger elution volume than that for procarboxypeptidase A, consistent with a reduction in molecular weight following activation. When human carboxypeptidase A, purified from pancreatic juice, was chromatographed on the same column in the presence of serum, it eluted in a single peak in the same location as the carboxypeptidase A which had been activated in serum. The molecular weight of human carboxypeptidase A is 34 700 (Peterson et al., 1976), and the chromatographic behavior of serum procarboxypeptidase A is consistent with its having a molecular weight that is approximately 5000–10 000 greater than that of the active enzyme. This molecular weight range for human procarboxypeptidase A is similar to that of the dogfish zymogen (Lacko & Neurath, 1970) as well as subunit I of bovine procarboxypeptidase A (Freisheim et al., 1967b). There was no evidence for the presence of a much higher molecular weight species (90 000) characteristic of bovine procarboxypeptidase A-S_6 (Yamasaki et al., 1963).

Further evidence of the precursor nature and tissue origin of the activity measured in serum was obtained by determinations in patients without a pancreas and in those with pancreatitis. The absence of procarboxypeptidase A in the serum of individuals without a pancreas certainly suggests a pancreatic origin as does the large increases observed in pancreatic inflammation. While other tissues contain carboxypeptidases which might be present in serum, none, other than the pancreatic enzyme, is known to exist as a precursor that can be activated by trypsin (McDonald & Schwabe, 1977). The marked variation in the degree of zymogen activation suggests that the enzyme:proenzyme ratio might provide additional and useful information about the biochemical events in pancreatitis.

The activity in the eluate from the affinity resin is characteristic of carboxypeptidase A. It is completely inhibited by the chelating agent 1,10-phenanthroline but not by the nonchelating analogue 1,7-phenanthroline typical for this zinc metalloenzyme. In addition to catalysis of the release of C-terminal phenylalanine from FAPP, the synthetic peptide substrate used in this assay, the enzyme also exhibits esterase activity against BzGlyGlyOPhe and is inhibited by L-phenylalanine but not by L-arginine. No activity could be detected when BzGlyArg, a carboxypeptidase B substrate, was used. Thus, the activity under study with this assay differs completely from carboxypeptidase N, a serum enzyme with catalytic properties similar to those of carboxypeptidase B (Erdös et al., 1967).

The amount of procarboxypeptidase A present in normal serum would be approximately 5.3 ng/mL on the basis of the specific activity of purified human pancreatic carboxypeptidase A toward FAPP (Peterson et al., 1982). This concentration is comparable to amounts of the other pancreatic proteolytic enzymes estimated in normal serum by radioimmunoassay: anionic trypsinogen, 5.5 ng/mL (Largman et al., 1978); carboxypeptidase B, 10.4 ng/mL (Geokas et al., 1974); cationic trypsinogen, 26 ng/mL (Geokas et al., 1979); elastase, 71 ng/mL (Geokas et al., 1977).

While other investigators have used radioimmunoassay methodology to suggest that the precursors of the pancreatic proteolytic enzymes were present in serum, evidence derived from these techniques could only be inferential or indirect since these methods depend on the immunologic properties of the enzymes (Brodrick et al., 1979). Because the precursor and active forms of these enzymes are not always immunologically distinct from each other, one might expect cross-reaction between antibodies to the precursor form and the active form of the enzymes (Barrett, 1965; Arnon & Neurath, 1970). Also, while alteration in immunologic constituents following addition of an activator to serum would be consistent with a change from a precursor to an active enzyme, it could also be explained by other modifications of the antigen by the activating agent. This assay for procarboxypeptidase A and carboxypeptidase A has the major advantage of demonstrating an unequivocal appearance or increase in activity following addition of an activating agent which is by definition the characteristic feature of zymogen activation.

The relatively large amount of trypsin required for activation of procarboxypeptidase A in serum is of interest. In the absence of serum, activation of purified procarboxypeptidase A requires only a small, catalytic amount of trypsin. Typically, with trypsin:zymogen ratios of 1:10 or 1:100 (w/w), complete activation is achieved within 30 min (Petra, 1970). However, the trypsin:zymogen ratio for complete activation of procarboxypeptidase A in serum is approximately 50 000. The most obvious explanation for the requirement for such a large excess of trypsin may lie in the presence of trypsin inhibitors. Indeed, the range of trypsin concentrations found to be required for procarboxypeptidase A activation, 0.5 (2.3×10^{-5} M) to 2.0 mg/mL (9×10^{-5} M), is in the same order of magnitude as the two most abundant serum trypsin inhibitors, α_1-antitrypsin (5×10^{-5} M) and α_2-macroglobulin (0.5×10^{-5} M). Other factors which influence the amount of active trypsin present (e.g., differing modes of inhibition, inhibitor capacity, association constants, and the potential for multiple competing protein interactions) also contribute to the high trypsin:zymogen ratio required for activation. At even higher concentrations, 4 mg/mL (18×10^{-5} M), some carboxypeptidase A activity is lost, presumably due to proteolytic destruction.

While the presence of procarboxypeptidase A in human serum of normal individuals as well as elevations in patients with pancreatitis seems certain, its route of entry into serum remains unknown. Lampel & Kern (1977) have presented evidence for a direct route of transport from the acinar cell into the circulation with electron micrographs showing extrusion of secretory proteins through the basolateral membrane of the acinar cell. Potential connections between the pancreatic duct and intrapancreatic capillaries and lymphatics have been demonstrated by using dye markers injected into the duct, indicating a second possible transport route (Anderson & Schiller, 1968; Bockman et al., 1971). It is highly unlikely that this enzyme and its precursor are transported from the intestine into serum, since, if this were the case, one would expect to find a larger percentage of the zymogen activated.

The biologic significance of low concentrations of precursors to the pancretic proteolytic enzymes in serum is unknown. However, the concentrations of some precursors in the blood coagulation and complement systems which play a critical or controlling role in hemostasis and immunologic competence are of the same order of magnitude (Masson, 1976). Genetic and acquired disorders altering either the quality or the quantity of these regulatory serum precursors have been described and, while uncommon, can have a profound influence on normal homeostasis (Zimmerman et al., 1971; Stenflo et al., 1974; Rosen et al., 1971; Harpel et al., 1975). Hence, it is possible that even low concentrations of precursor molecules in serum can be significant. Previously, study of the pancreatic zymogens in serum and their states of activation has been hindered by the lack of adequate and simple analytic methods. With the availability of methods such as those used and described in this report, new avenues of investigation are possible which might reveal the physiologic importance of these precursor forms as well as how they might interact with other serum proteins in various human diseases.

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Registry No. FAPP, 83661-95-4; procarboxypeptidase A, 37237-55-1; carboxypeptidase A, 11075-17-5; trypsin, 9002-07-7; chymotrypsin, 9004-07-3; plasmin, 9001-90-5; subtilisin, 9014-01-1; urokinase, 9039-53-6.

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Rotational Diffusion of *Escherichia coli* RNA Polymerase Free and Bound to Deoxyribonucleic Acid in Nonspecific Complexes†

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ABSTRACT: We have studied the rotational diffusion of *Escherichia coli* RNA polymerase free in solution and bound nonspecifically to DNA fragments. The rotational motion was measured by the decay in anisotropy of the triplet–triplet absorption by using as probes either the liganded enzyme inhibitor Rose Bengal or eosin 5′-isothiocyanate conjugated to the protein. The time resolution extended from 10 ns to 1 ms. Free RNA polymerase (holoenzyme) at high salt concentration (1 M NaCl) is monomeric and diffuses at 5 °C with a rotational correlation time of 0.66 μs, corresponding to an equivalent hydrodynamic sphere with a radius of 7.4 nm. These values and the known molecular weight are most compatible with a nonspherical shape, e.g., an oblate ellipsoid with an axial ratio of about 3. In 0.1 M NaCl, the holoenzyme is dimeric and has a rotational correlation time of 2 μs. The decay of anisotropy is at least biexponential upon binding RNA polymerase to calf thymus DNA or to poly[d(A-T)]. The fast component with half of the amplitude has decay kinetics comparable to those seen with the free monomeric enzyme. The slow component has a rotational correlation time of about 14 μs and is independent of DNA chain length in the range >180 base pairs. Both rotational correlation times decrease with temperature, and the relative amplitudes change such that the faster component dominates at higher temperature. The rotational relaxation of the enzyme–DNA complexes is discussed in terms of alternative models involving rigid rod–sphere diffusion, conformational changes in the enzyme and/or DNA, sliding motions of the protein along the DNA, and torsional-bending motions of DNA envisioned as a deformable rod.

The transcription of DNA is effected through a sequence of reactions involving different modes of interaction between RNA polymerase and its double-helical template:

(a) Formation of the prerecognition complex: The enzyme binds nonspecifically to a region of the DNA by an outside purely electrostatic mechanism leading to the release of counterions from the DNA (Shaner et al., 1983). These binary complexes have been visualized by electron microscopy (Williams, 1977; Kadesch et al., 1980) and characterized thermodynamically by chromatographic (de Haseth et al., 1978), sedimentation (Lohman et al., 1980; Revzin & Woychik, 1981), and nitrocellulose filter binding (Melançon et al., 1982) techniques.

(b) Nonspecific translocation: The enzyme migrates on the DNA by a random-walk process which probably involves one of or a combination of various mechanisms (Berg et al., 1981; Barkley, 1981; Berg & Ehrenberg, 1982; Fried & Crothers, 1983): "sliding", a one-dimensional diffusion; "hopping", a series of spatially correlated microscopic dissociations and reassociations; and "intradomain transfer", direct transfer of the protein from one segment of a randomly coiled (i.e., long) DNA molecule to another.

(c) Promoter recognition: RNA polymerase encounters and rapidly identifies a promoter sequence either by interactions of complementary matrices of hydrogen-bond donors and acceptors (von Hippel & Bear, 1983) and/or by recognition of specific sequence-imposed patterns of twist angles (Kabsch et al., 1982); conformational changes and associated protonation reactions of the enzyme are involved (Strauss et al., 1980). The contact region extends over about 30 base pairs upstream starting near position −10 (relative to the initiation site) and is restricted primarily to one side of the double helix (Siebenlist et al., 1980).

(d) Promoter activation and (e) transcription (initiation, elongation–translocation, termination): These processes will not be considered further here.

The purpose of our investigation was to probe by direct physical techniques the dynamic properties of the protein and DNA partners in the binary and ternary complexes described above. We have restricted ourselves initially to the nonspecific interactions, step a above. The method of transient absorption

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