Human Skin Fibroblast Collagenase

ASSESSMENT OF ACTIVATION ENERGY AND DEUTERIUM ISOTOPE EFFECT WITH COLLAGENOUS SUBSTRATES*

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The activation energy and solvent-deuterium kinetic isotope effect for human skin fibroblast collagenase were studied on different collagenous substrates. Values for activation energy (E_a) were determined on the a1 and a2 polypeptide chains of denatured collagen (12,900 calories, 13,300 calories, respectively), collagen in solution (42,290 calories), and collagen in native fibrillar form (101,050 calories). The energy dependence of catalysis thus increased markedly with formation of the collagen triple helix and further with the assembly of collagen monomers into a fibrillar structure.

The substitution of deuterium for hydrogen in solvent buffer (D_2O:H_2O = 0.9) slowed fibrillar collagenolysis 9-fold and degradation of solution monomers 2-fold. Enzyme-substrate binding (K_s) and enzyme-substrate dissociation (K_d) were not affected by deuterium. These results indicate that proton transfer is involved in the slow step of collagen degradation. Evidence from this study and previous kinetic data strongly suggest that K_d or actual peptide bond cleavage, represents the rate-limiting step of collagen degradation by fibroblast collagenase.

The activation energy and solvent-deuterium kinetic isotope effect for other collagenolytic enzymes were also investigated. In each case, the magnitude of the activation energy was directly proportional to that of the observed deuterium isotope effect, indicating an interrelationship between the accessibility of water molecules to the site of peptide bond hydrolysis in collagen and the energy requirements of enzymatic action. The data also suggest that the unusually large dependence of fibrillar collagen degradation upon both energy and hydrogen transfer are properties specific to mammalian collagenases, all of which catalyze only a single cleavage in native collagen, and may relate directly to the geographic location of the site of this cleavage in the collagen molecule.

Collagenases are characterized by the ability to initiate the specific degradation of the native triple helical collagen molecule. Studies designed to investigate the kinetics of collagenase action are essential in order to gain a better understanding of the mechanism by which this class of enzymes performs its unique function in nature. In a recent report characterizing the action of human skin fibroblast collagenase on fibrillar collagen (1), we have shown that collagenase binds very tightly to collagen fibrils and appears to remain bound to this substrate throughout ongoing collagen degradation at 37 °C, independent of subsequent dilution with buffer or the addition of exogenous collagen as a competitor. Thus, during the degradation of fibrillar collagen no equilibrium appears to exist between collagenase molecules bound to the fibrillar substrate and the external buffer of the reaction mixture. Studies of enzyme binding as a function of collagenase concentration indicated that only 10% of the total number of collagen molecules present in a fibrillar substrate gel are initially accessible to enzyme for binding prior to degradation of the substrate (1). The results were consistent with the available molecules occupying the surface of each fibril. The remaining 90% of collagen molecules, presumably located within the interior of the fibrils, apparently become accessible to the enzyme only after subsequent substrate catalysis.

In an accompanying paper (2), we have examined the collagen substrate specificity of human skin fibroblast collagenase as a function of both collagen type and species of substrate origin. Measurements of the basic kinetic parameters, K_m and k_cat, were performed on collagen types I–V from several animal species, using collagen in solution as substrate. Collagen types I, II, and III of all species examined were successfully attacked by fibroblast collagenase and the measured enzyme-substrate affinity was similar in all cases, K_m = 0.7-2.1 x 10^6 M. In contrast, large differences in catalytic rates were evident, ranging from 565 h⁻¹ for the homologous human type III substrate to 1.0 h⁻¹ for human type II collagen. Significant rate differences were also observed between collagens of the same type but of different species of origin. Human skin fibroblast collagenase was most specific for the homologous type I and III collagens.

In this communication, we have investigated the action of human skin fibroblast collagenase with respect to both temperature dependence of activity and solvent-deuterium isotope effect. These parameters have been studied utilizing fibrillar collagen, collagen in solution, and denatured collagen as substrates, in order to provide a more detailed understanding of the mechanism of collagenase action and specifically to help define the rate-limiting step of collagen degradation. For comparison, the collagenolytic proteases from the crustacean, Uca pugilator, and from the bacterium, Clostridium histolyticum, which are chemically unrelated to fibroblast collagenase, were also studied.

MATERIALS AND METHODS

Reagents—Acrylamide and bis-acrylamide were purchased from Eastman. Sodium dodecyl sulfate (99% pure) and deuterium oxide (99.8%) were obtained from Gallard-Schlesinger. Tris base, bovine pancreatic trypsin (type III), and soybean trypsin inhibitor were used.

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processed from Sigma. All other chemicals used were reagent grade.

Culture Methods—Normal human skin fibroblasts (CRL 1187) were purchased from American Type Culture Collection. The cells were grown in the presence of 20% fetal calf serum and the medium was harvested as described by Bauer et al. (3).

Purification of Collagenases—Human skin fibroblast collagenase was purified to homogeneity from serum-containing growth medium by a combination of carboxymethylcellulose and Ultrogel Aca-44 chromatography, as described by Stricklin et al. (4, 5). Pure collagenase from the hepatopancreas of the fiddler crab, U. pugilator, was prepared according to methods described by Eisen et al. (6). Bacterial collagenase (form III; protease-free) from C. histolyticum was purchased from Sigma. All other chemicals used were reagent grade.

Activation of Collagenase—The activation of fibroblast procollagenase was accomplished proteolytically by the addition of trypsin at 25°C for 10 min. Further tryp tic action was prevented by adding an 8-fold molar excess of SBTI. Maximal collagenase activity was ensured by performing a trypsin titration on each batch of enzyme. Crustacean and bacterial collagenases did not require proteolytic activation.

Assay Procedures: Determination of $E_A$ on Fibrillar Collagen—Guinea pig skin type I collagen, prepared in this laboratory as previously described by Groves (7), was the source of all collagen substrates employed in this study. The activation energy of each collagenase on fibrillar collagen was determined by measuring the rate of degradation of fibrillar substrate as a function of temperature. The temperature of the water bath incubator was controlled by a constant temperature circulator (Fisher Model 73) and monitored with a Fisher mercury thermometer with 1/10°C subdivisions. In each assay, 50 µl of a 0.4% solution of native, reconstituted [10°C] glycine-labeled collagen of specific activity 25,000 cpm/mg was allowed to gel at 37°C overnight to permit completion of the aggregation process to occur. Following incubation of such collagen gels with enzyme, the reaction mixtures were centrifuged at 10,000 x g and the supernatant fraction was counted in a liquid scintillation spectrometer.

At each temperature studied, samples were stopped at several time points to ensure linearity of reaction velocity with time and thus measurement of an initial velocity. Collagenase activity was then graphed as an Arrhenius plot of log reaction velocity versus 1/temperature (K) and the activation energy ($E_A$) calculated by the equation

$$1Nk_0 = \frac{E_A}{R} \left(\frac{1}{1/T_1} - \frac{1}{1/T_2}\right)$$

Determination of $E_A$ on Collagen in Solution—Degradation of collagen in solution by fibroblast collagenase was measured in 4°C increments over the temperature range of 16–36°C. At each temperature, 0.1 µg of collagenase was incubated with 20 µg of collagen in a total reaction mixture volume of 100 µl (buffer = 0.05 M Tris, 0.1 M CaCl₂, 0.2 M NaCl, pH 7.5). SBTI (1 mg/ml) was used in each reaction mixture to maintain enzyme stability at all temperatures studied. Degradation of collagen was always <20% of the initial amount present, thereby enabling measurement of an initial velocity (2). Incubation times varied from 3 min (36°C) to 4 h (16°C). The reaction process was stopped with EDTA, sodium dodecyl sulfate sample buffer added, and aliquots of each sample were then subjected to polyacrylamide gel electrophoresis using 8% gels cast in slab molds. Following staining with 1% Coomassie blue, reaction velocity was quantitated by measuring the formation of single cleavage TCA products (% degradation $= \frac{A(37°C)-A(10°C)}{A(10°C)} + a[a]$) using a spectrophotometer equipped with a gel scanning linear transport device. The results were then graphed as an Arrhenius plot and $E_A$ was calculated from the formula given above.

$$Determination of E_A$ on the a1 and a2 Peptide Chains of Gelatin—Separation of the a1 and a2 peptide chains of denatured guinea pig skin collagen was accomplished by carboxymethylcellulose chromatography, as originally described by Pies et al. (8). Pure human skin fibroblast collagenase initiates multiple cleavages in both gelatin chains at a low rate, including a cleavage indistinguishable by polyacrylamide gel electrophoresis from the 3/4-3/4 cleavage produced in native collagen (details of this gelatolytic activity will be published in a future communication). In the case of the a2 chain, this 3/4-3/4 cleavage could be isolated as a single catalytic event, prior to any subsequent cleavages, by stopping the reaction process after only 10–20% of the substrate present had been degraded. The activation energy of fibroblast collagenase on the a2 chain of gelatin was there-

\[ The \ abbreviation \ used \ is: \ SBTI, \ soybean \ trypsin \ inhibitor. \]
the resultant Arrhenius plot, whose slope indicates an \( E_A \) of 101,050 calories. This activation energy is extremely high when compared with most enzyme-catalyzed reactions and, as a result, fibrillar collagen degradation by fibroblast collagenase would be expected to increase approximately 200-fold for each 10 °C increase in temperature.

The Arrhenius energy characterizing the action of fibroblast collagenase on collagen in solution was measured over the temperature range of 16–36 °C. Short incubation periods (<5 min) were utilized at the higher temperatures in order to avoid spontaneous gelation with conversion to the fibrillar form of substrate. Quantitation of TC\(^2\) product formation was accomplished by running the final reaction mixtures on sodium dodecyl sulfate-polyacrylamide slab gels, staining with 1% Coomassie blue, and then scanning with a spectrophotometer equipped with a linear transport device (2). At each temperature studied, collagen degradation was linear with time over the entire assay period, thus ensuring measurement of an initial velocity. As shown in Fig. 1B, the resultant Arrhenius plot indicated an \( E_A \) of 49,200 calories. While this activation energy is considerably less than that which characterized the degradation of fibrillar collagen, cleavage of collagen monomers is nevertheless an unusually energy-dependent process. Collagenase activity on this form of substrate increased 16-fold per 10 °C.

As previously reported (4), human skin fibroblast collagenase purified to homogeneity exhibits a low level of proteolytic activity against gelatin. The activation energy of fibroblast collagenase on the \( \alpha_1 \) and \( \alpha_2 \) polypeptide chains of denatured type I collagen was examined from 35–41 °C, a temperature range high enough to prevent refolding of gelatin chains into a nonrandom secondary structure (10, 11) but yet low enough to maintain enzyme stability. Arrhenius energies of 13,900 and 13,300 calories were obtained for the \( \alpha_1 \) and the \( \alpha_2 \) chains, respectively (Fig. 2, A and B). The 2-fold change in reaction velocity per 10 °C indicated by these Arrhenius energies is far more typical of most enzyme-catalyzed reactions than the extraordinary temperature dependence which characterized the cleavage of native triple helical collagen both in fibrillar form and as solution monomers.

The activation energy of pure crab collagenase and of bacterial collagenase on fibrillar collagen is shown in Fig. 3. An Arrhenius plot of fibrillar collagen cleavage by the crustacean enzyme, over the temperature range of 35–39 °C, indicated an \( E_A \) of 24,300 calories (Fig. 3A). The activation energy of clostridial collagenase, assayed under the same conditions, was 19,400 calories (Fig. 3B). These values indicate that fibrillar collagen degradation by the crustacean and bacterial enzymes is far less energy dependent than is the case for human skin fibroblast collagenase.

In order to provide further insight into the reaction mechanism of human skin fibroblast collagenase, the solvent-deuterium kinetic isotope effect for this enzyme was examined. Fig. 4 illustrates the rate of fibrillar collagen degradation by fibroblast collagenase. In each sample, 100 \( \mu \)l of a collagenase solution of concentration \( = 20 \mu \)g/ml were incubated at 37 °C with 200 \( \mu \)g of \(^{14}C\)-labeled fibrillar collagen. Increasing amounts of deuterium oxide were incorporated into the solvent buffer of the reaction mixtures such that the ratio of \( \text{D}_2 \text{O}:\text{H}_2 \text{O} \) in total solvent buffer was 0.00 (0), 0.30 (30), 0.60 (60), and 0.90 (90). The reaction process was stopped after 30, 60, and 90 min by centrifuging the samples at 10,000 \( \times g \) and the supernatant fraction was counted in a liquid scintillation spectrometer. Activity is shown following subtraction of the appropriate blanks.
**Human Skin Fibroblast Collagenase**

**TABLE I**

| Effect of D2O on collagenase binding |
|-------------------------------------|
| 100 µl of a collagenase solution of concentration = 20 µg/ml was bound to 200 µg of 14C-labeled fibrillar collagen in the presence of either 90% D2O or 100% H2O in total solvent buffer. Enzyme activity was measured in 1°C increments over the temperature range of 36-38°C. At each temperature, 100 µl of a collagenase solution of concentration = 120 µg/ml was incubated with 200 µg of 14C-labeled fibrillar collagen. Log reaction velocity was arbitrarily set equal to 1.0 for the lowest temperature and values at the other temperatures were normalized accordingly. Ea was calculated from the Arrhenius plot using the formula shown under "Materials and Methods." |

| Table II |

**Comparison of the activation energy and deuterium isotope effect on fibrillar collagen degradation for several collagenases**

Values for Ea of human fibroblast, crab, and bacterial collagenase on fibrillar collagen were obtained as described in Figs 1A and 3. A and B. kH/kD represents the ratio of fibrillar collagen degradation in solvent buffer containing 100% H2O versus 90% D2O.

| Collagenase      | Ea (kcal/mol) | kH/kD |
|------------------|---------------|-------|
| Human fibroblast | 101.050       | 9.0   |
| Crab hepatopancreas | 24.300   | 2.6   |
| C. histolyticum  | 19.400       | 1.7   |

To define which step(s) in the degradation of fibrillar collagen was so markedly affected by deuterium, enzyme-substrate binding, enzyme-substrate dissociation, and enzymatic hydrolysis were independently examined for evidence of proton transfer. Enzyme-substrate binding was studied by incubating active collagenase with fibrillar collagen at 25°C in solvent buffer containing either 90% D2O or 100% H2O. Incubations were performed for increasing lengths of time up to 20 min, a period known to ensure completion of the binding process (1). At this temperature, due to the high activation energy of fibroblast collagenase, binding occurs without measurable substrate degradation (1). Following enzyme binding, the respective substrate gels were washed, resuspended in new buffer, and then incubated at 37°C. As shown in Table I, enzyme-substrate binding at 25°C was not affected by the presence of either deuterium or hydrogen; collagenase activity, however, was critically dependent upon the isotope composition of the suspending buffer during collagen degradation at 37°C. Quantitation of binding in D2O and H2O by calculation of the rate constant, kH, resulted in an identical value, 1.2 × 10³ M⁻¹ s⁻¹, for both solvents. Therefore, enzyme-substrate binding exhibited no discernible kinetic isotope effect. Substrate cleavage subsequent to binding, however, was slowed markedly in the presence of the heavier deuterium isotope.

Evidence for proton transfer in dissociation of the enzyme-substrate complex was next investigated. Fibroblast collagenase was bound to fibrillar collagen in buffer containing either D2O or H2O, as described above. Following completion of binding, the gels were washed and resuspended in identical buffer at 25°C for 1 h. Aliquots of each reaction mixture were then incubated with new collagen gels (solvent buffer = H2O) at 37°C to assay for the presence of dissociated enzyme. No significant collagenase activity was observed, consistent with little, if any, dissociation of enzyme from fibrillar substrate, regardless of whether bound enzyme was suspended in D2O or H2O (not shown). These results indicated that dissociation of the enzyme-substrate complex, like enzyme-substrate binding, did not exhibit any discernible kinetic isotope effect. Thus, only enzymatic hydrolysis showed evidence of proton transfer, since this step alone was slowed by the substitution of deuterium for hydrogen in solvent buffer. Interestingly, fibrillar collagen degradation by crab collagenase and clostridial collagenase was less profoundly affected by solvent deuterium than was the case for fibroblast collagenase. As illustrated in Table II, fibrillar collagenolysis by the crustacean and bacterial enzymes was slowed 2.6-fold and 1.7-fold, respectively, by the presence of 90% deuterium in solvent buffer.

To assess the deuterium isotope effect for the cleavage of collagen in solution by human skin fibroblast collagenase, values for the Ks and Vmax were determined (2) in solvent buffer containing 90% D2O versus 100% H2O. The resultant data, plotted according to the method of Lineweaver and Burk, are shown in Fig. 5. The substitution of deuterium for hydrogen in solvent buffer resulted in a 50% reduction in Vmax, with no accompanying change in Ks. Thus, the degradation...
formation are described in Fig. 1 of Ref. attempted to measure such aggregation in this study, and it is step of enzyme catalysis in each case. change in the structure of the reactants or in the rate-limiting temperature ranges investigated, indicating no significant may be indistinguishable in collagen. of collagen monomers by fibroblast collagenase was slowed by the heavier deuterium isotope, but to a lesser extent characterized the cleavage of fibrillar collagen (2-fold versus 9-fold).

**DISCUSSION**

This study examines the activation energy of human skin fibroblast collagenase on different forms of collagenous substrates. The values of $E_a$ on the $\alpha$ and $\alpha^\prime$ polypeptide chains of denatured collagen, collagen in solution, and collagen in native fibrillar form were 13,900, 13,300, 49,200, and 101,050 calories, respectively. Arrhenius plots were linear over the temperature ranges investigated, indicating no significant change in the structure of the reactants or in the rate-limiting step of enzyme catalysis in each case.

Recent work has demonstrated that the structure of collagen in solution consists of a mixture of single molecules and higher molecular weight aggregates (16, 17). We have not attempted to measure such aggregation in this study, and it is possible that the extent of microaggregate formation could affect the exact value of $E_a$ on this form of substrate. In our determination of $E_a$, however, samples were incubated for different lengths of time and at different temperatures, conditions under which the level of collagen aggregation would be expected to change. Yet, linear Arrhenius plots always resulted, even when measured in $1^\circ$C increments (not shown), again suggesting that such microaggregates may be indistinguishable to collagenase from collagen monomers (2).

The increase in the activation energy of fibroblast collagenase which accompanies an increasing level of organization in the collagen substrate itself is of particular interest. Nearly all reported values for the Arrhenius energy of enzyme-catalyzed reactions are 10–20,000 calories (14), indicating that reaction velocity changes 1.7–3.0-fold per $1^\circ$C. While such an activation energy characterizes fibroblast collagenase degradation of gelatin $\alpha$ chains, cleavage of native triple helical collagen molecules in solution was a far more energy dependent process, $E_a \approx 50,000$ calories, while further ordering of collagen into fibrils raised the Arrhenius energy to 100,000 calories. This very high activation energy for fibrillar collagen, the physiologic form of substrate, may be of considerable significance to the animal organism. For each $2^\circ$C increase in body temperature, collagenase activity would be expected to increase approximately 3-fold. A $5^\circ$C change in temperature, which not uncommonly accompanies fibroblast illnesses and local inflammation (18), would result in a 14-fold increase in the rate of fibrillar collagen degradation, while a change of $10^\circ$C, albeit most unlikely, would increase collagenase activity 200-fold.

A similar substrate-dependent process was evident when deuterium was substituted for hydrogen in solvent buffer. Whereas cleavage of collagen monomers by fibroblast collagenase decreased 2-fold in the presence of 90% deuterium oxide, when collagen was in the fibrillar form, the rate of degradation was slowed 9-fold. This kinetic isotope effect for fibrillar collagen cleavage is considerably greater than the 2–3-fold reduction in activity reported for most enzyme-catalyzed reactions where peptide bond hydrolysis is rate-limiting (12–15). Although the nature of the rate-limiting step of collagenolysis has not been previously defined for any collagenase, it seems clear that proton transfer must be implicated at the slow step of fibrillar collagen degradation by fibroblast collagenase. Since enzyme-substrate binding ($k_1$) and enzyme-substrate dissociation ($k_2$) are both unaffected by solvent deuterium, the results are most consistent with actual enzymatic hydrolysis, represented by the rate constant, $k_2$, as the rate-determining step of the reaction process. While a smaller, more typical deuterium isotope effect was observed for the cleavage of collagen molecules in solution by fibroblast collagenase, this 2-fold reduction in $V_{max}$ without any accompanying change in $K_m$ is also consistent with product formation as the rate-limiting step.

In a previous study (1), we reported the equilibrium constant of dissociation, $K_d$ ($k_{-1}/k_1$), for human skin fibroblast collagenase. This constant, determined on guinea pig skin type I collagen fibrils, has a value of $9.5 \times 10^{-5}$ M. Although the Michaelis constant, $K_m$ ($k_{-1}/k_1$), could not be measured on fibrillar collagen due to the solid nature of this substrate, the $K_m$ of fibroblast collagenase on collagen in solution form is similar for all susceptible collagens (2), including guinea pig type I collagen. This value, $1 \times 10^{-5}$ M, suggests that $K_m \approx K_d$, indicating that the ratio $k_2/k_1$ must be negligible, and further implicating peptide bond cleavage ($k_2$) as the rate-limiting step of collagen degradation. Additionally, as reported in the accompanying paper (2), large differences in the $V_{max}$ ($k_{cat}$) of fibroblast collagenase are found for collagen substrates of nearly identical affinity ($K_m$). Thus, although the collagen molecule is large, extremely asymmetric, and contains only a single catalytic site for human fibroblast collagenase, there is no evidence of any difficulty in obtaining effective collisions between enzyme and substrate.

In view of the activation energy and deuterium isotope effect which characterize the action of fibroblast collagenase on collagen fibrils and solution monomers, it appears that water is involved at the rate-limiting step of collagen degradation, and, with increased ordering of the collagen substrate, additional energy is required to properly position and/or utilize this water. Since the native collagen molecule exists as a triple helix with a hydrophobic core, the interposition of water molecules inside of this helical structure may become increasingly difficult as the substrate assumes higher orders of organization. Thus, in the case of the collagen fibril, the water of hydration must traverse a liquid-solid phase boundary in addition to gaining access into the interior of the triple helix. However, the nature of the collagen substrate alone does not determine the activation energy and solvent-deuterium kinetic isotope effect for all collagenolytic enzymes. The
degradation of fibrillar collagen by both clostridial and crab collagenases was characterized by a lower Arrhenius energy (19,400 and 24,300 calories, respectively) and smaller kinetic isotope effect (1.7- and 2.6-fold inhibition by D2O, respectively), compared to the values for human skin fibroblast collagenase on this same substrate (101,080 calories and 3.6-fold, respectively). Interestingly, however, in each case the magnitude of the Arrhenius energy was directly proportional to that of the deuterium isotope effect (Table II), lending further support to the possible interrelationship between the accessibility of water molecules to the site of peptidebond hydrolysis in collagen and the energy requirements of enzymatic action.

While the activation energy and kinetic isotope effect for bacterial and crustacean collagenases on fibrillar collagen were much smaller than for human fibroblast collagenase on this same substrate, it should be noted that these three collagenases are chemically and functionally distinct from one another. Only the fibroblast enzyme is a member of the group of specific vertebrate collagenases, which are all metalloenzymes that catalyze only a single cleavage in the native collagen molecule (19). The site of this cleavage, which has been determined for tadpole and a mammalian tumor collagenase, is a Gly-Ile bond (775–776) in the α1 chain (20, 21) and a Gly-Leu bond in the α2 polypeptide chain and results in typical 3- and 4-length products, TCA and TCB (22). By contrast, the cleavage pattern of crab collagenase, although also a metalloenzyme, catalyzes multiple cleavages in the native collagen molecule and, in addition, manifests a very different bond specificity. The bacterial collagenase cleaves the Y-Gly bond in sequences such as Gly-Pro-Y-Gly-Pro-Z or Gly-Pro-Y-Gly-Z-Hyp, resulting in the formation of numerous NH2-terminal glycine residues in the collagen chains (23). Of interest in comparing the bonds broken by human skin and clostridial collagenases is the geographical position of the bond cleaved in each case. For the human skin fibroblast enzyme, the nucleophilic attack by a water molecule during hydrolysis is at the carboxyl carbon of glycine, which is positioned most closely to the hydrophobic center of the triple helix. For the bacterial enzyme, on the other hand, this addition of water occurs at a carbonyl group located on the outside of a turn of the collagen helix (24). It is possible, then, that this relatively more accessible location of the bond for hydrolysis is related to the lower activation energy and deuterium isotope effect for the bacterial collagenase. The higher values of these parameters for the human enzyme may be the effect of the hydrophobic location of the glycine carbonyl in the triple helix. Crab collagenase is a chymotrypsin-like serine protease which catalyzes multiple cleavages in the native collagen molecule (6, 25). The collagen bond specificity of this enzyme is presently not known.

There is evidence suggesting that an unusually high activation energy characterizes not only human skin fibroblast collagenase but also other vertebrate collagenases which catalyze only a single 3- or 4-fold cleavage in native collagen. Hayashi et al. (26) have reported the activation energy of tadpole collagenase on types I, II, and III collagen monomers in solution ($E_A = 41,000, 39,000,$ and $63,000$ calories, respectively). These values are similar to the activation energy of fibrillar collagenase on type I collagen monomers ($E_A = 49,200$ calories). In addition, Harris and McCroskery (18) have reported a 4-fold increase in the rate of cleavage of cartilage collagen fibrils by crude human rheumatoid synovial cell collagenase at 36 °C compared to 33 °C (from their data, $E_A$ calculated on this basis would be 85,000 calories). Finally, our preliminary experiments indicate that rat uterus collagenase has an $E_A = 75,000$ calories on fibrillar collagen. Following an exhaustive search of the literature, we have been unable to find any other class of enzymes which are characterized by such a high activation energy on their physiologic substrates. Therefore, an unusually high activation energy on native collagen may be a property which is common to specific vertebrate collagenases (20) and, as discussed above, is likely to relate to the difficulty in bringing water molecules to the site of the 3- or 4-length cleavage initiated by these enzymes in the native collagen molecule.

The enzymatic cleavage of native collagen by human skin fibrillar collagenase represents an enzymatic process, which, in certain respects is different from most enzyme-catalyzed reactions described to date. The unique physical structure of the collagen molecule, so necessary for its functional role in the animal organism, has probably presented nature with equally unique problems regarding its degradation. Studies designed to further investigate the extreme dependence of this process upon both energy and hydrogen transfer will be vital toward ascertaining a more complete understanding of the kinetics of collagen degradation by human skin fibrillar collagenase.

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