Proteolysis of Elastin–Ligand Complexes. Stimulation of Elastase Digestion of Insoluble Elastin by Sodium Dodecyl Sulfate†

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ABSTRACT: Sodium dodecyl sulfate stimulates the rate of digestion of insoluble ligament elastin as measured by solubilization or by peptide-bond hydrolysis. This stimulation depends upon the detergent concentration and on the preincubation conditions. Maximal stimulation of proteolysis of about 6-fold is obtained when the insoluble elastin is preincubated with as much as 1% sodium dodecyl sulfate and then isolated by centrifugation and resuspended in detergent-free medium before addition of elastase. In contrast, the enzyme is inactivated at 0.03% sodium dodecyl sulfate when assays employ a blocked alanine ester as substrate (Visser, L., and Blout, E. R. (1969), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 28*, 407) or if the enzyme is preincubated with the detergent before addition to elastin. Thus, while elastase is very sensitive to sodium dodecyl sulfate, these studies suggest that the detergent binds to insoluble elastin yielding a substrate complex more susceptible to proteolysis and which serves to protect elastase against inactivation. This conclusion is supported by direct binding studies with [^{35}S]sodium dodecyl sulfate and insoluble elastin, by enzyme binding studies revealing that the sodium dodecyl sulfate–elastin complex has a higher affinity for elastase than does elastin, and by circular dichroism studies with α-elastin which indicate that the detergent induces a marked conformational change in this soluble elastin fraction. The stimulation of elastolysis could result from an increase in the anionic character of elastin by the bound detergent thus enhancing the binding of enzyme to the substrate, and a detergent-induced conformational change in the insoluble substrate altering the accessibility of peptide bonds to the enzyme. The present findings are considered in relationship to the known ability of elastic fibers to bind hydrophobic, detergent-like ligands *in vivo* and the potential of such ligands to render structural elastic fibers more susceptible to proteolytic attack.

Elastin is an unusual protein by virtue of its insolubility, its content of lysine-derived cross-links, and its general resistance to proteolysis by many mammalian proteases (Franzblau, 1970). Various nonenzymatic techniques have now been successfully employed to solubilize this protein to fragments which can be used to facilitate the investigation of its physical-chemical properties. Such techniques have included boiling with 40% urea (Hall, 1951), heating in dilute oxalic acid (Adair *et al.*, 1951; Partridge *et al.*, 1955), and long-term exposure to basic alcoholic solutions (Robert and Poullain, 1963).

Among the mammalian proteases, pancreatic elastase is unusual in that it readily hydrolyzes and solubilizes insoluble elastin (Balo and Banga, 1950; Lewis *et al.*, 1956). Using a purified preparation of elastase, Hall and Czerkowski (1961) proposed that elastolysis proceeds in two stages, the first of these yielding primarily large molecular weight fragments. These fragments, referred to as α-elastin, are further degraded in the second phase. The second phase of the elastolytic reaction was inhibited by 0.5 mM sodium dodecyl sulfate added to the reaction mixture. This resulted in the accumulation of α-elastin which was shown to be homogeneous both by electrophoretic and sedimentation patterns. It is of greater interest, however, that this concentration of detergent caused a twofold increase in the rate of the initial phase of elastolysis.

In apparent contrast to these results, it has recently been shown that sodium dodecyl sulfate at similarly low concentrations readily inactivates highly purified elastase when enzyme activity is measured using the *p*-nitrophenyl ester of *tert*-Boc-L-alaninate[1] (NBA) as substrate (Visser and Blout, 1971). Using highly purified elastase, we find evidence that these effects can be understood in terms of the relative binding affinities of elastase and elastin for sodium dodecyl sulfate and for each other. These data, further, may bear upon the known ability of elastin to bind hydrophobic materials *in vivo*.

Experimental Section

Materials. Sodium dodecyl sulfate was obtained from Fisher Scientific Co. while [^{35}S]sodium dodecyl sulfate was a product of New England Nuclear Corp. (specific activity of 1.41 Ci/mole at time of purchase). Corrections were made as required for radioactive decay during the experimental period. Highly purified elastase (>99.8%, batch 24) was obtained from the Whatman Co., Maidstone, Kent, England. The chromophoric elastase substrate, *p*-nitrophenyl *tert*-Boc-L-alaninate, was obtained from Schwarz BioResearch and recrystallized from an ethanol–H_2O mixture.

Methods. Bovine *Ligamentum nuchae* elastin was prepared according to the procedure of Partridge *et al.* (1955). Solubilized α-elastin was prepared by successive extractions of the ligament elastin powder with 0.25 N oxalic acid, according to Partridge *et al.* (1955). Protein solubilized in the fourth and fifth extractions was used as high molecular weight α-elastin.

† From the Department of Biochemistry, Boston University, School of Medicine, Boston, Massachusetts 02118. *Received May 1, 1972.* This research was supported by grants from the National Institutes of Health (AM-07697 and HL-13262) and the American Heart Association (70 852). The data presented are taken in part from the Ph.D. dissertation of R. E. J. (in preparation). R. E. J. is grateful for the National Science Foundation Predoctoral Traineeship No. GZ 2019.

[1] Abbreviations used are: NBA, *p*-nitrophenyl *tert*-Boc-L-alaninate; CD, circular dichroism.

Enzyme Assays. ESTER HYDROLYSIS. Assay of elastase activity was performed with NBA as substrate at pH 6.5 in 0.01 M potassium phosphate buffer at 25°. The rate of *p*-nitrophenol accumulation was followed in a Gilford recording spectrophotometer at 400 nm thermostatted at 25° (Visser and Blout, 1969).

ELASTIN SOLUBILIZATION. Elastolysis of insoluble *L. nuchae* elastin was assayed by the spectrofluorometric method of Quinn and Blout (1970). Incubations were performed at 37° in 0.05 M ammonium acetate buffer adjusted to pH 8.45 with sodium hydroxide. Fluorescence was measured with an Aminco Bowman spectrophotofluorometer, Model 4-8202. The wavelength of excitation was 320 nm while emission was followed at 390 nm. Separate studies established that sodium dodecyl sulfate increased the fluorescent yield of the solubilized elastin by 10–20%. Correction factors were derived from these experiments at the sodium dodecyl sulfate concentrations employed and were applied to all fluorescent assay data.

The supernatant obtained from centrifugation of elastin digested in the presence of low concentrations of sodium dodecyl sulfate (less than 0.1%) spontaneously coascervated at room temperature, consistent with their content of α-elastin. To eliminate artifacts in the fluorescence readings arising from turbidity, each supernatant was treated with 0.05 ml of 10 N sodium hydroxide which was found to solubilize and totally clarify the coacervated solution.

Ninhydrin Assay. Proteolysis of oxalic acid solubilized α-elastin by elastase was followed by quantitation of the appearance of free amino groups by the ninhydrin method of Rosen (1957). Incubations were at 37° in 0.01 M potassium phosphate buffer (pH 8.45).

pH-STAT ASSAY. Assays of proteolysis by the pH-Stat method employed a Radiometer automatic titrator Model TTT-11 coupled to a Model 28 pH meter and recording unit. The reaction vessel contained 2 ml of a water suspension of 10 mg of elastin adjusted to pH 8.45, thermostatted at 37°, continuously stirred, and flushed with nitrogen gas.

Binding Studies with [^{35}S]Sodium Dodecyl Sulfate. Insoluble ligament elastin was incubated with [^{35}S]sodium dodecyl sulfate previously diluted 1:10 with nonradioactive sodium dodecyl sulfate to give a specific activity of 0.14 Ci/mole. Incubations were done at 37° for 1 hr in 0.05 M ammonium acetate adjusted to pH 8.45 with NaOH. Following incubation, the suspended elastin was centrifuged and the radioactivity remaining in the supernatant was determined by liquid scintillation spectrometry and compared to an identical solution of [^{35}S]sodium dodecyl sulfate in buffer but which did not contain elastin.

Circular Dichroism. Circular dichroic spectra of solutions of soluble elastin were recorded at 27° in a Cary Model 61 recording circular dichroism spectropolarimeter. Protein was at a concentration of 0.1 mg/ml and cells of 1.0- to 10.0-mm path length were employed. All spectra were compared to base lines obtained with the specific solvent being used in the study. Mean residue ellipticities were calculated using mean residue weights of 108 for elastase and 87 for soluble elastin.

Results

Studies with Insoluble Elastin. Insoluble ligament elastin (10 mg) was incubated with 20 μg of elastase in the presence or absence of sodium dodecyl sulfate (0.1%) and the accumulation of fluorescent, soluble material was followed. A separate incubation mixture was used for each time point obtained. As will be noted, the order of additions has a marked effect on the results of the assay. In the experiment of Figure 1, the effect of detergent was determined by preincubating the elastin substrate with sodium dodecyl sulfate and starting the reaction by the addition of elastase. As shown (Figure 1), solubilization is considerably enhanced in the presence of sodium dodecyl sulfate. Total solubilization of the sodium dodecyl sulfate treated elastin occurred 3 to 4 hr earlier than it did in the control. It is also apparent that the initial lag in solubilization of the control is eliminated in the presence of sodium dodecyl sulfate. This lag in the appearance of soluble elastin fragments is characteristic of assays which measure solubilization as the index of the digestion of elastin and reflects the initial binding and early proteolytic events (Robert and Robert, 1970).

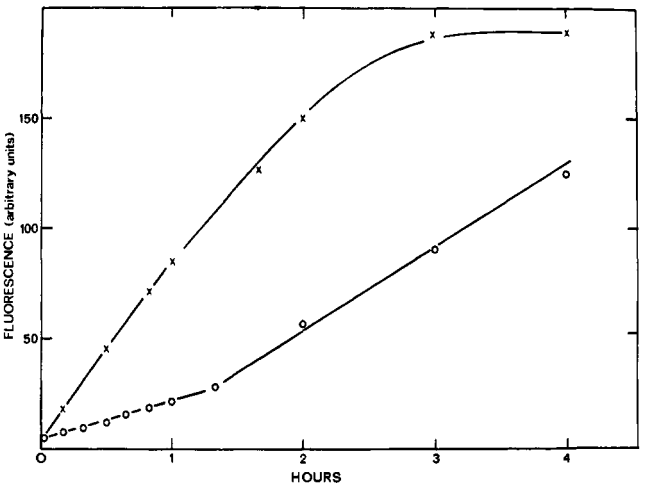

FIGURE 1: Time study of the solubilization of ligament elastin by elastase at 37°. (O) Control; (×) incubation in the presence of 0.1% sodium dodecyl sulfate.

The rate of solubilization of elastin and the apparent sensitivity of elastase to the detergent are both dependent upon the preincubation conditions and the sodium dodecyl sulfate: elastin ratio (Figure 2). Thus, adding fresh enzyme to suspensions of elastin preincubated in varying concentrations of sodium dodecyl sulfate results in a bell-shaped solubilization profile characterized by an optimum sodium dodecyl sulfate concentration at 0.15%. The stimulation of elastolysis at the optimum is about 3.5-fold that of the control. Detergent concentrations in excess of this optimum value result in inhibition of elastolysis.

In marked contrast to these results, preincubation of elastase in these same concentrations of sodium dodecyl sulfate for 60 min at 37° before adding the enzyme to the substrate leads to essentially total inactivation of elastase at 0.02–0.03% sodium dodecyl sulfate and no apparent stimulation of elastolysis (Figure 2).

Effect of Sodium Dodecyl Sulfate on the Hydrolysis of NBA and Soluble Elastin. In contrast to the effects seen with insoluble elastin as substrate, the elastase-catalyzed esterolysis of the alanine ester (NBA) is very sensitive to sodium dodecyl sulfate with total inactivation occurring at a detergent concentration of about 0.02% (Figure 3). The results are essentially the same regardless of whether elastase is first preincubated with sodium dodecyl sulfate or not. Further, it should be noted that the sensitivity of the enzyme to sodium dodecyl sulfate in this case is the same as observed in the preincuba-

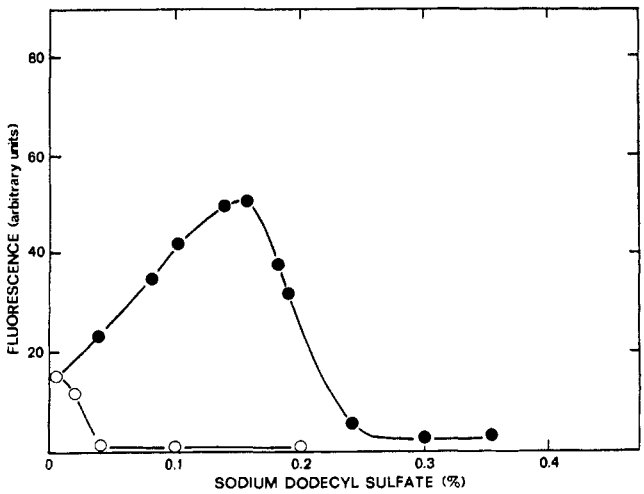

FIGURE 2: Effect of sodium dodecyl sulfate concentration and preincubation conditions on solubilization of ligament elastin by elastase. The points represent fluorescence measurements after 1-hr incubations at 37°. (●) Elastin preincubated with sodium dodecyl sulfate, reaction started with untreated elastase; (○) elastase preincubated 1 hr at 37° with specified concentrations of sodium dodecyl sulfate, reaction started with this pretreated elastase added to elastin also in sodium dodecyl sulfate.

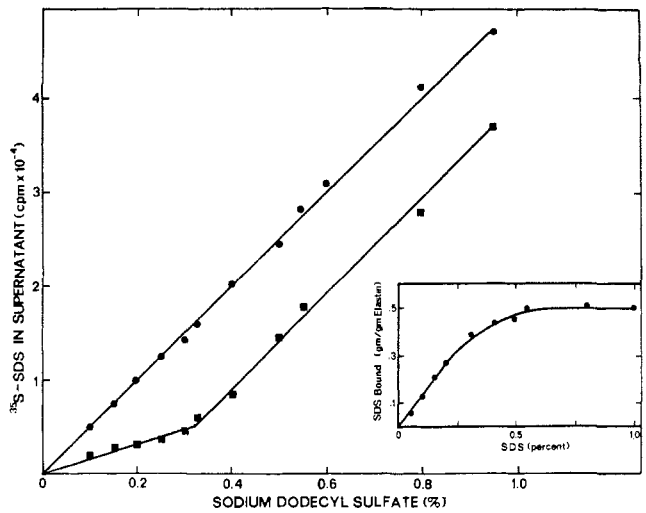

FIGURE 4: Binding of [^{35}S]sodium dodecyl sulfate to 10 mg of insoluble ligament elastin at 37°. (●) Counts per minute in original [^{35}S]sodium dodecyl sulfate solution; (■) counts per minute in supernatant after centrifuging out (1000*g*, 5 min) elastin–sodium dodecyl sulfate complex. *Inset:* binding curve obtained by calculation from data in Figure 4.

tion experiment (Figure 2) in which the pretreated enzyme was assayed with insoluble elastin.

Elastolytic activity toward soluble α-elastin is also progressively inhibited by increasing concentrations of sodium dodecyl sulfate (Figure 3). In this case, however, the enzyme is apparently protected from inactivation since its proteolytic activity is still expressed up to approximately 0.35% sodium dodecyl sulfate. Unlike insoluble elastin, the rate of digestion of α-elastin is not stimulated at any concentration of sodium dodecyl sulfate tested (Figure 3).

Binding of Sodium Dodecyl Sulfate to Elastin. The data thus far described suggest that the stimulation of elastolysis by sodium dodecyl sulfate and the increased resistance of the enzyme to sodium dodecyl sulfate both relate to the binding of the detergent by the elastin substrate. The degree of binding of sodium dodecyl sulfate to insoluble elastin was tested under assay conditions as a function of the concentration of sodium dodecyl sulfate (Figure 4).

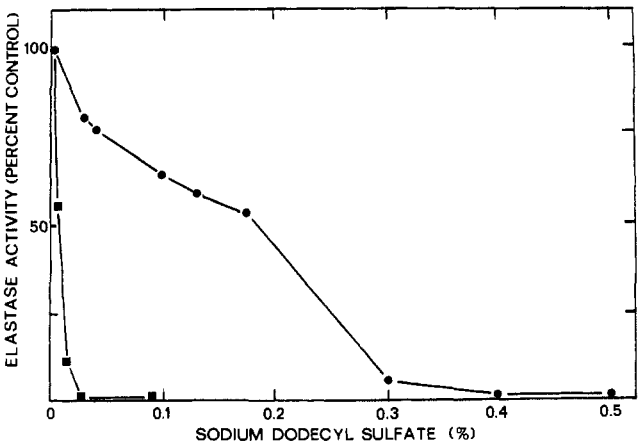

FIGURE 3: Effect of sodium dodecyl sulfate on the elastase-catalyzed hydrolysis of the ester substrate (NBA) and of soluble α-elastin. (■) Ester substrate; (●) soluble elastin substrate.

Insoluble elastin (10 mg) was incubated at the specified concentrations of [^{35}S]sodium dodecyl sulfate in 2 ml of 0.05 M ammonium acetate buffer (pH 8.45) for 1 hr with shaking at 37°. The suspended elastin was then centrifuged and the radioactivity of the supernatants and of control solutions of [^{35}S]sodium dodecyl sulfate in buffer but lacking elastin were separately determined. As shown (Figure 4), the difference between the curves represents the sodium dodecyl sulfate bound to the pelleted elastin. As also shown in Figure 4 (inset), the binding curve plateaus between 0.35 and 0.5% yielding an apparent maximum binding ratio of approximately 0.5 of detergent/g of elastin.

Binding of Elastase to Elastin. Previous studies have shown that elastase can bind to elastin yielding a sedimentable enzyme–substrate complex (Robert and Robert, 1970; Gertler, 1971). It was of interest to examine the effect of detergent on the formation of this complex (Figure 5).

In the experiment presented in Figure 5 (control) 10 mg of elastin was preincubated at 4° with 20 μg of elastase for 2 min in 2 ml of 0.05 M ammonium acetate buffer (pH 8.45). The pellet was isolated by centrifugation at 4°, resuspended in fresh buffer, and incubated at 37° for the times indicated to allow solubilization to proceed. The supernatant obtained by centrifuging the original suspension was also assayed for elastase activity by adding to it 10 mg of fresh elastin and then incubating this suspension at 37°, as well. As a control, a duplicate elastin–elastase mixture was incubated at 4° and then at 37° without separating the pellet from the suspension. The rates of solubilization during the 37° incubation of elastin were taken in each case as reflective of the relative proportions of elastase present in the original suspension and in the resulting pellet and supernatant. The results indicate that, under the stated conditions, approximately one-third of the elastase originally added is bound to the pelleted elastin, while two-thirds can be accounted for in the elastin-free supernatant.

The effect of sodium dodecyl sulfate on this distribution of free and bound enzyme was explored employing basically the same protocol. The elastin was preincubated with 0.15%

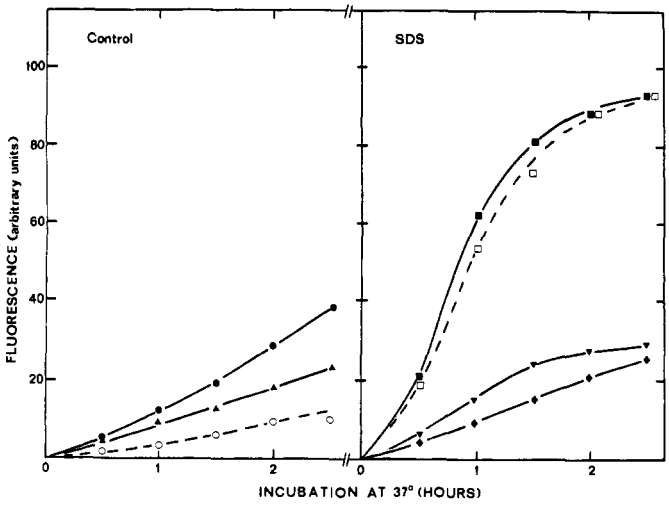

FIGURE 5: Effect of sodium dodecyl sulfate on the binding of elastase to elastin. *Control* (left), solubilizing enzyme activity: (●) in original suspension; (○) in pellet resuspended in fresh buffer; (▲) in supernatant. SDS (right), solubilizing enzyme activity: (■) in original suspension; (□) in pellet resuspended in fresh buffer; (▼) in pellet resuspended in fresh buffer containing 0.15% sodium dodecyl sulfate, (◆) in supernatant.

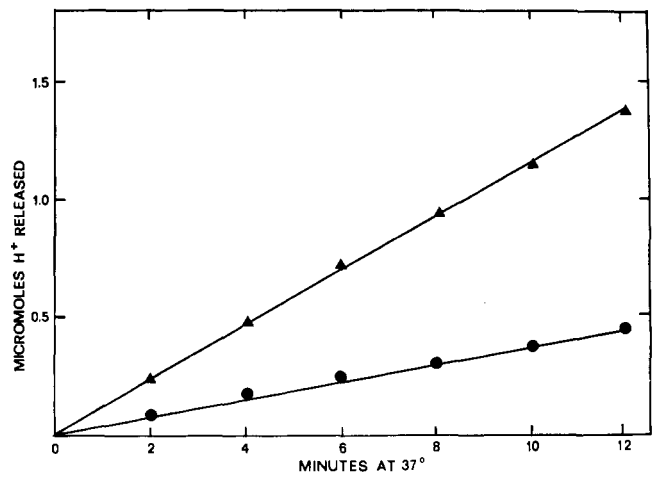

FIGURE 6: Effect of sodium dodecyl sulfate on peptide-bond hydrolysis in insoluble elastin (10 mg) by elastase (20 μg). (●) Control; (▲) elastin in 0.1% sodium dodecyl sulfate.

sodium dodecyl sulfate in the same volume and buffer as above prior to the addition of enzyme. After addition of enzyme and incubation at 4°, the pellet was isolated and resuspended either in fresh buffer which was free of both sodium dodecyl sulfate and enzyme or in buffer which contained sodium dodecyl sulfate at a concentration of 0.15% (Figure 5). Estimating at the 1-hr assay times, the pellet which was isolated and then resuspended in enzyme-free and sodium dodecyl sulfate free buffer is solubilized at approximately 85% of the rate of the original suspension. The supernatant isolated after removal of the pellet solubilizes a previously untreated 10-mg sample of elastin added to it at about 15% of the rate of the original suspension.[2] The close similarity between the activities of the pellet suspended in sodium dodecyl sulfate free buffer and of the original suspension in 0.15% sodium dodecyl sulfate suggests both that the pellet binds most of the enzyme present and that the sodium dodecyl sulfate which binds to the elastin resulting in stimulation of elastolysis remains bound during pellet separation and resuspension procedures. This is strengthened by the finding, also shown in Figure 5, that resuspension of the sodium dodecyl sulfate–elastin–enzyme pellet in a new 2-ml aliquot of 0.15% sodium dodecyl sulfate results in a markedly lower elastolytic rate as compared to the original suspension in 0.15% sodium dodecyl sulfate. This would be expected if the pellet retained the sodium dodecyl sulfate bound to it from the original 0.15% suspension and then additional sodium dodecyl sulfate would result in the inhibition of elastolysis previously observed at 0.25 to 0.35% sodium dodecyl sulfate (see Figure 2). Comparison of the results of both experiments of Figure 5 suggests, therefore, that sodium dodecyl sulfate facilitates the binding of enzyme to elastin.

pH–Titration Studies of Elastoylsis. The enzymatic digestion of elastin can be readily followed by pH-Stat titration of protons released upon peptide-bond hydrolysis. As shown (Figure 6), the addition of 20 μg of elastase to an aqueous suspension of 10 mg of elastin results in the immediate release of protons with time. As in the case of the solubilization studies, 0.15% sodium dodecyl sulfate increases the rate of proteolysis of elastin 3- to 4-fold.

It was observed in the elastase binding experiment that sodium dodecyl sulfate pretreated elastin which was isolated as a pellet and then resuspended in sodium dodecyl sulfate free buffer still displays the properties of the sodium dodecyl sulfate activated elastin substrate. This was explored further, isolating elastin from a series of suspensions which were preincubated with varying concentrations of sodium dodecyl sulfate, resuspending the pellets in water, and assaying the substrate properties of the pretreated elastin upon the addition of elastase by the pH-Stat method. The assays were compared to pH-Stat assays done with the original suspensions of elastin in the presence of these same concentrations of sodium dodecyl sulfate (Figure 7). The velocity–concentration profile obtained with the original suspensions is very similar to that obtained by the solubilization assay. Thus, hydrolysis is maximally stimulated 3- to 4-fold over the control at 0.15% while additional sodium dodecyl sulfate results in inhibition of elastolysis. In marked contrast, the activities obtained with the isolated pellets do not show an inhibitory phase at the higher concentrations of sodium dodecyl sulfate, but continue to increase past 0.15% sodium dodecyl sulfate to a plateau level between 0.4 and 1.0% sodium dodecyl sulfate of 6-fold stimulation of hydrolysis. This curve relating the substrate properties of elastin to sodium dodecyl sulfate concentration compares favorably to the concentration dependency of [^{35}S]sodium dodecyl sulfate binding to elastin (see Figure 4, inset).

These pH-Stat studies indicate that the enhancement of solubilization by sodium dodecyl sulfate reflects the primary catalytic event, that is, the enhancement of hydrolysis of peptide bonds. However, while the initial rate of proteoly-

[2] While the activity of the supernatant is about 15% of the control suspension, it is higher than would be expected considering that the substrate is elastin which had not been pretreated with sodium dodecyl sulfate. However, as revealed by the sodium dodecyl sulfate binding experiment (see Figure 4), this supernatant still contains about 30% of the sodium dodecyl sulfate originally added to the initial elastin suspension, and so, some stimulation of elastolysis is to be expected. Furthermore, the original detergent–elastin–elastase suspension was preincubated at 4° for 2 min, conditions which likely account for the apparent resistance of elastase to free sodium dodecyl sulfate in the supernatant.

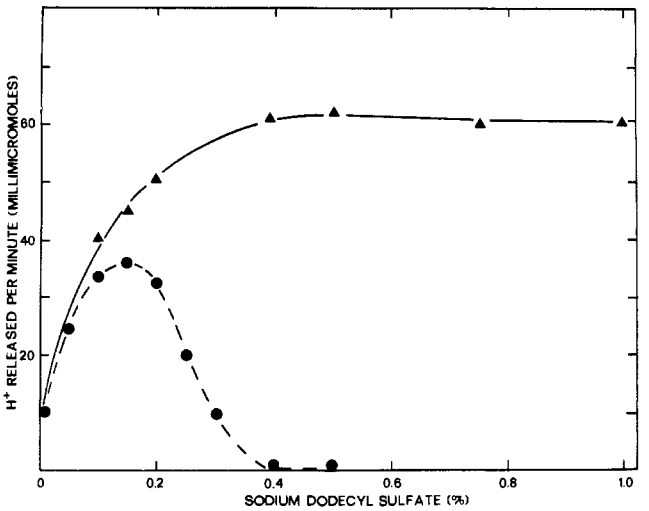

FIGURE 7: Peptide bond hydrolysis in elastin suspended in sodium dodecyl sulfate and in the isolated sodium dodecyl sulfate–elastin complex. (●), elastase (20 μg) added to elastin (10 mg) suspended in 2 ml of designated concentrations of sodium dodecyl sulfate; (▲), elastase added to elastin isolated from sodium dodecyl sulfate suspensions and resuspended in 2 ml of water.

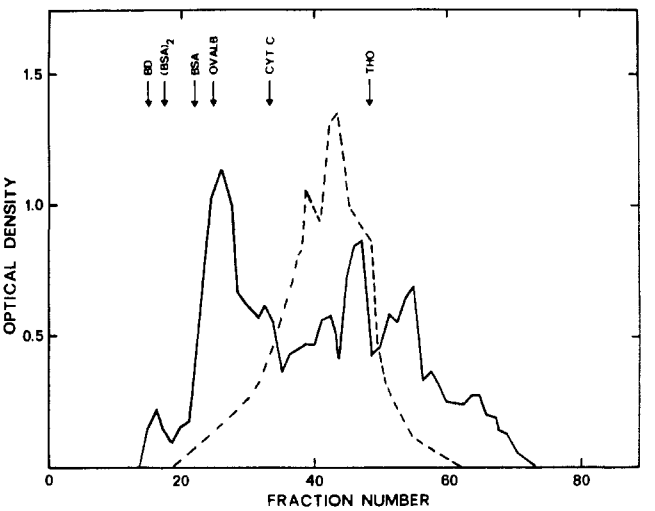

FIGURE 9: Gel exclusion chromatography of elastin digests. Fifty milligrams of insoluble elastin was digested at 37° for 18 hr with 100 μg of elastase in 0.05 M ammonium acetate (pH 8.45), in the presence (—) or absence (– – –) of 0.15% sodium dodecyl sulfate. The digests were concentrated to 2 ml and applied to a 79 × 2.5 cm column of 10% agarose (Bio-Gel A 0.5m). The eluting buffer was 0.02 M sodium phosphate (pH 7.0). Fractions of 8.3-ml volume were collected, and analyzed for optical density at 250 nm. Abbreviations and molecular weights of known proteins are as follows: BSA = bovine serum albumin, 55,400; $(BSA)_2$ = BSA dimer, 110,800; Ovalb. = ovalbumin, 45,000; Cyt *c* = cytochrome *c*, 12,400. BD = Blue Dextran, THO = tritiated water.

sis of elastin is stimulated, the total number of elastin peptide bonds which are attacked by elastase is significantly reduced in the presence of sodium dodecyl sulfate (Figure 8). As shown, carrying the digestion of equal amounts of elastin to completion in the presence and absence of sodium dodecyl sulfate reveals that approximately 15% fewer bonds are split in the presence of sodium dodecyl sulfate. Additional elastase was added at the points indicated by the arrows to determine whether the plateau regions actually represented completion of the enzymatic digestion. There was no further release of protons either in the presence or absence of sodium dodecyl sulfate indicating that proteolysis was maximal in each case.

Chromatography of Elastin Digests. The overnight digests of elastin incubated in the presence or absence of 0.15% sodium dodecyl sulfate were each chromatographed through a column of 10% agarose (Figure 9). The elution positions of proteins of known molecular weight which were also chromatographed through this column are indicated. A plot of elution volumes *vs.* the logarithm of the molecular weight of these proteins yielded a straight line indicating the ability of the column to resolve by virtue of solute size (Andrews, 1964). The elastin digested in the absence of sodium dodecyl sulfate is composed of protein fragments having a broad distribution of comparatively small molecular weights with the major products occurring over a molecular weight range of 2000–10,000. In contrast, the digest in sodium dodecyl sulfate shows the accumulation of a prominent peak with a molecular weight of about 30,000 g/mole with lesser amounts of smaller species distributed throughout the chromatogram. The high molecular weight band did not have a significant amount of sodium dodecyl sulfate associated with it, as detected by radioactivity measurements for bound [^{35}S]sodium dodecyl sulfate in separate experiments. Furthermore, the addition of sodium dodecyl sulfate to the control elastase digest just before application of this digest to the column did not alter the chromatographic behavior from that shown in Figure 9. Thus, these chromatographic profiles are not significantly affected by the detergent and indicate that large fragments accumulate in elastase digests of elastin when sodium dodecyl sulfate is present.

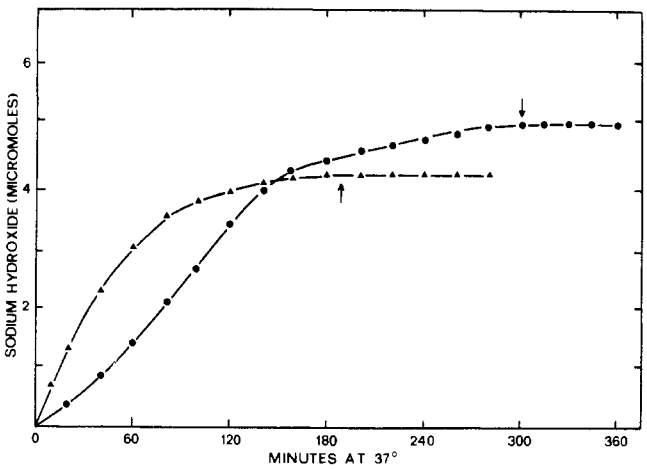

FIGURE 8: Effect of sodium dodecyl sulfate on the digestion of elastin to completion by elastase. (●) Control; (▲) elastin suspended in 0.15% sodium dodecyl sulfate. Additional aliquots of elastase (10 μg) were added at the points indicated by the arrows.

Circular Dichroism Studies. The far-ultraviolet CD spectrum of α-elastin in water and in 0.15% sodium dodecyl sulfate is shown in Figure 10. The spectrum in water shows two prominent negative cotton effects at 201 and 220–230 nm, respectively. This spectrum is not typical of a completely randomly oriented polypeptide and, with particular reference to the band at 220–230 nm, has been interpreted to reflect the presence of a small amount of α-helical structure in this protein (Mammi *et al.*, 1968). The spectrum in sodium dodecyl sulfate is quite different from that in water. Thus, the band at 201 nm is red shifted to 205 nm while the nega-

tive band at higher wavelength becomes intensified and shows a distinct center at 222 nm. In addition, a strong positive band at about 190 nm is generated. This change in the spectrum is consistent with the induction of a conformational change in α-elastin by sodium dodecyl sulfate which leads to a higher degree of α-helix content.

Discussion

The general resistance of elastin to proteolysis by mammalian enzymes is an important feature of this structural protein. This resistance doubtlessly contributes to the stability and longevity of elastic fibers in connective tissues. Although elastase does readily attack elastin, the present study indicates that elastin is rendered even much more susceptibile to digestion by elastase in the presence of the detergent, sodium dodecyl sulfate.

It is clear that the stimulating effect is not due to sodium dodecyl sulfate directly increasing the intrinsic catalytic ability of elastase. Thus, elastase activity toward the ester substrate is destroyed by very low concentrations of the detergent. This finding is in agreement with the previous studies of Visser and Blout (1971) who also showed that the detergent causes a marked conformational change in the enzyme. The present studies further indicate that low concentrations of sodium dodecyl sulfate will also inactivate the proteolytic activity of the enzyme against insoluble elastin, but only if the enzyme is first preincubated with the detergent. Thus, elastin will not reverse the deleterious effect of sodium dodecyl sulfate on the enzyme.

It is apparent, then, that it is the interaction of sodium dodecyl sulfate with elastin which results in the enhancement of proteolysis. Indeed, the binding experiment employing [^{35}S]sodium dodecyl sulfate indicates that insoluble elastin will bind up to 0.5 g of detergent/g of protein under the conditions of the assay. Further, the degree of binding of sodium dodecyl sulfate coincides well with the generation of the activated substrate property of the protein. However, the binding experiment also shows that at any given concentration of sodium dodecyl sulfate up to 0.35%, approximately 30% of the detergent remains unbound. Therefore, at 0.15% sodium dodecyl sulfate, the optimum level for elastolysis of 10 mg of elastin, there is the equivalent of 0.015% bound and 0.045% sodium dodecyl sulfate free in the supernatant. Taken at face value, this result would seem to be inconsistent with the sensitivity of elastase to this concentration of detergent established by the preincubation experiment and the esterase assays. Further, this result does not seem to be explicable in terms of the sodium dodecyl sulfate monomer:micelle ratio since the unbound detergent would appear to be primarily in the monomeric state at the ionic strength and sodium dodecyl sulfate concentrations employed in these studies (Reynolds and Tanford, 1970). Presumably, monomeric sodium dodecyl sulfate could interact with elastase as it does with a variety of proteins (Reynolds and Tanford, 1970). It would seem, therefore, that this apparent resistance of elastase is due to a significant time dependency of the interaction of sodium dodecyl sulfate with elastase and/or a comparatively rapid binding of the enzyme to its protein substrate resulting in a complexed form of the enzyme which is protected from sodium dodecyl sulfate inactivation. In this regard, we have performed preliminary studies (H. M. Kagan, unpublished data, 1972) in which we have observed that the changes in the low uv circular dichroic spectrum of porcine elastase induced by adding 0.1% sodium dodecyl

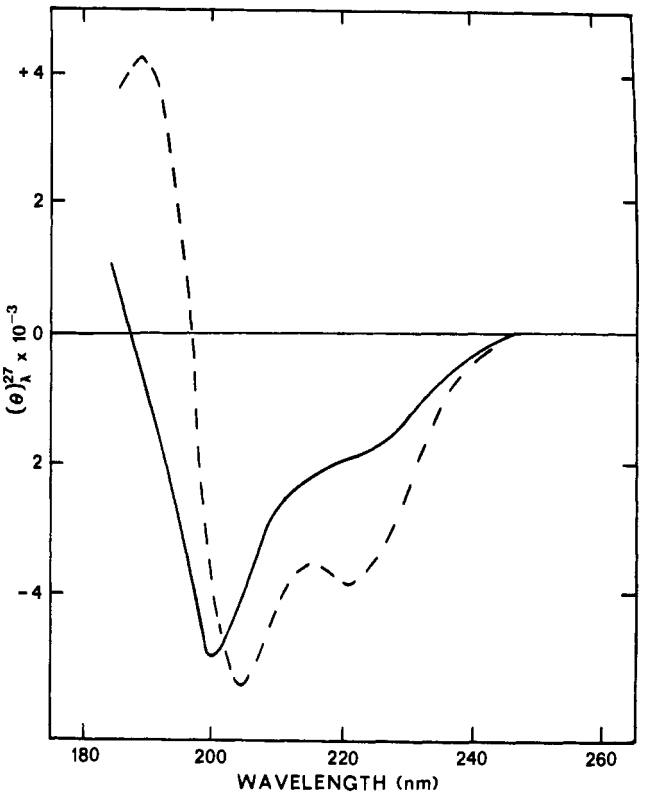

FIGURE 10: Circular dichroic spectra of α-elastin. (——) In water; (---) in 0.15% sodium dodecyl sulfate.

sulfate exhibit a significant time dependency. In its simplest terms, therefore, resistance of elastase to the free sodium dodecyl sulfate in the assay mixture may reflect a higher affinity between the enzyme and substrate than between the enzyme and detergent.

The binding of elastase to elastin is unusual in that the enzyme is sufficiently strongly absorbed to allow the isolation of the enzyme–substrate complex simply by centrifuging. The studies of Robert and Robert (1970) indicate the existence of specific enzyme fixation sites on elastin fibers from which elastase diffuses very slowly and only after some proteolysis has occurred. The present studies reveal that the sodium dodecyl sulfate–elastin complex has an increased ability to bind the enzyme. This property of the sodium dodecyl sulfate–elastin complex may be an essential factor in increasing the susceptibility of the complex to elastase digestion.

The findings of Hall and Czerkowski (1961) and of Gertler (1971) both indicate that elastase is absorbed to elastin by electrostatic attraction between the negatively charged carboxylate groups of elastin and positively charged groups of the enzyme. The binding of sodium dodecyl sulfate to elastin would be expected to result in an increase in the anionic character of the protein as its sodium dodecyl sulfate complex. The apparent increase in the affinity of the complex for elastase is consistent, therefore, with the proposed mechanism of enzyme–substrate interaction. Experiments to substantiate this are in progress.

The circular dichroic spectrum of soluble α-elastin undergoes a dramatic change upon the addition of sodium dodecyl sulfate. The CD spectrum of the sodium dodecyl sulfate complex is, in fact, similar to that reported by Urry *et al.* (1969) for films of coacervated elastin and with those of a trifluoro-

ethanol solution of α-elastin reported by Urry *et al.* (1971). Each of these CD spectra is similar to those of α-helical proteins (Beychok, 1967). The ability of the detergent to convert α-elastin from a mostly random conformation to one possessing a higher order of structure raises the possibility that sodium dodecyl sulfate induced conformational changes may also occur in insoluble elastin which could have an effect both on the affinity of the protein for elastase as well as on the accessibility of peptide bonds to proteolysis. It is of interest in this regard that Kornfeld-Poullain and Robert (1968) have observed that a variety of organic solvents stimulate the rate of solubilization of elastin by potassium hydroxide. The efficiency of these solvent molecules in this process increased with the number of methylene groups per molecule. These investigators concluded that the interaction between these stimulating agents and elastin was largely hydrophobic and suggested that the solvents induced a conformational change in elastin enhancing access of peptide bonds to hydroxide ions.

While sodium dodecyl sulfate stimulates the initial elastolytic rate, significantly fewer bonds are hydrolyzed in the insoluble protein in the presence of the detergent. This observation coincides with the results of gel chromatography indicating the accumulation of large molecular weight fragments of elastin in the elastase digest of the suspension in sodium dodecyl sulfate. This is in agreement with the report of Hall and Czerkowski (1961) that α-elastin accumulates in the presence of the detergent. Further, their suggestion that a second phase of elastolysis in which the breakdown of α-elastin to smaller fragments occurs is inhibited by sodium dodecyl sulfate is also substantiated by the present observation that the proteolysis of α-elastin is, indeed, inhibited by the detergent. The fact that this inhibition extends over a much higher range of sodium dodecyl sulfate concentration than is required to inactivate the unprotected enzyme likely reflects the ability of α-elastin to bind the detergent, hence reducing its effective concentration. However, it should be noted that the chromatographic analysis of the elastin digest in sodium dodecyl sulfate reveals that there is a considerable amount of small molecular weight species present in addition to the high molecular weight material. This result is not surprising considering that digestion of α-elastin is only partially inhibited at the sodium dodecyl sulfate concentration used (0.15%). Recognition should also be made of the likelihood that α-elastin produced during elastolysis may differ from α-elastin obtained by oxalic acid hydrolysis, as used in the present studies.

In summary, the binding of sodium dodecyl sulfate to elastin exerts a profound effect on the susceptibility of the insoluble protein to digestion by elastase. This effect could stem from both an increase in negative charge on the resulting elastin complex allowing enhanced binding of elastase as well as from a detergent-induced conformational change in the protein substrate. While the mechanistic basis for these stimulating effects remain to be further elucidated, the impact of a similar phenomenon *in vivo* could be considerable. Thus, while sodium dodecyl sulfate is not a physiologically important compound, its property of separated polar and apolar regions is shared by fatty acids, phospholipids, and other naturally occurring compounds, many of which, including fatty acids and phospholipids, are known to bind to elastic fibers *in vivo* (Kramsch *et al.*, 1971). Our recent studies indicate that certain of these physiological compounds do, indeed, cause a similar marked enhancement in elastolytic rates when added to elastin suspensions. It is of interest in this regard that atherosclerotic arterial walls show evidence of deposition of cholesterol, phospholipids, and other fat-soluble substances at elastic membranes (Kramsch *et al.*, 1971) as well as apparent fragmentation of these membranes (Adams and Tuquan, 1961). Among others, Loeven (1969) has raised the possibility that proteolysis of elastic fibers may play a role in weakening of arterial walls and plaque formation in this disease. An effect such as described in the present study in which the deposition of detergent-like lipid molecules on elastin fibers renders the fibers more susceptible to enzymatic digestion could be of significance in such disease mechanisms.

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