THE SOLUBILITY AND PROPERTIES OF A PURIFIED ICHTHYOCOL IN SALT SOLUTIONS OF NEUTRAL pH

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Heretofore most studies dealing with the actions of enzymes on collagen have been carried out with the substrate in solid phase, for example, in suspension. However, in the course of an investigation undertaken in this laboratory employing a bacterial collagenase for the determination of some features of structure of a purified ichthyocol, the kinetic methods used, such as the measurement of changes of viscosity and optical rotation, required that the substrate initially be in solution without denaturation in a medium of neutral pH. Thus it was necessary to find methods by which to disperse collagen under neutral conditions and to redetermine some of the definitive physical and chemical properties of the solubilized protein in order to rule out the possibility that denaturation or gelatinization had occurred.

Extraction of collagen from skins and from hide powder by socalled neutral salts, but under conditions predisposing to the denaturation of the protein, have been described (1). More recently, Gross, Highberger, and Schmitt (2); Harkness, Marko, Muir, and Neuberger (3); Neuberger (4); and Jackson and Fessler (5) have reported the extraction of undenatured collagen from native tissues by certain salt solutions under conditions of neutral or slightly alkaline pH. The present authors, however, desired to begin their study with an already purified and well characterized preparation. For this reason the collagen selected was the citrate-extracted ichthyocol of carp swim bladder, some of the physical and chemical properties of which have already been established in acidic solution by Gallop (6, 7), Cohen (8), and Boedtker and Doty (9, 10). Additional chemical properties of this collagen have been determined and are included in the present report; these serve to define even more precisely the nature of the starting material used in this study.

Materials

Citrate-extracted ichthyocol, referred to hereafter simply as ichthyocol, was prepared as described by Gallop (6). The wet fibrils which formed when the acid-dispersed collagen was dialyzed against 0.02 M Na_2HPO_4 solution, were harvested by centrifugation, washed with distilled water three times and each time collected by centrifugation, suspended in water, and

then dried from the frozen state. The lyophilized ichthyocol was kept in the refrigerator at approximately 2 to 5°C., and was shown to be stable under these conditions as determined from the constancy of optical rotatory and viscous properties of solutions made from it periodically over one year's time.

The chemicals employed were the best quality which could be purchased, and, when available, specifically of the analytical reagent grade.

The arginase used for the analysis of arginine was obtained from the Worthington Biochemical Co., as was the crystalline trypsin. The collagenase employed was that elaborated by *Clostridium histolyticum*. Crude preparations were furnished by Drs. Joseph Seifter and George Warren of the Wyeth Institute for Medical Research and by Dr. LeRoy Klein of Boston University. From these materials a purified collagenase, free of non-specific proteinases, was obtained by methods reported elsewhere (11, 12).

Methods

Chemical Methods.—Most of the chemical analyses on collagen were performed after conversion of the protein to gelatin by heating at 70°C. for 10 minutes. In the case of collagen in solid phase, this was done after the material was brought to a definite volume with distilled water. The purpose of this step in analysis was either to have solutions of relatively low viscosity to insure accurate pipetting, or to have material in an accurately measurable volume.

Total nitrogen was determined by micro-Kjeldahl analysis using the procedure of Ma and Zuazaga (13). Protein *per se* was determined by a biuret procedure (14) or, when a more sensitive method was required, by the procedure of Lowry *et al.* (15). The total carbohydrate of the ichthyocol was measured by an anthrone method (16) and expressed in terms of the quantity of glucose which, under the conditions of the reaction, produced an equivalent amount of color. Hexuronic acid and hexosamine analyses were kindly performed by Dr. LeRoy Klein using respectively the methods of Fishman and Green (17) and Boas (18).

In preparation for the determination of the several amino acids studied, with the exception of hydroxyproline, an acid hydrolysate of ichthyocol was made by placing an aliquot of the protein in 6 N HCl in a sealed tube and heating at 100°C. for 18 hours. At the end of this time the mixture was neutralized with NaOH and brought to a definite volume with distilled water; suitable aliquots were then removed for analysis.

Since it has been observed by the present authors and by others (19) that hydroxyproline is partially destroyed by treatment with hot HCl, a separate basic hydrolysate of the protein was prepared for analysis of this amino acid as follows. An aliquot of the collagen was made 1.25 N with respect to NaOH and heated in a covered test tube at 100°C. for 8 hours. At the end of this time the material was transferred quantitatively to a suitable volumetric flask and diluted to the mark with water; aliquots were then removed for analysis.

Glycine was determined by the chromotropic acid method of Alexander, Landwehr, and Seligman (20); and *serine*, after oxidation by periodate, was measured as formaldehyde by the same method. *Proline* was determined by the procedure of Chinard (21). For the analysis of *arginine*, advantage was taken of the fact that the Chinard method measures ornithine as well as proline. Thus a second aliquot of the neutralized acid hydrolysate was incubated with a purified preparation of arginase causing a quantitative conversion of arginine to ornithine. The Chinard reaction was then performed on this sample to yield a value for proline plus ornithine. The previously determined proline was then subtracted and a figure for ornithine thus obtained. From this latter value, the content of arginine was determined by a simple calculation. *Hydroxyproline* was measured by a modification of the method of Neuman and Logan (22, 23).

Physical Methods.—All solutions subsequently examined for viscosity or optical rotation were first cleared by centrifugation for one hour in a Spinco model L preparative ultracentrifuge at 60,000 times gravity or greater.

Viscosity measurements were made at 20.0°C. in Ostwald-Fenske viscometers with flow times of about 70 seconds with water at this temperature. No study of gradient dependence of the intrinsic viscosity was made. Determinations of optical rotation were performed in one decimeter cells using the Keston polarimeter attachment (Standard Polarimeter Co., New York) to the Beckman DU spectrophotometer. In one case measurements were made using a Rudolf Model 75 precision polarimeter. Sedimentation studies were performed using a Spinco Model E ultracentrifuge.

Methods for Studying Solubilization.—An approximately 0.4 per cent suspension of collagen, the exact concentration of which was determined subsequently by micro-Kjeldahl analysis, was made by homogenizing a weighed amount of lyophilized ichthyocol for 4 minutes at 0 to 2°C. with a Teflon-glass homogenizer. The temperature was carefully controlled in order to prevent gelatinization by heat arising during the homogenization process. The suspending medium was either distilled water, 0.1 M sodium acetate solution, or 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer of pH 7.0, depending upon the nature of the study and the methods of analysis to be used. In each case the resulting suspension was uniform and easily pipetted with only small differences among similar aliquots as determined by biuret analysis.

While keeping all solutions in an ice bath, 2 ml. aliquots of the well mixed ichthyocol suspension were delivered into a series of centrifuge tubes, and to each tube was then added 2 ml. of a given concentration of the agent under study. The contents of the tubes were mixed, and all tubes were placed in an ice bath in a refrigerator for 18 hours. Thus the temperature was maintained between 0 and 2°C. during the course of the experiment. At the end of this time the undispersed collagen was separated by centrifugation at 0 to 2°C. at 2500 R. P. M. using an International refrigerated centrifuge. The supernates were decanted and saved for analysis. The precipitates were carefully resuspended and washed with cold distilled water and separated again by centrifugation. The supernates previously obtained were analyzed for nitrogen by micro-Kjeldahl except in those cases in which nitrogen was part of the agent being tested. In all instances the washed precipitates were converted to gelatin and subjected to analysis by the biuret procedure. Thus both an index of the quantity of ichthyocol dissolved and of that remaining undissolved were obtained.

RESULTS

Chemical Constituents of Ichthyocol.—Table I shows the results of a partial chemical analysis of purified citrate-extracted ichthyocol. Such an analysis, except for the hexosamine and hexuronic acid determinations, has been carried out on three separate preparations; the reported values are reproducible and serve to characterize the starting material used in the present and subsequent physicochemical and enzymatic studies. In general, the contents of the several amino acids as given in the table are similar to those reported for other fish collagens (24) except that one of the values for hydroxyproline reported here is significantly higher. This difference is probably explained by the fact that in this case the amino acid was determined after basic hydrolysis of the protein, under which conditions hydroxyproline is not destroyed.

Solubilization by Salt Solutions at Neutral pH.—Table II shows the degree of solubilization or dispersion of a fixed amount of ichthyocol in varying concentrations of different salts held in a constant volume. Such studies have been made with four separate preparations of ichthyocol with almost identical results. In the particular experiment summarized in the table, sodium acetate, as indicated, was frequently used to maintain the pH of the solutions in an ap-

proximately neutral range. However, detailed studies in the absence of sodium acetate using salt solutions adjusted to pH 7 with HCl or NaOH were also made, and some of the results are included in the table. Similar experiments were also performed using salt solutions buffered at pH 7.0 with 0.05 M Tris buffer, and again comparable results were obtained, although the analyses were carried out by the biuret method since the buffer contained nitrogen. When sodium acetate was used to maintain pH, analyses were performed both by biuret and Kjeldahl procedures.

TABLE I

Partial Chemical Analysis of the Ichthyocol Used in These Experiments

Substance	Amount per 100 gm. of protein
	gm.
Total nitrogen	17.0
Carbohydrate (equivalent to glucose)	0.60
Hexosamine	0.16
Hexuronic acid	0.08
Glycine	19.0
Proline	18.4
Hydroxyproline*	7.4
Hydroxyproline‡	9.7
Arginine	9.5
Serine§	4.5

* After acid hydrolysis of the protein.

‡ After basic hydrolysis of the protein.

§ Uncorrected for hydroxylysine and carbohydrate.

In all cases in which the biuret determination was used for measuring undissolved collagen and micro-Kjeldahl analysis used for determining the ichthyocol in solution, the sum of the two results closely approximated the amount of protein added at the start.

Examination of Table II shows the following:

Potassium chloride, sodium chloride, sodium sulfate, sodium acetate, sodium phosphate buffer, sodium ethylenediaminetetraacetate, sodium citrate, or sodium borate exerted very little or no solubilizing effects on ichthyocol under the conditions of the experiment. It is also seen that sodium acetate itself had no solubilizing action.

Ammonium chloride in a concentration of 1 M slowly dispersed the ichthyocol so that in 18 hours the protein was almost completely in solution. In lower concentrations this salt had no significant solubilizing effect.

Potassium thiocyanate in a concentration of 0.5 M did not dissolve ichthyocol in 18 hours, but in a concentration of 2.0 M caused the immediate and com-

TABLE II

The Degree of Solubilization of Ichthyocol in Various Salt Solutions at 0 to 2°C.

Salt	Final molarity	In the presence of 0.1 M sodium acetate		In the absence of 0.1 M sodium acetate	
		Final pH	Protein dissolved*	Final pH	Protein dissolved*
			gm./100 ml.		*gm./100 ml.*
Potassium chloride	1.0	6.9	0.01		
	0.5	6.8	0.01		
	0.3	6.9	0		
	0.1	6.9	0		
Sodium chloride	1.0	6.9	0.01		
	0.5	7.0	0.02		
	0.3	7.0	0		
	0.1	7.0	0		
Sodium sulfate	0.3	6.9	0.01		
	0.1	7.1	0.02		
Sodium acetate	1.0			7.6	0.02
	0.5			7.4	0.01
	0.3			7.4	0
	0.1			7.3	0
Sodium phosphate	0.5	7.6	0.03	7.5	0.04
	0.3	7.6	0.02	7.4	0.03
	0.1	7.7	0.01	7.6	0
Sodium EDTA	0.17	6.9	0.01		
	0.035	6.4	0		
Sodium citrate	0.5	7.3	0.01		
	0.3	7.3	0		
	0.15	7.3	0		
Sodium borate	0.06	7.4	0.01	7.6	0.01
	0.03	7.8	0.01	7.8	0
Ammonium sulfate	1.0	6.4	0.02		
	0.5	6.4	0.01		
Ammonium chloride	1.0	6.4	0.13	6.4	0.15
	0.5	6.5	0.03	6.4	0.06
	0.3	6.6	0	6.4	0.02
	0.1	6.9	0	6.4	0
Potassium thiocyanate	2.0	7.1	0.17‡		
	1.0	7.1	0.09		
	0.5	7.0	0		
	0.3	7.0	0		
Magnesium chloride	1.0	6.2	0.17‡		
	0.5	6.4	0.17‡		
	0.3	6.6	0.16		
	0.1	6.8	0.03		
Calcium chloride	1.0	6.6	0.17‡		
	0.5	6.6	0.17‡		
	0.3	6.7	0.16		
	0.1	7.0	0.02		
Magnesium thiosulfate	1.0	6.7	0.17‡		
	0.5	6.7	0.17‡		
	0.3	6.9	0.17‡		
	0.1	6.9	0.08		
	0.05	6.9	0.02		
Sodium thiosulfate	1.0	7.0	0.02	6.3	0.03
	0.5	7.1	0.04	6.3	0.04
	0.25	7.0	0.06	6.4	0.17§
	0.1	6.9	0.03	6.5	0
Magnesium sulfate	1.0	6.4	0.11		
	0.5	6.4	0.12		
	0.3	6.6	0.16		
	0.1	6.8	0.04		

* Determined by micro-Kjeldahl and/or biuret analysis.

‡ Dissolved immediately and completely.

§ Completely dissolved after 18 hours.

plete solubilization of the protein. However, as is well known, this salt promotes the rupture of hydrogen bonds with the consequent denaturation of collagen. Thus, in this instance, different from all others included in the table, solubilization was achieved by gelatinization; and, only in this case, removal of the salt by dialysis resulted in the formation of a gel in the cold.

Magnesium chloride, calcium chloride, and magnesium thiosulfate in final concentrations of 0.3 to 1 M caused the immediate and complete solution of ichthyocol.

Sodium thiosulfate and magnesium sulfate each showed an optimum intermediate range of concentrations for complete solution of the collagen. In the case of sodium thiosulfate the optimum occurred at 0.25 M and with magnesium sulfate at 0.3 M. Sodium thiosulfate showed a unique and unexplained difference in behavior depending upon the presence or absence of sodium acetate. While the optimum molarity appeared to be the same under both conditions, the degree of solubilization by sodium thiosulfate was markedly decreased in the presence of sodium acetate.

The results reported in Table II were obtained after the mixtures of ichthyocol and salt solution had been maintained at 0–2°C. for 18 hours. However, in the instances involving calcium and magnesium chlorides, each in concentrations of 0.5 and 1 M, complete solubilization occurred immediately upon mixing. In these cases and those in which full solution occurred at the end of 18 hours, the quantity of ichthyocol dissolved should be considered only as a minimum figure and not as an expression of the full capacity of these salts to solubilize the collagen. Thus, in other experiments in which larger amounts of collagen were added at the start, solutions of the protein containing as much as 400 mg. per 100 ml. were obtained with 0.5 M calcium or magnesium chloride.

Some experiments were performed in which the degree of solubilization was estimated by visual inspection rather than by chemical analysis, and it was learned that 0.5 M and 1 M barium chloride solutions behaved in the same manner as similar solutions of calcium or magnesium chlorides; *i.e.*, they caused the immediate and complete solution of the ichthyocol added. Potassium ferrocyanide and potassium ferricyanide at 0°C. were observed to act in a manner similar to sodium thiosulfate and magnesium sulfate, in that an optimum range of concentrations was exhibited with respect to solubilization of ichthyocol.

The Characterization of Ichthyocol in Neutral Solutions.—The solutions of ichthyocol obtained with the various salts, except that given by potassium thiocyanate as explained previously, were highly viscous. The viscosity fell precipitously when the solutions were heated, indicating that the protein was converted to gelatin. No separation of a solid phase occurred when the solutions were brought to 37°C., as has been reported in the case of other collagens extracted under neutral conditions (25, 5).

Since calcium chloride, magnesium chloride, and sodium thiosulfate had been found to be among the most effective solubilizing agents for ichthyocol under neutral conditions, solutions of the protein prepared with them were used in the characterization studies summarized below. In all cases the solutions were buffered at pH 7.0 with either 0.05 M Tris buffer or 0.05 M sodium maleate buffer, both of which maintained the pH unchanged. Final protein concentrations were determined by the biuret or Lowry methods when Tris was used and by micro-Kjeldahl analysis when sodium maleate was the buffer.

1. Preparation and Stabilization of the Solutions.—An aqueous uniform suspension of the ichthyocol in water, approximately 0.3 per cent in concentration, was mixed at 0°C. with an equal volume of cold solution of the desired

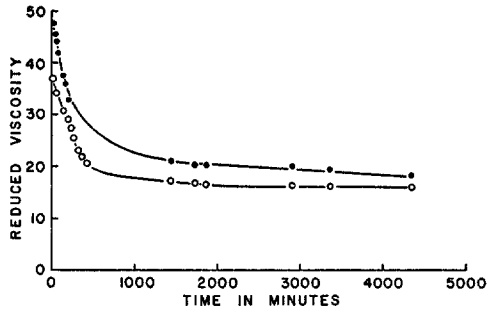

FIG. 1. The change in reduced viscosity with time of solutions of ichthyocol in 0.5 M calcium chloride (●) and magnesium chloride (○) buffered with Tris at pH 7.0.

salt contained in the buffer. Both salt and buffer were in twice the concentration finally desired. After mixing, and when the collagen appeared visually to be in solution, time was allowed for further dispersion of the protein. That this was necessary is shown by the results plotted in Fig. 1, in which it is seen that ichthyocol dispersed in calcium or magnesium chloride continues to undergo a decrease in reduced viscosity until a stable high value is reached. Routinely, therefore, the solutions were made one day previous to their anticipated use and kept in the refrigerator.

2. Intrinsic Viscosity.—Fig. 2 shows a typical plot of reduced viscosity versus concentration of ichthyocol obtained with solutions of that protein in 0.5 M calcium chloride, 0.5 M magnesium chloride, and 0.25 M sodium thiosulfate. It can be seen that the intrinsic viscosity in each case has the high value of 16. The slopes of the curves leading to this value, however, are dissimilar, which probably can be explained by differences in ionic strength of the solutions and by protein-salt interactions.

3. Optical Rotation.—Numerous measurements with solutions of ichthyocol in 0.5 M calcium chloride buffered at pH 7.0 and in sodium thiosulfate adjusted

to pH 7.0 showed specific optical rotations with an average value of −320 ± 30°. When these solutions were heated for 10 minutes at 70°C., the specific rotations fell to an average of −110 ± 20°.

4. Sedimentation.—Preliminary studies of the collagen in solution with sodium thiosulfate and with calcium chloride have been made in the ultracentrifuge. In all cases the material showed a single hypersharp peak similar to that obtained with ichthyocol in citrate buffer at pH 3.7. After heating, the material showed a pattern almost identical with that described for parent gelatin prepared at pH 3.7 (7), including the presence of a small but definite

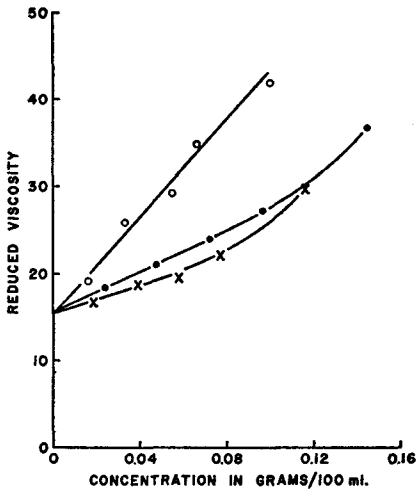

FIG. 2. The reduced viscosities of ichthyocol in 0.5 M calcium chloride (○), 0.5 M magnesium chloride (●), and 0.25 M sodium thiosulfate (×), each buffered at pH 7.0 with sodium maleate. The abscissa represents the concentration of ichthyocol, and the viscosity extrapolated to zero concentration is the value for intrinsic viscosity.

second component. Further analysis of the patterns has not as yet been carried out. Typical sedimentation diagrams of ichthyocol in neutral solution and of the gelatin derived from it are shown in Fig. 3.

5. Reconstitution.—The collagen dissolved in neutral solutions was completely reconstituted into fibers by dialysis against cold running tap water or 0.02 M sodium phosphate (dibasic). These were examined by phase contrast and light- and dark-field microscopy, and appeared to resemble the fibers reconstituted from acid-dispersed ichthyocol. As yet the material has not been studied with the electron microscope.

Action of Enzymes.—The effects of trypsin and of a purified collagenase from *Clostridium histolyticum* on ichthyocol in neutral solution were followed viscometrically at 20.0°C. Thus it was established that this protein in solution to

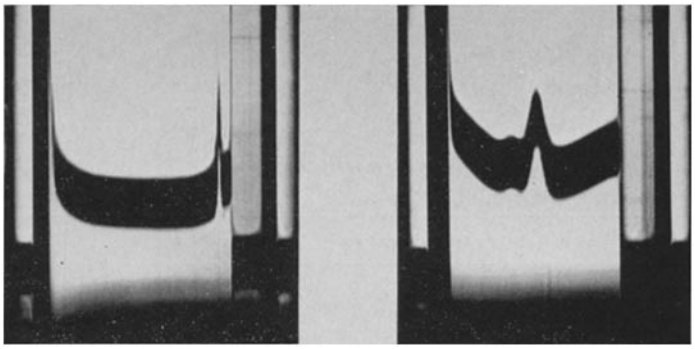

FIG. 3. Sedimentation patterns of ichthyocol and its derived gelatin as determined in neutral solution. The picture on the left was made with 0.12 per cent ichthyocol in 0.25 M sodium thiosulfate at pH 7.0, at a temperature of 15°C., 40 minutes after the rotor was at full speed, using a bar angle of 60°. The picture on the right was made with 0.4 per cent gelatin in 0.25 M sodium thiosulfate at pH 7.0, at a temperature of 33°C., 116 minutes after the rotor was at full speed, using a bar angle of 50°. The speed was 59,780 R. P. M.

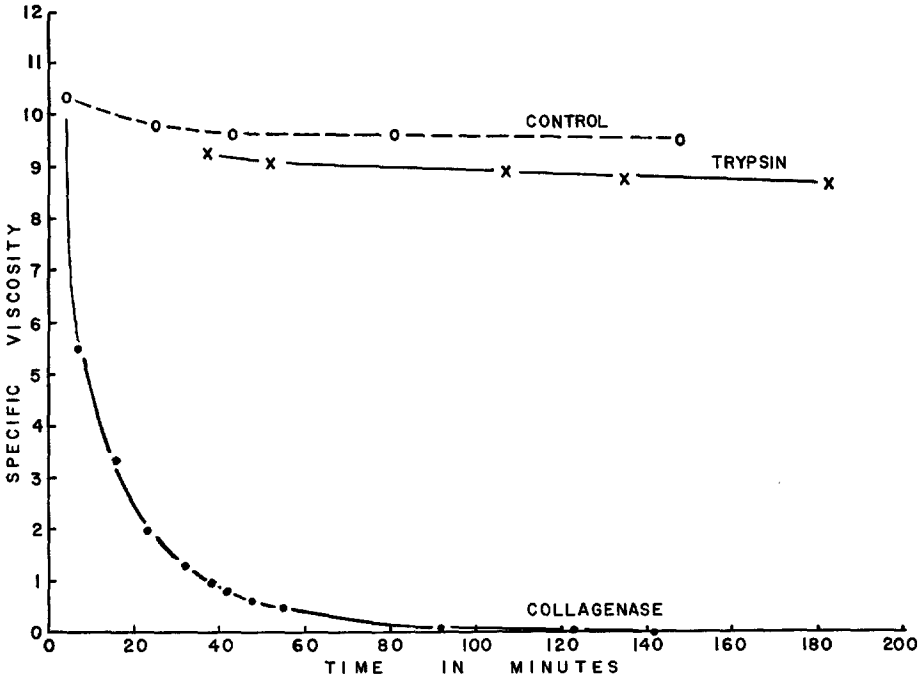

FIG. 4. The change in specific viscosity with time of neutral solutions of ichthyocol as influenced by trypsin and collagenase. See text for explanation.

the extent of 0.2 per cent with 0.5 M calcium chloride, 0.5 M magnesium chloride, or 0.25 M sodium thiosulfate, each buffered at pH 7.0 with Tris, was not degraded by trypsin in a concentration of 100 micrograms per ml. The same solutions of

TABLE III

Comparison of Some of the Properties of Ichthyocol in Neutral Solution with Those of Ichthyocol in Acidic Solution and of Its Derived Gelatin

Property	In neutral solution	In acidic solution	Gelatin
Molecular weight		340,000‡	125,000‡ 138,000‡ 70,000§
Sedimentation constant	*	2.85 S‖ 2.96 S‡	3.31 S§ 3.48 S‡ 3.77 S‡
Intrinsic viscosity	12 to 16	10 to 14‡ ‖	0.34§ 0.44 to 0.55‡
Specific optical rotation	−320 ± 30°	−350 ± 30°¶	−110 ± 20°¶
Particle dimensions			
Diameter		13.6 A‡	
Length		3,000 A‡	
Gelation at low temperatures	Does not set	Does not set	Sets
Reconstitution under defined conditions	Forms fibers	Forms fibers	Does not form fibers
Action of enzymes			
Trypsin	Does not attack	—	Attacks
Collagenase	Attacks	—	Attacks

* Gives a single hypersharp peak in the ultracentrifuge resembling that given by ichthyocol in acidic solution.

‡ Taken from reference (10).

§ Taken from reference (7).

‖ Taken from reference (6).

¶ Taken from reference (8).

ichthyocol were rapidly degraded to material of low viscosity and molecular weight by collagenase. That the presence of salts in such large concentrations was not inhibitory of trypsin was established by studying suitable controls with gelatin dissolved in the same media, and it was found that this protein was very rapidly digested by trypsin. Fig. 4 shows a typical plot of specific

viscosity versus time, obtained, in this case, when the two enzymes were allowed to act at 20.0°C. on a 0.2 per cent solution of ichthyocol in 0.25 M sodium thiosulfate at pH 7.0. It can be seen that the collagen in the control mixture slowly underwent a small degree of denaturation (gelatinization) as evidenced by a slight drop in viscosity of the solution. The addition of trypsin caused a further very slight fall in viscosity which can be attributed to the action of this enzyme on the thermally formed gelatin. However, the addition of collagenase caused a striking reduction in viscosity as the substrate was rapidly converted to molecules of smaller size and axial ratio.

DISCUSSION

Table III presents a summary of some of the properties of ichthyocol as previously determined in acid solution as compared with those of the same material measured in neutral solution. Some of the characteristics of the gelatin derived from the collagen are also included in the table for comparison. The correspondence of the properties of the collagen in acid solution with those observed in neutral solution is striking, and the differences between gelatin and ichthyocol in neutral solution are equally noteworthy. It is therefore apparent that purified ichthyocol dissolved in solutions of calcium or magnesium chloride or of sodium thiosulfate under conditions of neutral pH, is undenatured, and retains the characteristics of the original material. It should be pointed out that the collagen in neutral solution, like that in acid solution (6, 10), tends to be unstable when kept at room temperature for extended periods of time. This was demonstrated by the slow decline in viscosity of neutral solutions of the protein at 20.0°C.

The availability of methods for dissolving ichthyocol under neutral conditions without denaturation has made it possible to study the actions of certain enzymes on the dissolved protein in contrast to the protein in solid phase. Further, the high viscosity of these solutions has provided the basis of a method for studying the degradation of collagen by these enzymes. Thus it has been possible to show that crystalline trypsin is without effect on ichthyocol in solution as it is known to be on the same protein in suspension. Since the same enzyme rapidly digests gelatin in solution, additional evidence is thereby provided showing that the solubilization of ichthyocol occurs without gelatinization. In contrast to trypsin, the bacterial collagenase has been found to degrade ichthyocol in neutral solution as well as in suspension.

SUMMARY

1. Purified citrate-extracted ichthyocol obtained from carp swim bladders has been further characterized with respect to its content of certain amino acids and carbohydrate substances.

2. The degree of solubilization or dispersion of ichthyocol by solutions of

certain salts maintained in the range of neutral pH and at a temperature of 0–2°C. has been determined.

3. While a number of salts of monovalent cations had no significant solubilizing effects on ichthyocol, ammonium chloride in a concentration of 1 M did cause solution of the protein.

4. Sodium thiosulfate in a range of concentrations caused the solubilization of ichthyocol but was most effective in an intermediate concentration of 0.25 M.

5. Several salts of divalent cations, in particular the chlorides of calcium, magnesium, and barium, and magnesium thiosulfate in concentrations ranging from 0.3 to 1 M caused the immediate and complete solubilization of the ichthyocol.

6. Solutions of ichthyocol in calcium chloride, magnesium chloride, and sodium thiosulfate buffered or adjusted to pH 7.0, were studied with respect to intrinsic viscosity of the protein, optical rotation, ultracentrifugal sedimentation, and reconstitution into fibers. It was found in each case that the original characteristics of the collagen, as determined previously in acid solution, were maintained when the protein was dissolved in salt solutions of neutral pH. No evidence of denaturation or gelatinization could be found when ichthyocol was solubilized under the stated conditions.

7. Collagen in neutral solution with sodium thiosulfate, calcium chloride, or magnesium chloride was not attacked by trypsin as determined viscometrically at 20.0°C., but was rapidly degraded by a purified bacterial collagenase.

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