because of the effect of counter ions the apparent low w actually represent a low (less than stoichiometric) $\bar{z}$.

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The Molecular Weights of the α Chains of Chicken Bone Collagen by High-Speed Sedimentation Equilibrium*

Elton P. Katz, Camille J. Francois, and Melvin J. Glimcher

ABSTRACT: High-speed sedimentation equilibrium studies were made of the $\alpha 1$, $\alpha 2$, and $\alpha 3$ chains of chicken bone collagen obtained by free-flow electrophoresis of a neutral pH, KSCN, and LiCl extract. All three components were found to be molecularly homogeneous. The molecular weights found were: $\alpha 1$, 92, 100; $\alpha 2$, 91, 200; and $\alpha 3$, 101,000. This gives a molecular weight of the chicken bone macromolecule of 284,000.

A model has been proposed, relating the unequal chain lengths for the α components of the chicken bone collagen macromolecule to certain of its distinctive physicochemical properties.

Although the molecular structure, macromolecular aggregation state, and amino acid composition of bone collagens are qualitatively similar to those of the collagens of unmineralized soft tissues (Eastoe, 1956; Piez and Likins, 1960; Glimcher and Krane, 1968), the collagen as it is organized in bone, possesses certain characteristic physicochemical properties which distinguish it from most of the soft tissue collagens (Glimcher and Katz, 1965; Glimcher *et al.*, 1965). For example, in contrast to the collagens of most other tissues, very little of the collagen of demineralized bone, even in young, rapidly growing animals, can be extracted in solutions normally used to extract the undenatured protein from unmineralized soft tissues (Glimcher and Katz, 1965). On the other hand, unlike the insoluble collagen of bovine Achilles tendon, for example, a large fraction of chicken bone collagen can be extracted as gelatin at neutral pH and in the cold by certain salts known to denature the protein (Glimcher and Katz, 1965). Chicken bone gelatin, like the gelatin obtained from the collagen of soft tissues, contains α components (the single-chain units making up the triple-stranded collagen molecules) as well as the higher molecular weight β-dimer and γ-trimer components formed by covalently cross-linking α chains (Glimcher and Katz, 1965; Francois and Glimcher, 1967a). However, the proportion of α chains in the chicken bone gelatin was found to be unusually high as compared with other normal tissue collagens, especially in the first extracts which

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contained 40–60% of the total collagen present in the bone tissue.[1]

Isolation of the α fractions of gelatin from mixtures containing the α, β, and γ components by CM-cellulose chromatography has not proved very satisfactory, for the repeated chromatography necessary to resolve the α components results in some hydrolysis of the polypeptide chains at the temperature and pH required for the CM-cellulose chromatography (Piez *et al.*, 1963; Lewis and Piez, 1964a,b; Piez, 1965).

However, fractionation of the α components of chicken bone gelatin has been achieved and under milder conditions, by first separating the α components from the higher molecular weight material by molecular sieving, and then fractionating the respective α components by free-flow electrophoresis in urea (Francois and Glimcher, 1967a). The three components obtained by these methods have been designated α1, α2, and α3, respectively, on the basis of their electrophoretic and chromatographic behavior as compared with the α components of codfish skin collagen, and the terminology introduced for the α subfractions of codfish gelatin by Piez (1965).

This communication is concerned with the determination of the molecular weights of the respective α species of chicken bone gelatin obtained by molecular sieving and free-flow electrophoresis, using the high-speed sedimentation equilibrium method of Yphantis (1964).

Experimental Section

Materials. The α1, α2, and α3 components of chicken bone gelatin used in these experiments were freeze-dried aliquot samples of the same fractions obtained from a neutral pH, LiCl, and KSCN extract (Glimcher and Katz, 1965; Francois and Glimcher, 1967a). The fractionation procedures and amino acid analyses have been previously reported (Francois and Glimcher, 1967a). The protein samples were dissolved in 1 M $CaCl_2$–0.1 M Tris buffer (pH 7.4) to give approximately 0.01–0.02% protein solutions and then dialyzed against solvent for 24 hr at room temperature.

Sedimentation Equilibrium. In the high-speed sedimentation method where the concentration at the meniscus is negligible (Yphantis, 1964), the number-average molecular weight, M_n, and the weight-average molecular weight, M_w, of the protein are given by the relations

$$\frac{\omega^2(1 - \bar{V}\rho)}{RT} \frac{\int C(x)\mathrm{d}(x^2/2)}{C(x)} = \frac{1}{M_n} + A(M_n)C(x) \qquad (1)$$

$$\frac{\omega^2(1 - \bar{V}\rho)}{RT}\left(\frac{\mathrm{d} \ln C(x)}{\mathrm{d}(x^2/2)}\right)^{-1} = \frac{1}{M_w} + 2A(M_w)C(x) \qquad (2)$$

where $A(M_w)$ and $A(M_n)$ are nonideality coefficients, if $\bar{V}$ and $C(x)$ are both determined when the protein is in a state of osmotic equilibrium with respect to the solvent (water and supporting electrolyte; Casassa and Eisenberg, 1961). If the protein is molecularly homogeneous

$$M_w = M_n; \; A(M_w) = A(M_n) \qquad (3)$$

and the nonideality coefficient (sometimes reported as B_1) is related to the second virial coefficient (Casassa and Eisenberg, 1961).

High-speed sedimentation equilibrium experiments were performed in a Spinco Model E ultracentrifuge employing interference optics aligned at the mid plane of a 12-mm cell, and utilizing a multicell rotor, filled-epon, double-sector centerpieces, and sapphire windows. Photographs of the concentration boundary after 20–24 hr of sedimentation were analyzed. No changes in the fringe shift across the cell could be detected for longer sedimentation times. Fringe displacements were determined on a Gaertner microscope using the average of measurements on five to seven fringes starting with 0.100-mm intervals at the top of cell and decreasing to approximately 0.025-mm intervals as the bottom of the cell was approached. The interference patterns were read to approximately 0.050 mm of the cell bottom. Cell and optical system distortion corrections were made after the method of Johnson *et al.* (1959). Data reduction was performed on a PDP-1 computer using a program whereby d ln $C(x)/\mathrm{d}x_2$ was determined from the least-squares slope of ln $C(x)$ and four unweighted data points about x. The integral in the number-average calculation was evaluated by summation of rectangular increments. The intercept and slope of eq 2 for the respective experiments were obtained by unweighted least-squares analysis using only the data for concentrations greater than one fringe displacement (Yphantis, 1964). In the case of the number-average slopes and intercepts, least-square analyses were performed using only data greater than two fringe displacements since the lower concentration points are more susceptible to systematic error.

The value of $\bar{V}$ = 0.695 obtained both by the pycnometric and the density gradient column method for a 1% solution of codfish gelatin after dialysis against 1 M $CaCl_2$ (pH 7; Piez, 1965) was used in the calculations. This value was also reported for ichthyol gelatin in 2 M KSCN (Boedkter and Doty,

[1] These findings have been challenged by Miller *et al.* (1967), who found (1) that less than 20% of the bone collagen from 3-week-old chicks could be extracted in guanidine hydrochloride in 1 week, and (2) broadened elution peaks in the region of the α fractions during molecular sieving, and low recovery of protein when the crude, unsieved extracts were chromatographed directly on CM-cellulose. They interpreted these results as indicating the presence of both high molecular weight components, and species of lower molecular weight than the α components, the latter due to hydrolysis. However, guanidine salts extract chicken bone collagen much more slowly than does LiCl or KSCN (Glimcher and Katz, 1965) so that part of the discrepancy between our own findings and those of Miller *et al.* (1967) may be explained on the kinetics of the extraction phenomenon using guanidine hydrochloride as the reagent. Moreover, in our study the bone was finely powdered prior to decalcification and extraction, whereas Miller *et al.* (1967) used small pieces of bone, which would also tend to diminish the rate of protein extraction. Although Miller *et al.* (1967) presented no *direct* evidence either for the presence of high molecular weight aggregates or low molecular weight fragments, it is possible that the protein was hydrolyzed in their experiments, particularly since the chick bones used in their experiments were decalcified in 0.5 M acetic acid for 3 weeks prior to extraction with guanidine hydrochloride. However, since the authors presented no data regarding either the solubility properties or the possible degradation of bone collagen using LiCl or KSCN as solvents under the conditions we described, we do not believe that their data are applicable to our own experiments in which the latter substances were used as reagents. Moreover, examination of LiCl and KSCN extracts of bone collagen by CM-cellulose chromatography and molecular sieving on Sepharose columns, has failed to demonstrate evidence of significant amounts of higher molecular weight aggregates or of a heterogeneous molecular weight population of molecules in the α fraction due to the presence of species of molecular weight lower than the α components.

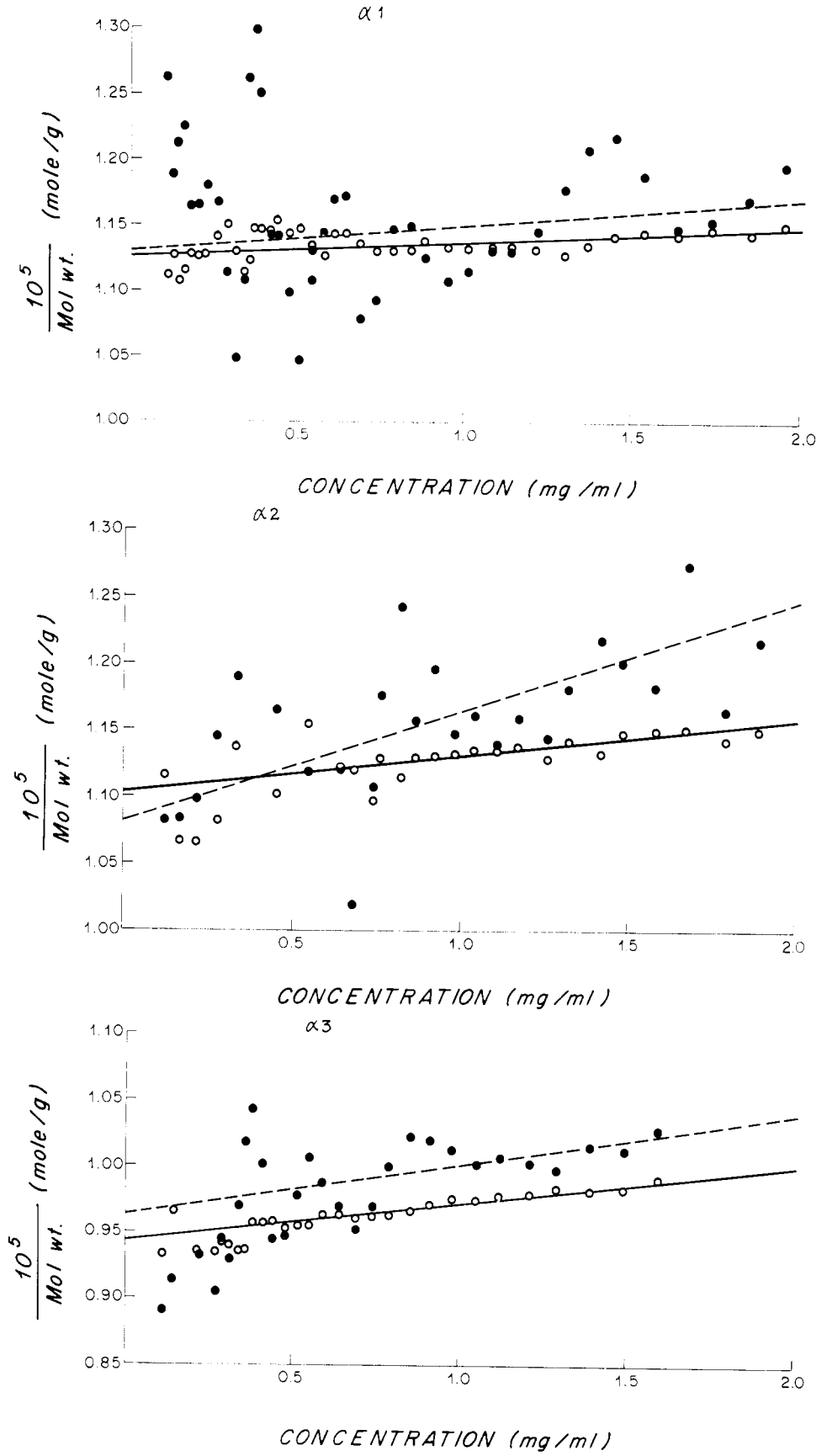

FIGURE 1: Plots of the reciprocal molecular weight against concentration for the α chains of chicken bone gelatin in 1 M $CaCl_2$–0.1 M Tris buffer (pH 7.4) determined at 20,000 rpm at 25°. (O—O) Number-average and (O---O) weight-average points. The lines drawn are from a least-square analysis using data greater than 0.25 mg/ml for the weight-average and 0.5 mg/ml for the number-average points, a, α1; b, α2; c, α3.

TABLE I: Molecular Weight and Nonideality Coefficient of the α Chains of Chicken Bone Collagen in 1.0 M $CaCl_2$–0.1 M Tris Buffer, pH 7.4, at 25°.

Speed (rpm)	Mol Wt (10^5 g/mole) M_w	M_n	Nonideality Coef (10^{-4} ml/g^2) $A(M_w)$	$A(M_n)$
		$\alpha 1$		
19,160	0.884	0.887	1.1	0.9
20,000	0.917	0.940	3.1	4.2
24,630	0.944	0.951	3.0	3.0
Av	0.915	0.926	2.4	2.7
Standard deviation	0.030	0.034	1.1	1.6
		$\alpha 2$		
19,160	0.925	0.910	4.3	3.2
20,000	0.910	0.914	4.3	2.8
24,630	0.908	0.909	4.5	4.9
Av	0.914	0.911	4.4	3.6
Standard deviation	0.010	0.002	0.1	1.1
		$\alpha 3$		
19,160	1.037	1.061	1.8	2.9
20,000	1.006	0.974	3.1	2.4
24,630	0.969	0.999	3.7	5.0
Av	1.004	1.011	2.9	3.1
Standard deviation	0.034	0.045	1.4	1.6

1956). The density of 1 M $CaCl_2$–0.1 M Tris buffer (pH 7.4) at 25°, ρ, is 1.089.

Results

The data obtained from sedimentation equilibrium experiments at a speed of 20,000 rpm for the $\alpha 1$, $\alpha 2$, and $\alpha 3$ components of chicken bone gelatin are shown in Figures 1a–c, respectively, in the form of reciprocal molecular weight *vs.* concentration plots. The plots of such data for other experimental conditions were similar to these. In all cases the weight-average points show considerably more scatter than the number-average points due to the differential calculation of the former *vs.* the integral calculation of the latter.

Molecular weights, calculated from the intercepts, and the nonideality coefficients obtained from the slopes from this experiment as well as others, are shown in Table I for $\alpha 1$, $\alpha 2$, and $\alpha 3$ components, respectively. Very good agreement between the M_w and M_n values was found for any given experiment. The differences that occur between M_n and M_w either from a particular experiment or in the averaged values are not significant. (An analysis of variance performed on the nine M_n and M_w determinations of Table I show that the precision of either an M_n or an M_w determination is approximately 3%, and that the standard error of either M_n or M_w averages is approximately 1.8%.) The nonideality coefficients $A(M_w)$ and $A(M_n)$ from any one experiment are only in fair agreement. The variation in values from experiment to experiment is most likely due to the inaccurate choice of the respective zero concentration base line, which affects the slope far more than the intercepts. The values of the respective nonideality coefficients obtained by averaging, however, are in excellent agreement for each of the materials, indicating a very high level of molecular homogeneity in all cases. The differences between the respective A averages of the three α components are not statistically significant.

TABLE II: The Molecular Weights of the $\alpha 1$, $\alpha 2$, and $\alpha 3$ Components of Chicken Bone Gelatin Computed as the Average Value of the Weight-Averaged and Number-Averaged Molecular Weights.

$\alpha 1$	92,100
$\alpha 2$	91,200
$\alpha 3$	100,800
$\alpha 1 + \alpha 2 + \alpha 3$	284,000
$A \times 10^4$	3.2

The molecular weight values obtained by averaging together the weight-average and number-average values for each of the components are listed in Table II for comparison. The value of 3.2×10^{-4} for the nonideality coefficient shown in this table is the average of all the coefficients in Table I. This value is in very good agreement with 3.0×10^{-4} found both by osmometry and light scattering of whole ichthycol gelatin in 2 M KSCN at 20° (Boedkter and Doty, 1956), 3.5×10^{-4} found by sedimentation equilibrium for calfskin γ component in both 2 M KSCN at 30° and in 0.1 M KCl (pH 6.5) at 40° (Veis *et al.*, 1962), and a value of 3.6×10^{-4} which we have calculated from the data reported on the $\alpha 1$ component of ratskin obtained by sedimentation equilibrium and carried out in potassium acetate buffer (pH 4.8) at 40° (Lewis and Piez, 1964a,b). The molecular weight values for the $\alpha 1$ and $\alpha 2$ components listed in Table II are virtually identical. The $\alpha 3$ value is approximately 10% higher than these. A t test for significance shows that the probability of obtaining this difference by chance is less than 0.01. The possibility that the high value of the molecular weight of $\alpha 3$ compared with the $\alpha 1$ or $\alpha 2$ components may be due to differences in the partial specific volume for these three species is remote. The partial specific volumes of gelatins with quite different amino acid compositions are indistinguishable from each other (Boedkter and Doty, 1956; Piez, 1965; Heaps *et al.*, 1966). Moreover, in the case of the chicken bone gelatins, the amino acid composition of the $\alpha 1$ chain is extremely close to that of the $\alpha 3$ chain (Francois and Glimcher, 1967a).

We conclude therefore that the $\alpha 3$ chain of chicken bone gelatin has a molecular weight higher than that of $\alpha 1$ and of $\alpha 2$.

This finding correlates well with the electrophoretic properties of the α components. The order of mobility in urea solutions in free-flow electrophoresis is $\alpha 2 > \alpha 3 > \alpha 1$ (Francois and Glimcher, 1967a). This is fundamentally a charge effect as attested to by the chromatographic properties of the α fractions on the ion-exchange CM-cellulose columns (Francois and Glimcher, 1967a,b; Piez, 1965). The order of mobility in the more size-sensitive, acrylamide gel electrophoreses of these

components in urea solutions, is $\alpha 2 > \alpha 3 \simeq \alpha 1$ (Francois and Glimcher, 1967a,b), indicating that the greater charge of the $\alpha 3$ component with respect to $\alpha 1$ has been masked by its greater mass.

There have been two previous studies of the molecular weights of the α chains of gelatin by sedimentation equilibrium methods, both using fractions obtained from chromatography on CM-cellulose columns. In a study of ratskin gelatin only two α fractions were resolved, $\alpha 1$ and $\alpha 2$ (Lewis and Piez, 1964a,b). In the study of codfish skin gelatin, an $\alpha 3$ component was resolved from the $\alpha 1$ elution region (Piez, 1965). With the possible exception of the ratskin $\alpha 1$ component, all of the fractions were reported to be heterogeneous. Since an $\alpha 3$ component has also been reported in calfskin gelatins (Francois and Glimcher, 1967b; Heidrich and Wyston, 1965), it is possible that the ratskin $\alpha 1$ fraction might have contained some $\alpha 3$. The M_w values reported for the ratskin $\alpha 1$ fraction was 98,000 with a 5,000 increment uncertainty based on a $\bar{V}$ of 0.705. It has been proposed that a $\bar{V}$ value of 0.695 should have been used (Heaps *et al.*, 1966). This would lower M_w to 95,000 which would bring it into excellent agreement with a value obtained by averaging the $\alpha 1$ and $\alpha 3$ components of chicken bone gelatin. The ratskin $\alpha 2$ component was also reported to have an M_w equal to 95,000 (if based on $\bar{V} = 0.695$). The $\alpha 2$ samples, however, were reported to contain some cross-linked material which would elevate M_w somewhat. On the other hand, the sedimentation coefficient of $\alpha 2$, which would be less affected by the presence of β components than the sedimentation equilibrium values, was found to be about 3% less than that for $\alpha 1$ (and/or $\alpha 3$) (Lewis and Piez, 1964a,b). The α chains of the codfish skin collagen were found to be very labile under the same fractionation conditions used for ratskin. As a result the heterogeneity of the fractions was quite marked. The 95,000 value for the molecular weight in 1 M $CaCl_2$ ($\bar{V} = 0.695$) reported for the $\alpha 1$, $\alpha 2$, and $\alpha 3$ chains was an estimate based on the respective total cell contents (Piez, 1965).

On the assumption that a chicken bone collagen macromolecule contains one $\alpha 1$, one $\alpha 2$, and one $\alpha 3$ chain, its molecular weight is 284,000. This value is about 8% higher than the most recent determination of the molecular weight of calfskin collagen obtained by physicochemical means (Davison and Drake, 1966), but about 6% lower than the value obtained from electron microscopy and X-ray diffraction data (Hodge and Petruska, 1963).

Discussion

Boedtker and Doty (1956) originally proposed a number of models for the collagen macromolecule in which one or more chains were of unequal linear length, and postulated that the additional "tails" played an important role in the end-to-end aggregation of the macromolecules in the collagen fibrils. More recently it has been shown that a number of proteolytic enzymes release peptides (telopeptides) from undenatured collagen macromolecules (Rubin *et al.*, 1963, 1965), apparently leaving the triple-standard part of the molecule intact. It has been proposed that the telopeptides are not an integral part of the triple-stranded structure (Rubin *et al.*, 1963, 1965). Studies by Deyl *et al.* (1967) have demonstrated that there are a large number of such telopeptides in calfskin and that they are components of both the $\alpha 1$ (and/or $\alpha 3$) and $\alpha 2$ chains (Rosmus *et al.*, 1967) suggesting that the α strands may be branched-chain polymers. The finding that the $\alpha 3$ chain of chicken bone gelatin has approximately 100 more residues than the other two therefore supports very strongly the point of view that not all of the collagen molecule can be in a triple-stranded conformation, and that there are tails and/or polypeptide side chains to the rigid main body of the tropocollagen molecule. A 9800 molecular weight increment of the $\alpha 3$ chain can be taken as a lower limit estimate to the telopeptide content of chicken bone collagen (since the $\alpha 1$ and $\alpha 2$ chains may also contain telopeptides). This lower limit estimate is already two-thirds of the 15,000 value calculated for the telopeptide content of calf skin collagen (Drake *et al.*, 1966).

Telopeptides in soft tissue collagens have been implicated to some extent in the *intramolecular* covalent cross-linking (Drake *et al.*, 1966) and in the *intermolecular* cross-linking (Kühn *et al.*, 1966) of the α chains, both of which are important factors in determining the physicochemical and mechanical properties of a tissue. The paucity of *intramolecular* covalent cross-links in chicken bone collagen rule out this mode of action for its telopeptides. Nevertheless, there are a number of highly characterizing properties of chicken bone collagen which taken together show that intermolecular bridging of some sort is occurring, so that it is quite possible that this is accomplished by means of intermolecular telopeptide–telopeptide association or telopeptide–chain association. Four of these distinctive properties are: (1) Most of the chicken bone collagen can be extracted from the tissue in its denatured state by certain neutral salt solutions, but the collagen is only very sparingly extractable as the undenatured protein in solvents and under conditions normally employed to dissolve the undenatured protein from unmineralized soft tissues (Glimcher and Katz, 1965). (2) Unlike most soft tissue collagens, the chicken bone collagen that is extracted in an *undenatured* state is unstable in acetic acid solutions in the cold, and spontaneously precipitates on standing in the cold as native-type fibrils (Glimcher *et al.*, 1965). (3) Demineralized chicken bone collagen in the tissue does not swell in acid solutions as do other insoluble tissue collagens, such as bovine Achilles tendon, and acid-extracted skin (Glimcher and Katz, 1965). (4) After heat denaturation, the chicken bone collagen in the tissue recovers both its molecular structure and its macromolecular aggregation state in cold salt solutions at neutral pH, as evidenced by the return of both the wide and low-angle X-ray diffraction patterns (L. O. Bonar and M. J. Glimcher, unpublished data).

There is common to all four of these phenomena an intermolecular interaction component which is characteristic of chicken bone. The nature of this interaction can most easily be seen in the reversible thermal-denaturation phenomenon. There are two requirements for reversibility in the case of the thermal denaturation of collagen when in a fibrous solid state. The first is that the conformation of the tropocollagen molecule returns to its original state after denaturation, and the second is that the denatured molecules are returned to their original crystalline (~700-Å axial repeat) array. In effect, to meet these requirements there must be some means present in chicken bone collagen either to keep particular α chains in lateral register when in the melted state, or the formation of such "bonds" is greatly and specifically facilitated on cooling. In any event, *specific* lateral interactions are necessary, for on cooling, not only is the triple-stranded helix re-formed, but also the lateral relationships of adjacent macromolecules in the

fibril. Quite a different phenomenon is seen in bovine Achilles tendon, for example. In this instance, as a result of heat denaturation, the individual α chains of the collagen macromolecule in the melt evidently become indiscriminately entangled with the chains from adjacent molecules, for on cooling and reelongation, while a fraction of the α chains reform in the triple-stranded helical configuration, there is no return of the regularity in the lateral packing of collagen macromolecules, *viz.*, the native, axial repeating period (~700 Å) is irreversibly lost (L. O. Bonar and M. J. Glimcher, unpublished). This occurs despite the fact that the α chains of the Achilles tendon collagen are extensively covalently cross-linked, as evidenced by the increased shrinkage temperature of bovine Achilles tendon, and by the fact that the collagen in this tissue is essentially insoluble in the denaturing salts used to extract chicken bone collagen, even at very elevated temperatures and for prolonged periods of time (Glimcher and Katz, 1965). It would therefore appear that the presence of stable, covalent chemical bonds joining chains is by itself neither a necessary nor a sufficient condition for the type of reversible thermal denaturation demonstrated by chicken bone collagen.

The extraordinary *intermolecular* interaction properties of chicken bone collagen are also revealed in its swelling properties. Swelling in collagen fibers is due to its polyelectrolyte composition and the phenomenon of Donnan equilibrium. At acid pH values where the collagen molecule becomes highly charged Donnan swelling (or dissolution if possible) occurs in soft tissue collagens but not chicken bone collagen. In addition X-ray diffraction studies (L. O. Bonar and M. J. Glimcher, unpublished) reveal that the strong ~15-Å equatorial reflection in chicken bone collagen is maintained at low pH as compared with the gradual loss of this reflection of bovine Achilles tendon as it progressively swelled at increasingly lower pH values. This demonstrates that special constraints have been imposed between the collagen molecules in chicken bone which prevents swelling.

The presence of strong and specific intermolecular telopeptide interaction at points along the length of the collagen macromolecule which would facilitate the maintenance of a specific lateral-packing arrangement could also be the explanation for the unusual tendency of chicken bone collagen to reassociate in solution, under acid conditions and in the cold, to such an extent that precipitation of fibrils occurs (Glimcher *et al.*, 1965). A significant finding in this regard is that although the crystallization of chicken bone collagen into the native aggregation state (~700-Å axial repeat) was easily accomplished by conventional means, the aggregation of the macromolecules into segment long-spacing aggregates using ATP was not possible, and the precipitates which did form were found to consist of native- (~700-Å axial repeat) type fibrils (Glimcher *et al.*, 1965). This would also be consistent with either a far greater preference of chicken bone collagen compared with other collagens for the specific quarter-stagger lateral-packing characteristic of native-type fibrils, or the maintenance in solution of lateral and longitudinal polymers already in the characteristic, quarter-stacking packing arrangement (Glimcher *et al.*, 1965).

The great tendency of chicken bone gelatin to aggregate and gel at concentrations and at temperatures at which most soft tissue collagens remain dissociated, may similarly be directly related to such bridging interactions.

There are of course other physicochemical factors which must contribute to the phenomena observed in chicken bone collagen, and some of these have already been discussed, particularly in regard to the swelling and extractability properties (Glimcher and Katz, 1965). It is clear, however, that the dissimilarity in total chain length that is found in the chains constituting chicken bone collagen suggests a means of intermolecular interactions for collagen that cannot be discounted.[2] The relative importance of this factor in a number of other phenomena implicated by the model of intermolecular interaction presented here is now under investigation.

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[2] Since the submission of this paper, Müller and Kühn (1968) and Stark and Kühn (1968) have presented chemical evidence that there are three distinct α chains in acid-soluble calfskin collagen. They suggest that the difference between the $\alpha 1$ and $\alpha 3$ chains is primarily in the nature of peptide side chains, rather than in the amino acid composition of the main polypeptide chains. Their findings and their interpretation concerning calfskin collagen agree with the hypothesis presented in the present paper that the $\alpha 1$ and $\alpha 3$ chains of chicken bone collagen also differ in their molecular weights, and that this difference may be due to differences in the number and composition of the telopeptides.

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The Effects of Pressure on Actomyosin Systems*

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ABSTRACT: The interactions of actin with myosin, heavy meromyosin, and subfragment 1 were investigated under pressure from 1 atm up to 4000 kg/cm². The results obtained by the change in the intensity of transmitted light through a pressure cell showed that, in the absence of adenosine triphosphate, turbid solutions of myosin B, actomyosin, and heavy actomeromyosin became transparent with increasing pressure finally to give constant intensities of transmitted light at *ca.* 2000 kg/cm², indicating some change in the state of molecules in a solution under pressure. When dissociation of actomyosin into actin and myosin under pressure is assumed, pressure effects on actomyosin should be the same as on the individual component. However, experiments for denaturation of F-actin, heavy meromyosin, and heavy actomeromyosin by pressure demonstrated that heavy meromyosin plus pressure-treated F-actin (at 3500 kg/cm²) had less adenosine triphosphatase activity than F-actin plus pressure-treated heavy meromyosin and pressure-treated heavy actomeromyosin, suggesting that heavy meromyosin protected denaturation of F-actin. In the presence of adenosine triphosphate, high magnesium adenosine triphosphatase activity of heavy actomeromyosin, and actosubfragment 1 decreased sharply to magnesium adenosine triphosphatase of heavy meromyosin, and subfragment 1 in the pressure range from 1 atm to 700 kg/cm². Calcium-activated adenosine triphosphatase of heavy actomeromyosin and actosubfragment 1, on the other hand, showed an increase in activity by pressure, the increase which was due to the pressure effect for adenosine triphosphatase of heavy meromyosin, and subfragment 1. Summarizing those results, the pressure effects on actomyosin systems are accounted for in terms of depolymerization or dissociation of protein components, and adenosine triphosphate plays an important role in interactions of actin and heavy meromyosin.

Many investigations have been carried out on interactions between actin and myosin, which would be a key reaction in muscle contraction. Characteristic properties of actomyosin systems, *e.g.*, Mg-activated ATPase activity, superprecipitation, and dissociation of actin and myosin on the addition of ATP, etc., may originate from these interactions. Measurements of volume changes seem to be useful for the elucidation of the nature of the interactions.

Baskin (1964), using dilatometer, found that a volume of a suspension of myofibrils was decreased or increased on the addition of a low or a high concentration of ATP, the result which was confirmed by Hotta and Terai (1966). However, any definite result which indicates a volume change arises from the interactions between actin and myosin has not been reported so far. This kind of experiment was tried once by Szent-Györyi (1951). Noguchi *et al.* (1964) who intended to measure directly a volume change accompanied with the interaction between myosin B and ATP could not find any definite change. We have tried to measure a volume change induced by mixing of actin and heavy meromyosin with dilatometers of the same type as Noguchi *et al.* used, but any conclusive result could not be obtained either. The accuracy of our apparatus was *ca.* 100 ml/mole of heavy meromyosin, and when the volume change be smaller than this value, it could not be detected.

Compared with direct measurements of a volume change with dilatometry, pressure experiments are much more sen-

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