metry, the unpaired electron occupies a degenerate orbital. Averaging over the ten hydrogen-bonded positions one can obtain average Hückel spin densities, $\langle\zeta\rangle$, and excess charge densities $\langle\epsilon\rangle$. For the radical anion, $\langle\zeta\rangle$ is 0.0630 in the planar configuration (β_{io} = 1.00), decreases slightly to a minimum value of 0.0616 for β_{io} = 0.72, and is 0.0667 for β_{io} zero.

For corannulene, one is not sure of the σ framework for the carbon atoms with bonded hydrogen, but if we use the McConnell and Colpa–Bolton relationships, $a^{M} = 27\zeta$ and $\alpha^{CB} = -27\zeta + 12.8\epsilon$, as discussed by Snyder and Amos,[4] we find for the planar configuration $a^{M} = -1.70$ and $a^{CB} = -1.66$; if $\beta_{io} = 0.72$, $a^{M} = -1.66$ and $a^{CB} = -1.63$. The calculation for the nonplanar configuration including the excess charge effect gives the best agreement with the experimental value $|a| = 1.56$. The agreement between the calculated and experimental values is somewhat surprising considering the nature of the approximations that have been made. However, in the case of coronene anion radical, which is alternate and planar, the calculated splitting using average Hückel spin and charge densities is -1.49, compared with an experimental value of -1.47.[5]

Segal, *et al.*,[6] have found, in agreement with Stone's theory,[7] that the g values of hydrocarbon radicals can be fitted very well by $\Delta g = g - g_0 = (31.9 - 16.6\lambda) \times 10^{-5}$. Here g_0 is the free-electron value and λ is the Hückel energy level coefficient of the unpaired electron orbital. Segal found that the g value of radicals such as coronene with the unpaired electron in a degenerate orbital do not conform to this relationship, but corannulene does. For corannulene the experimental value of Δg is 38×10^{-5} and the calculated values for planar (β_{io} = 1.00) and nonplanar (β_{io} = 0.72) are 40×10^{-5} and 38×10^{-5}, respectively.

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On the Dissociation and Reassociation of the Polypeptide Chains of Tropomyosin and Paramyosin[1]

Sir:

The molecules of tropomyosin and paramyosin are similar. In benign media, both consist of two α-helices arranged side by side and twisted slightly about one another.[2-4] Thus, although they differ significantly in amino acid composition, the only gross difference in molecular form is in the over-all molecular length.

Further questions immediatedly arise. Among these are: (1) How many individual polypeptide chains make up each molecule? Is there one long chain with a hairpin turn between long helical segments, or two completely helical chains held side by side, etc.? (2) Are there any covalent cross-linkages (in particular, disulfide bonds) between the two helical segments? (3) Are the covalent cross-linkages, if any, necessary to maintain the native conformation?

Two of these questions have already been answered for tropomyosin.[3,5] We report here experimental results which answer all three for both proteins. Except where otherwise noted, preparative methods and physical techniques were as reported earlier.[2,3]

Tropomyosin. A molecule of tropomyosin has a mass of 74,000 amu.[3] In a denaturing medium in which the helix content (measured by optical rotatory dispersion) is zero, the same mass is observed, and the other solution properties are not those of a linear, random coil of that mass.[3] Clearly, then, this molecule has one or more covalent cross-linkages. This agrees with chemical studies, which indicate one disulfide bond/molecule.[6] In a medium that would reduce disulfide linkages, the molecular mass is about one-half the value given above.[5] Thus, there are two polypeptide chains in tropomyosin, as it is usually prepared, held together by at least one disulfide linkage.

To investigate further, we prepared tropomyosin samples that had all the disulfide linkages reduced to sulfhydryl, and then all sulfhydryls were masked with acetyl groups. This was accomplished by dissolution of native tropomyosin in a denaturing medium (5 *M* guanidine hydrochloride), reduction with β-mercaptoethanol, acetylation with iodoacetic acid, dialysis, and lyophilization. This lyophilized material ("modified" tropomyosin) was dissolved in both benign and denaturing aqueous media, and several macromolecular properties of these solutions were determined. Specifically, measurements were made of intrinsic viscosity, intrinsic sedimentation coefficient, and (light scattering) molecular weight. In parallel, measurements of intrinsic viscosity and sedimentation coefficient were made on solutions of unmodified tropomyosin. Results for both kinds of material are summarized in Table I (rows labeled TM).

The data in Table I confirm our earlier experiments on unmodified tropomyosin in both media. We have also confirmed that the molecular weight of unmodified tropomyosin, in a medium that is both reducing and denaturing, is one-half that value. It is also plain from the table that the properties of modified and unmodified tropomyosin are quite different from one another in the denaturing medium, but are indistinguishable in the benign medium.

Most striking is the drop in molecular weight (by a factor of two) suffered by the modified protein when transferred from the benign to the denaturing medium and its complete recovery upon transfer back to the benign medium.

Paramyosin. A paramyosin molecule has a mass of 220,000 amu.[2] The molecule is extraordinarily stable; optical rotatory dispersion experiments show appreci-

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Table I. Data for Modified and Unmodified Tropo- and Paramyosin in Various Media[a]

	Medium[b]	$[\eta]^u$	$[\eta]^m$	$10^{13}[s_{20,w}]^u$	$10^{13}[s_{20,w}]^m$	$10^{-3}\ M^u$	$10^{-3}\ M^m$
TM	$KCl_{1.0}K[PO_4]_{0.1}$ (7.3)	34	34	2.59	2.68	74.2[c]	77.2
TM	$GuHCl_{5.0}KCl_{0.6}$-$K[PO_4]_{0.06}$ (6.1)	45[c]	33	2.38		76.2[c]	39.5
PM	$KCl_{1.0}K[PO_4]_{0.1}$ (7.4)	207	206	3.1	2.99	220[d]	200
PM	$GuHCl_{7.5}KCl_{0.2}$-$K[PO_4]_{0.03}$ (7.4)	103	65.6	4.4	2.63		{108[e] 92[f]

[a] Columns whose headings contain superscript m (u) refer to the property obtained when the modified (unmodified) protein is dissolved in the medium given. Intrinsic viscosities are in cc/g; $[s_{20,w}]$ in sec^{-1}. [b] To designate aqueous solvent media, the chemical formula for each constituent (other than water) is given with its molarity as a subscript, followed by parenthetical specification of the pH (ref 3). [c] Reference 3. [d] Reference 2. [e] From the empirical relation: $[\eta] = 0.716r^{0.66}$ as given in C. Tanford, K. Kawahara, and S. Lapanje, *J. Am. Chem. Soc.*, **89**, 729 (1967). [f] From the Scheraga–Mandelkern equation with $\beta' = 2.5 \times 10^6$, as given in H. A. Scheraga and L. Mandelkern, *ibid.*, **75**, 179 (1953).

able helix content, at 25°, up to a guanidine concentration of 7.0 *M*.[7,8] Chemical studies have revealed no disulfide bonds.[6]

To investigate the polypeptide chain make-up of this protein, we prepared modified paramyosin in the same way as tropomyosin, the only difference being that the guanidine concentration of the paramyosin solvent was higher (7.5 *M*). Measurements of the same macromolecular properties were made (Table I, rows labeled PM). Two differences in technique used in the paramyosin study are noteworthy: (1) molecular weights in the benign medium were measured by the Archibald procedure; (2) since it is very difficult to determine the molecular weight in 7.5 *M* guanidine by absolute measurements (because of strong nonideality), we have resorted to use of the intrinsic viscosity–molecular weight relationship for proteins[9] and have confirmed this value by use of the sedimentation–viscosity relationship for random coils.[10]

The results for paramyosin are analogous to those for tropomyosin. Modified and unmodified paramyosins show, for example, markedly different intrinsic viscosities in the denaturing solvent, that of the unmodified protein being larger. Since destruction of cross-linkages in a single-chain random coil would produce the opposite effect,[9] this observation suggests some dissociation of chains in the modified protein. The molecular weights confirm this: the mass of the modified protein molecule in guanidine is one-half that of the native protein. That this chain separation is reversible is apparent from comparison of the data for the modified and unmodified proteins in benign media; they are indistinguishable.

Several conclusions can be drawn from the experimental results: (1) the molecules of both tropomyosin and paramyosin contain two individual polypeptide chains; (2) in both proteins, at least as they are usually prepared, the chains are joined by at least one disulfide linkage (which has *not* been detected chemically in paramyosin[6]); and (3) the disulfide cross-bridging is, in both cases, unnecessary for maintenance of the native conformation—even after all disulfides have been reduced and the resulting sulfhydryls acetylated, the double α-helix is recovered in benign media.

The last of the three conclusions noted above should not be stated without some equivocation. There is a chance that the two chains are unanimously parallel, or unanimously antiparallel, in the unmodified double-helical molecules, but that after modification and redissolution in benign medium a mixture of double helical molecules results, some having parallel chains and some antiparallel. The measurements reported here are responsive only to comparatively gross changes in molecular shape and cannot resolve this ambiguity. However, if a coiled coil owes its stability to knobs-into-holes packing, as has been suggested,[11] it seems quite unlikely that any side-to-side packing other than that characteristic of the native protein would be very stable.

Needless to say, several questions remain; among these is whether the two chains within an individual molecule are identical or different, and, if the latter, whether they can be chemically separated. If so, it would be particularly interesting, for the fundamental theory of stability of protein conformations, to see if a system containing only one of the chains would form an α-helix in a benign medium. Thus far, no instance of a single protein α-helix has been reported.

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Relief by Modification of Carboxylate Groups of the Calcium Requirement for the Activation of Trypsinogen

Sir:

The activation of bovine trypsinogen involves the removal of a highly anionic hexapeptide from the N-terminal region of the molecule.[1,2] MacDonald and Kunitz[3] had shown earlier that this process is greatly enhanced by divalent cations such as calcium. These ions seem to exert a directing influence by both promoting the formation of active trypsin from trypsinogen and precluding the formation of an "inert protein." Thus in the presence of calcium ions proteolysis is restricted to the single peptide bond between lysine-6 and isoleucine-7, all other lysyl and arginyl bonds being resistant to tryptic cleavage. In the absence of calcium ions proteolysis is retarded and becomes relatively

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