Fibers for X-ray diffraction were prepared by stretching thin strips of gel. Elongations of up to 300% were obtained at a relative humidity (RH) of 98%. Under the conditions used to prepare pure κ-carrageenan gels and mixed carrageenan–carob gels, pure carob gum samples did not gel. Carob fibers were prepared by stretching strips cut from a dried film (RH = 98%). The wavelength used was 1.54 Å and fibers were dusted with calcite for calibration. The interior of the diffraction camera was maintained at RH = 98% and flushed continually with helium to reduce scattering. Diffraction patterns were recorded on film. Diffraction patterns obtained at different relative compositions are shown in Figure 4a–d. The diffraction pattern obtained for pure carob gum under nominally identical conditions is shown in Figure 4e. The quality of this pattern can be substantially improved by annealing but Figure 4e is felt to represent the type of pattern that one might expect to observe in a mixed-gel system. For all the compositions studied the X-ray fiber diffraction patterns obtained for mixed gels are, within the limits of experimental accuracy, qualitatively and quantitatively identical with the published photographs[22] obtained for pure κ-carrageenan. Since the nature of the unit cell and its dimensions are unchanged, this implies that the carob gum has not been incorporated into the κ-carrageenan junction zones within the mixed gel. This is in complete contrast to observations on the synergistic interactions leading to mixed gels of isotactic and syndiotactic PMMA where the diffraction pattern obtained from the mixed gel is distinctly different from the patterns obtained from gels of either pure component. Although weak but definite diffraction patterns were obtained for carob gum alone, there was no indication of a carob gum diffraction pattern superimposed on a carrageenan pattern nor was there any detectable modification of the carrageenan pattern for the mixed gels. This would seem to suggest that most of the carob gum molecules have not been oriented by the stretching of the gel network. Thus a specific molecular interaction involving parallel alignment of both the carrageenan and the carob gum within the junction zones of the gel (Figure 1) is unlikely.

Mechanical studies demonstrate the drastic changes induced by adding carob gum to κ-carrageenan. Diffraction studies directed toward testing two predictions implicit in the currently accepted model for the synergistic interaction have failed to reveal any evidence of carob–carrageenan interaction. The model shown in Figure 1 could be modified to accommodate the present results. The mixed gels could be considered to contain large aggregates or microcrystalline carrageenan regions linked by surface attachment of carob molecules. One could further suppose no preferential alignment of surface attached carob molecules or that the mannan attachment regions are small. However, such a picture is more akin to a composite gel structure than a discrete molecular interaction. Further experiments aimed at testing the currently accepted model are urgently required.

Registry No. Carob gum, 9000-20-8; κ-carrageenan, 11114-20-8.

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α-Helix-to-Random-Coil Transition of Two-Chain, Coiled Coils. Experiments on the Thermal Denaturation of α-Tropomyosin and β-Tropomyosin

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Some time ago, an equilibrium statistical mechanical theory was developed for the thermally induced α-helix-to-random-coil transitions of two-chain, α-helical, coiled-coil proteins.[1] This theory was applied, with apparent success, to a 43-residue synthetic polypeptide chain analogue of tropomyosin,[2] to α-tropomyosin itself,[3,4] and to two well-characterized fragments of α-tropomyosin.[5] Realization of the theory requires input information comprising (1) the amino acid sequence of the polypeptide chain under investigation, (2) values of the parameters σ and $s(T)$ that embody the short-range interactions for each amino acid type, and (3) a measurement of helix content vs. temperature, which, with the theory, provides the value of a third parameter (w) that measures the "long-range", i.e., helix–helix, interaction as a function of temperature. In past applications, requirement (1) was satisfied by extant data,[6] (2) by algorithms[2,3] chosen to fit extant data,[7] and (3) by extant or new circular dichroism (CD) measurements.[2-5]

Although these applications seem promising, the case for the theory cannot be considered proved. Moreover, the theory itself has recently been expanded to include the effects of both loop entropy and out-of-register structures,[8-10] both of which were assumed to be absent in the original treatment. For these reasons, we thought it desirable to extend the data base available for testing the expanded theory by making measurements at pH 7.4 of CD vs. temperature over a wide range of protein concentration for β-tropomyosin, a genetic variant of the same chain length as α-rtropomyosin and whose sequence is also known.[6] Furthermore, we have reinvestigated α-tropomyosin over the same large range of protein concentration

0024-9297/84/2217-2445$01.50/0

as the original CD measurements[3] (also at pH 7.4) and extended their range to lower temperature to produce a more complete picture of its thermal stability. We report these results below along with comparisons that can be made without recourse to the theory for two-chain molecules. A detailed fit of these data to the latter theory is in progress and will occupy some time, so comments on that will not be attempted here.

Materials and Methods

Preparation of α-tropomyosin from rabbit cardiac muscle and manipulation of both proteins were as previously described.[3] The β-tropomyosin was obtained from rabbit skeletal tropomyosin by ion exchange chromatography;[11] no α-chains appeared on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Circular dichroism measurements were made with the same precautions with respect to temperature measurement and control, base line monitoring, and reversibility as before.[3] Calculation of fraction helix from CD was also as previously described.[3] All measurements were on non-cross-linked protein; i.e., the samples were reduced with dithiothreitol (DTT) and kept in solutions containing protective amounts of DTT. In all cases the medium is $(NaCl)_{500}(NaP_i)_{50}(DTT)_x(7.4)$,[12] with $0.5 \leq x \leq 1$ mM. The complete reversibility observed makes clear that no cross-linking occurred by air oxidation during the somewhat lengthy thermal denaturation determinations. As an added check, we measured a few freshly reduced samples after rapidly bringing them to an elevated temperature. These results agreed well with those recorded at the same elevated temperature in the routine protocol.

In an investigation in which absolute values of helix content for two different proteins are to be compared, it is essential to be sure that determinations of protein concentrations are accurate. In this laboratory, we have for some years employed absorbance at 277 nm to determine tropomyosin concentration with a numerical value of 0.314 cm^2 mg^{-1} for the extinction coefficient, a value originally determined with rabbit skeletal tropomyosin through micro-Khjeldahl determinations of nitrogen content.[13] Since α-tropomyosin and β-tropomyosin have the same number of tyrosines per molecule, nearly the same molecular weight, and nearly the same conformation, it would seem likely that the same value could be used for them as for the skeletal protein, which is an almost equimolar mixture of $\alpha\alpha$ and $\alpha\beta$ species. Nevertheless, to justify this routine use of the same extinction coefficient for all our tropomyosin samples, we checked our concentration measurement using the method of Edelhoch.[14]

In Edelhoch's method, the tyrosine concentration is determined by dissolving the protein in denaturing medium (6 M guanidinium chloride (GdmCl)) buffered at pH 6.5 and recording the change in absorbance (at 295 nm) resulting from titration to pH 12.5. The method is useful because essentially only tyrosines titrate in that pH range and the change in tyrosine extinction coefficient at 295 nm is very large, has been accurately measured, and is the same for all proteins in the unfolded state.[14] Since the tyrosine content of both α- and β-tropomyosin is well-known,[6] the protein concentration can easily be calculated from the data.

In the present context, the absorbance (277 nm) of a reduced protein solution in benign medium, $(KCl)_{500}(KP_i)_{50}(7.4)$, was measured and the "routine" protein concentration calculated by our usual method. A 0.400-mL aliquot of this solution was then added to a 2.00-mL volumetric flask containing 1.50 mL of $(GdmCl)_{8000}(KCl)_{250}(KP_i)_{25}(6.5)$. Dilution to the mark with water then provided a solution of the unfolded protein (at the proper pH) whose "routine" concentration could be deduced from its history and whose absorbance (295 nm) was measured. The volume of 45% KOH required to bring the solution to pH 12.5 was also known from a preliminary experiment, so that the absorbance (295 nm) measured after addition of base could be corrected for the small, additional dilution.[15] Thus, the protein concentration deduced from our original routine absorbance method and from Edelhoch's method were compared. When this procedure was carried out on α- and β-tropomyosin, agreement between our routine value and the value from the Edelhoch method was within 2% for each protein. We conclude that use of absorbance at 277 nm with an extinction coefficient of 0.314 cm^2 mg^{-1} provides correct values for the concentration of both the α- and β-proteins.

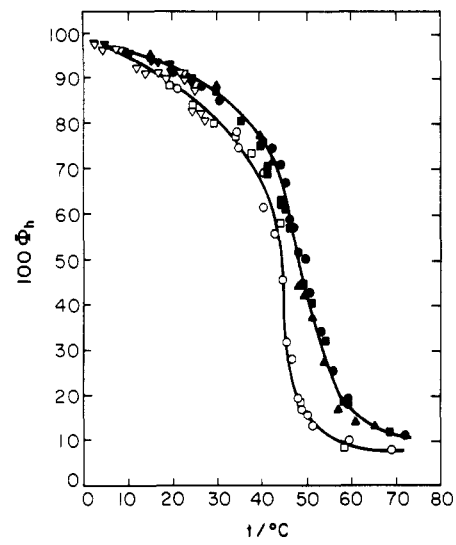

Figure 1. Percent helix vs. Celsius temperature for reduced α-tropomyosin in $(NaCl)_{500}(NaP_i)_{50}(DTT)_x(7.4)$. Filled symbols: 5.2 mg mL^{-1}, x = 1.0 mM. Open symbols: 0.0044 mg mL^{-1}, x = 0.5 mM. Variously shaped symbols designate different runs. Solid curves are spline curves.

The use of protein solutions as dilute as 0.0044 mg ml^{-1} raises questions concerning adsorbtion of protein to the walls of the CD cells. The danger is that the adsorbed material may be an appreciable fraction of the total, thus reducing the effective concentration in the cell. We guard against this by rinsing the cell with the solution preliminary to loading the actual sample. However, we have never found any difference in CD between these solutions and those loaded directly into a dry cell. In the present instance, this effect is apparently immaterial.

Results and Discussion

Results of our augmented study of the thermal denaturation of α-tropomyosin are shown in Figure 1 for two extreme concentrations of protein. The spline curves through the data differ only slightly from our prevous values because of the extension of the data to lower temperatures. The effect of protein concentration as previously observed is in evidence, although it is again apparent that careful temperature measurements and a wide range of concentration (for Figure 1, a 1200-fold concentration range) are required for its demonstration. This "mass action" effect must be caused by the dissociation of the two chains in the transition.

Corresponding data for β-tropomyosin over a slightly narrower range of concentration (500-fold) are shown in Figure 2. The results are qualitatively rather similar to the α-tropomyosin data. The concentration effect is clearly seen here as well, although the difference is somewhat less, almost certainly because the range is somewhat smaller. In neither reduced α- nor in reduced β-tropomyosin in the range 0–70 °C do we see evidence in our data for small transitions in addition to the principal transition, as has been reported for reduced skeletal tropomyosin (at ~7 °C)[15] and reduced α-tropomyosin (at ~30 °C, and 1 M ionic strength).[16]

Comparison of the stability of the double α-helix in α_2 and in β_2 molecules is most directly made for comparable concentrations. Such a direct comparison is shown in Figure 3, where data for intermediate, almost identical concentrations of each protein are displayed. It is clear that the two-chain, α-helical, coiled coil is appreciably more stable in α_2 than in β_2 molecules. This conclusion has previously been reached, but only very limited data were

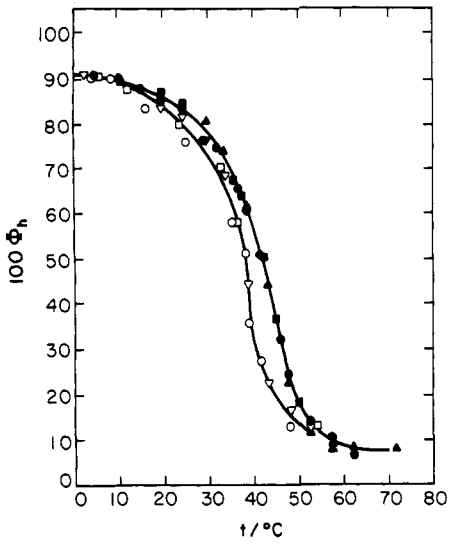

Figure 2. Percent helix vs. Celsius temperature for reduced β-tropomyosin in $(NaCl)_{500}(NaP_i)_{50}(DTT)_x(7.4)$. Filled symbols: 4.72 mg ml^{-1}, x = 1.0 mM. Open symbols: 0.0100 mg mL^{-1}, x = 0.5 mM. Variously shaped symbols designate different runs. Solid curves are spline curves.

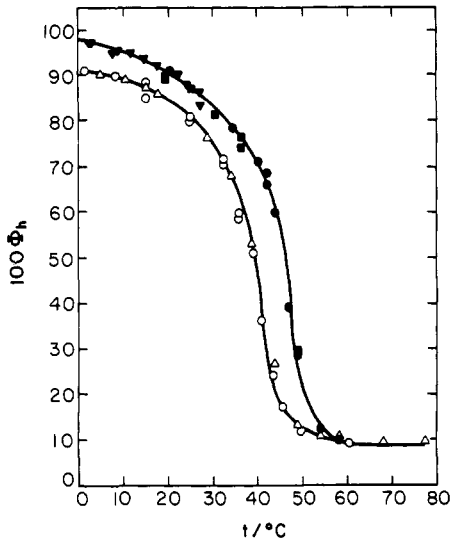

Figure 3. Percent helix vs. Celsius temperature for reduced tropomyosins in $(NaCl)_{500}(NaP_i)_{50}(DTT)_{0.5}(7.4)$. Filled symbols: α-tropomyosin at 0.104 mg mL^{-1}. Open symbols: β-tropomyosin at 0.100 mg ml^{-1}. Variously shaped symbols designate different runs. Solid curves are spline curves.

presented to support it.[17] The present more detailed study not only confirms this conclusion but provides a data base broad enough to support a detailed test of the theory of the α-helix-to-random-coil transition in these molecules.

In advance of an attempt to fit these data to the extant theory,[10] it is premature to speculate on the results. However, one conclusion can be stated because it is independent of the two-chain theory. The observed difference in stability between α- and β-tropomyosin cannot result from differences in the short-range (σ and $s(T)$) interactions. We have been aware for some time that the helix content predicted from the helix–coil theory for *single* chains of α-tropomyosin and β-tropomyosin at 30 °C are almost identical.[18] We have extended the same calculation to cover the entire experimentally accessible temperature range (0–80 °C) and find that predicted differences never exceed a fraction of a percentage point in helix content. It is thus immediately apparent (and dependent only on the theory for single chains) that observed differences between the thermal denaturation curves of the α_2 and β_2 molecules must arise from interactions other than those of short range. Whether these non-short-range differences are, in fact, the helix–helix interactions which are the only such interactions introduced in the two-chain theory,[1] whether the two-chain theory in its more complete form[10] treats them correctly, and whether sense can be made of the observed differences in terms of the 39 out of 284 amino acid sites (11 of which are at the hydrophobic, helix–helix contact surface) at which α- and β-tropomyosin chains differ remain open questions.

Acknowledgment. This study was supported by Grant No. GM-20064 from the Division of General Medical Sciences, U.S. Public Health Service.

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Halato-Telechelic Polymers. 10. Effect of the Ionic End Groups on the Glass Transition Temperature

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The incorporation of ions induces drastic modifications in the physicomechanical properties of organic polymers.[1-3] The obvious technical importance of the subject has promoted an in-depth investigation of both the supermolecular structure and the thermal transitions of ion-containing polymers and, more especially, of ionomers. It is worth recalling that ionomers usually result from the incorporation of relatively few ionic groups into nonpolar polymers by the random copolymerization of common organic monomers with ionizable comonomers.

Eisenberg[3] has reviewed the effect of ions on the glass transition temperature (T_g) of ionomers based mainly on styrene,[4] butadiene,[5] ethyl acrylate,[6] and ethylene.[7] The glass transition temperature increases with increasing salt (metal acrylate or methacrylate) content and, although no meaningful correlation exists as yet, the effect would seem more pronounced as the T_g of the host material decreases.[3] Some experimental results[8] support that cross-linking by

0024-9297/84/2217-2447$01.50/0