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Hybrid Formation between Collagen and Synthetic Polypeptides†

Eckhart R. Heidemann,* Brian S. Harrap,‡ and Hans D. Schiele

ABSTRACT: The α1 chain of calf-skin collagen can form hybrids in the presence of synthetic polypeptides, *e.g.*, $(Pro\text{-}Ala\text{-}Gly)_n$, $(Pro\text{-}Gly\text{-}Pro)_n$, and, to a very much lesser extent, $(Pro\text{-}Ser\text{-}Gly)_n$. The extent of the hybridization reaction increases with an excess of the synthetic polypeptide and also with its molecular weight. The hybrid may be isolated by molecular sieve chromatography and identified by amino acid analysis. There is no evidence for hybrid formation between these polypeptides and the two-chain α-helical tropomyosin. Interaction between the α chain and the synthetic polypeptides is also indicated from optical rotation data. The hybridization experiments show that a single type of synthetic polytripeptide containing the collagen fold can be recognized and accepted to form a common triple helix structure by the many different types of tripeptides which make up the α1 chain of collagen. This hybrid-forming capacity apparently depends both on the nature of the tripeptide in the polymer and the sequence in the collagen chains. Since a collagen chain cannot bind $(Pro\text{-}Ser\text{-}Gly)_n$ to give a collagen-like structure it appears that the collagen chain cannot induce a structural fold in the chain $(Pro\text{-}Ser\text{-}Gly)_n$.

The polypeptide chains of collagen represent a primary structure composed of the following four types of tripeptides (Traub and Piez, 1971)

Gly- (Hyp) Pro-(Hyp) Pro
I
Gly- (Hyp) Pro-X
II
Gly-X(Hyp) Pro
III
Gly-X-X
IV

where X is any amino acid other than Gly, Hyp, or Pro.

The sequence determination of the CNBr peptide, α1-CB6,[1] from calf-skin collagen has shown that the distribution of the tripeptides I–IV, respectively, in this peptide is in the ratio 10:20:20:50 (Mark *et al.*, 1970). The distribution is approximately the same in some of the relatively small peptides of the collagen chains, as for example, in α1-CB4 and α1-CB5 from rat skin (Butler, 1970). The proportion of types I–III is, however, higher in the peptides α1-CB2 and α2-CB2 from chick skin and other sources of collagen (Highberger *et al.*, 1971).

The formation of the collagen triple helix has been investigated for many examples of synthetic polypeptides with known sequences, representing the four types of tripeptides defined above: type I (Yonath and Traub, 1969); type II (Traub and Yonath, 1967); type III (Andreeva *et al.*, 1967); and type IV (Doyle *et al.*, 1970). Triple helix formation in water occurs very readily with type I polytripeptides (Engel *et al.*, 1966), moderately well with type II, and somewhat less readily with type III (Heidemann and Bernhardt, 1967a,b). This difference between II and III was confirmed by Doyle *et al.* (1971). However, it does not seem possible to induce triple helix formation in type IV polytripeptides, *e.g.* $(Gly\text{-}Ala\text{-}Ala)_n$ (Doyle *et al.*, 1970). The tendency for triple helix formation depends mainly on the nature of the amino acids in the type IV unit. It is also possible, however, that the insertion of types I–III units into appropriate places in the polypeptide chain in the vicinity of the type IV tripeptides can promote the formation of triple helix in the type IV unit, *i.e.*, the presence of neighboring triple helix stimulators may give rise to this structure in type IV units. This has been demonstrated by Segal (1969) for polyhexapeptides composed of two different types of tripeptides. The combination of types IV and I in $(Gly\text{-}Ala\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ gives a triple helix for the whole chain. It may be for these reasons that small CNBr peptides such as α1-CB2 can form triple helices *in vitro* (Piez and Sherman, 1970) even though this peptide has two type IV tripeptide units out of a total of 12. The formation of triple helices from collagen chains or from their CNBr peptides seldom proceeds 100% *in vitro* to give discrete compounds be-

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[1] The nomenclature used for the CNBr peptides is reviewed by Traub and Piez (1971).

cause of the lack of sufficient specificity in helix fold reaction. The following observations may be made.

(1) The aggregation of refolded collagen chains on cooling will in general be initially fairly unspecific (Harrington and Rao, 1970). This is more likely to be so when the difference between the temperature of refolding and the melting temperature of the collagen (T_m) is large because the chains are then more likely to assemble without their termini in register or to fold back in the case of low concentration. In this case each tripeptide unit assembles as well as possible with comparable units from neighboring chains by forming short-range triple helices.

(2) $\alpha 2$ chains are able to form triple helices *in vitro* although in nature they do not form a triple helix of the type $(\alpha 2)_3$. Tkocz and Kühn (1969) found that $(\alpha 2)_3$ of calf-skin collagen is markedly less stable than $(\alpha 1)_3$. Moreover, helix formation in the $\alpha 2$ chain of rat-skin collagen is very much slower, under comparable conditions of concentration and temperature, than in the $\alpha 1$ component (Piez and Carillo, 1964). The structure $(\alpha 2)_3$ formed *in vitro* may itself be regarded as a hybrid and a similar description could be applied to $(\alpha 1)_3$ of most species.

(3) The virtual impossibility of re-forming discrete triple helix compounds from denatured CNBr or hydroxylamine peptides[2] is due to the nonspecific assembly of the tripeptide units from different molecules. The partially folded chain is not able to discern a specific pattern along neighboring chains which would enable it to interact with two neighboring chains to form a triple helix with the chain termini in register. The only distinguishing feature of a collagen chain is that it is a series of types I–IV tripeptides with Gly in each first position.

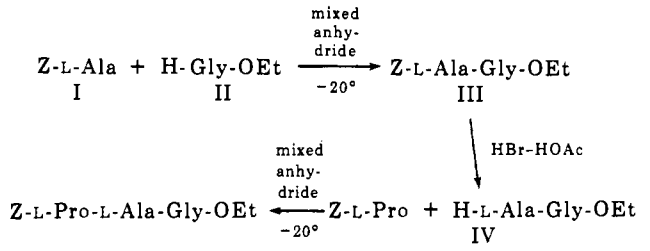
This article reports the results of experiments carried out to investigate the formation of hybrid triple helices between $\alpha 1$ chains and synthetic polytripeptides. The tendency toward hybrid formation between collagen chains and synthetic polymers composed of types I–III tripeptides appears to depend upon the ability of each synthetic polypeptide itself to form triple helices.

Experimental Section

Preparation of Collagen Chains. Solutions (1 mg/ml) of acid-soluble calf-skin collagen (Heidemann and Heinrich, 1970) in 1 M NaCl, containing 0.05 M Tris and 0.05 M $CaCl_2$ and adjusted with 6 N HCl to pH 8.0, were used for the cleavage of the telopeptides with α-chymotrypsin. The enzyme (Serva, Heidelberg, 11,000 *N*-acetyl-L-tyrosine ethyl ester units/mg) was added to the collagen in the ratio 1:10 and incubated for 120 hr at 15°. The cleavage reaction was interrupted by adding phenylmethanesulfonyl fluoride (Merck, Darmstadt). The isolation and purification of $\alpha 1$ chains was carried out by carboxymethylcellulose chromatography (Piez *et al.*, 1963). The isolated $\alpha 1$ chain is designated as the $\alpha 1$ chain.

Preparation of Tropomyosin B. Rabbit tropomyosin B was extracted from the leg and dorsal muscles and purified as described by Bailey (1948) with the modifications of Woods (1967).

Synthesis of Synthetic Polypeptides. The synthesis of Pro-Ala-Gly was performed according to the method of Jäger (1966)



The pure tripeptide was obtained after unblocking by alkaline saponification and catalytic hydrogenation. The tripeptide Pro-Ser-Gly was synthesized as described by Heidemann and Nill (1969a) and Pro-Gly-Pro according to Engel *et al.* (1966).

Polymerization of the tripeptides was performed by the methods of Jäger (1966) and Heidemann and Bernhardt (1967b) and as described in detail by Heidemann and Nill (1969b). After the reaction ethyl ether was added and the solid washed with ether and dried over P_2O_5. A second polymerization step was carried out by either the tetraethyl pyrophosphite or hydroxysuccinimide activated ester method as described by Heidemann and Meisel (1972). In some cases further monomer was added at the second polymerization step. The resultant polymer (0.2–0.3 g dissolved in 3 ml of 0.05 M acetic acid) was chromatographed on a column of Sephadex G-10 (Pharmacia, Copenhagen) (3.2 × 95 cm, 250 ml/hr, V_0 = 250 ml) and rechromatographed on a column of Sephadex G-50 (3.2 × 83 cm, 60 ml/hr, V_0 = 210 ml) in 0.05 M acetic acid at room temperature. Pooled fractions of the eluate were rechromatographed three times on Sephadex G-50 to gain a very narrow molecular weight distribution.

The determination of molecular weight was made on a column of Bio-Gel P-150 (Bio-Rad Laboratories, Richmond, Calif., 100–200 mesh, 2 × 75 cm, 8–10 ml/hr, V_0 = 65 ml) and a column of agarose A 1.5m (Bio-Rad Laboratories, Richmond, Calif., 200–400 mesh, 1.6 × 112 cm, 15 ml/hr, V_{tot} = 249 ml), both eluted with a Tris–$CaCl_2$ (pH 7.5) buffer, as described by Piez (1968). The standards used to calibrate the column were the collagen derivatives β_{11}, $\alpha 1$, $\alpha 1$-CB8, $\alpha 1$-CB7, $\alpha 1$-CB3, $\alpha 1$-CB4, $\alpha 1$-CB5, and $\alpha 1$-CB2. The separation of the hybrids was also made on a Bio-Gel P-150 column (100–200 mesh, 3.2 × 85 cm, 30 ml/hr, V_0 = 180 ml) thermostated to 10°.

Collagen-Fold Formation and Helix–Random Coil Transition. In order to increase the probability of multichain interactions on refolding (*e.g.*, triple helix formation between three different chains) (Harrington and Rao, 1970) the solutions of collagen and polypeptides were studied at the high concentration of 1 mg/ml in acetic acid (pH 3.7). Before the induction of collagen fold formation on cooling, the solution was heated for 15 min at 40° to break down any unspecified aggregation. The solution was cooled in one step to 25° and then in steps of 3°, allowing equilibrium to be established at each new temperature. Attainment of equilibrium was followed by measurement of the optical rotation in a thermostated cuvet using a spectropolarimeter (LEP ± 0.005°, Zeiss, Oberkochen). The helix–random coil transition was followed in the same way except that the temperature was then increased in steps of 3° over the range 4–40°.

Calculation of the Degree of Hybridization. The hybrid formed between collagen $\alpha 1$-chymotrypsin chains and the synthetic polypeptide in question was separated from an ex-

[2] An exception to this is found in a hydroxylamine peptide which occurs near the C terminus of $\alpha 2$-CB3/5 of calf-skin collagen. This forms a discrete compound which, after SLS[3] formation and phosphotungstic acid staining, is visible in the electron microscope (Lange, 1970).

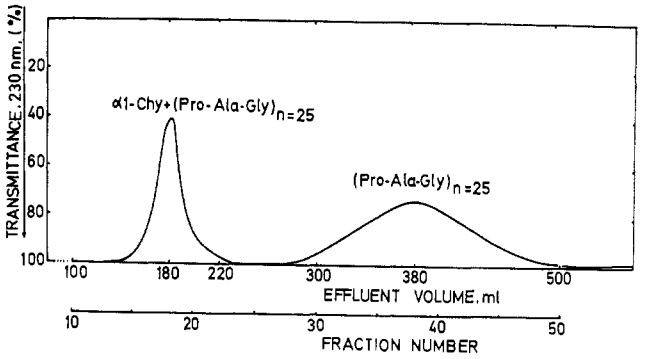


FIGURE 1: Separation of products of refolding of a mixture of α1-chymotrypsin and (Pro-Ala-Gly)$_{25}$ on a Bio-Gel P-150 gel permeation column (3.2 × 85 cm; 30 ml/hr; V_0 = 180 ml).

cess of the latter by chromatography on the 10° thermostated Bio-Gel P-150 column previously described. Amino acid analysis (single column procedure on a Biocal BC 200 analyzer, Biocal, Munich) of the hydrolyzed hybrid fraction gave the increase in the total amino acid content of those amino acids, with the exception of glycine, which were introduced into the hybrid from the synthetic polypeptide. Since the glycine content is 33% in both the α1-chymotrypsin and the synthetic polypeptides it was not possible to use the glycine content as a means of determining the degree of hybridization. The latter was calculated from the contents of Pro and Ala for (Pro-Ala-Gly)$_n$[3] and of Pro and Ser for (Pro-Ser-Gly)$_n$; for (Pro-Gly-Pro)$_n$ only the Pro content could be used.

As an example of the calculation let us consider the case where one molecule of α1-chymotrypsin hybridizes with x molecules of (Pro-Ala-Gly)$_{25}$. To a first approximation α1-chymotrypsin contains 1000 residues of which (from analysis) 334 are Gly, 118 are Ala, and 133 are Pro. Thus, in the hybrid

$$\text{mol \% Pro} = [(133 + 25x)/(1000 + 75x)]/100$$

$$\text{mol \% Ala} = [(118 + 25x)/(1000 + 75x)]/100$$

By inserting for Pro the analytically determined value of 18% and for Ala 16.8%, one calculates in both cases x = 4.0. This calculation can be refined by taking an amino acid not influenced by hybridization, *e.g.*, Gly as an internal standard and comparing the integrated area of its peak with those of the amino acids introduced from the synthetic polypeptide.

Results and Discussion

Hybridization Experiments. The α1-chymotrypsin chain of collagen has a mol wt of nearly 100,000, with nearly 1000 residues (Rauterberg and Kühn, 1971; see also Stark and Rauterberg, 1971). On cooling a mixture of α1-chymotrypsin and (Pro-Ala-Gly)$_{25}$ in aqueous solution the types of compounds which may be formed are (i) hybrids from both components in different ratios of composition and (ii) and (iii) unhybridized triple helices from α1-chymotrypsin and (Pro-Ala-Gly)$_{25}$, respectively. If this mixture is chromatographed at 10° on a P-150 Bio-Gel permeation column those compounds with mol wt >100,000 will be eluted in the first peak (Figure 1). This peak will contain both the hybrids and any pure α1-chymotrypsin triple helices. The molecular weights of components in this peak could range from 112,000 (one-chain α1-chymotrypsin + two (Pro-Ala-Gly)$_{25}$) to 300,000 ((α1-chymotrypsin)$_3$). Although these could not be separated from each other on such a column they would be separated from the excess of unhybridized (Pro-Ala-Gly)$_{25}$ triple helices and from the synthetic polypeptide in the random form (peak 2 in Figure 1). Data obtained under conditions of collagen fold formation from α1-chymotrypsin with each of the synthetic polypeptides (Pro-Ala-Gly)$_n$, (Pro-Ser-Gly)$_n$, and (Pro-Gly-Pro)$_n$ at different collagen:synthetic polypeptide ratios are shown in Tables I and II. From Table I it is clear that if the two components are cooled separately and then mixed the degree of hybrid formation is insignificant since it is possible to separate almost completely the components from each other by gel filtration. Clearly, for both species present, their capacity to form weak intermolecular bonds is fully satisfied by participating by themselves either in the triple helix structure or in associations between like molecules.

TABLE I: Hybridization Experiments between α1-Chymotrypsin and the Synthetic Polypeptides in Which the Components Were Mixed in the Cold after Refolding Separately.[a]

Synthetic Polypeptide	W[b]	Fraction No.	X[a,c]
(Pro-Ala-Gly)$_{25}$	1:8	14-17	0.16
	1:8	18-19	0.18
	1:8	20-22	0.18
	1:10	18-19	0.20
(Pro-Gly-Pro)$_{25}$	1:8	14-17	0.18
	1:8	18-19	0.21
	1:8	20-22	0.20
	1:10	17-19	0.22
(Pro-Ser-Gly)$_{25}$	1:8	14-17	0.15
	1:8	18-19	0.16
	1:8	20-22	0.15
	1:10	18-19	0.19

[a] The degree of hybridization (X) was evaluated from amino acid analysis of fractions resulting from chromatography on Bio-Gel P-150 at 10° as described in the text. [b] W = ratio of α1-chymotrypsin to synthetic polypeptides (on mixing). [c] Molecules of synthetic polypeptides:α1-chymotrypsin.

From Table II, which refers to conditions of collagen fold formation under which hybridization can occur, several points emerge. (1) As the ratio of synthetic polypeptide:collagen increases an interesting proportion of the synthetic polypeptide is incorporated in the hybrid. (2) Similar values are obtained for the composition of the hybrid for different pooled sets of tubes across the chromatographic peak. It is therefore unlikely that a series of hybrids of differing composition is present at any one overall collagen:synthetic polypeptide ratio (w). (3) For both the (Pro-Ala-Gly)$_n$ and (Pro-Gly-Pro)$_n$ systems a greater proportion of the polypeptides with n = 25 was incorporated into the hybrid than those with n = 12. This is in agreement with the greater structure-forming capacity of the higher molecular weight polypeptides in the absence of collagen (Heidemann and Bernhardt, 1967b). (4) Earlier observations of Engel *et al.* (1966) showed that (Pro-Gly-Pro)$_n$—with two imino acids in the tripeptide unit—forms very well a collagen-like triple helix. This folding seems to be more readily formed than (Pro-Ala-Gly)$_n$, as

[3] Abbreviations used are: Pro-Ala-Gly, L-prolyl-L-alanyl-glycyl; Pro-Ser-Gly, L-prolyl-L-seryl-glycyl; Pro-Gly-Pro, L-prolyl-glycyl-L-prolyl; SLS, segment-long-spacing preicpitate of collagen.

TABLE II: Hybridization Experiments between α1-Chymotrypsin and the Synthetic Polypeptides in Which the Components Were Mixed in the Randomly Coiled State before Refolding.[a]

Synthetic Polypeptide	W[b]	Fraction No.	X[a,c]
(Pro-Ala-Gly)$_{25}$	1:1	14-17	1.0
	1:1	18-20	1.1
	1:1	21-23	1.0
	1:2	18-20	1.3–1.4
	1:8	18-20	3.2–3.4
	1:10	15-17	3.8–3.9
	1:10	18-20	3.7–4.0
	1:10	21-23	3.8–3.9
(Pro-Ala-Gly)$_{12}$	1:8	14-17	1.1
	1:8	18-19	1.0
	1:8	20-22	1.1
(Pro-Gly-Pro)$_{25}$	1:1	14-17	1.1
	1:1	18-19	1.3
	1:1	20-22	1.2
	1:2	17-19	1.7
	1:8	14-17	3.6
	1:8	18-20	3.6
	1:8	21-22	3.7
(Pro-Gly-Pro)$_{12}$	1:8	14-16	1.5
	1:8	17-19	1.4
	1:8	20-22	1.4
(Pro-Ser-Gly)$_{25}$	1:1	14-17	0.26
	1:1	18-19	0.28
	1:1	20-22	0.28
	1:2	17-19	0.28
	1:8	15-17	0.58
	1:8	18-19	0.59
	1:8	20-22	0.59

[a] The degree of hybridization (X) was evaluated from amino acid analysis of fractions resulting from chromatography on Bio-Gel P-150 at 10°, as described in the text. Where two values are given for X these represent the results from two replicate experiments. [b] W = ratio of α1-chymotrypsin to synthetic polypeptides (on mixing). [c] Molecules of synthetic polypeptides: α1-chymotrypsin.

TABLE III: Comparison of the Amino Acid Composition for Rabbit Tropomyosin B with That for the Contents of the First Peak Obtained from P-150 Bio-Gel Chromatography of a Refolded Mixture of Tropomyosin B and (Pro-Ala-Gly)$_{25}$.[a]

Amino Acid	Tropomyosin B (Rabbit)	Tropomyosin B/(Pro-Ala-Gly)$_{25}$ W = 1:8
Asp	101	100
Thr	30	29
Ser	43	42
Glu	258	258
Pro	10	14[b]
Gly	24	29[b]
Ala	119	124[b]
Cis	6	5
Val	33	30
Met	20	18
Ile	37	35
Leu	109	106
Tyr	20	19
Phe	7	7
Lys	127	126
His	9	9
Arg	49	49
Σ	1002	1000

[a] Residues per 1000 residues of tropomyosin B. [b] It is evident that Pro, Ala, and Gly are the only three residues to increase in content. If we calculate the degree of hybridization as described for the collagen systems we only obtain (per 1000 residues of tropomyosin B) X = 0.16 from Pro, 0.2 from Gly, and 0.3 from Ala.

judged by the slopes of their respective [α] *vs.* t curves in the helix–coil transition. One might expect therefore that (Pro-Gly-Pro)$_n$ would hybridize more readily than (Pro-Ala-Gly)$_n$. Although Table II shows a slight trend in this direction for the 1:1 and 1:2 mixtures it is not significant. Even these differences are not apparent in the 1:8 and 1:10 mixtures. Apparently (Pro-Ala-Gly)$_n$ is sufficiently in excess in these systems to induce a high degree of hybridization. (5) In the mixtures of collagen with (Pro-Ser-Gly)$_n$ very little structure can be detected even at the higher mixture ratios (1:8). This is consistent with previous observations (Heidemann and Nill, 1969b) that (Pro-Ser-Gly)$_n$ gives very unstable helical structures.

Influence of α-Helical Structures. Tropomyosin B, a two-chain protein with a high α-helical content, which can be reversibly converted to the randomly coiled state by heating (Woods, 1969), was used to provide additional evidence that the hybridization of the above synthetic polypeptides with collagen is dependent on the establishment of the collagen triple helical conformation. This protein and (Pro-Ala-Gly)$_{25}$ were mixed in the ratio 1:8 in the denatured state (pH 3.7, 80°) and cooled according to a similar procedure to that described for the collagen hybrids. The resultant solution was separated on the same P-150 Bio-Gel column as used for the collagen system.

Table III shows a comparison of the amino acid analysis for tropomyosin and the contents of the first peak from the P 150 column. The close correspondence between the two sets of data indicates that this protein undergoes no significant hybridization in the presence of a high proportion of collagen like polytripeptide and emphasizes the specific role of the tripeptide structure in forming hybrids between collagen and synthetic polypeptides.

Optical Rotation Temperature Profiles. Figure 2 shows the denaturation profiles for α1-chymotrypsin (curve 1) and (Pro-Ala-Gly)$_n$ (mol wt 18,900, curve 4) taken separately. The profile for a 1:1 mixture of these separately refolded components (*i.e.*, mixed after cooling) is shown in curve 2. The optical rotation values in curve 2 are an arithmetic mean of those in curves 1 and 4, further indicating that there is insignificant interaction between the components mixed in this way. If, however, the components are mixed in the denatured state to give a 1:1 mixture before cooled, the temperature profile of the refolded product (curve 3) shows a shift with respect to curve 2 in the temperature range of helix formation, 5–36°. Clearly, the high order of the refolded α1-chymotrypsin triple helix, as measured by $\Delta[\alpha] = |[\alpha]_{\text{helix}}|-$

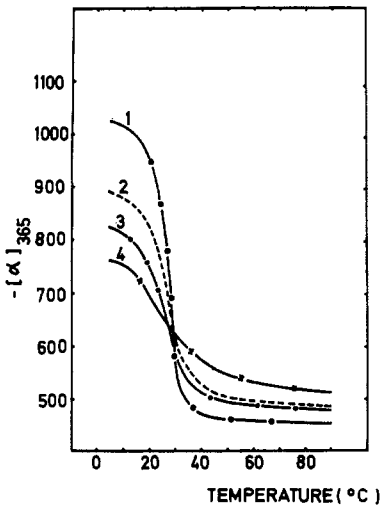


FIGURE 2: Denaturation curves of: (1) α1-chymotrypsin refolded; (2) α1-chymotrypsin–(Pro-Ala-Gly)$_n$ mixed after refolding; (3) α1-chymotrypsin–(Pro-Ala-Gly)$_n$ mixed in randomly coiled state and then refolded; (4) (Pro-Ala-Gly)$_n$ refolded.

$[\alpha]_{\text{random coil}}$, has been interfered with to a considerably greater extent by hybridization than by simple mixing with refolded synthetic polypeptide. Thus, from the curves in Figure 2 we see that for α1-chymotrypsin (curve 1) the values of $-[\alpha]_{365}$ for the refolded and random coil forms are 1020 and 450°, respectively, giving a value of 570° for $\Delta[\alpha]$. For (Pro-Ala-Gly)$_n$ alone (curve 4) the corresponding values for $-[\alpha]_{365}$ are 760 and 520°, with $\Delta[\alpha] = 240°$. From curve 2, where the 1:1 mixture was made after refolding of the single components, the values for $-[\alpha]_{365}$ are 890 and 485° so that the value of 405° for $\Delta[\alpha]$ is the arithmetic mean of the values from curves 1 and 4. On the other hand, when the two components were mixed in the random coil form and then refolded (curve 3) the values for $-[\alpha]_{365}$ are 820 and 480°, with a value for $\Delta[\alpha]$ of 340°. The difference, $\Delta[\alpha]_{\text{curve 2}} - \Delta \times [\alpha]_{\text{curve 3}} = \Delta[\alpha]_d = 65°$ (d = disturbance), between the value of $\Delta[\alpha]$ for the simple 1:1 mixture of refolded components and that for the product resulting from mixing before refolding measures the disturbance due to hybridization and provides a further indication of the degree of hybridization. The values of $\Delta[\alpha]_d$ for the three series of polypeptides of differing molecular weights are collected in Table IV. The values for $\Delta[\alpha]_d$ increase with the molecular weight of the polypeptide, agreeing with the increased tendency toward hybridization observed above. The much smaller influence of (Pro-Ser-Gly)$_n$, as compared to the other two polypeptides, also agrees with the data in Table I.

TABLE IV: Hybridization as Indicated by $\Delta[\alpha]_d$ Values (See Text) for Mixtures of α1-Chymotrypsin with (Pro-Ala-Gly)$_n$, (Pro-Gly-Pro)$_n$, and (Pro-Ser-Gly)$_n$.

Synthetic Polypeptide	Av Mol Wt	$\Delta[\alpha]_d$
(Pro-Ala-Gly)$_n$	3,200	15
	6,100	55
	18,900	65
(Pro-Gly-Pro)$_n$	3,200	20
	6,100	70
	12,500	85
(Pro-Ser-Gly)$_n$	3,400	10
	6,200	15
	18,100	15

Conclusions

The chromatographic separation data and the optical rotation *vs.* temperature data both clearly indicate the formation of hybrids when the mixtures of either of the synthetic polypeptides (Pro-Ala-Gly)$_n$ or (Pro-Gly-Pro)$_n$ with α1-collagen chains are cooled. The rate determining step in collagen folding at concentrations above 0.1 mg/ml is the formation of a hydrogen-bonded nucleus through interaction of peptide segments belonging to three different chains (Harrington and Rao, 1970). Hydrogen bonds between the chains can only occur between segments which already contain the collagen fold. The nucleus formation from prefolded segments can therefore be regarded as a special case of protein–protein interaction.

Our experiments may be used as a guide to the relative tendencies of the synthetic polypeptides to form the collagen-like triple helix in solution. This tendency is much less for (Pro-Ser-Gly)$_n$ than for the other two polypeptides studied and agrees with the observation of Nill (1969) that none of the serine-containing polypeptides (Pro-Ser-Gly)$_n$, (Ser-Pro-Gly)$_n$, (Pro-Gly-Ser)$_n$, (Ser-Gly-Pro)$_n$, (Gly-Pro-Ser)$_n$, or (Gly-Ser-Pro)$_n$ gives a triple helix structure in aqueous solution. Brown *et al.* (1972) found a similar result for (Pro-Ser-Gly)$_n$. Furthermore, our experiments confirm that (Pro-Ser-Gly)$_n$, a chain of type II, cannot itself readily form a collagen fold, and therefore cannot easily be built into a triple helix together with collagen chains. This also implies that the collagen chains cannot stimulate the collagen fold in the neighboring chains of (Pro-Ser-Gly)$_n$. It has been shown furthermore that there is no tendency for hybrid formation between an α-helical polypeptide chain and a collagen-like polytripeptide.

These reactions confirm the introductory remarks that the tripeptide character of the collagen chains, with Gly at the first position of each tripeptide unit, provides the basis for recognition by other synthetic or natural polytripeptides which have a similar structural basis. In previous experiments with synthetic polytripeptides with known collagen-like sequences of the types I, II, and III, the triple helical conformation was formed only between three identical chains. Even in the collagen triple helix itself similar types of tripeptide units are fixed side by side, since collagen is composed of two α1 chains and one α2 chain whose sequences seem to be generally homologous over quite large regions of the chain.

The above hybridization experiments show, however, that Pro-Ala-Gly and Pro-Gly-Pro units can apparently be recognized and accepted by a large number of different tripeptide units of the α1-collagen chains and be stabilized by parallel alignment and hydrogen bonding.

Thus, the triple helix may be less specific with respect to the type of tripeptide units situated side by side than has previously been thought.

Acknowledgment

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Infrared Spectroscopic Studies on the Conformation of Myosin in Films†

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ABSTRACT: The infrared spectra of myosin films prepared under a variety of conditions have been studied. The precipitating conditions determine whether β structure as well as the α helix is observed. In films which have been prepared by precipitation from low ionic strength aqueous media (which approach physiological conditions) the myosin appears to be a mixture of random and α-helical conformation. A very high degree of enzymatic activity is retained by the myosin in the dried films indicating no major irreversible changes on precipitation and dehydration under N_2. Lower activity is observed from films which have been prepared by lyophilizing myosin from solution at high ionic strength. In films which have been prepared by precipitation in aqueous media at low pH, the infrared spectra indicated a significant proportion of β structure has been induced by the pH change. The observed conformations also depend on the precipitating media when organic solvents were used to prepare films. If pure methanol or a 50:50 mixture of methanol and chloroform were used in the precipitating media, β structure was observed while with pure $CHCl_3$ there was no indication of β structure. The possible role of β structure in muscular contraction is of course purely speculative, but an extension of length on formation of β structure is known for other polymeric systems. In considering possible mechanisms of muscular contraction, the burst of hydrogen ions produced by the hydrolysis of ATP under physiological conditions could result in a localized region of low pH. The presence of β structure at low pH suggests that an $\alpha \rightarrow \beta$ conversion should also be considered in postulating possible mechanisms for movement in the flexible hinge region of myosin to allow cross-bridge movement.

Infrared absorption spectra of polypeptides and proteins have been used in the study of conformation for a number of years. There have been numerous reviews of the correlation between spectra and conformation (Elliot *et al.*, 1962; Miyazawa *et al.*, 1967; Timasheff and Gorbunoff, 1967; Susi, 1969). The amide I band (1600–1700 cm^{-1}) which is due to the C=O stretching mode, the amide II band (1500–1550 cm^{-1}) which is due to the hybridization of the N–H bending and C–N stretching modes, as well as the amide IV band (600–700 cm^{-1}) which arises from the CONH groups have been used for conformational diagnosis. Polypeptides also exhibit other strong infrared bands such as the amide A (3300 cm^{-1}) and

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