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REVIEW ARTICLE

Synthesis, Structure, and Biological Properties of Sequential Polypeptides

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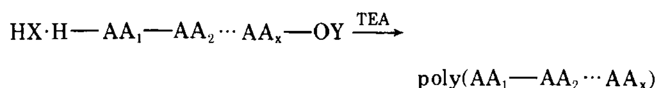
Keyphrases □ Polypeptides, sequential—review of synthesis, structure, optical integrity, enzyme models, immunochemical properties □ Collagen silk fibroin—structural models, conformation studies, and helix stability, similarity of sequential polypeptides □ Antibodies, polypeptide produced—specificity, review □ Peptides—synthesis, structure, and biological properties of sequential polypeptides □ Protecting groups, peptide synthesis—review □ Immunochemical properties—sequential polypeptides, review

Investigators have been interested in sequential polypeptides for a number of reasons. The synthesis of these materials was for a long time very difficult and time consuming. These problems have been alleviated somewhat by the techniques of modern peptide chemistry. However, even now there can arise valid questions concerning the molecular weight and optical purity of certain preparations. The field has in recent years tended to veer toward the utility of sequential polypeptides for structural and biological studies. This may be due to the maturation of this speciality or, more likely, to the reluctance of federal agencies to fund anything that is not applicable to an immediate problem. Notwithstanding the rationale of this shift in emphasis, the field of sequential polypeptides has borne, and should continue to bear, useful information about protein structure and biological functions.

SYNTHESIS

A review in 1958 (1) on polyamino acids also included a small number of synthetic sequential polypeptides which had been prepared up to that date. Since then the volume of literature concerning the synthesis of sequential polypeptides has increased tremendously, and this information is collected in Table I.

In simplistic terms, the synthesis of sequential polypeptides involves the self-condensation of a preformed monomer as exemplified in Scheme I, where



$$HX\cdot H—AA_1—AA_2\cdots AA_x—OY \xrightarrow{TEA} poly(AA_1—AA_2\cdots AA_x)$$

Scheme I

TEA = triethylamine, HX = strong acid salt, AA = amino acid residue, and Y = ester group. Essentially, this method of synthesis was first introduced by Fischer in 1906 (2). Fischer used alkyl esters for his C-terminal activation, so naturally his polymerization conditions required heat and long reaction times to produce only low molecular weight polypeptides. Today the most popular method for the synthesis of sequential polypeptides is still through the use of preformed peptide monomers, but they possess C-terminal esters which are highly activated toward aminolysis. By these means, polymerization can be

performed under very mild conditions. The synthesis of such monomers is achieved by the normal techniques of peptide synthesis, which were reviewed by Kapoor (3).

Historically, Wieland and Schafer (4) in 1952 reported that amino acid thiophenyl esters were capable of forming peptide linkages in aqueous solution. These esters were soon applied to the synthesis of sequential polypeptides (5), and their success prompted other workers to look for activated esters that also possess easier handling, crystallization, and storage characteristics. Of the numerous activated esters available, the pentachlorophenyl (6), the *p*-nitrophenyl (7), and the *N*-hydroxysuccinimido (8) esters are the most widely used. The application of the *p*-nitrophenyl activated ester for the synthesis of sequential polypeptides was described by Kovacs *et al.* (9) who prepared [β-Asp(OBzl)] for structural studies. Almost simultaneously, DeTar *et al.* (10), using the self-condensation of a tripeptide *p*-nitrophenyl ester, prepared the sequential polypeptide poly[Asp-(OMe)-Gly_2]. This procedure has been used extensively by a number of workers; DeTar and coworkers (11–16) further explored the utility of the *p*-nitrophenyl activated ester method for the preparation of models for enzyme active sites (15, 16) and also for investigations into the optical integrity of the polymers produced by this polymerization method. Stewart and coworkers (17–25) used the *p*-nitrophenyl ester method extensively for the synthesis of polypeptides for structural studies. Other examples also were demonstrated (26–37), and some workers (28–37) have produced a wealth of literature using this activated ester for polymerizing their polymers which were designed principally as models of silk fibroin and collagen. Many investigators (38–46) have used the *p*-nitrophenyl activated ester method for the synthesis of their sequential polypeptides.

Pentachlorophenyl activated esters were first introduced by Kupryszewski and coworkers (6, 47) for peptide synthesis. In 1965, Kovacs and coworkers (48, 49) began investigating the use of pentachlorophenyl esters for the synthesis of sequential polypeptides. The pentachlorophenyl derivatives frequently have higher melting points (50), better recrystallization characteristics, and higher rates of aminolysis than the corresponding *p*-nitrophenyl derivatives. For these reasons, several workers in this field prefer this activated ester for the synthesis of high molecular weight polypeptides. The utility of this method was extended (50–53), and this method was successfully applied to prepare a series of polytetrapeptides (54–70) for studies on the antigenicity of proteins and peptides and for investigating antibody specificity. Other examples are illustrated in the literature (43, 71–74). The pentachlorophenyl ester method of polymerization was used extensively for the synthesis of a large number of collagen models (34, 36, 75–86).

Along with the pentachlorophenyl activated esters, the pentafluorophenyl and the trichlorophenyl esters have received some attention. The pentafluorophenyl esters are considerably more reactive than the pentachlorophenyl esters, whereas the trichlorophenyl esters are slightly less so (87, 88). The increased reactivity of the pentafluorophenyl esters makes them useful as alternatives for the backing-off procedure of Goodman and Stueben (89); however, their low melting points and ease of hydrolysis can be unfavorable characteristics (90, 91). Both the pentafluorophenyl (92) and the trichlorophenyl (28, 32, 34, 36, 93–98) activated esters were used for the synthesis of sequential polypeptides. Sometimes, because of the low solubility of many polypeptide active ester monomers possessing relatively large molecular weights, the desirability of the improved solubility of these trichlorophenyl esters may make them valuable intermediates for the synthesis of sequential polypeptides.

In 1965, Katchalski (99) reported the use of the *N*-hydroxysuccinimido ester for the synthesis of poly-(Tyr-Ala-Glu). This ester is highly reactive and can be used in aqueous media; the by-product of its reaction, *N*-hydroxysuccinimide, is water soluble and easily removable. The average molecular weight of the polypeptide, poly(Tyr-Ala-Glu), was found to be 20,000 (99), but subsequent preparations have increased this value to over 60,000 (100). This activated ester has been gaining popularity for the synthesis of sequential polypeptides (46, 101–108).

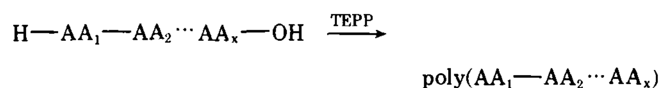
In 1968, Johnson and Jacobs (109) reported the use of a new amino acid carboxyl protecting group, the 4-(methylthio)phenyl ester. These esters are easily prepared by the dicyclohexylcarbodiimide method (110). The attractive feature of this protective ester is its facile conversion, by oxidation and without racemization (111), to the activated 4-(methylsulfonyl)phenyl ester. This method of protection and then subsequent activation was applied to the synthesis of the linear polypeptide poly(Lys-Gly)Gly (112).

In 1968, the use of the *o*-hydroxyphenyl esters for peptide synthesis was reported (113), and this activated ester was applied to the preparation of sequential polypeptides (114–118). The reactivity of this ester is due to intramolecular base catalysis, which accelerates aminolysis without, it is claimed, the occurrence of racemization. An advantage of this method is that it can be used as a protective ester in the unreactive, *o*-benzyloxyphenyl ester form. Activation is achieved by fission of the benzyl ether by acidolysis (114–116) or hydrogenolysis (117).

All of these methods of preparing sequential polypeptides employ a high concentration of the polymerizing unit in a polar solvent and in the presence of an organic base. Such conditions minimize cyclization of the polymerizing unit and favor linear intermolecular polymerization. However, these conditions can also favor early precipitation of the polypeptide from solution. Some polypeptides prepared by this method have also shown discrepancies between end-group assays and molecular weights obtained by other methods (119). This deficiency of free amino groups may be due to the formation of very large cyclic polypeptides since the growing polypeptide chain always carries the activating moiety. To circumvent these possible defects in the normal method of pre-

paring sequential polypeptides, it has been shown that polymerization can be conducted in dilute solution if a partially blocked monomer is added. This monomer, the C-terminal end of which is blocked, can act as a nucleus for growth of the polymer chain. The resulting polymer does not carry the activating moiety and thereby eliminates the possibility of cyclization of the polypeptide. The dilute conditions of polymerization allow greater solution of the polypeptide as it is formed, which should allow higher molecular weight polymers to be obtained when compared to the conventional procedure (54–70, 120, 121).

Although the activated ester method is by far the most popular procedure for the synthesis of sequential polypeptides, it is by no means the exclusive procedure for the preparation of such materials. Some investigators still use the tetraethyl pyrophosphite procedure for polymerization as illustrated in Scheme II, where TEPP = tetraethyl pyrophosphite, and AA = amino acid residue.



Scheme II

The advantage of this process resides in the comparative ease of synthesis of the monomer to be polymerized since the extra step of forming the activated ester is eliminated. However, it was reported (122) that this procedure produces phosphorus-containing polymers. Reservations are also held concerning the optical integrity of those amino acid residues activated by this method, provided they are not glycyl or prolyl moieties. Notwithstanding these criticisms, the method has been employed successfully (28, 123–130).

Dicyclohexylcarbodiimide and the mixed anhydride methods have also found application in the preparation of sequential polymers (123, 131–134). However, due to their undesirable side reactions (135), which can lead to early termination of polymerization, these two methods have not been as widely adopted as the activated ester method. Before this last mentioned method was developed, the acid chloride (136, 137), azide (138), the later hydrazide oxidation (139, 140) methods were also used to prepare polypeptides of low molecular weight. The acid chloride method was recently resurrected by Ridge *et al.* (141) for the preparation of polydepsipeptides. Reasonable molecular weights were reported in this paper.

Application of the Merrifield (142) method of solid phase peptide synthesis has been applied to the preparation of sequential polypeptides (143–145). In these cases, the repeated coupling of blocks of peptides to an insoluble support has shown promise. Although time consuming, this method is most probably the best for the synthesis of polymers of a narrowly defined molecular weight.

The molecular weight of the polypeptides prepared by the solution method has been of concern to many workers (34, 52). In general, the pentachlorophenyl activated ester method is superior to the other methods of activation. Kovacs *et al.* (52) compared various activated esters by the synthesis of the polymers poly(γ-D-Glu-Gly) and poly(γ-Glu-Gly) through the mixed anhydride, carbodiimide, the pentachlorophenyl ester methods. This last method proved to be the best. Similarly, Shibnev *et al.* (34) made a comparison of several of the activated esters in the preparation of the polymers poly(Gly-Hyp_2), poly(Gly-Pro-Hyp), and poly(Gly-Ala-Hyp). It was concluded that the molecular weight of the polypeptides obtained varied with the method of activation. It was found that the pentachlorophenyl ester produced polypeptides with the highest molecular weights, followed in the order of: *N*-hydroxysuccinimido, 2,4,5-trichlorophenyl, 2,4,6-trichlorophenyl, *p*-nitrophenyl, pentafluorophenyl, and 8-hydroxyquinolyl esters. Another factor that plays a large role in the molecular weight of the resulting polymer is the purity of the polymerizing unit. In the experience of the author, it has been necessary to use extensive chromatography to ensure an analytically pure polymerizing unit before polymerization is considered. Also the solubility of the resulting polypeptide in the medium in which it is being produced is another important parameter affecting the molecular weight of the polymer obtained.

Optical integrity is one main concern in peptide chemistry. There is a risk of racemization upon polymerization with the majority of activated esters (12–16, 90, 97, 146–149) mentioned in this review if the C-terminal amino acid of the polymerizing unit is not a glycine or an imino acid. Even small amounts of racemization can have far-reaching effects on the behavior of polypeptides. The backing-off procedure overcomes some of these difficulties by allowing information of the *N*-blocked peptide activated esters without racemization of the C-terminal amino acid residue (89, 150–154). Use of the *N*-hydroxysuccinimido ester (155–157) would appear to be racemization free, as exemplified by the synthesis of poly(Tyr-Ala-Glu) (99, 100); however, one report (158) throws doubt on this assertion. A similar claim of racemization-free synthesis is made for the *o*-hydroxyphenyl esters (114–118). However, the detection of small quantities of racemate is extremely difficult with present methods. Thus, to substantiate this claim, this method will have to be scrutinized fully by a number of investigators.

STRUCTURE

Sequential polypeptides have been used extensively for structural studies, and this is a continuing process. Since collagen possesses a high content of glycyl, prolyl, and hydroxyprolyl residues, it has prompted the synthesis of sequential polypeptides as models for this protein. These models possess, like the protein (159–161), a glycine moiety at every third residue as well as one or both of the imino acids proline and hydroxyproline.

It has been shown that poly(Gly-Pro-Hyp) (124, 162–167), poly(Gly-Pro_2) (168–173), poly(Gly-Pro-

Ala) (169, 171–178), poly(Gly-Hyp_2) (126, 171, 178), poly(Gly-Hyp-Pro) (170, 178), and poly(Gly-Pro-Lys) (178) possess collagen-like X-ray patterns. It would appear that all of these polymers have helical parameters close to those of collagen, and they possibly have a similar conformation with three polypeptide chains wound about a common axis. It has been suggested that the structure of poly(Gly-Pro_2) probably represents some 30–50% of those regions of collagen that consist of the sequence (Gly-Pro-X), where X can be an amino or imino acid (170, 179, 180). In general, it would appear that polytripeptides of the form poly(Gly-$Imino_2$) or poly(Gly-Imino-Amino) readily assume the triple-helix conformation (181). However, polytripeptides of the form poly(Gly-Amino-Imino), including poly(Gly_2-Pro) (182), poly(Gly-Ser-Pro) (183), and poly(Gly-Ala-Pro) (101), have been found to form structures of a different type in which the chains, rather than being wound around each other, are hydrogen bonded together to form sheet-like aggregates. In contrast, X-ray studies (178) have shown that poly(Gly-Ala-Hyp) has a collagen-like conformation. It is suggested that the sequence (Gly-Amino) is a source of instability in the triple-helix structure of collagen. Further support is given to such a conclusion by the deuterium–hydrogen exchange rate of a number of sequential polypeptides (184). Results showed that the extent of exchange in the solid state in the sequences (Gly-Ala-Pro) and (Pro-Gly-Ala) is higher than in (Gly-Pro-Ala). Thus, this latter sequence possesses a more stable structure than the former two sequences.

The molecular conformation of collagen has every third residue near the axis of the triple helix where there is room only for the smallest of the amino acids, namely glycine. Thus, it is not too surprising that the sequential polymers poly(Ala-Pro_2) and poly(Ala-Hyp_2) do not form collagen-like structures (127). Indeed, appreciable amounts of imino acid residues are required to prevent these sequential polymers from assuming α-helix or β-pleated sheet conformations as have been found for poly(Gly-Ala_2), poly(Gly-Ala-Phe), and poly[Gly-Ala-Glu(OEt)]. The four polyhexapeptides poly(Gly-Ala-Pro-Gly-Pro_2), poly(Gly-Pro-Ala-Gly-Pro_2), poly(Gly-Ala-Pro-Gly-Pro-Ala), and poly(Gly-Ala_2-Gly-Pro_2) were shown (102, 185) to possess collagen-like X-ray patterns. These polyhexapeptides all contain alanine and are, therefore, sterically less restricted than poly(Gly-Pro_2). By comparing the denaturation temperatures of poly(Gly-Ala-Pro-Gly-Pro_2), poly(Gly-Pro-Ala-Gly-Pro_2), and poly(Gly-Pro_2), Segal (102) and Segal *et al.* (185) suggested that substitution of an alanyl for a prolyl residue in position 2 has a greater destabilizing effect than substitution in position 3. This tends to be confirmed by the observations that poly(Gly-Ala-Pro) and poly(Gly-Ser-Pro) do not give collagen-like X-ray patterns when prepared by evaporation from aqueous solution whereas poly(Gly-Pro-Ala) and poly(Gly-Pro-Ser) do. Segal *et al.* (185) suggested that the amino acid residue in position 2 may be able to twist outward and thereby facilitate hydrogen bonding to water, thus accounting for the observed instability of (Gly-Amino-Pro) sequences compared with (Gly-Pro-Amino).

Several studies (162, 186) suggested that hydroxyprolyl residues do not play any special role in stabilizing the molecular conformation of collagen. However, this suggestion was questioned by Sakakibara *et al.* (145) who compared the solution properties of the homogeneous polypeptides $(\text{Pro-Hyp-Gly})_n$ and $(\text{Pro}_2\text{-Gly})_n$, where in each case $n = 5$ and 10. It was found that all four of the polytripeptides form the triple-helix conformation, which is analogous to that found in collagen. However, a comparison of their maximum rotation temperatures showed that these values were considerably higher for each hydroxyproline polypeptide than for the corresponding proline analog. From these results it has been concluded that the hydroxyprolyl residues stabilize the triple-helix conformation of collagen. To resolve these conflicting reports on the role of the hydroxyproline residues in collagen, it has been suggested (145) that previous workers (162, 186) were using polymers that were far larger and more heterogeneous in size. It is known (161) that the stability of the triple helix of a synthetic polytripeptide is dependent upon its length. Thus, the use of heterogeneous polymers gave only qualitative results.

Anderson *et al.* (187) investigated some model tripeptides which resemble the polar regions of collagen; preliminary characterization by X-ray diffraction, IR spectrometry, circular dichroism measurements, and optical rotatory dispersion measurements indicated that poly[Ala-Glu(OEt)-Gly] as obtained from an aqueous suspension has an antiparallel β-sheet conformation while poly(Ala-Gly_2) and poly[Glu(OEt)-Gly_2] possess random conformations.

All stereoisomers of poly(Ala-Gly-Pro), as well as a number of closely related polymers containing modifications to the prolyl residue, have been synthesized (73). Thus, proline has been replaced by thiazolidine-4-carboxylic acid, piperidine-2-carboxylic acid, and azetidine-2-carboxylic acid (74). The conformation of these materials has not been reported so far, however; it will be of interest to learn of their stabilities and to note any correlations with the conclusions obtained from previous work (102, 185).

Evidence for the mode of chain association in poly(Gly-Pro_2) sequences was obtained by Kobayashi *et al.* (144) who used polymers of defined molecular weight. The molecular weight determined for the polypeptides $(\text{Gly-Pro}_2)_{10}$, $(\text{Gly-Pro}_2)_{15}$, and $(\text{Gly-Pro}_2)_{20}$ are in good agreement with values calculated for the corresponding trimers. Berg *et al.* (188) concluded from the titration behavior of such homologous polymers that the three *N*-terminal ends are close to each other and at the same end of the molecule. Thus, it appears that the three strands of the molecule are aggregated parallel to each other with little overlap. A more impressive demonstration of the triple-helix structure of a small synthetic collagen model was shown by Sakakibara *et al.* (189). It was shown by X-ray diffraction that the unit cell of $(\text{Pro}_2\text{-Gly})_{10}$ consisted of 12 chains of the oligopep-

tide arranged on four triple-helix structures.

Silk fibroin is characterized by the high composition of glycine, alanine, and serine. This has prompted the synthesis of a number of sequential polypeptides as models of silk fibroin (20, 32, 36, 38, 92, 190, 191). The polypeptides poly(Ala-Gly) (36, 38, 92, 190), poly(Ala-Gly_2) (38), and poly(Gly-Ser-Gly) (20) were all found to form β-structures similar to that found in silk fibroin.

Homopolymers and random copolymers can exist in well-recognized conformations such as the α-helical. Similar studies have been conducted on the molecular conformation of sequential polypeptides. The nature of these polymers lends itself to the process of modifying one or more residues within the repeating chain and thereby observing the effects of the substitution on the conformation of the molecule. A similar process is used for locating the active sites of the biological functions of these materials (as illustrated in the next section). Fraser *et al.* (192) extensively studied the effects of nonhelix-forming amino acid residues on the α-helix conformation adopted by the γ-ethyl ester of polyglutamic acid. For this purpose, a number of sequential polypeptides were synthesized in which valyl (17, 18), S-benzyl-cysteinyl (17, 19), glycyl (22), and *O*-acetyl-seryl (24) residues were incorporated at regular intervals and in varying proportions in the primary structure of poly[Glu(OEt)]. It was found that although valyl and S-benzyl-cysteinyl residues reduce the stability of the α-helix, they do not preclude α-helix formation even when present in considerable proportions (193). However, large molar concentrations of glycyl and *O*-acetylseryl residues completely inhibited α-helix formation (194). Another study, investigating the effects of the outer portions of the side chains on the helicity of a polymer, was conducted with homopolypeptides related to poly-γ-benzylglutamate (195). It was shown that interactions between the outer portions of residues well removed from the main chain can also have an important influence on the stability of the α-helix.

In other secondary structural studies, poly[Glu(OBzl)-Tyr(OAc)] (71) was shown to have a right-handed helix with some deviation, which may be caused by the acetyl group, and poly(Ala-Phe-Gly) (43) was shown to have a possible α-helix; NMR measurements on this material did not reveal any side-chain interactions involving the phenylalanyl residues. Poly(Met_2-Ala) (40, 41) possesses a β-structure but tends to form an α-helix with increasing molecular weight. A series of sequential polypeptides of general molecular formula poly[Ala-$(Gly)_{1-3}$] (72) showed varying structures depending upon their workup, such as precipitation or evaporation from various solvents. These structures were reminiscent of the β-structure, or polyglycine II, or a new and not fully elucidated conformation as characterized by IR and X-ray diffraction. When two-thirds of the lysyl residues in the α-helix formed by polylysine are replaced by alanine such as in poly(Lys-Ala_2) (107), a polymer results that forms a more stable α-helix when fully ionized (107). Presumably there are fewer side-chain interactions in this latter polymer than in polylysine. The structure of the antigen poly(Tyr-Glu-Ala-Gly)Gly (57, 59) has been shown to be of the β-turn conformation (196) and is most definitely not α-helical. The conformation of this antigen is stable to temperatures ranging from 25 to 56° and in pH from 2.5 to 10.0 (197), thus indicating the very stable nature of this particular conformation. The mechanism of hydrogen-bonding collapse has been investigated for some time (198, 199). Ridge *et al.* (141) synthesized the sequential polypeptide of leucine and L-2-hydroxy-4-methylpentanoic acid and came to the conclusion that one in three or four peptide linkages was protonated before the α-helix conformation collapsed to a random coil.

From the preceding comments, it is apparent that sequential polypeptides can be of great use for the elucidation of new conformations occurring in proteins. Possibly polymers of well-defined and narrow molecular weight distributions may offer even more information for structural elucidation.

BIOLOGICAL

The use of synthetic sequential polypeptides for biological studies is advantageous, because once the biological activity of one synthetic material has unequivocally been demonstrated, many analogs of varying size, charge, and conformation can be prepared and tested to elucidate the molecular aspects of the biological phenomenon. The low antigenicity of collagen has been an obstacle to many investigators in studying the antigenic sites of this protein. In spite of this deterrent, Schmitt *et al.* (200) and Davison *et al.* (201) demonstrated that the antigenic sites of this protein lay in regions that are open to proteolytic attack and that these regions are high in tyrosine content. These areas were most probably the nonhelical portions of the *N*-terminal and C-terminal regions of collagen. Michaeli *et al.* (202) found that rat or guinea pig skin collagen produced antibodies in the rabbit which were directed primarily against the α-2-chain. Further evidence for this was found when an *N*-terminal fragment of this chain, produced by cyanogen bromide treatment, was a very effective inhibitor of the antigen–antibody reaction. Similarly other investigators (203–205), using peptides, located antigenic determinants at the C-terminal ends of the α-1- and α-2-chains of calf and rat skin collagen. Furthmayer and Timpl (206) found that the antigenic determinants of the α-1-chain of human skin collagen were located at both the *N*- and C-terminal ends of this particular chain. When denatured collagen is used as the antigen, additional antigenic sites can be demonstrated, thus supporting the conclusion that only the nonhelical regions of native collagen are significantly antigenic. It is thus not too surprising that many synthetic sequential polypeptides used for structural studies are usually very weakly antigenic and do not cross-react with collagen. However, notwithstanding these pitfalls, the polymers poly(Pro-Gly) and poly[Pro-Gly-Hyp(Ac)] have been shown (207) to be immunogenic

and their antibodies appear to be exclusively directed against determinants that predominantly consist of proline. Poly(Pro-Gly-Pro) was also found (208) to be immunogenic in guinea pigs and rabbits. In a passive cutaneous anaphylaxis test, cross-reactions were observed between poly(Pro-Gly-Pro) and fish, rat, and guinea pig collagen. It was suggested (208) that the sequential polymer cross-reacts immunologically with collagen by virtue of the triple-helix conformation common to both substances.

Sequential polypeptides were used as enzyme models by DeTar and Vajda (15), who synthesized the polytripeptide poly(Gly-Ser-Gly) which contains those amino acid residues that are found at the active site of hydrolytic enzymes. However, this polymer did not show any detectable catalytic activity toward the hydrolysis of *p*-nitrophenyl acetate (15). Poly(Asp-Ser-Gly) (16) also contains the sequence that occurs at the active site of several hydrolytic enzymes, and the acetylated polymer poly[Asp-Ser(Ac)-Gly] (16) is a model of the acetylated enzymes which are intermediates in the hydrolysis process. The rate of hydrolysis was found to be comparable to that of certain simple serine derivatives and to be much slower than that of the acylated enzymes (16).

Another enzyme system that has been studied is the protocollagen proline hydroxylase system. This enzyme hydroxylates the peptidyl proline in collagen to hydroxyproline. To ascertain the specificity of this enzyme's activity, sequential polypeptides have been used as the substrate. Kivirikko and Prockop (209) showed that this enzyme does not hydroxylate free proline, the simple peptide Gly-Pro-Pro, or polyproline. However, a small rate of hydroxylation was observed with the tripeptide Pro-Pro-Gly (210), and a far larger rate of hydroxylation was observed with the sequential polymer poly(Pro-Gly-Pro) (209). It was speculated that protocollagen proline hydroxylase is specific for prolyl residues that follow glycyl moieties. This concurs with the positions in which hydroxyproline is found in collagen. Further evidence supports this conclusion (211) in that protocollagen proline hydroxylase did not hydroxylate (Glu-Pro-Gly)$_2$, (Glu-Pro-Gly)$_3$, [Glu(OMe)-Pro-Gly]$_4$-OMe, or Pro-Pro-Gly-[Glu(OMe)-Pro-Gly]$_2$-OMe. Indeed, the Glu-Pro-Gly sequence, which is not hydroxylated, did not inhibit hydroxylation of the other proline residues in Glu-Pro-Gly-Leu-Pro-Gly-Pro-Pro-Gly.

Continuing the investigation of hydroxylation by protocollagen proline hydroxylase, Kivirikko *et al.* (212) investigated a series of peptides of the general formula X-Pro-Gly to study the effect of chain length, conformation, and amino acid sequence in the substrate. A series of peptides containing the sequence X-Pro-Gly was synthesized using the Merrifield (142) method, which produced essentially homogeneous products. When using the random coil forms of the peptides (Pro$_2$-Gly)$_n$, where n = 5, 10, 15, and 20, it was found that all the peptides were hydroxylated; however, their Michaelis constants, K_m, decreased markedly with increasing chain length. Hydroxylation of the polypeptide (Pro$_2$-Gly)$_{10}$ in its triple-helix conformation (189) did not prevent hydroxylation. Although the reaction was slower than the random coil form, both conformations had similar K_m values when expressed in molar concentrations. The hydroxylation of (Pro$_2$-Gly)$_{10}$ in a triple-helix conformation is consistent with previous results, which suggested that protocollagen can be hydroxylated in both the native and random coil forms.

Sequential polypeptides have found great use in investigations concerning the molecular aspects of antigenicity and antibody specificity. The capsular polypeptide obtained from *Bacillus anthracis* is composed exclusively of residues of D-glutamic acid linked by γ-peptide bonds (213). This material precipitates antibodies elicited by the encapsulated organism. A specificity for the residues of D-glutamic acid was demonstrated by the inability of a sample of poly-α-L-glutamic acid either to precipitate with antiserum or to inhibit precipitation of antiserum with the homologous anthrax polypeptide (214). To investigate the role of the carboxyl group of the native polyglutamic acid in the precipitin reaction with the immune serum against anthrax, the following polymers were synthesized: poly(γ-D-Glu-Gly) (52), poly(γ-Glu-Gly) (52), poly(γ-D-Glu-β-Ala) (50), poly(γ-Glu-β-Ala) (50), poly(γ-D-Glu-γ-Abu) (215), and poly(γ-Glu-γ-Abu) (215). When antiserum to the encapsulated organism was cross-reacted with poly(γ-D-Glu-Gly), poly(γ-Glu-Gly), poly(γ-D-Glu-β-Ala), and poly(γ-Glu-β-Ala), no precipitation was observed (214). However, inhibition of the precipitin reaction was observed with all of these polypeptides, the best inhibitors being those polymers containing D-glutamyl residues. Interestingly, it was found that the polymers poly(γ-D-Glu-γ-Abu) and poly(γ-Glu-γ-Abu) both produced precipitates with antiserum to the capsular material of *B. anthracis.* The ability of the L-isomers of the polymer poly(γ-Glu-γ-Abu) to produce such a precipitin reaction was unexpected (215).

The protective effect of glutathione against radiation prompted the synthesis of polyglutathione, polyasparthione, and related sequential polypeptides (53). The antiradiation abilities of such materials have yet to be reported. However, such materials would be considered to have a high probability to be antigenic.

The synthetic polypeptide poly(Tyr-Ala-Glu) has been used to investigate the role of the conformation in immunogenicity and antigenic specificity (216). It was shown that this polymer possesses an α-helix structure under physiological conditions and that antibodies produced against it do not cross-react with a branched polymer consisting of the tripeptide (Tyr-Ala-Glu) attached to the backbone polymer poly(DL-Ala)...Lys. This latter branched polymer exists as a random coil conformation. It was shown that the antigenic determinants of the α-helix polymer were conformation dependent, whereas the branched polymer possessed sequential determinants (106, 216–221).

The molecular aspects of antigenicity have also

been studied using synthetic polypeptides of a known primary structure. It has been found that poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C-OEt (57, 59) gives a precipitin reaction with its antiserum (222). The role of the aromatic moiety on the antigenicity of this material has been studied by the use of various analogs of this antigen. It has been found that the polymer deficient in tyrosine, *i.e.*, poly(Glu-Ala-Gly)Gly-OEt (54), is very weakly antigenic (223) and that its antigenicity is enhanced by the inclusion of the tyrosyl residue, confirming the observation made some time ago that the tyrosyl residue enhances the antigenicity of a molecule. The role of the aromatic residue also has been studied by use of the polymers poly(Phe-Glu-Ala-Gly)Gly-1-^{14}C-OEt (60), poly[Tyr(Me)-Glu-Ala-Gly]Gly-1-^{14}C-OEt (64), and poly(Ala-Glu-Ala-Gly)Gly-1-^{14}C-OEt (65). Both polymers containing an aromatic moiety have been shown to be antigenic (60, 64), whereas the polymer devoid in such a residue has not (65).

Sequential polypeptides have been used to study the specificity of antibodies produced by an antigen. Thus, using the antigen poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C-OEt (57, 59) as a model, it has been shown that the antigenic determinants are most probably conformation dependent since the small peptides (Tyr-Glu-Ala-Gly)$_n$Gly, where $n = 1$–3, do not cross-react with these antibodies nor inhibit the precipitate of these antibodies with their homologous antigen. Further evidence for the conformational dependency of the antigenic determinants was shown by the use of the polymer poly(Tyr-γ-Glu-Ala-Gly)Gly-OMe (66). By maintaining the same order of amino acids as the antigen but producing a polymer with a different conformation due to the γ-peptide linkage, it was shown that there was no cross-reaction with the anti-poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C-OEt serum, nor was there any inhibition of the precipitin reaction of this antiserum and its homologous antigen (66). Further work on the specificity of antibody to poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C-OEt has been conducted with respect to the alanyl residue. The polypeptides poly(Tyr-Glu-Gly$_2$)Gly-OMe (61) and poly(Tyr-Glu-Val-Gly)Gly-1-^{14}C-OEt (58) were used for this purpose. It was found that the former material did not cross-react with the antiserum; however, the latter material removed all of the antibody as shown by absorption experiments. It is thought that the polymer poly(Tyr-Glu-Gly$_2$)Gly-OMe does not have the same conformation as the antigen and so its determinants are not correctly oriented for cross-reaction. However, it is capable of covering part of the active site on the antibodies since it can cause inhibition of the precipitin reaction of the antiserum and its homologous antigen. The polymer poly(Tyr-Glu-Val-Gly)Gly-1-^{14}C-OEt (58) has the alanyl residue replaced by the sterically larger valyl residue. Thus, it could be expected that if the alanyl moiety is part of the antigenic determinant, then the antibody receptor site would be too small to accommodate the valyl residue and some change in the precipitating ability of this valyl polymer should be observable. However, poly(Tyr-Glu-Val-Gly)Gly-1-^{14}C-OEt (58) causes complete precipitation of antibody, thus indicating that this polymer has the same conformation as the antigen and that the alanyl residue, although important for maintaining the conformation of the molecule, is not part of the active site.

The role of the tyrosyl residue and the specificity of the antibodies for this residue have been investigated. For this purpose the polypeptides poly(Phe-Glu-Ala-Gly)Gly-1-^{14}C-OEt (60), poly[Tyr(Me)-Glu-Ala-Gly]Gly-1-^{14}C-OEt (64), poly(Trp-Glu-Ala-Gly)Gly-OMe (67), and poly(Ala-Glu-Ala-Gly)Gly-1-^{14}C-OEt (65) have been used, using absorption and cross-reaction techniques (67). It was found that the first three polymers cross-react with antiserum to poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C-OEt; however, none precipitated all of the antibodies. Modification of the tyrosyl residue to *O*-methyltyrosyl precipitated 75% of the antibody, modification to phenylalanyl precipitated 54%, and modification to tryptophanyl precipitated 40% of antibodies, whereas the alanyl moiety gave no precipitate. This latter polymer did cause inhibition of the precipitin reaction (224).

The role of the glutamyl residue in the specificity of antibodies produced by the antigen poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C ethyl ester was studied with the use of the polymers poly(Tyr-Asp-Ala-Gly)Gly-OMe (69) and poly[Tyr-(γ-Gly)-Glu-Ala-Gly]Gly-OMe (70). Both polymers represent changes in the distance of the side-chain carboxyl groups from the backbone polypeptide. It was observed that both polymers gave precipitin reactions with antiserum to poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C-OEt; however, in both cases the amount precipitated was markedly reduced. From these experiments it has been concluded that antibodies to the antigen poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C-OEt are dependent upon the conformation of the antigen and they also possess specificities for the phenolic hydroxyl group of the tyrosyl moiety, the aromatic ring of the same amino acid residue, and the γ-carboxyl group of the glutamyl residue. Similar investigations have concerned the specificity of the antibodies produced by the antigens poly(Phe-Glu-Ala-Gly)Gly-1-^{14}C-OEt (60, 225) and poly(Tyr-Glu-Val-Gly)Gly-1-^{14}C-OEt (226, 227).

It is evident that polypeptides possessing a known repeating sequence of amino acids have been useful as structural models for proteins, and some can also imitate their biological properties as well. In this latter respect, these sequential polypeptides may possibly find their greatest use as vehicles to study the molecular aspects of various biological phenomena. This undoubtedly will continue to include the use of polymers for enzyme and immunochemical studies. However, in the next few years it could also include the use of polymers for studies on at least one of the components of complement and on the chemistry of membranes. This latter area could provide a wealth of information concerning the selective transport of ions, *etc.*, across membranes and the peptidyl requirements of the membrane itself to allow such a process to occur.

Table I contains, hopefully, all of the sequential polypeptides that have been reported since the review by Katchalski and Sela in 1958 (1). The long hours required to prepare these sequential polypeptides is readily recognized, so apologies are extended to anyone whose work has inadvertently been omitted. All of the compounds listed are of the L-configuration unless otherwise designated. The abbreviated nomenclature follows the IUPAC-IUB Commission on Biochemical Nomenclature Revised Recommendations on Abbreviated Nomenclature of Synthetic Polypeptides (Polymerized Amino Acids) (228), as well as the tentative Abbreviated Designation of Amino-acid Derivatives and Peptides (229). Additional abbreviations which appear are: Glycol = glycolic acid, 2-Hy-4-mepent = L-2-hydroxy-4-methylpentanoic acid, Azet = L-azetidine, Pipec = piperidine, Thz = thiazolidine, OPib = *p*-iodobenzyl, OPcp = pentachlorophenyl, OPfp = pentafluorophenyl, *o*-ONp = *o*-nitrophenyl, TEPP = tetraethyl pyrophosphite, OPyr = 3-hydroxypyridine, S.P. = solid phase method, OHp = o-hydroxyphenyl, M.A. = mixed anhydride, OTcp = trichlorophenyl, $OMSO_2P$ = 4-methylsulfonylphenyl, [O] = oxidative method, Cl = acyl chloride method, ODnp = dinitrophenyl, and Res = residues.

Table I—Synopsis of Synthetic Sequential Polypeptides

Polypeptide	Method of Preparation	Average Molecular Weight[a]	Yield, %	Purpose of Preparation[b]	Reference
(D-Ala-Ala)$_n$	ONp	—	78	A	17
(Ala-Gly)$_n$	ONp	12,000[4]	80	A, C	17, 32, 36, 190
(Ala-Gly)$_n$	OTcp	—	60	C	32, 36
(Ala-Gly)$_n$	OPfp	12,000	30–40	A, C	92
(Ala-Gly)$_n$	S.P.	n = 5, 10, 20	—	A, C	230
(Ala-Gly)$_n$	OPcp	14,000	—	A, D	72, 231
(Ala-Gly)$_n$	DCC	—	—	A	232
(β-Ala-Phe)$_n$	OHp	3400[4]	39	A	116
(β-Asp)$_n$	ONp	8800[4]	—	A	9
(β-Asp)$_n$	OPcp	—	—	A	49
[Asp(OBzl)-Glu(OBzl)]$_n$	ONp	<100 Res[4]	57	A, D	23
[Cys(Bzl)]$_n$	ONp	60–80 Res[4]	44	A, D	21
[Cys(Bzl)-Glu(OEt)]$_n$	ONp	—	68	A	17, 19, 193
[Cys(Bzl)-Gly]$_n$	ONp	60–80 Res[4]	65	A, D	21
(Gln-Ala)$_n$	OHp	10,000	—	A, C	233
(γ-D-Glu-β-Ala)$_n$	OPcp	10,000[3]	48.5	A, E	50, 214
(γ-Glu-β-Ala)$_n$	OPcp	10,000[3]	74	A, E	50, 214
(γ-Glu-γ-Abu)$_n$	OPCp	—	49	A, E	215
(D-Glu-γ-Abu)$_n$	OPcp	12,300[3]	64	A, E	215
(Glu-γ-Abu)$_n$	OPcp	12,500[3]	62	A, E	215
(γ-D-Glu)$_n$	OPcp	8000[3]	89	A	234
(γ-Glu)$_n$	OPcp	9000[3]	89	A	234
[γ-Glu(OMe)-Ala]$_n$	OHp	10,000	—	A, C	233
[Glu(OBzl)]$_n$	ONp	—	27	A	15
[Glu(OBzl)-D-Glu(OBzl)]$_n$	ONSu	—	42	D	105
[Glu(OBzl)-D-Glu(OBzl)]$_n$	ONp	12,000–23,000	—	A, D	235
[Glu(OBzlNO$_2$)-Glu(OBzl)]$_n$	ONp	<100 Res[4]	93	A, D	23
[Glu(OBu^t)-D-Glu(OBu^t)]$_n$	DCC	12,000	69	A, D	105, 236
[Glu(OMe)-Glu(OEt)]$_n$	ONp	70–80 Res[4]	68	A, D	21
[Glu(OMe)-γ-Glu(OMe)]$_n$	DCC	—	—	A, D	131
[Glu(OPib)-Glu(OBzl)]$_n$	ONp	<100 Res	88	A, D	23
[Glu(OBzl)-Gly]$_n$	ONp	<100 Res	90	A, E, D	15, 23, 39
[Glu(OEt)-Gly]$_n$	ONp	—	87	A, D	17, 194
[Glu(OMe)-Gly)$_n$	OPcp	—	—	A	85
[Glu(OMe)-Gly]$_n$	OTcp	—	—	A	85
(Glu-Gly)$_n$	ONp	5000–10,000[4]	55	A, D, E	15, 39
(γ-D-Glu-Gly)$_n$	M.A.	1100[3]	97	A, E	52, 214
(γ-D-Glu-Gly)$_n$	DCC	1100[3]	84	A, E	52, 214
(γ-D-Glu-Gly)$_n$	OPcp	11,500[3]	86	A, E	52, 214
(γ-Glu-Gly)$_n$	M.A.	1100[3]	81	A, E	52, 214
(γ-Glu-Gly)$_n$	DCC	1100	48	A, E	52, 214
(γ-Glu-Gly)$_n$	OPcp	2800[3]	84	A, E	52, 214
[Glu-(OBzl)-Glycol)$_n$	ONp	—	16	D	25
[D-Glu(OBzl)-Leu]$_n$	ONSu	13,500[2]	51	A, D	158
[Glu(OBzl)-Lys(Z)]$_n$	ONp	<100 Res[4]	78	A, D	23
[Glu(OBzl)-Tyr(Ac)]$_n$	OPcp	—	13	D	71
(Gly-Ala)$_n$	ONp	—	—	C	32
(Gly-Ala)$_n$	OTcp	—	48	C	32, 36
(Gly-Ala)$_n$	OHp	12,000	—	A	116
[Gly-Glu(OBzl)]$_n$	OPcp	—	—	A	85
[Gly-Glu(OBzl)]$_n$	OTcp	—	—	A	85
(Gly-Glycol)$_n$	ONp	—	71	D	25
[Gly-Lys(Tos)]$_n$	OTcp	—	—	A	78, 85
[Gly-Lys(Tos)]$_n$	OPcp	—	—	A	85
(Hyp-Gly)$_n$	ONp	8000[3]	—	A	11, 13, 237
(Leu-2Hy-mepent)$_n$	Cl	24,000[4]	38	A, D	141
(Lys-Ala)$_n$	ONp	19,000	—	A, D	43
(Lys-Gly)$_n$Gly-1-^{14}C-OEt	OMSO$_2$P	11,000[2]	16	A	112
[Lys(Z)-Glu(OBzl)]$_n$	ONp	<100 Res[4]	93	A, D	23
[Lys(Tos)-Gly]$_n$	OTcp	—	—	A	85
[Lys(Tos)-Gly]$_n$	OPcp	—	—	A	85

Table I—*(Continued)*

Polypeptide	Method of Preparation	Average Molecular Weight[a]	Yield, %	Purpose of Preparation[b]	Reference
[Lys(Z)-Gly]$_n$	ONp	<100 Res	97	A, D	23
[Lys(Tos)]$_n$	ONp	—	—	E	31
(Met-Ala)$_n$	ONp	15,000	—	A, D	42
(Phe-Gly)$_n$	ONp	—	—	A	10
(Pro-Ala)$_n$	ONp	3000	—	A, D	238
(Pro-Gly)$_n$	ONp	13,200	—	A, D, E	11, 13, 207, 238, 237, 239, 232
(Pro-Gly)$_n$	TEPP	—	—	A	232
(Pro-Gly)$_n$	ODnp	—	—	A	232
(Pro-Gly)$_n$	[O]	—	—	A	232
(Sar-Gly)$_n$	ONp	—	67	A	240
[Ser(Ac)-Glu(OMe)]$_n$	ONp	14 Res[4]	61	A, D	24
[Tyr(Me)-Glu(OEt)]$_n$	ONp	70–80 Res[4]	84	A, D	21
[Val-Glu(OEt)]$_n$	ONp	70–80 Res[4]	75	A, D	21
[Val-Glu(OMe)]$_n$	ONp	—	86	A	17, 18
(Ala$_2$-Gly)$_n$	ONSu	2200–4500[3]	92	A, B	103
(Ala$_2$-Gly)$_n$	ONp	35,000[1]	40	A, C	38, 241, 242
(Ala$_2$-Gly)$_n$	OPcp	14,000	—	A, D	72, 231
(Ala$_2$-Glycol)$_n$	ONp	—	68	D	25
[Ala-Glu(OEt)-Gly]$_n$	ONp	9000[4]	—	A, D	187
(DL-Ala-Gly$_2$)$_n$	ONp	30,000[1]	87	A	17
(Ala-Gly$_2$)$_n$	ONp	30,000[1]	40	A, C, D	38, 46, 187, 241
(Ala-Gly$_2$)$_n$	ONSu	40 Res	—	A, D	46
(Ala-Gly$_2$)$_n$	OPcp	14,000	—	A, D, C	72, 231
(Ala-Gly$_2$)$_n$	*o*-ONp	40 Res	—	A	242
(Ala-Gly-Pro)$_n$	OPcp	9000	70	A	73
(D-Ala-Gly-D-Pro)$_n$	OPcp	12,700	53	A	73
(Ala-Gly-D-Pro)$_n$	OPcp	3700	41.5	A	73
(D-Ala-Gly-Pro)$_n$	OPcp	4300	30	A	73
(Ala-Gly-Pro)$_n$	TEPP	12,000[4]	—	A, B	28, 127
(Ala-Gly-Lys)$_n$	OPcp	n = 10	—	A	82
[Ala-Gly-Lys(Z)]$_n$	OPcp	—	64	A	74
(Ala-Gly-Thz)$_n$	OPcp	10,000[1]	61.5	A, D	74
(Ala-Hyp$_2$)$_n$	TEPP	6000[3]	—	A, B	127
(Ala-Phe-Gly)$_n$	OPcp	—	—	D	43
(Ala-Pro-Gly)$_n$	ONSu	5000–14,000[3]	81	A, B	101, 104, 174
(Ala-Pro-Gly)$_n$	ONp	5000–14,000[3]	60	A, B	11, 13, 104
(Ala-Pro-Gly)$_n$	TEPP	—	—	A	232
(Ala-Pro$_2$)$_n$	TEPP	9450[4]	—	A, B	127
(Asp-Cys-Gly)$_n$	OPcp	6000[3]	60	A, E	53
(β-Asp-Cys-Gly)$_n$	OPcp	7000[3]	60	A, E	53
[Asp(OMe)-Gly$_2$]$_n$	ONp	19,000[1]	70	A	10, 14
[Asp(Im)-Gly$_2$]$_n$	ONp	5000–10,000[1]	55	A	14
[Asp(OMe)-Phe-Gly]$_n$	ONp	—	—	A	10
[Asp(OMe)-Ser-Gly]$_n$	ONp	3000–11,000	84	A	16
(Asp-Ser-Gly)$_n$	ONp	3000–11,000[3]	—	A, E	16
[Asp-Ser(OAc)-Gly]$_n$	ONp	3000–11,000[4]	—	A, E	16
{Cys(Bzl)-[Glu(OEt)]$_2$}$_n$	ONp	90 Res[4]	92	A, D	19, 21, 193
[Cys(Bzl)-Gly$_2$]$_n$	ONp	—	40	A	17
(Glu-Ala$_2$)$_n$	OHp	3000[1]	62	A	117
(Glu-Ala-Glu)$_n$	OPcp	20,000[4]	94	A	48, 51
(Glu-Ala-Gly)$_n$	OPcp	10,000	75	A	54
(γ-Glu-Ala-Gly)$_n$	OPcp	13,500[3]	44	A	54
[Glu(OEt)-Cys(Bzl)-Glu(OEt)]$_n$	ONp	60 Res[4]	92	A, D	17, 19, 193
(γ-Glu-Cys-Gly)$_n$	OPcp	9000[3]	59	A, E	53
(Glu-Cys-Gly)$_n$	OPcp	16,000[3]	58	A, E	53
[Glu(OBzl)]$_n$	OHp	11,000[1]	50	A, D	118
{[Glu(OEt)]$_2$-Gly}$_n$	ONp	50–100 Res[4]	95	A, D	22, 194
{[D-Glu(OBzl)]$_2$-Leu}$_n$	ONSu	12,100–21,600[2]	—	A	158
(Glu-Gly-Ala)$_n$	ONp	8700[4]	—	A	35
(Glu-Gly-Ala)$_n$	OPcp	6780[4]	—	A	35
[Glu(OBzl)-Gly-Ala]$_n$	ONp	—	35	A	37
[Glu(OEt)-Gly$_2$]$_n$	ONp	7000[4]	72	A, D	17, 187
[Glu(OMe)-Gly-Lys(Tos)]$_n$	OTcp	—	—	A	78
[Glu(OBzl)-Gly-Lys(Tos)]$_n$	ONp	—	—	A	78
[Glu(OBzl)-Gly-Lys(Tos)]$_n$	OPcp	—	—	A	78
(Glu-Ser-Gly)$_n$	ONp	5000–10,000[4]	45	A, D, E	15, 39
[Glu(OBzl)-Ser-Gly]$_n$	ONp	—	—	A, D, E	15, 39
[Glu(OMe)-Ser(Ac)-Glu(OMe)]$_n$	ONp	40–100 Res[4]	100	A, D	24
[Glu(OMe)-Val-Glu(OMe)]$_n$	ONp	—	92	A	17, 18
(Gly-Ala-Hyp)$_n$	TEPP	13,500[4]	—	A, B	127, 178
(Gly-Ala-Hyp)$_n$	OPcp	25,000	—	A	34
(Gly-Ala-Pro)$_n$	TEPP	10,000[4]	—	A, B	127
(Gly-Azet-Ala)$_n$	OHp	2000[2]	27	A, D	74
[Gly-Glu(OBzl)-Lys(Tos)]$_n$	OPcp	3528	—	A	81
(Gly$_2$-Lys)$_n$	OTcp	n = 9	—	A, E	98
(Gly$_2$-Phe)$_n$	OPcp	20,000[3]	49	A, E	48, 51
(Gly$_2$-Phe)$_n$	ONp	—	—	A	12, 13

(continued)

Table I—(*Continued*)

Polypeptide	Method of Preparation	Average Molecular Weight[a]	Yield, %	Purpose of Preparation[b]	Reference
(Gly$_2$-Phe)$_n$	OHp	5000[4]	45	A	116
(Gly$_2$-Pro)$_n$	TEPP	—	—	A, B	127
[Gly$_2$-Lys(Tos)]$_n$	ONp	10,000	—	A	31
(Gly-His-Gly)$_n$	OTcp	—	—	A	93
(Gly-Hyp-Gly)$_n$	OPyr	4000	39	A	243
(Gly-Hyp$_2$)$_n$	OPcp	157,800[1]	89	A, B	34, 75, 76, 171, 178
(Gly-Hyp$_2$)$_n$	ONSu	—	—	B	34
(Gly-Hyp$_2$)$_n$	OTcp	16,000	48	A, B	34, 96
(Gly-Hyp$_2$)$_n$	TEPP	25,000[4]	—	A, B	126, 127
(Gly-Hyp-Gly)$_n$	TEPP	—	—	A	127
[Gly-Lys(Tos)-Gly]$_n$	OTcp	<11,000[4]	66	A	86
[Gly-Lys(Tos)-Gly]$_n$	OPcp	5000[4]	40	A	86
{Gly-[Lys(Tos)]$_2$}$_n$	OTcp	21,000	—	A	84, 94
{Gly-[Lys(Tos)]$_2$}$_n$	OPcp	21,000	—	A	84
{Gly-[Lys(Tos)]$_2$}$_n$	ONp	3000	—	A	31, 94
{Gly-[Lys(Z)]$_2$}$_n$	OTcp	—	—	A	244
(Gly-Orn-Gly)$_n$	DCC	10,800[2]	55	A	245
(Gly-Orn$_2$)$_n$	[O]	1500[2]	22	A	245
(Gly-Orn$_2$)$_n$	OTcp	2100[2]	25	A	245
(Gly-Pipec-Ala)$_n$	OHp	2000[2]	15	A, D	74
(Gly-Pro-Ala)$_n$	OHp	12,000[7]	66	A	114
(Gly-Pro-Ala)$_n$	ONp	15,000[3]	—	A	26, 172, 175
(Gly-Pro-Ala)$_n$	ONSu	20,000	—	A	108
(Gly-Pro-Ala)$_n$	OTcp	4300[4]	23	A	28
(Gly-Pro-Ala)$_n$	TEPP	2000	—	A	127
[Gly-Pro-Glu(OBzl)]$_n$	ONp	2500	30	A, B	33
(Gly-Pro-Gly)$_n$	OTcp	9000	53	A, B	28, 34, 96
(Gly-Pro-Gly)$_n$	ONp	6000[3]	31	A, B	28, 34, 26, 172
(Gly-Pro-Gly)$_n$	ONSu	11,000	—	A, B	34, 108
(Gly-Pro-Gly)$_n$	OPfp	—	—	B	34
(Gly-Pro-Gly)$_n$	OQu	3200	—	A	246
(Gly-Pro-Hyp)$_n$	OTcp	20,000	—	A, B	34, 96
(Gly-Pro-Hyp)$_n$	OPcp	100,000	57	A, B	34, 79
(Gly-Pro-Hyp)$_n$	TEPP	36,000	—	A, B	124, 125, 127,162 167, 232
(Gly-Pro-Hyp)$_n$	OPfp	—	—	B	34
(Gly-Pro-Hyp)$_n$	ONSu	—	—	B	34
(Gly-Pro-Hyp)$_n$	ODnp	—	—	A	232
(Gly-Pro-Leu)$_n$	TEPP	6000	—	A, B	123, 127
(Gly-Pro-Leu)$_n$	ONSu	4000–6000	81	A, B	108
(Gly-Pro-Lys)$_n$	TEPP	—	—	B	127
[Gly-Pro-Lys(Tos)]$_n$	ONp	7000–10,000	—	A, B, E	30, 94
[Gly-Pro-Lys(Tos)]$_n$	OTcp	7000–10,000	—	A, E	94
[Gly-Pro-Lys(Z)]$_n$	ONp	—	—	A	244
(Gly-Pro$_2$)$_n$	ONp	3000	—	A, B	29
(Gly-Pro$_2$)$_n$	TEPP	11,700[4]	—	A, B	127, 168
(Gly-Pro$_2$)$_n$	OPcp	100,000	—	A, B	83
(Gly-Pro-Ser)$_n$	TEPP	3000	45	A, B	129
(Gly-Pro-Tyr)$_n$	TEPP	3500[4]	—	A, B	127
(Gly-Ser-Ala)$_n$	[O]	3000[3]	50	A	140
(Gly-Ser-Gly)$_n$	ONp	4700[3]	—	A, E	16
(Gly-Ser-Gly)$_n$	OPcp	3000	50	A, C	36, 77
(Gly-Ser-Hyp)$_n$	OPcp	6000	—	A, B	80
(Gly-Ser-Pro)$_n$	TEPP	3000[2]	60	A	129
(Gly-Ser-Pro)$_n$	OPcp	2500	—	A, B	80
(His-Gly$_2$)$_n$	ONp	4000[4]	—	A	10, 13
[His(ImZ)-Gly$_2$]$_n$	OTcp	—	—	A	93
(Hyp-Glu-Gly)$_n$	OTcp	8500	—	A, B, E	95, 97
[Hyp-Glu(OBzl)Gly]$_n$	OTcp	8500	90	A, B	97
(Hyp-Pro-Gly)$_n$	ONp	10,400	—	A, D	238
(Hyp-Ser-Gly)$_n$	OTcp	6130	35.3	B, E	95, 97
(DL-Leu-Gly$_2$)$_n$	–Cl	$n = 5$	—	A	247
(Leu-Gly-Pro)$_n$	TEPP	8700[4]	—	A, B	127
[Leu$_2$-Asp(OBzl)]$_n$	ONp	—	—	A, D	248
(Leu$_2$-Asp)$_n$	ONp	—	—	A, D	249
(Leu$_2$-2Hy-4-mepent)$_n$	–Cl	32,000[4]	45	A, D	141
(Leu-Orn-Leu)$_n$	ONSu	4000–10,000[3]	90	A, E	250
(Leu-Orn-Leu)$_n$	TEPP	2700[3]	90	A, E	250
(Lys-Ala$_2$)$_n$	OHp	3000[1]	62	A	115, 117
(Lys-Ala$_2$)$_n$	ONSu	13,000	—	A, D	251
(Lys-Ala-Gly)$_n$	OPcp	13,100[5]	81	A	55
(Lys-Arg-Ala)$_n$	OTcp	5300	—	A	252
(Lys-Arg-Gly)$_n$	OTcp	7000	—	A	252
[Lys(Z)-Gly-Ala]$_n$	OPcp	—	55	A	82
(Lys-Gly-Ala)$_n$	OPcp	$n = 9$	—	A	82
(Met$_2$-Ala)$_n$	ONp	6000	—	A, D	40, 41
(Phe-Pro-Gly)$_n$	ONp	—	70	D	44
(Pipec-Ala-Gly)$_n$	OPcp	—	16	D	74
(Pro-Ala-Gly)$_n$	OPcp	—	34	A	73

(*continued*)

Table I—*(Continued)*

Polypeptide	Method of Preparation	Average Molecular Weight[a]	Yield, %	Purpose of Preparation[b]	Reference
(Pro-Ala-Gly)$_n$	ONSu	—	16	A	73
(Pro-Ala-Gly)$_n$	TEPP	15,000[2]	—	A, B	128, 173, 232
(Pro-Ala-Gly)$_n$	ONp	12,400[4]	81	A ,B	27, 73, 253
(Pro-Ala-Glycol)$_n$	ONp	1400	90	A, B, D	25
(Pro-Gly-Ala)$_n$	TEPP	—	—	D	184
[Pro-Gly-Hyp(Ac)]$_n$	TEPP	2700	—	A, B, E	170, 207, 239
(Pro-Gly$_2$)$_n$	ONp	—	—	A	17
(Pro-Gly$_2$)$_n$	TEPP	3500[4]	—	A, B	170, 182
(Pro-Gly$_2$)$_n$	[O]	1400[4]	—	A	139
(Pro-Gly-Pro)$_n$	TEPP	1000–12,000[3]	64	A, B, E	99, 122, 170, 179, 208, 209, 253
(Pro-Gly-Ser)$_n$	TEPP	2800[2]	50	A	129
(Pro-Hyp-Gly)$_n$	ONp	40,000[3]	—	A	11, 13
(Pro-Hyp-Gly)$_n$	S.P.	n = 5, 10	—	A, B	145
(Pro-Leu-Gly)$_n$	TEPP	6600	—	A	123
(Pro-Leu-Gly)$_n$	DCC	6600	—	A	123
(Pro-Phe-Gly)$_n$	ONp	—	74	D	44
(Pro$_2$-Gly)$_n$	S.P.	n = 10, 15, 20	—	A, B	143, 144, 189, 212
(Pro-Ser-Gly)$_n$	ONp	16,000[4]	65	A, B	27, 253
(Pro-Ser-Gly)$_n$	TEPP	5000[2]	60	A	129
(Pro-Ser-Gly)$_n$	ONSu	5100[2]	32	A	129
(Pro-Ser-Gly)$_n$	OPcp	4000	50	A, B	183
(Ser-Gly-Pro)$_n$	TEPP	2500[2]	40	A	129
(Ser-Pro-Gly)$_n$	TEPP	3900[2]	54	A	129
(Ser-Pro-Gly)$_n$	ONp	10,000[3]	—	A	11, 13
(Tyr-Ala-Glu)$_n$	ONSu	60,000[3]	63	A, D, E	99, 100, 106, 216–221, 254
[Tyr(Me)-Ala-Gly]$_n$	ONp	70–80 Res[4]	84	A, D	21
(Val-Pro-Gly)$_n$	ODnp	109,000[3]	—	A, B	232
(Val-Pro-Gly)$_n$	[O]	—	—	A	232
(Ala$_3$-Gly)$_n$	ONp	20,000[1]	40	A, C	38, 241, 242
(Ala$_2$-Gly$_2$)$_n$	OPcp	10,500	—	A, D	231
(Ala-Glu-Ala-Gly)$_n$Gly-1-^{14}C-OEt	OPcp	<100,000[2]	14	A, E	65
(Ala-Gly$_3$)	OPcp	14,000	—	A, D	72, 231
[Glu(OEt)-Cys(Bzl)-(Glu(OEt))$_2$]$_n$	ONp	60 Res[4]	82	A, D	19, 21
(Glu$_2$-Ala-Gly)$_n$Gly-1-^{14}C-OEt	OPcp	260,000[5]	78	A	56
{[Glu(OEt)$_2$-Cys(Bzl)-Glu(OEt)}$_n$	ONp	48 Res[4]	92	A, D	19, 21, 193
{[Glu(OEt)]$_3$-Gly}$_n$	ONp	50–100 Res[4]	95	A, D	22, 194
{[D-Glu(OBzl)]$_3$-Leu}$_n$	ONSu	11,400–23,000[2]	56	A	158
{[Glu(OMe)]$_2$-Ser(Ac)-Glu(OMe)}$_n$	ONp	40–100 Res[4]	100	A, D	24
{[Glu(OMe)]$_2$-Val-Glu(OMe)]$_n$	ONp	—	93	A, D	17, 18
(Glu-His-Lys-Tyr)$_n$	DCC	15,500[4]	53	A	132, 133
(Glu-Tyr-Ala-Gly)$_n$Gly-1-^{14}C-OEt	OPcp	100,000[2]	32	A, E	63
(Gly$_2$-Hyp-Gly)$_n$	ONp	17,000[3]	—	A, D	11, 13, 237, 238
(Gly$_2$-Pro-Gly)$_n$	ONp	6700	—	A, D	237, 238
{Gly$_2$[Lys(Tos)]$_2$}$_n$	ONp	10,000	—	A, E	31
(Leu$_3$-2-Hy-4-mepent)$_n$	Cl	46,000[3]	42	A, D	141
(Phe-Asp-Val-Gly)$_n$Gly-OMe	OPcp	1–5 × 10,000[6]	6	A, E	255
(Phe-Glu-Ala-Gly)$_n$Gly-1-^{14}C-OEt	OPcp	<100,000[2]	41	A, E	60, 62, 225
(Phe-Glu-Val-Gly)$_n$Gly-OMe	OPcp	1–5 × 10,000[6]	21	A, E	255
[Pro-Gly-Hyp(OAc)-Pro]$_n$	ONp	—	—	E	239
(Trp-Glu-Ala-Gly)$_n$Gly-OMe	OPcp	3000[6]	22	A, E	67
(Tyr-Asp-Ala-Gly)$_n$Gly-OMe	OPcp	<100,000[6]	27	A, E	69
(Tyr-Glu-Ala-β-Ala)$_n$Gly-OMe	OPcp	<100,000[6]	30	A, E	224
(Tyr-γ-Glu-Ala-Gly)$_n$Gly-OMe	OPcp	20,000[2]	27.8	A, E	66
[Tyr(Me)-Glu-Ala-Gly]$_n$-Gly-1-^{14}C-OEt	OPcp	100,000[2]	24	A, E	64
(Tyr-Glu-Ala-Gly)$_n$Gly-1-^{14}C-OEt	OPcp	100,000[5]	78.6	A, E	57, 59, 222
(Tyr-Glu-Gly$_2$)$_n$Gly-OMe	OPcp	100,000[2]	58	A, E	61
[Tyr-(γ-Gly)-Glu-Ala-Gly]Gly-OMe	OPcp	<100,000[6]	27	A, E	70
(Tyr-Glu-Val-Gly)$_n$Gly-1-^{14}C-OEt	OPcp	155,000[2]	82	A, E	58, 226, 227
[Tyr-(ϵ-Glu)-Lys-Ala-Gly]$_n$Gly-OMe	OPcp	20,000[2]	28	A, E	68
(Ala$_2$-Gly$_3$)	OPcp	40 Res	—	A, D	231
{Glu(OEt)-[Cys(Bzl)]$_2$-[Glu(OEt)]$_2$}$_n$	ONp	60–80 Res[4]	100	A, D	19, 21, 193
[Glu(OEt)-Cys(Bzl)-Glu(OEt)-Cys(Bzl)-Glu(OEt)]$_n$	ONp	60–80 Res[4]	94	A, D	19, 21, 193
{[Glu(OEt)]$_3$-Cys(Bzl)-Glu(OEt)}$_n$	ONp	60–80 Res[4]	100	A, D	19, 21, 193
{[Glu(OEt)]$_4$-Gly}$_n$	ONp	50–100 Res[4]	96	A, D	22, 194
{[D-Glu(OBzl)]$_4$-Leu}$_n$	ONSu	31,500[2]	57	A	158
{[Glu(OMe)]$_3$-Ser(Ac)-Glu(OMe)}$_n$	ONp	40–100 Res[4]	100	A, D	24
{[Glu(OMe)]$_3$-Val-Glu(OMe)}$_n$	ONp	—	94	A, D	17, 18
(Ala$_3$-Gly$_2$)	OPcp	40 Res	—	A, D	231
(Ala$_2$-Gly-Pro$_2$-Gly)$_n$	ONSu	2000–12,000[3]	—	A, B	102, 185
(Ala-Gly-Ala-Gly-Ser-Gly)$_n$	ONp	16,000[4]	96	A, C	20, 191
(Ala-Pro-Gly-Pro-Ala-Gly)$_n$	ONSu	2000–12,000[3]	—	A, B	102, 185
[Cys(Bzl)-Gly$_2$-Glu(OMe)$_n$-Val-Glu(OMe)]$_n$	ONp	—	65	A	17
{[Glu(OMe)]$_3$-Val-Val-Glu(OMe)}$_n$	ONp	70–80 Res[4]	89	A, D	21

(continued)

Table I—*(Continued)*

Polypeptide	Method of Preparation	Average Molecular Weight[a]	Yield, %	Purpose of Preparation[b]	Reference
{[Glu(OEt)]$_2$-Gly-Glu(OEt)$_3$}$_n$	ONp	50–100 Res[4]	98	A, D	22, 194
(Pro-Ala-Gly-Pro$_2$-Gly)$_n$	ONSu	2000–12,000[4]	—	A, B	102, 185
(Leu-Glu-Lys-Ala-Glu-Ala-Gly)$_n$	ONp	6000[2]	33	A	256
(Leu-Glu-Lys-Ala-Glu-Ser-Gly)$_n$	ONp	11,000[2]	34	A	256

[a] Method of molecular weight estimation is given by superscript number as follows: [1], viscosity; [2], gel filtration; [3], ultracentrifugation; [4], end-group assay; [5], osmometry; and [6], Diaflo membrane. [b] A = synthetic study, B = collagen model, C = silk fibroin model, D = conformation study, and E = biological study.

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RESEARCH ARTICLES

Cholesterol Solubility in Model Bile Systems: Implications in Cholelithiasis

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Abstract ☐ Studies of cholesterol solubility in a physiologically realistic model bile system as a function of its (crystalline) form revealed significant behaviorial differences in the rate of solution and the approach to equilibrium. The solubility of the equilibrium species, hydrated cholesterol, and of fresh human gallstones was found to be approximately 5 mole %. Anhydrous and coprecipitated cholesterol samples attained metastable solubility values. The lag time for nucleation and growth to occur in these systems could account for lab-to-lab variations in previous studies and could be of extreme importance *in vivo* when lithogenic bile is present. These studies were extended to measurement of the solubility as a function of the bile salt-lecithin ratio. Evaluation of these data with respect to available clinical data reveals that many normal individuals have bile that is supersaturated with respect to cholesterol. The principles governing the maintenance of supersaturation in these persons are discussed and suggestions for future studies are offered.

Keyphrases ☐ Cholesterol solubility in model bile systems—relationship to solid-state (crystalline) properties and bile salt-lecithin ratio, implications in cholelithiasis ☐ Gallstones and cholesterol-supersaturated bile in man—implications of cholesterol solubility in model bile systems, solution rate and equilibrium considerations ☐ Bile salt-lecithin ratio—relationship to cholesterol solubility, model

Cholesterol is the major component of gallstones found in western man. This water-insoluble lipid is primarily excreted in the bile. The nature of the interactions between cholesterol and the other biliary lipids, namely bile salts and phospholipids, has been the subject of many investigations. Transport of the cholesterol has been shown to be mediated by a lecithin-bile salt micelle system. Overloading this system can result in cholelithiasis, which is the deposition of cholesterol as gallstones, a disease state that has been estimated to affect some 15 million Americans. "More than 300,000 gallbladders are removed annually because of cholelithiasis while medical, surgical, and hospitalization expenses ascribed to gallstone disease total almost 1 billion dollars a year" (1).

The functions of bile almost seem paradoxical; on the one hand it is involved with intestinal digestion and absorption of lipids, and on the other it is responsible for the excretion of the otherwise water-