94. The Construction of New Proteins

Part III[1])

Artificial Folding Units by Assembly of Amphiphilic Secondary Structures on a Template

by **Manfred Mutter***, **Eva Altmann**, **Karl-Heinz Altmann**, **René Hersperger**, **Pjotr Koziej**, **Kurt Nebel**, **Gabriele Tuchscherer**, and **Stéphane Vuilleumier**

Institut für organische Chemie, Universität Basel, CH-4056 Basel

and **Hans-Ulrich Gremlich**

E. Merck, D-6100 Darmstadt

and **Klaus Müller**

F. Hoffmann-La Roche & Co. AG, CH-4002 Basel

(21.III.88)

A new general strategy for the construction of artificial proteins with predetermined tertiary structure is presented. Amphiphilic α-helix and β-sheet-forming oligopeptides are assembled on a multifunctional template molecule which directs the peptide blocks to adopt characteristic folding topologies. The design, synthesis, and conformational properties of these template-assembled synthetic proteins (TASP) are exemplified for $\beta\alpha\beta$-, α-helix-bundle- and β-barrel-like tertiary structures using specially designed oligopeptides as template molecules. In contrast to linear polypeptide chains of comparable molecular weights, these conceptually novel macromolecules are readily accessible to chemical synthesis and exhibit excellent solubility in a number of solvents. Experimental evidence is provided for a template-induced intramolecular folding to secondary and tertiary structures in aqueous solutions. This approach opens new prospects for the chemical construction of biomacromolecules with tailor-made structural and functional properties.

Introduction. – The construction of new proteins is a challenging goal in present peptide and protein chemistry. Progress in our understanding of the rules that govern protein folding and topology has stimulated efforts to design polypeptide sequences with predetermined conformation and tailor-made chemical, biological, and catalytic properties. One of the major hurdle stems from our still limited understanding of the relationship between primary sequence and conformation ('folding code') of a linear polypeptide chain [1]. Natural polypeptide sequences have evolved that are able to fold into tightly packed structures with optimized intramolecular interactions, a situation hardly met by *de novo* design of proteins. On the other hand, X-ray diffraction studies of a large number of proteins have revealed common topological features such as the assembly of α-helices and β-sheets to characteristic folding units [2]. Because these super-secondary structures are not bound to a specific amino-acid sequence, the folding

[1]) Part II: [4].

code appears to be highly degenerate. Taken together, these observations were the conceptual starting point for the design of new proteins by linking amphiphilic α-helices and/or β-sheets *via* loop sequences to generate linear polypeptides with a tendency for folding into predetermined folding topologies [3–7].

Despite some encouraging results, the *de novo* design of proteins appears to be limited by the competition between intramolecular interactions (process of folding) and intermolecular aggregation of the amphiphilic peptide segments resulting in poor solubility of the target molecules. To overcome this intrinsic problem, we have recently proposed a new strategy for the construction of the tertiary structures [8]. Rather than trying to match the complex folding mechanism of a linear polypeptide chain, we use template molecules that direct blocks of amphiphilic secondary structures to predetermined chain topologies. Based on today's synthetic peptide chemistry as well as on knowledge about the energetics of peptide conformational stability and protein topology, the design, synthesis, and conformational properties of several prototypes of this new generation of macromolecules are reported in the present article.

Results and Discussion. – 1. *Design of Template-Assembled Synthetic Proteins (TASP).* Tertiary structures are constructed by assembling peptide blocks with a high potential for taking up a secondary structure on a multifunctional carrier molecule (template). As shown schematically in *Fig. 1*, amphiphilic α-helix- and β-sheet-forming oligopeptides are attached to the template which favours a specific packing of the peptide units to predetermined topologies, *e.g.* βαβ-, 4-helix-bundle-, or β-barrel-like folding patterns. The spatial constraints of these branched macromolecules result in a higher volume density compared to an unfolded linear polypeptide chain of equal number of amino-acid residues; these template-enhanced intramolecular interactions act as a major driving force for the folding of the template-attached amphiphilic peptide blocks into a globular structure (similar to the postulated hydrophobic collapse) in which the hydrophobic residues make minimal contact with the solvent (H_2O). In principle, multifunctional molecules exhibiting limited conformational flexibility (*e.g.* specially designed linear oligopeptides) as well as conformational constrained (*e.g.* cyclic) molecules can be used as templates. In this exploratory study, we have focused on multifunctional oligopeptides as template molecules for the following reasons: (*i*) they are readily accessible to chemical synthesis; (*ii*) orthogonal main and side-chain protection provides synthetic flexibility; thus, peptide blocks can be attached by segment condensation or stepwise synthesis (*Scheme 1*), and peptide vectors can be assembled with parallel or antiparallel orientations; (*iii*) templates with tailor-made spatial geometry and conformational constraints can be generated by incorporation of nonnatural amino acids and peptide mimetics. As verified by computer-assisted molecular modelling, templates suitable for the adjacent assembly of α-helices and β-sheets may consist of two antiparallel peptide segments linked by one or two β-turns, loops, or turn mimetics [9]. A prototype for a semirigid template molecule is shown in *Fig. 2*. The conformation of the cyclic decapeptide cyclo-(Pro-Lys-Ala-Lys-D-Phe-)$_2$ was modelled in analogy to the known structure of gramicidin S [10], in which two antiparallel β-sheet segments are linked by two β-turns of type II′ at Pro-D-Phe. Assuming a low-energy conformation for our template molecule, the ε-amino groups of the four lysine side chains can be properly spaced for the construction of tertiary structure as depicted in *Fig. 1*. For example, variation of the lysine side-

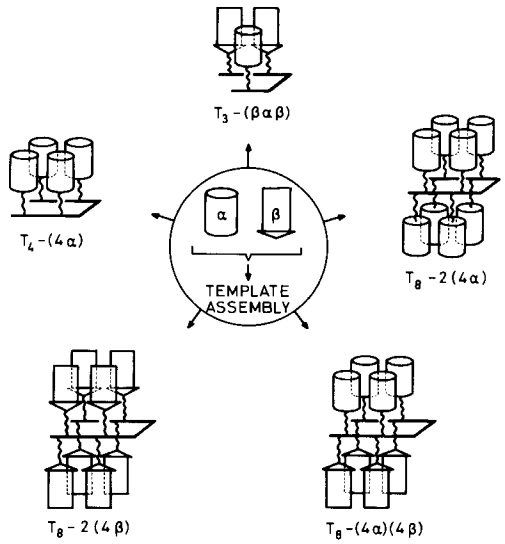


Fig. 1. *Schematic representation of template-assembled proteins.* T_n, T = Template molecule, n = number of attachment sites on T; α, β, amphiphilic peptide blocks with tendency for helix (α) and β-sheet (β) secondary-structure formation. Different examples of synthetically accessible folding topologies are shown.

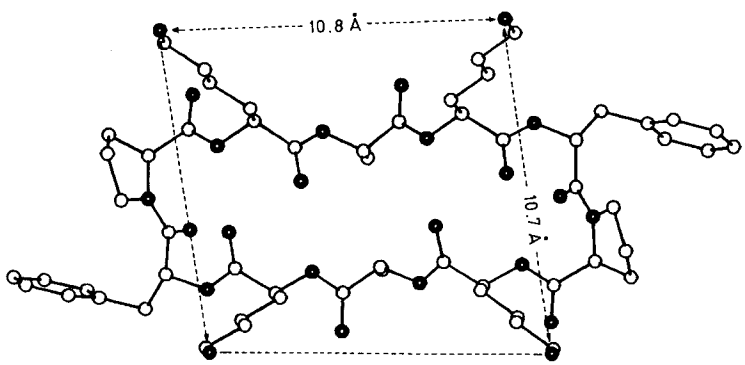


Fig. 2. *Drawing of a computer-generated model of the cyclic decapeptide analogue of gramicidin S, cyclo(-Pro-Lys-Ala-Lys-*D*-Phe-)$_2$, as a prototype for a semirigid template molecule in a low-energy conformation.* The distances r between the attachment sites (the ε-amino groups of lysines) allow the construction of a 4-helical-bundle TASP (see *Fig. 3*). Modelling and energy minimization were performed using the proprietary RIMG (*Roche* Interactive Molecular Graphics) system [30].

chain conformation results in distances r between the ε-amino groups ranging from 4 to 14 Å. In particular, distances r of 10 Å (*Fig. 2*) result in a low-energy conformation which is suitable for the construction of a 4-helix-bundle protein (*Fig. 3*).

Acyclic templates with the general features of the one depicted in *Fig. 2* were chosen for the construction of $\beta\alpha\beta$ (TASP I in *Table 1*), 4-helix-bundle (TASP II, III), and β-sheet (TASP IV) topologies, because the synthesis of cyclic peptides is not yet amenable to standard polymer-supported peptide synthesis methodology, which we considered the method of choice for a first screening of the usefulness of our strategy. The design of the

Scheme 1. *General Scheme for the Synthesis of Template-Assembled Proteins T_4-(2α)(2β)* (see *Fig. 1*). aa = trifunctional amino acid (*e.g.* lysine), R = C-terminal protecting group and/or polymeric support, Y_n = side chain protecting group, α',β' = side chain protected α-helical or β-sheet peptide block, respectively.

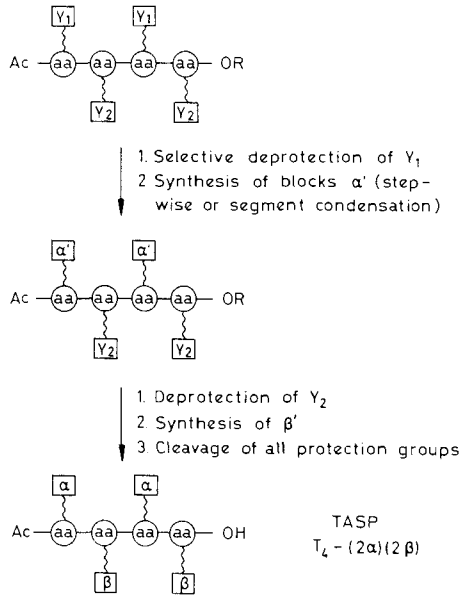


Table 1. *Primary Sequences of the Different Template-Assembled Synthetic Proteins* (TASP) *Described*

TASP	Postulated topology[a])	Primary sequences[b])	
I	T_3-($\beta_I\alpha_I\beta_I$)	T_3:	Ac-Lys(α_I)-Pro-Lys(β_I)-Lys(β_I)-Polymer
		β_I:	Ac-(Thr-Val)$_3$-
		α_I:	Ac-Glu-Ala-Leu-Abu-Ala-Glu-Leu-Abu-Glu-Leu-Glu-Ala-
II	T_4-(4 α_{II})	T_4:	Ac-Lys(α_{II})-Lys-Lys(α_{II})-Pro-Gly-Lys(α_{II})-Glu-Lys(α_{II})-OH
		α_{II}:	H-Asp-Ala-Ala-Thr-Ala-Leu-Ala-Asn-Ala-Leu-Lys-Lys-Leu-
III	T_8-2(4 α_{III})	T_8:	Ac-[Lys(α_{III})]$_4$-Pro-Gly-[Lys(α_{III})]$_4$-ε Ahx-Gly-OH
		α_{III}:	Ac-Glu-Ala-Leu-Ala-Lys-Glu-Leu-Ala-Ala-Lys-Leu-
IV	T_8-2(4β_{IV})	T_8:	Ac-[Lys(β_{IV})]$_4$-Pro-Gly-[Lys(β_{IV})]$_4$-ε Ahx-Gly-OH
		β_{IV}:	Ac-(Val-Lys)$_4$-

[a]) *Cf. Fig. 1.*

[b]) Conventions as in *Fig. 1*; the α and β peptide blocks are linked to the template through amide-bond formation between their C-terminal carboxyl group and the ε-amino groups of the template lysine side chains. Abu = 2-aminobutyric acid, ε Ahx = 6-aminohexanoic acid (spacer), Ac = N-terminal acetyl group, H = N-terminal free amino group, OH = C-terminal carboxylic group.

α-helix and β-sheet peptide blocks was based on experimental data on the relationship between primary-structure and secondary-structure formation [11–13], empirical conformation predictions [14], and computer-assisted molecular-modelling studies similar to those done previously [3][4][15].

As indicated by molecular-modelling studies, the template Lys-Pro-Lys-Lys in TASP I (*Table 1*) can adopt a low-energy conformation with a β-turn of type I' for Pro-Lys, in

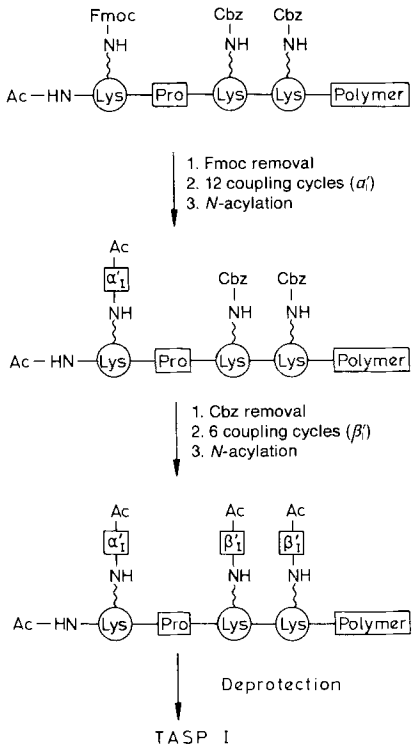
agreement with the high probability for this pair to occur at positions $i + 1$ and $i + 2$ of turns in proteins [16]. The templates used for TASP II–IV may exist in a low-energy conformation similar to that of the cyclic decapeptide of *Fig. 2*, with the β-turn of type II for Pro-Gly connecting the antiparallel β-sheet segments.

The design of a template-assembled protein by computer-assisted molecular modelling is shown in *Fig. 3* for the example of a 4-helix-bundle topology (TASP II analogue, see *Table 1* and legend to *Fig. 3c*). Due to the conformational characteristics of the oligomethylene side chain of lysine, a considerable directional persistence perpendicular to the plane of the template is predicted [17]; furthermore, sufficient torsional flexibility of the lysine (tetramethylene)amino units allows the attached peptide blocks to orient themselves for optimal packing and solvation, *i.e.* the template is expected to enhance and stabilize tertiary-structure formation. Indeed, the design of templates which directs the folding to predetermined topology seems a prerequisite for the construction of complex globular-like structures. Simple cross-linking of peptide chains appears appropriate only if the peptide chains exhibit an intrinsic tendency to fold in a particular overall structure, as proposed for the triple-helix formation of collagen models [18] or in β-barrel topologies [7].

2. *Synthesis and Solution Properties.* One of the most attractive features of TASP molecules is their accessiblility to chemical synthesis. In contrast to globular proteins, the tertiary structures are formed by individual oligopeptides of medium size (6–10 residues for β-structure blocks, 10–20 residues for helical blocks), which are assembled either simultaneously or successively on a template *(Scheme 1)*. From a synthetic point of view, the template corresponds to a multifunctional carrier with specifications of capacity and average distance between attachment sites similar to those of soluble or insoluble supports in conventional peptide synthesis [19]. Consequently, the synthesis of TASP molecules follows the same rules as those of conventional peptide synthesis; however, the strategy for the construction of these branched peptides is much more efficient and less subject to the limitations (*e.g.* solubility and coupling problems, see below) encountered in the synthesis of linear peptides of comparable size [20].

TASP I–IV were synthesized by standard protocols [19] applying both soluble (TASP I) and insoluble (TASP II–IV) polymeric supports. Orthogonal main and side chain functional group protection strategies give access to a number of ways to build up 'artificial proteins'. For example, selectively cleavable protecting groups at the attachement sites in the template tetrapeptide allowed us to successively synthesize first the α-helix and then the β-sheet peptide blocks in TASP I *(Scheme 2)*. Having previously prepared linear folding units [4][21] as well as α-helical [12] and β-sheet blocks [13] of similar or identical molecular weight and amino-acid composition, special attention was given to any noticeable differences in physico-chemical properties between linear and template-assembled peptides. Remarkably, the fully protected template-assembled TASP-I peptide exhibited high solubility in CH_2Cl_2, DMF, AcOH during all steps of the synthesis while the analogous linear $\beta\alpha\beta$-peptide of comparable number of residues [4][21] was poorly soluble. Furthermore, no chain-length-dependent coupling problems could be detected during the synthesis of TASP I, in contrast to a significant decrease in the coupling kinetics observed with growing chain length in the corresponding linear peptide [4][21]. Preliminary attenuated total reflection infrared (ATR-IR) and circular dichroism (CD) studies of the fully protected TASP I [22], indicating that the template-

Scheme 2. *Strategy for Polymer-Supported Synthesis of Type TASP I* (see *Table 1*). The polymer is polyethylene glycol monomethyl ether, $M_r = 5000$ [19].



assembled molecule adopts a pure random-coil conformation when the side chains are still protected, are in accord with these findings; in particular, the postulated secondary and tertiary structure (which are expected to induce dramatic changes in physico-chemical properties) are only established after deprotection of the side chains in the target molecule (see below).

Observations along these lines were also made in the solid-phase syntheses of TASP prototypes II–IV. Starting from regular polystyrene-based resins (capacity 0.1–0.4 mmol/g), both the α-helix-(TASP II, III) and β-sheet forming (TASP IV) peptides were assembled 'simultaneously' on the template. By this efficient strategy, the total number of required coupling steps is identical with the number of residues in the single peptide blocks themselves. For example, the synthesis of TASP III (molecular weight *ca.* 11000) starting from the oligopeptide template needed only 11 reaction cycles. Moreover, coupling yields were comparable to those observed for the synthesis of the corresponding single, unattached peptide.

These findings show that TASP molecules of even large molecular weight are readily accessible by efficient polymer-supported synthesis. However, whenever crystallizable products are the objective, segment condensation in solution *(Scheme 1)* is undoubtedly a more promising strategy. TASP I–IV proved to be readily soluble in H_2O over a wide range of experimental conditions (pH, temperature, and concentration variation,

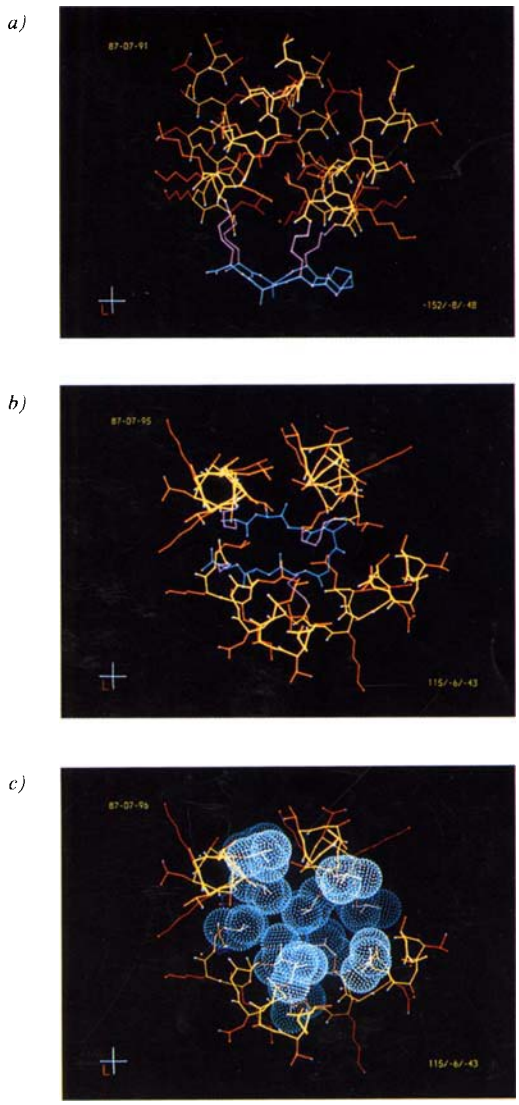


Fig. 3. *Computer-generated model of a template-assembled 4-helix-bundle protein* (analogue of TASP II, see *Table 1*). *a*) Side view of the molecule (peptide template in cyan, lysine side chains in magenta, helix backbones in yellow, amino-acid side chains of helices in red). *b*) View along one helix axis from the N-terminal end, showing that the helix axes are tilted with respect to each other. *c*) Same as *b*) with the inclusion of dotted *van der Waals* surfaces for all leucine side chains (3 per helix) showing the hydrophobic contacts in the interior of the 4-helix bundle. The octapeptide model template (Lys-Ala-Lys-Pro-Gly-Lys-Ala-Lys) was built with standard geometries for all amino acids, assuming a type-II β-turn for Pro-Gly and an antiparallel β-sheet structure for the two Lys-Ala-Lys segments. This template conformation persisted after full energy minimization. The terminal amino groups of the 4 lysine side chains were then linked to the C-terminal acyl units of pre-modelled amphiphilic helices (Ala-Ala-Thr-Ala-Leu-Ala-Asn-Ala-Leu-Lys-Lys-Leu). Keeping the template backbone structure fixed, the lysine side chains and the attached helices were then conformationally relaxed with respect to all degrees of freedom, resulting in an unstrained low-energy conformation. The modelling and energy minimization were performed using the proprietary RIMG system [30].

addition of salts and organic solvents). The pronounced solubility together with the greatly reduced tendency for aggregation of TASP molecules may be regarded as strong experimental evidence that the template enhances 'intramolecular aggregation' of the amphiphilic peptides to a globular structure, in which the hydrophilic residues are exposed to H_2O.

3. *Conformational Features of TASP I–IV.* TASP I–IV have been further characterized by CD (*Figs. 4* and *5*) and IR (*Table 2*) spectroscopy. Experimental data are in full agreement with the postulated secondary and tertiary structures. TASP I (*Fig. 4*) exhibits a negative *Cotton* effect at pH 7 ($\lambda = 218$–222 nm) in H_2O/CF_3CH_2OH 8:2 consistent with the presence of both an α-helix and β-sheet structure [23]. In addition, the solid-state IR-absorption bands (*Table 2*) are indicative of the presence of both helical ($\tilde{\nu} = 1655$ cm^{-1}) and β-sheet ($\tilde{\nu} = 1630$ cm^{-1}) blocks [24]. Preliminary investigations in D_2O by ATR-IR methods [22] indicate that the secondary structures are independent of dilution, in accord with an intramolecularly folded state of the molecule. Remarkably, the critical chain length for the onset of secondary-structure formation is significantly lower (6 residues) for the β-sheet block when it is attached to the template (see *Scheme 2*)

Table 2. *Ir-Absorption Frequencies of TASP I–IV in the Solid State* (Kbr)

TASP	$\tilde{\nu}$ (amide A) [cm^{-1}]	$\tilde{\nu}$ (amide I) [cm^{-1}]	Secondary-structure type [24]
I	3280	1630	β-sheet
	3300	1655	α-helix
II	3300	1660	α-helix
III	3320	1665	α-helix
IV	3295	1635	β-sheet

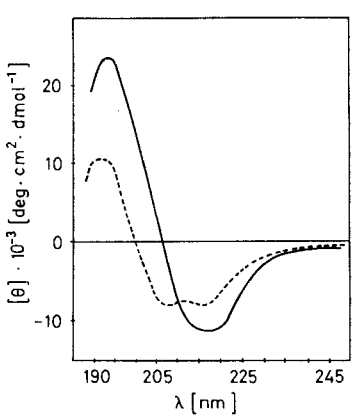


Fig. 4. *CD spectra of TASP IV in H_2O at pH 10.1 ($c = 10^{-3}$ M; ——) and of TASP I in H_2O/CF_3CH_2OH 8:2, ($c = 10^{-4}$ M; – – –). See Table 1.*

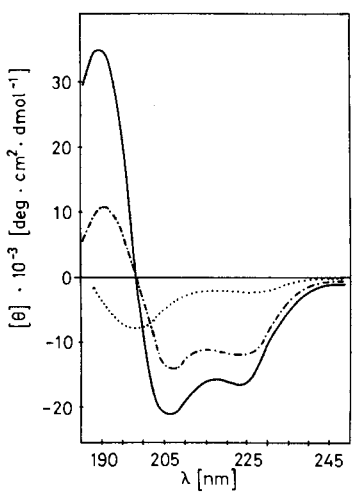


Fig. 5. *CD spectra of TASP II in salt-free H_2O ($c = 10^{-3}$ M; ——) and of template-unattached 13-mer peptide at pH 7 ($c = 10^{-3}$ M) in H_2O (· · · · ·) and in 0.4 M sodium dodecyl sulfate (· — · — · — ·). See Table 1.*

compared to the individual peptide block alone [13], an observation pointing to the secondary-structure-inducing effect of the template.

The template effect is even more dramatic for the prototypes TASP II–IV. As shown in *Fig. 5*, TASP II exhibits a CD spectrum in aqueous buffer typical for an α-helix conformation (negative *Cotton* effects $\lambda = 222$ nm ($n - \pi^*$) and $\lambda = 207$ nm ($\pi - \pi^*$), crossover at $\lambda = 198$ nm, positive CD at $\lambda = 190$ nm), whereas the corresponding template-unattached 13-mer peptide adopts predominantly random-coil conformation under identical experimental conditions (negative CD at $\lambda = 197$ nm, crossover at $\lambda = 190$ nm, *Fig. 5*). Interestingly, the CD spectra (not shown) of the template itself point to a predominantly random-coil conformation under identical experimental conditions.

Moreover, sodium dodecyl sulfate (SDS) addition to the 13-mer peptide *(Fig. 5)* induces the transition from a disordered to a helical conformation, whereas no significant changes in the spectra are observed for the template-assembled 4-helix bundle (not shown). We consider this as a clear indication that the α-helix blocks in TASP II are stabilized by hydrophobic contacts between interacting blocks in the core of the bundle similar to the long-range interactions found in folded proteins [25]. Interestingly, the overall shape of the CD spectra of the bundle structure without SDS *(Fig. 5)* and of the single 13-mer peptide with SDS *(Fig. 5)* are quite similar; however, considerably higher ellipticities are observed for the template-assembled bundle. More detailed investigations of the behaviour of TASP II under a variety of conditions are under way; according to the experiments with SDS described above, preliminary results indicate remarkable stability of TASP II with respect to dilution, salt addition, and heating; moreover, TASP II exists as the monomer in aqueous solution, as shown by size-exclusion chromatography studies.

Therefore, the template acts as a 'concentration' enhancer for the α-helix blocks even in dilute solutions. It is interesting to compare our findings with experimental evidence of the concentration-dependent self-aggregation of *de novo* designed single amphiphilic helices to 4-helix-bundle-like proteins in aqueous buffer solutions [5]; further support for the tendency of α-helices, when linked together by loop sequences, to fold predominantly into this topological arrangement was provided recently by *Ho* and *DeGrado* [26].

The present data on the conformational and solution properties of TASP II can only be rationalized if a 4-helix-bundle-like conformation resulting from a template-induced intramolecular 'collapse' [27] of the α-helix sequences is assumed (*cf. Fig. 3*). If our model is correct, a parallel arrangement of the template-attached helices does not decrease the overall stability of the postulated tertiary structure decisively, as might have been assumed from the less favourable dipole-dipole interactions between the helix blocks compared to those of the more common antiparallel arrangement in natural protein 4-helical bundles [28]. Our spectroscopic data support the general idea that the intramolecular folding of template-assembled amphiphilic peptides to a particular folding topology is clearly dominated by the number and spatial orientation of attachment sites on a specific template molecule.

TASP III and IV were designed as structural prototypes of binding proteins and consist of two potentially independent domains on opposite sites of a multifunctional template molecule. The CD (*Fig. 4*, TASP IV) and IR spectra (*Table 2*) are consistent with the postulated structures, exhibiting typical *Cotton* effects for predominantly α-helical (TASP III) and β-sheet (TASP IV) conformations in H_2O. Similarly to the findings

for TASP II, the corresponding single-chain peptide blocks show predominantly random-coil conformations under the same experimental conditions (not shown).

Conclusions. – In the present article, the foundations of our new concept for the construction of artificial proteins are established by the design, synthesis, and conformational analysis of several prototypes of TASP molecules. Though an ultimate proof of the hypothetical tertiary structures will be the topic of forthcoming work (mainly focussing on NMR and X-ray investigations of TASP molecules synthesized by solution methods [33]), we have achieved considerable experimental evidence that the template assembly of the amphiphilic α-helices and β-sheets may represent a valuable concept for the construction of tertiary structures. By comparison to other current strategies for the *de novo* design of proteins, the novel 'construction plan' presented here shows some distinct advantages:

1) The most critical hurdle in protein design – our limited knowledge of the folding mechanisms which direct a polypeptide chain into a unique tertiary structure – is overcome by the template-induced folding in TASP molecules. Due to the new architecture of the polypeptide, the folding into a 'rigid' tertiary structure is strongly enhanced from a thermodynamic point of view.

2) The above considerations are of utmost importance with respect to the 'insertion' of functional properties into a given tertiary structure. Due to the increased tendency for intramolecular folding (exemplified by the much lower tendency for intermolecular aggregation compared to linearly assembled amphiphilic polypeptides), the overall structure is expected to be less sensitive to amino-acid substitution ('overdetermined structure') similar to the situation in natural proteins ('degenerate folding code').

3) The strategy is applicable to the design of a wide variety of folding topologies; peptide building blocks can be designed independently of templates and can be assembled in a modular fashion.

4) Template-assembled proteins are readily prepared by the techniques of peptide synthesis combining current potential in molecular design with organic synthesis. In contrast to DNA recombinant methods ('protein engineering'), this approach for the construction of new proteins can take advantage of the chemical tools (*e.g.* unusual amino acids, peptide mimetics, crosslinks) for structure stabilization or incorporation of functional properties.

5) Template-assembled proteins may be ideal objects for biophysical studies on domain interactions, providing new insights into the folding mechanism of proteins, which might be difficult to obtain otherwise [29].

We may conclude that adoption of a tertiary structure is not bound to the complex folding mechanism of linear polypeptide chains selected over millions of years. Having gained access to the chemical construction of predetermined tertiary structures by the template approach, the incorporation of functional properties in these synthetic macromolecules is now a most fascinating prospect.

The support of these investigations by the *Swiss National Science Foundation* is gratefully acknowledged. We also thank Professor *J. Lambris*, Basel Institute of Immunology, for his collaboration on the automated peptide synthesis, and Professors *K. Kirschner* and *J. P. Rosenbusch*, Biocenter, Basel, for valuable comments.

Experimental Part

Synthesis of the Peptides. All solvents and reagents used were of highest purity available, and, in the case of liquids, they were freshly destilled and dried over molecular sieves. Amino-acid derivatives were of L-configuration and purchased from *Novabiochem* (Switzerland). Before use, they were tested for homogeneity by TLC in different solvents.

TASP I was synthesized according to the liquid-phase procedure [31] using polyethylene glycol monomethyl ether ($M_r = 5 \cdot 10^3$) as C-terminal soluble support. Details of the synthesis will be published elsewhere [32].

The stepwise syntheses of TASP II–IV were carried out according to the general principles of the solid-phase procedure manually (TASP III, IV) and with a peptide synthesizer (*Applied Biosystems 430 A*) on polystyrene-based PAM resins (capacity 0.1–0.4 mmol/g).

The acid-labile Boc protecting group was used for N^{α}-protection; the side chains of the trifunctional amino acids were protected as following: Boc-Lys(Fmoc)-OH, Boc-Lys(Cbz)-OH, Boc-Thr(Bzl)-OH, Boc-Glu(OBzl)-OH, Boc-Asp(OBzl)-OH.

All coupling steps were performed in CH_2Cl_2 using a 3-fold excess of preformed symmetrical anhydrides (TASP I, II) or *N,N*-diisopropylcarbodiimide (DICI)/hydroxybenzotriazole (HOBt) (TASP III, IV) and were then recoupled in DMF as solvent; each coupling was for 1 h. Coupling of the C-terminal residue of the helices/β-sheets to the ε-NH_2 group of the lysines was achieved using $(i\text{-}Pr)_2EtN$ (1 equiv.) to guarantee complete deprotonation of the ε-NH_2 groups of the template lysines. Each coupling step was tested for completion by ninhydrine and fluorescamine reactions.

Removal of the base-labile N^{α}-Fmoc protecting group was obtained by treatment with 20% piperidine/CH_2Cl_2 (30 min, r.t.). The final protected peptide resin was treated with the high HF method to cleave the peptide from the resin support and simultaneously the remaining side-chain protecting groups.

The crude peptides were purified by gel chromatography on *Sephadex LH 20* (MeOH/AcOH 2:8) and lyophilized. According to TLC, anal. HPLC, amino-acid analysis, and *Edman* degradation, the target peptides were established to be > 90% pure. For the preliminary conformational investigations, no further purification was performed.

Conformational Studies. CD spectra: *Yobin Yvon Mark V (Instruments AG)* circular dichrometer; concentration of peptide solutions in the range of 10^{-4}M and 10^{-3}M peptide, quartz cells with pathlengths of 0.01, 0.05, and 0.1 cm; ellipticities expressed as mean residue ellipticities. IR spectra: *Perkin-Elmer 781* IR spectrophotometer; solid state: film on KBr pellet, using 2.0 mg of peptide/100 mg KBr.

REFERENCES

[1] T.E. Creighton, 'Proteins', W.H. Freeman and Company, New York, 1987, p. 159.
[2] J.S. Richardson, *Adv. Protein Chem.* **1981**, *34*, 167; C. Chotia, *Ann. Rev. Biochem.* **1984**, *53*, 537.
[3] M. Mutter, *Angew. Chem. Int. Ed.* **1985**, *24*, 639; M. Mutter, K.-H. Altmann, K. Müller, S. Vuilleumier, T. Vorherr, *Helv. Chim. Acta* **1986**, *69*, 985.
[4] M. Mutter, K.-H. Altmann, T. Vorherr, *Z. Naturforsch., B* **1986**, *41*, 1315.
[5] D. Eisenberg, W. Wilcox, S.M. Eshita, P.M. Pryciak, S.P. Ho, W.F. DeGrado, *Proteins* **1986**, *1*, 16.
[6] R. Moser, R.M. Thomas, B. Gutte, *FEBS Lett.* **1983**, *157*, 247; E.T. Kaiser, F.J. Kezdy, *Proc. Natl. Sci. USA* **1983**, 80; W.J. Kullmann, *J. Med. Chem.* **1984**, *27*, 106; B. Rajashekar, E.T. Kaiser, *J. Biol. Chem.* **1986**, *261*, 13617; E.T. Kaiser, F.J. Kezdy, *Ann. Rev. Biophys. Chem.* **1987**, *16*, 561.
[7] B.W. Erickson, S.B. Daniels, P.A. Reddy, C.G. Unson, J.S. Richardson, D.C. Richardson, in 'Current Comm. in Mol. Biology', Eds. R. Fletterick and M. Zoller, Cold Spring Harbor Laboratory, New York, 1986, p. 53.
[8] M. Mutter, in 'Peptides: Chemistry and Biology', Ed. G.R. Marshall, Escom, Leiden, 1988, p. 349.
[9] G.D. Rose, L.M. Gierasch, J.A. Smith, *Adv. Prot. Chem.* **1985**, 37.
[10] S.E. Hull, R. Karlsson, P. Main, M.M. Woolfson, E.J. Dodson, *Nature (London)* **1978**, *275*, 206.
[11] C. Toniolo, G.M. Bonora, S. Salardi, M. Mutter, *Macromolecules* **1979**, *12*, 620.
[12] M. Mutter, K.-H. Altmann, A. Flörsheimer, J. Herbert, *Helv. Chim. Acta* **1986**, *67*, 786.
[13] K.-H. Altmann, A. Flörsheimer, M. Mutter, *Int. J. Pept. Protein Res.* **1986**, *27*, 314.
[14] P.Y. Chou, G.D. Fasman, *Adv. Enzymol.* **1978**, *47*, 45; M. Schiffer, A.B. Edmundson, *Biophys. J.* **1967**, *7*, 121; E.T. Kaiser, F.J. Kezdy, *Science* **1984**, *223*, 249.
[15] T. Vorherr, K. Müller, Ph. D. thesis of T. Vorherr, University of Basel, 1987.

[16] A.S. Kolaskar, V. Ramabrahman, K.V. Soman, *Int. J. Pept. Protein Res.* **1980**, *16*, 1.
[17] J.P. Flory, 'Statistical Mechanics of Chain Molecules,' Interscience, New York, 1969.
[18] W. Germann, E. Heidemann, *Biopolymers* **1988**, *27*, 157.
[19] M. Mutter, E. Bayer, in 'Peptides: Analysis, Synthesis, Biology', Eds. E. Gross and J. Meienhofer, Academic Press, New York, 1980, Vol. 2, p. 285; G. Barany, B. Merrifield, *ibid.*, p. 3.
[20] M. Mutter, K.-H. Altmann, D. Bellof, A. Flörsheimer, J. Herbert, M. Huber, B. Klein, L. Strauch, Th. Vorherr, H.-U. Gremlich, in 'Peptides Structure and Function', Eds. C.M. Deber, V.J. Hruby, and K.D. Kopple, Pierce Chemical Company, Rockford, 1985, p. 397.
[21] M. Mutter, K.-H. Altmann, *Angew. Makromol. Chem.* **1986**, *145*, 211; K.-H. Altmann, Ph.D. Thesis, University of Basel, 1986.
[22] M. Mutter, K.-H. Altmann, G. Tuchscherer, H.-U. Gremlich, in preparation.
[23] P. Manavalan, W.C. Johnson Jr., *Nature (London)* **1983**, *305*, 831; N. Greenfield, G.D. Fasman, *Biochemistry* **1969**, *8*, 4108.
[24] S. Krimm, J. Bandekar, *Adv. Prot. Chem.* **1986**, *38*, 181.
[25] C.B. Anfinsen, H.A. Scheraga, *Adv. Prot. Chem.* **1975**, *29*, 205.
[26] S.P. Ho, W.F. DeGrado, *J. Am. Chem. Soc.* **1987**, *109*, 6751.
[27] D.A. Dolgikh, R.I. Gilmanshin, E.V. Brazhnikov, V.E. Bychkova, G.V. Semisotnov, S.Y. Venyaminov, O.B. Ptitsyn, *FEBS Lett.* **1981**, *136*, 311.
[28] W.G.J. Hol, P.T. van Duijnen, H.J.C. Berendsen, *Nature (London)* **1978**, *273*, 443; W.G.J. Hol, L.M. Halie, C. Sander, *ibid.* **1981**, *294*, 532; R.M. Sheridan, R.M. Levy, F.R. Salemme, *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 4545; K.R. Shoemaker, P.S. Kim, E.J. York, J.M. Stewart, R.L. Baldwin, *Nature (London)* **1987**, *326*, 563; S. Marquese, R.L. Baldwin, *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 8898.
[29] T.E. Creighton, *Nature (London)* **1987**, *326*, 547; R. Pain, *Trends Biochem. Sci.* **1987**, *12*, 309; and ref. cit. therein.
[30] K. Müller, H.J. Ammann, D.M. Doran, P. Gerber, G. Schrepfer, in 'Innovative Approaches in Drug Research', Ed. A.F. Harms, Elsevier Scientific Publishers, Amsterdam, 1986, p. 125.
[31] M. Mutter, R. Uhmann, E. Bayer, *Liebigs Ann. Chem.* **1975**, 901.
[32] K.-H. Altmann, M. Mutter, in preparation.
[33] M. Mutter, P. Koziej, in preparation.