48. Single-Centre Model for the Active Site of α-Chymotrypsin

by **Thomas Vorherr**, **Karl-Heinz Altmann**, and **Manfred Mutter***

Institut für Organische Chemie der Universität Basel, St. Johanns-Ring 19, CH-4056 Basel

Dedicated to Prof. *Rolf C. Schulz* on the occasion of his 65th birthday

(20.XI.85)

The polymer-bound heptapeptide H-Glu-Gly-His-Pro-Gly-Ser-Gly-PEGM was designed as a 'single-centre model' for the active site of α-chymotrypsin. The peptide was synthesized according to the general principles of the liquid-phase method for peptide synthesis, and its conformational properties were investigated by CD and IR spectroscopy in solution and in the solid state. In harmony with empirical prediction codes, experimental and theoretical conformational considerations, the peptide adopts a β-turn conformation stabilized by H-bonds involving the side chains of Glu, His, and Ser. The development of a H-bonded system similar to the active site of α-chymotrypsin leads to implications with respect to a possible catalytic activity of the model peptide.

Introduction. – In the last years, much work has been devoted to the construction of model systems imitating the unique catalytic properties of enzymes [1–3]. The accessibility of such synthetic enzymes ('synzymes') would provide a valuable tool in organic synthesis to overcome some of the limiting shortcomings in the use of natural enzymes, *e.g.* problems of stability and solubility. Furthermore, the study of these simple models contributes to a better understanding of the mechanism of enzyme activity.

Many attempts have been made to mimic the active site of α-Cht[1]) [4–7], one of the most extensively studied proteolytic enzymes [8] [9]. Functionalized polymers and polypeptides have been studied for mimicing enzyme activity [10–15]; however, the lack of structural order in these systems proved to be a serious limitation in the interpretation and evaluation of the models [16].

In a previous paper [17], we have described a 'single-centre model' as an approach for the elucidation of individual parameters contributing to enzyme-like activity. The model is based on amphiphilic block copolymers comprising a hydrophobic peptide block (containing a catalytically active group) and a hydrophilic PEG[1]) block. Due to its strongly solubilizing effect, the PEG chain enables the investigation of otherwise insoluble peptide sequences even in aqueous solution without exerting a significant influence on the conformation of the peptide block [18].

In the present paper, we wish to report about the design and conformational properties of a PEG-peptide serving as a single-centre model for the active site of α-Cht.

Design of the Sequence. – For the construction of a model similar to the active centre of α-Cht, the amino-acid residues His, Ser, and Asp involved in the chemical-reaction mechanism of α-Cht [8] [9] have to be taken under consideration. In the enzyme, these

[1]) Abbreviations used: α-Cht, α-chymotrypsin; Bu^t, *t*-butyl; Fmoc, 9-fluorenylmethoxycarbonyl; PEGM, polyethylene glycol monomethyl ether ($M_r = 5 \cdot 10^3$).

residues are hold in a fixed orientation towards each other due to the rigid tertiary structure of the whole molecule. Although small peptides are not able to fold in a tertiary structure [16], the incorporation of these trifunctional amino-acid residues into a backbone with a defined *secondary structure* may allow interactions between the essential functional groups similar to the enzyme.

For this purpose, we decided to incorporate His, Ser, and Glu into a peptide sequence with a high potential to form a β-turn conformation [19]; Asp was replaced by Glu for synthetic reasons. Pro and Gly were chosen for the central positions $i + 1$ and $i + 2$ of the β-turn as suggested by empirical prediction schemes [20] [21] and experimental investigations of model peptides [22] [23]. On the other hand, statistical analysis of globular proteins shows a high frequency of occurrence of His and Ser in turn positions i and $i + 3$, respectively [20]. Consequently, the sequence His-Pro-Gly-Ser seemed to be the appropriate choice for the central part of the target peptide.

The formation of a β-turn is additionally supported by the incorporation of Glu at position i-2, 'spacered' from His (position i) by a flexible Gly residue (position i-1). According to model building and molecular modeling, the formation of a plane H-bonded system involving the side chains of Glu, His, and Ser corresponds to a preferred conformation of the peptide chain, thus resembling the interactions of Asp, His, and Ser in the active centre of α-Cht.

In summary, empirical and theoretical considerations of conformational features resulted in the design of the polymer-peptide H-Glu-Gly-His-Pro-Gly-Ser-Gly-PEGM (schematic representation in *Fig. 1*), where Gly was incorporated as C-terminal amino acid to achieve efficient coupling to the polymeric support.

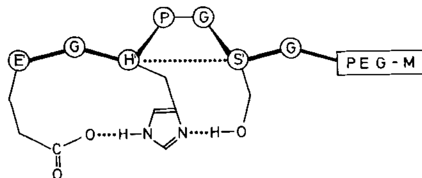
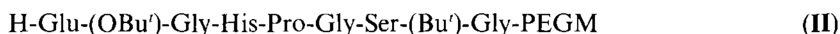


Fig. 1. *Schematic representation of the H-bond system within the designed single-centre model H-Glu-Gly-His-Pro-Gly-Ser-Gly-PEGM.* One letter abbreviations were used for amino-acid residues [29]. E', H', and S' mean E, H, and S, respectively, without side chain.

Conformational Studies. – Conformational studies were performed by means of CD spectroscopy in solution (CF_3CH_2OH, MeOH, H_2O) and IR spectroscopy in CH_2Cl_2 solution and in the solid state. To evaluate the influence of the side chains of Glu, His, and Ser on the conformation, the model peptide was investigated in the fully deprotected (**I**) as well as in the side-chain-protected state (**II**).

H-Glu-Gly-His-Pro-Gly-Ser-Gly-PEGM (**I**)


H-Glu-(OBu^t)-Gly-His-Pro-Gly-Ser-(Bu^t)-Gly-PEGM (**II**)

The CD spectrum of **I** in *CF_3CH_2OH* is shown in *Fig. 2*. The spectrum is characterized by a negative *Cotton* effect at 230 nm and a strong positive band at about 200 nm which is typical for peptides adopting a β-turn conformation [22]. In contrast, the spectrum of **II** (not shown) is dominated by a strong negative *Cotton* effect at 200 nm accompanied by a weak minimum at 220 nm indicating a predominantly unordered conformation of **II** in

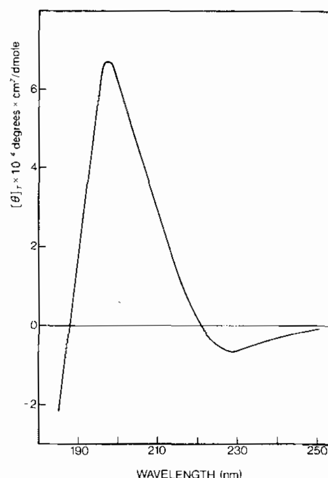


Fig. 2. *CD spectrum of H-Glu-Gly-His-Pro-Gly-Ser-Gly-PEGM in CF_3CH_2OH*

CF_3CH_2OH. As CF_3CH_2OH is known to favour intramolecular H-bonds, the development of a β-turn for **I** in this solvent is not unexpected. However, the lack of any ordered conformation in **II** indicates a decisive contribution of the free side chains of Glu and Ser to the formation of the turn. This observation is in full harmony with the assumption of a H-bonded system involving the side chains of Glu, Ser, and His stabilizing the secondary structure of the peptide.

Similar to the behaviour in CF_3CH_2OH, the CD spectrum of **II** in *MeOH* shows the typical features of peptides in an unordered conformation (strong negative *Cotton* effect at 200 nm; not shown). After removal of the side-chain-protecting groups, a CD curve results that shows a high portion of β-turn conformation for **I** even in MeOH *(Fig. 3)*.

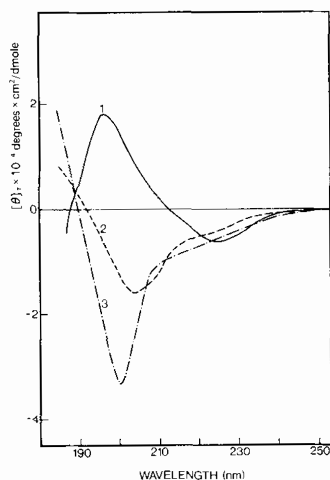


Fig. 3. *CD spectra of H-Glu-Gly-His-Pro-Gly-Ser-Gly-PEGM in MeOH* (–, 1) *and H_2O* (– – –, 2) *and of H-Glu(OBu^t)-Gly-His-Pro-Gly-Ser(Bu^t)-Gly-PEGM in H_2O* (–·–·–, 3)

Concerning the curve shape, the spectrum is very similar to that in CF_3CH_2OH, the negative *Cotton* effect being slightly blue-shifted to 224 nm. However, a remarkable decrease in the ellipticities is observed, probably due to a destabilization of the turn in MeOH compared to CF_3CH_2OH. Going from MeOH to *H_2O* as a solvent, the amount of secondary structure is drastically reduced even for peptide **I**. As seen from *Fig. 3*, the CD spectra for **I** and **II** are dominated by a strong negative *Cotton* effect at 204 and 200 nm, respectively, pointing to a predominantly unordered conformation.

It is well known, that the ability to stabilize β-turns is decreased in the order $CF_3CH_2OH > MeOH > H_2O$ [24]. Therefore, it can be assumed that peptide-solvent interactions in H_2O are competing with intramolecular H-bond interactions resulting in a destabilization of ordered structures.

The results of the conformational studies obtained by CD spectroscopy were further confirmed by IR spectroscopic investigations in the solid state and in CH_2Cl_2. In both cases, the IR spectra of peptide **I** in the amide-I region are characterized by absorption bands at 1695 and 1645 cm^{-1}. In agreement with theoretical calculations of *Bandekar* and *Krimm* [25], this IR pattern can be attributed to a type-I β-turn. Consequently, the development of a β-turn conformation induced by the formation of a H-bonded system is also probable in the solid state and in CH_2Cl_2 solution.

Conclusions. – The results of the conformational investigations demonstrate the ability of the designed model peptide to adopt an ordered backbone conformation under appropriate conditions.

The formation of a β-turn conformation is only observed for the fully deprotected peptide **I**, but not for the protected analogue **II**. Consequently, interactions between the free side chains of Glu, His, and Ser must be of essential importance for the development of the turn pointing to a similar arrangement of these functional groups as it is known for the active site of α-Cht. This proves the validity of the approach to attain specific interactions between the side chains of trifunctional amino acids incorporated into a peptide backbone adopting secondary structural features. However, no increased catalytic activity of peptide **I** compared to other functional model compounds could be observed in the hydrolysis of *p*-nitrophenyl acetate.

In order to achieve α-Cht-like catalysis, amino-acid sequences exhibiting a specific tertiary structure seem to be a fundamental prerequisite. Studies to construct this kind of polypeptides are in progress [16].

Experimental Part

Synthesis of the Peptide. All solvents and reagents used were of the highest purity available, and, in the case of liquids, they were freshly distilled and dried over molecular sieves. Amino-acid derivatives were of the L-configuration and purchased from *Bachem* (Switzerland). Before use, they were tested for homogeneity by TLC in various solvent systems. Polyethylene glycol monomethyl ether (HO-PEGM) with $M_r = 5 \times 10^3$ was a product of *Union Carbide* (U.S.A.). The conversion of HO-PEGM into 'amino-PEGM' (H_2N-PEGM) was described earlier [26].

Peptide synthesis followed the general principles of the liquid-phase method reviewed elsewhere [27]. The base-labile Fmoc protecting group was used for N^{α}-protection, the side chains of Ser and Glu were protected as their *t*-butyl ether and *t*-butyl ester, respectively. His was coupled as the Di-Fmoc derivative.

Coupling was achieved using DCC/HOBt activation in CH_2Cl_2 and 1.5- to 4-fold excess of the activated amino acid to be incorporated. Each coupling step was tested for completion by semi-quantitative ninhydrin

reaction [28]. In the case of the reaction of Fmoc-His(Fmoc) with N-terminal Pro, more than 90% completion were established by amino-acid analysis; remaining amino groups were irreversibly blocked by acetic anhydride.

Removal of the N^{α}-Fmoc protecting group was achieved by 50% piperidine/DMF (30 min) and controlled by UV in the range 250–350 nm. Side-chain-protecting groups were removed by 50% CF_3COOH/CH_2Cl_2 (2.5 h) under N_2 in the presence of anisole and 1,2-ethanedithiol.

Amino-acid analysis showed a degree of homogeneity higher than 90% for the polymer-bound peptide.

Conformational Studies. CD spectra were recorded on a *Jasco J 500 A* circular dichrometer. The concentration of peptide was 0.5–1.0 mg/ml, and quartz cells with path lengths of 0.01 and 0.05 cm were used. IR spectra were performed on a *Beckman* model *4420* spectrophotometer. For measurements in the solid state, a film on a KBr pellet was employed using 5.0 mg of PEG-peptide/100 mg KBr. The soln. measurements were carried out in a cell of 0.1-cm path length with compensation of the solvent. The concentration was 15 mg of PEG-peptide/ml. Bands are accurate to ±1 cm.

This work was supported by the *Swiss National Science Foundation*.

REFERENCES

[1] G.P. Royer, *Adv. in Catal.* **1980,** *29,* 197.
[2] T. Kunitake, Y. Okahata, *Adv. Polymer Sci.* **1976,** *20,* 159.
[3] R.M. Kellog, *Topics Curr. Chem.* **1981,** *101,* 111.
[4] R.M. Schultz, J.P. Huff, P. Anagnostaras, U. Olsher, E.R. Blout, *Int. J. Pept. Protein Res.* **1982,** *19,* 459.
[5] I. Photaki, M. Sakarellou-Daitsiotou, *J. Chem. Soc., Perkin Trans. 1* **1976,** 589.
[6] J.C. Sheehan, G.B. Bennet, J.A. Schneider, *J. Am. Chem. Soc.* **1966,** *88,* 3455.
[7] Y. Murakami, A. Nakano, K. Matsumoto, K. Iwamoto, *Bull. Chem. Soc. Jpn.* **1978,** *51,* 2690.
[8] J.J. Birkoft, O.M. Blow, *J. Mol. Biol.* **1972,** *68,* 187.
[9] A.A. Kosiakoff, S.A. Spencer, *Biochemistry* **1981,** *20,* 6462.
[10] C.G. Overberger, N.J. Vorchheimer, *J. Am. Chem. Soc.* **1963,** *85,* 951.
[11] H.C. Kiefer, W.I. Congdon, I.S. Scarpa, I.M. Klotz, *Proc. Natl. Acad. Sci. USA* **1972,** *69,* 2155.
[12] A. Everaerts, C. Samyn, G. Smets, *Makromol. Chem.* **1984,** *185,* 1897.
[13] E. Katchalski, G.D. Fasman, E. Simons, E.R. Blout, F.R.N. Gurd, W.L. Kaltun, *Arch. Biochem. Biophys.* **1960,** *88,* 361.
[14] H.J. Goren, T. Fletcher, M. Fridkin, E. Katchalski-Katzir, *Biopolymers* **1978,** *17,* 1679.
[15] B. Nakajima, N. Nishi, *Int. J. Biol. Macromol.* **1983,** *5,* 116.
[16] M. Mutter, *Angew. Chem.* **1985,** *97,* 639.
[17] M. Mutter, K.-H. Altmann, J. Schmitt, *Makromol. Chem. Rapid Commun.* **1985,** *6,* 785.
[18] M. Mutter, *Macromolecules* **1977,** *10,* 1413.
[19] J.S. Richardson, *Adv. Protein Chem.* **1981,** *34,* 167.
[20] P.Y. Chou, G.D. Fasman, *J. Mol. Biol.* **1977,** *115,* 135.
[21] A.S. Koloskar, V. Ramabrahman, K.V. Soman, *Int. J. Pept. Protein Res.* **1980,** *16,* 1.
[22] K. Bode, M. Goodman, M. Mutter, *Helv. Chim. Acta* **1985,** *68,* 705.
[23] S. Brahms, J. Brahms, *J. Mol. Biol.* **1980,** *138,* 149.
[24] R. Deslaurier, D.J. Evans, S.J. Leach, Y.C. Meinwald, E. Minasian, G. Nemethy, I.D. Rae, H.A. Scheraga, E.R. Somorjai, E.R. Stimson, J.W. van Nispen, R.W. Woody, *Macromolecules* **1981,** *14,* 985.
[25] J. Bandekar, S. Krimm, *Biopolymers* **1980,** *19,* 1.
[26] M. Mutter, *Tetrahedron Lett.* **1978,** *19,* 2839.
[27] M. Mutter, E. Bayer, in 'The Peptides', Eds. E. Gross and J. Meienhofer, Academic Press, New York, 1980, Vol. 2, p. 285.
[28] D.H. Spackman, W.H. Stein, S. Moore, *Anal. Chem.* **1958,** *30,* 1190.
[29] IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), *Pure Appl. Chem.* **1984,** *56,* 595.