BIOORGANIC CHEMISTRY 3, 133–140 (1974)

A Preferred Conformation in the Vasoactive Intestinal Peptide (VIP). Molecular Architecture of Gastrointestinal Hormones

MIKLOS BODANSZKY[1], AGNES BODANSZKY, YAKIR S. KLAUSNER,[2] AND SAMI I. SAID

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106, and Department of Internal Medicine, The University of Texas Southwestern Medical School, and V.A. Hospital, Dallas, Texas 75216

Received July 9, 1973

The ORD spectrum of the vasoactive intestinal peptide (VIP) in water indicates a preferred conformation with low helix content. Addition of organic solvents, especially of trifluoroethanol, results, even at low solvent concentration, in spectra with pronounced helical character. The readiness of shorter chains, with C-terminal sequences of VIP, to take up helical conformation under the effect of organic solvents parallels their biological activity. This suggests that an "active architecture" may be required for the interaction between hormone and receptor.

After the isolation of the vasoactive intestinal peptide (VIP) by Said and Mutt (*1*), its amino acid sequence (Fig. 1) was determined by Mutt and his associates (*2*).

We recently reported the synthesis of VIP, a single-chain octacosapeptide (*3*), and the availability of the synthetic intermediates prompted a study of their conformation through ORD–CD spectra. A similar "anatomy of a peptide" provided interesting information on the existence of a secondary–tertiary structure in secretin (*4*).

The ORD spectrum of VIP in water (Fig. 2) roughly resembles a spectrum calculated (*5*) for a peptide chain consisting of about 20% helix and 80% random coil.[3] In VIP, a chain built of 28 amino acids, this would mean a very short helical stretch, two turns or less. A careful comparison with a series of published (*5*) spectra calculated for combinations of helices, β-sheets, and random chains did not suggest significant participation of β-structure. The spectra—in water—of shorter chains, comprising C terminal portions of the molecule, are also shown in Fig. 2. The weak helical character of VIP is still discernible in the docosapeptide VIP_{7-28} but with decreasing chain length the spectra resemble more and more those of peptides in random coils.

[1] To whom correspondence should be addressed (CWRU).

[2] Present address: Dept. of Biological Chemistry, The Hebrew University, Jerusalem, Israel.

[3] The word "random" is used here to denote the lack of regular geometry in parts of a molecule, but does not necessarily mean that these parts are not present in a defined conformation.

A simple calculation of helix content, f_H ($[m]_{233} + 2520/12\,700$) used by Chen, Yang, and Martinez (cf. Ref. *5*) gives 14% for f_H. Because of the effect of terminal turns in short helices, a somewhat higher helical fraction should be assumed (cf. J. T. Yang in Poly-α-Amino Acids, Vol. I, G. D. Fasman, Ed., M. Dekker, New York, 1967, page 239). Therefore, the approximative figure of 20% is used as a semiquantitative expression for the characterization of the VIP molecule.

The effect of organic solvents (*6*) added in moderate amount to the aqueous solutions of secretin (*7*) provided some insight into the conformational freedom of different parts of its chain. A similar study was therefore undertaken on VIP and on the related

```
                1  2  3  4  5  (6) 7  8  9  (10) 11 12 (13) 14
VIP             HIS-SER-ASP-ALA-VAL-PHE-THR-ASP-ASN-TYR-THR-ARG-LEU-ARG-
Secretin        HIS-SER-ASP-GLY-THR-PHE-THR-SER-GLU-LEU-SER-ARG-LEU-ARG-
Glucagon        HIS-SER-GLN-GLY-THR-PHE-THR-SER-ASP-TYR-SER-LYS-TYR-LEU-

                15 16 17 18 19 20 21 (22) (23) 24 25 (26) (27) 28 29
VIP             LYS-GLN-MET-ALA-VAL-LYS-LYS-TYR-LEU-ASN-SER-ILE-LEU-ASN-NH2
Secretin        ASP-SER-ALA-ARG-LEU-GLN-ARG-LEU-LEU-GLN-GLY-LEU-VAL-NH2
Glucagon        ASP-SER-ARG-ARG-ALA-GLN-ASP-PHE-VAL-GLN-TRP-LEU-MET-ASP-THR
```

FIG. 1. The amino acid sequence of the porcine hormones, secretin, VIP, and glucagon. The positions occupied by hydrophobic residues are indicated by circles. The absence of proline, a helix-breaking residue, from all these peptides is noteworthy.

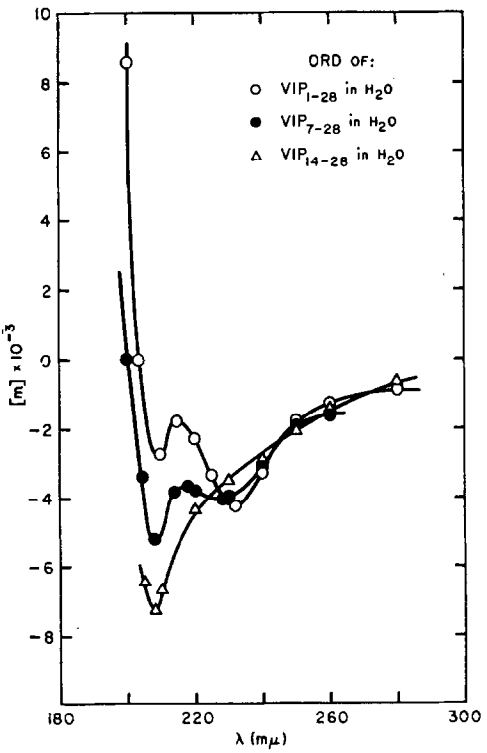

FIG. 2. ORD spectra of VIP, VIP_{7-28} (docosapeptide), and VIP_{14-28} (pentadecapeptide) in water.

shorter peptides as well. It should be noted that solvent effects on protein conformation can be fairly complex. Denaturation caused by the solvent might be followed by the development of a new architecture (*8*). The two processes are probably not sufficiently separated to allow a meaningful interpretation of the concomitant changes in physical

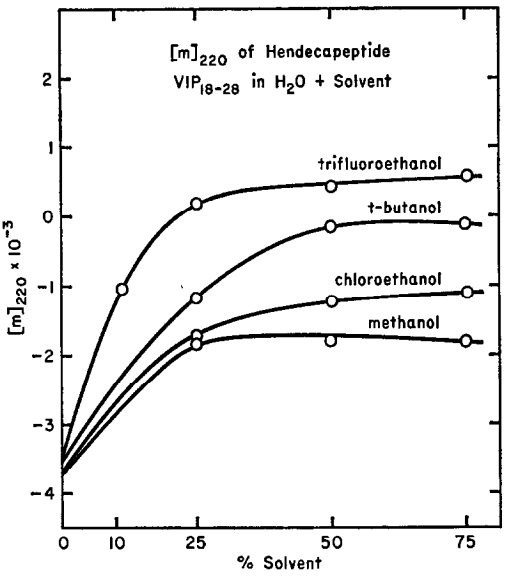

FIG. 3. The effect of different solvents on the ORD spectrum of the hendecapeptide VIP_{18-28} in water.

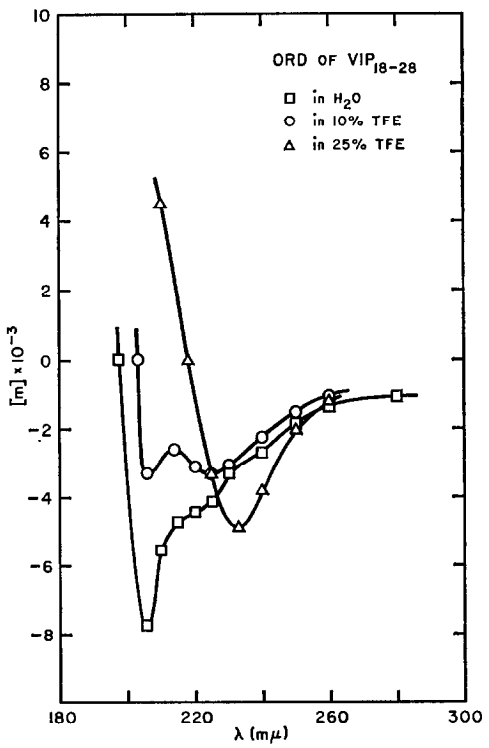

FIG. 4. The effect of trifluoroethanol on the ORD spectrum of the hendecapeptide VIP_{18-28} in water.

properties, e.g., in the ORD or CD spectra. A study of solvent effects on simple, not highly organized peptide chains seemed to be more auspicious: in some cases only the emergence of a preferred conformation will take place, in others there will be an enhancement of the already existing helicicity. These expectations were vindicated in the present investigations.

Since solvent effects depend also on the nature of the solvent used (*8*) a preliminary evaluation of the influence of different alcohols on the ORD spectrum of VIP_{18-28} was

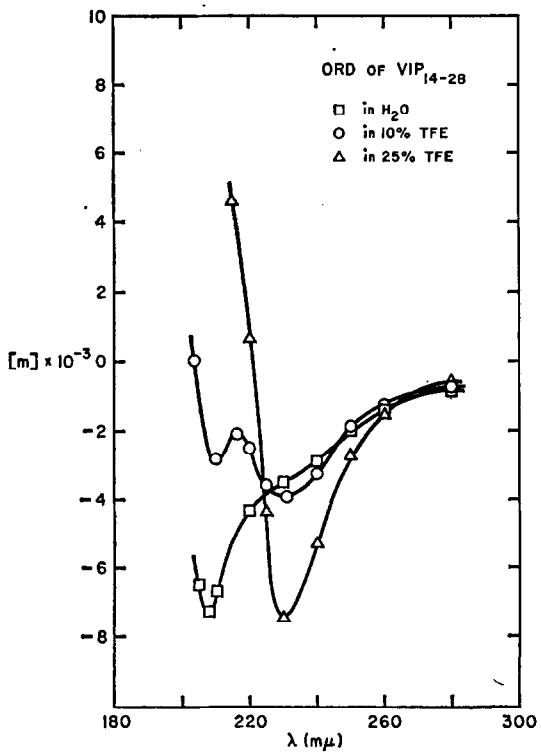

FIG. 5. The effect of trifluoroethanol on the ORD spectrum of the pentadecapeptide VIP_{14-28} in water.

carried out (Fig. 3). From the four solvents examined, trifluoroethanol (TFE) exerted the most pronounced effect on the conformation of the hendecapeptide and, therefore, this solvent was applied in moderate concentrations in the subsequent work. Helical spectra at high concentration of the organic solvent are rather commonplace: helices could be produced simply because water is removed from the environment of the peptide and with it the competition for potential intraturn H-bonds. The changes that occur under the less drastic influence of solvents added to a moderate concentration are more characteristic for individual peptides: a helix is produced if the peptide "cooperates."[4]

The shortest C terminal peptide investigated[5], the octapeptide VIP_{21-28}, exhibits

[4] Cf. Fig. 3 in Ref. 7 and also J. Hermans, Jr., and D. Puett, *Biopolymers* **10**, 895 (1971).

[5] The present study was based on peptides available by deprotection of synthetic intermediates. We hope to extend later these investigations to N-terminal sequences as well.

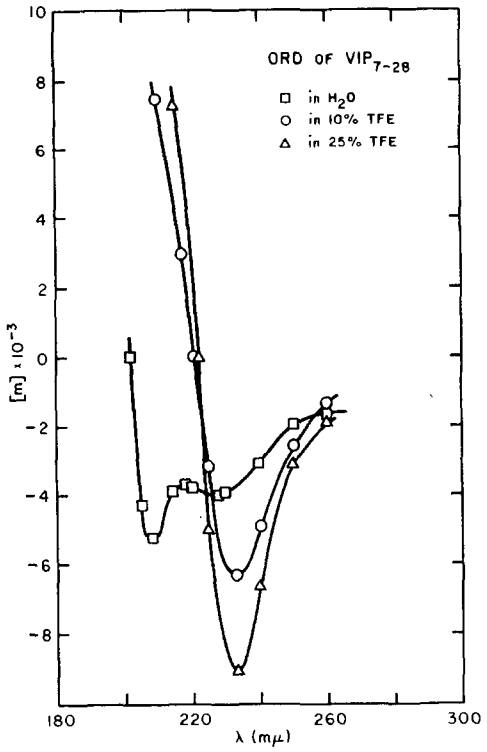

FIG. 6. The effect of trifluoroethanol on the ORD spectrum of the docosapeptide VIP_{7-28} in water.

only a shallow trough at about 225 nm in its ORD spectrum, and a substantial change toward helical character was not noted, not even at high concentration of TFE. The spectrum of the C terminal hendecapeptide VIP_{18-28} in water is that of a random coil (Fig. 4), but in this case the addition of as little as 10% TFE produces a spectrum that lies between that of VIP and VIP_{7-28}, both in water. The pentadecapeptide VIP_{14-28}, and the tetradecapeptide VIP_{15-28} as well, show even greater readiness to take up the conformation of VIP. An increase of the concentration of TFE to 25% produced typical helical spectra in these peptides, while 10% TFE can do the same with VIP_{7-28} and with VIP itself (Figs. 5–7).

DISCUSSION

Notwithstanding the extensive similarities in their sequences, in aqueous solutions VIP, secretin, and glucagon exhibit different ORD spectra. Hence their preferred conformation—if any—should be different in water. The moderately helical character of secretin (*4*), the even lower helix content of VIP, and the virtual absence of helicity in glucagon (*9*) are in good agreement with the Prothero rules (*10*). These rules and their extension by Ptitsyn (*11*) predict helical stretches in the C-terminal tetradecapeptide portion of secretin and in a part of VIP encompassing positions 12–23, but none in glucagon. Yet the slight, but definite overlap in the hormonal activities of VIP with those

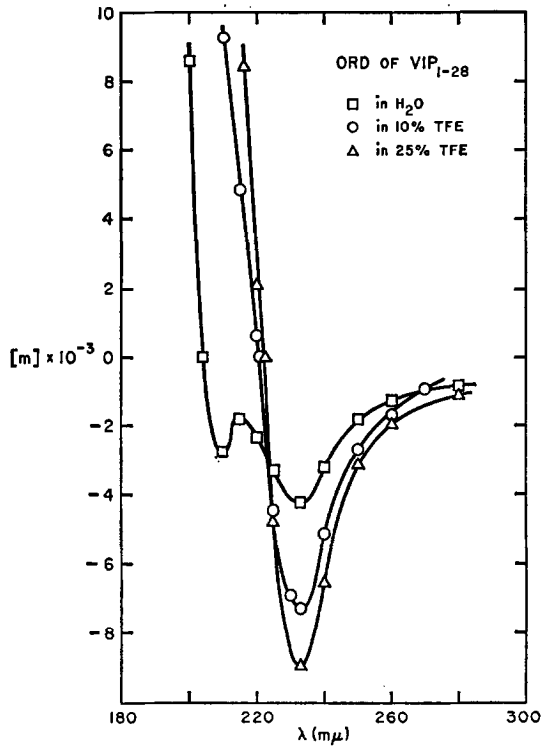

FIG. 7. The effect of trifluoroethanol on the ORD spectrum of VIP (the octacosapeptide VIP_{1-28}) in water.

of secretin (*1*) and of glucagon (*12, 13*) strongly suggests that the three closely related peptides (Fig. 1) might exist in a similar conformation when in contact with the same receptor. It is rather obvious that close similarity in geometry is required for close molecular fit, which is probably one of the prime prerequisites for triggering hormonal action. Therefore, it seemed to us that the conformation of these chains in water, in the absence of the receptor, is less important than their potential "active architecture" in which they exert biological activity. The assumption (*9*) that addition of organic solvents to aqueous solutions of peptide hormones can simulate the effect of the receptor molecules was tested on VIP.

The solvent (TFE) induced helicicity, not shown by the octapeptide VIP_{21-28}, is more and more pronounced with increasing chain length (*14*), It is interesting to note that the potency of these peptides in different pharmacological tests also increases in the same direction (*3*). The hendecapeptide VIP_{18-28} is about 1000 times less active than the parent molecule, an octacosapeptide. The pentadecapeptide VIP_{14-28} is about 50 times less potent than VIP. The tetradecapeptide VIP_{15-28} is much less active than the pentadecapeptide. The only difference, an additional arginine residue in the latter, might provide one more ionic bond between hormone and receptor. The 22-membered peptide VIP_{7-28} shows about a tenth of the potency of VIP itself. The parallelism between the biological effects of these peptides and their readiness to take up the conformation

of VIP under the influence of TFE indeed suggests the existence of an "active architecture" in VIP and in the related peptides. That the addition of 5–10% TFE to aqueous solutions of VIP and glucagon can produce similar ORD spectra to those of secretin in water (Fig. 8) could serve as an indication—certainly not as proof—that the presence of the organic solvent creates conditions that are similar to those at the receptor. Our studies provide some support for the existence of an "active architecture."

EXPERIMENTAL SECTION

Optical rotatory dispersion (ORD) spectra were measured at room temperature with a Cary Model 60 spectropolarimeter in fused quartz cells of 1 and 5-mm pathlengths (*7*). In several instances CD spectra were also recorded. The same Cary instrument with

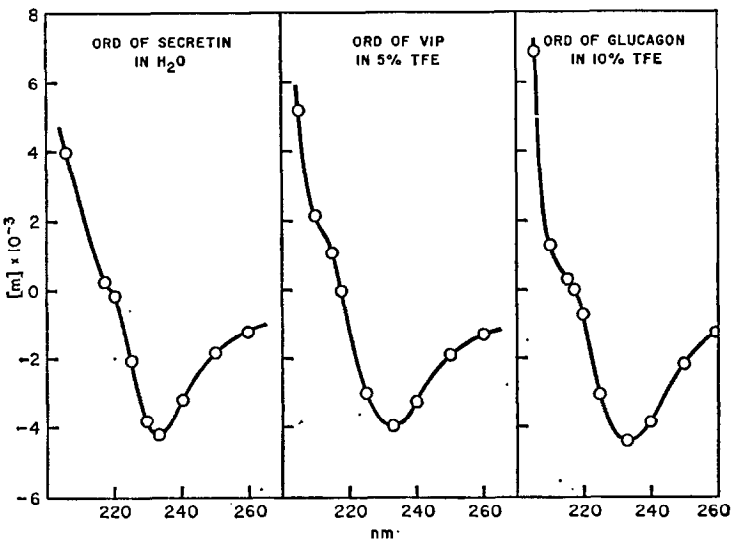

FIG. 8. ORD spectra of secretin in water, VIP in 5% trifluoroethanol–95% water, and glucagon in 10% trifluoroethanol–90% water.

Model 6001 CD attachment was used. In these studies the CD spectra offered no additional relevant information and, therefore, only the ORD spectra, more reproducibly observed on our instrument, are reported.

Solutions were prepared by dissolving weighed amounts of peptides in distilled water. Concentrations of the solutions were calculated from measurements of uv absorption of the aqueous stock solution ($\varepsilon_{275} = 1340$ for tyrosine) and/or from the recovery of quantitative amino acid analysis (*15*) of the solution after hydrolysis with 6N HCl for 16 hr at 110°C, on Beckman Spinco Model 120B analyser. For comparison, the observed data were expressed in terms of mean residue rotation $[m]$ in degrees cm^2 $decimole^{-1}$, defined by $[m]_\lambda = [\alpha]_\lambda \times M_{res}/100$, where M_{res} is the mean residue molecular weight obtained for each peptide by dividing the molecular weight by the number of amino acid residues. No corrections were made for refractive indices of the solvents.

ACKNOWLEDGMENTS

This study was supported by grants from the U.S. Public Health Service (NIH Center Award HL-14187 and NIH-AM-1273).

REFERENCES

1. S. I. SAID AND V. MUTT, *Science* **169**, 1270 (1970); S. I. SAID AND V. MUTT, *Eur. J. Biochem.*, **28**, 199 (1972).
2. V. MUTT, personal communication.
3. M. BODANSZKY, Y. S. KLAUSNER, AND S. I. SAID, *Proc. Nat. Acad. Sci., USA*, **70**, 382 (1973).
4. A. BODANSZKY, M. A. ONDETTI, V. MUTT AND M. BODANSZKY, J. *Amer. Chem. Soc.*, **91**, 944 (1969).
5. N. GREENFIELD, B. DAVIDSON, AND G. D. FASMAN, *Biochemistry*, **6**, 1634 (1967); cf. also Y. H. CHEN, J. T. YANG AND H. MARTINEZ, *Biochemistry*, **11**, 4120 (1972).
6. E. SCHECHTER AND E. R. BLOUT, *Proc. Nat. Acad. Sci. USA*, **51**, 695 (1964); R. F. EPAND AND H. A. SCHERAGA, *Biopolymers*, **6**, 1383 (1968); J. HERMANS, JR., *J. Amer. Chem. Soc.*, **88**, 2418 (1966); R. LUMRY AND S. RAJENDER *Biopolymers*, **9**, 1125 (1970); S. N. TIMASCHEFF, *Acc. Chem. Res.*, **3**, 62 (1970); G. J. HART, A. E. RUSSEL, AND D. R. COOPER, *Biochem. J.*, **125**, 599 (1971).
7. A. BODANSZKY, M. A. ONDETTI, AND M. BODANSZKY, *J. Amer. Chem. Soc.*, **94**, 3600 (1972).
8. T. T. HERSKOVITS, B. GADEGBEKU, AND H. JAILLET, *J. Biol. Chem.* **245**, 2588 (1970).
9. W. B. GRATZER, G. H. BEAVEN, H. W. E. RATTLE, AND E. M. BRADBURY, *Eur. J. Biochem.* **3**, 276 (1968).
10. J. W. PROTHERO, *Biophys. J.*, **6**, 367 (1966).
11. O. B. PTITSYN, *J. Mol. Biol.*, **42**, 501 (1969).
12. C. KERINS AND S. I. SAID, *Proc. Soc. Ex. Biol. Med.*, **142**, 1014 (1973).
13. S. I. SAID, L. P. BOSHER, J. A. SPATH, AND H. A. KONTOS, *Clin. Res.*, **20**, 29 (1972).
14. M. GOODMAN, A. S. VERDINI, C. TONOIOLO, W. D. PHILLIPS, AND F. BOVEY, *Proc. Nat. Acad. Sci. USA*, **64**, 444 (1969).
15. D. H. SPACKMAN, W. H. STEIN, AND S. MOORE, *Anal. Chem.* **30**, 1190 (1958).