

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY
(Organic Chemistry Division)

in conjunction with

International Union of Biochemistry
Academy of Sciences of the USSR
Academy of Sciences of the Uzbek SSR

FRONTIERS OF BIOORGANIC CHEMISTRY AND MOLECULAR BIOLOGY

Proceedings of the
International Symposium on Frontiers of Bioorganic
Chemistry and Molecular Biology
Moscow and Tashkent, USSR, 25 September - 2 October 1978

Editor

S. N. ANANCHENKO

USSR Academy of Sciences, Shemyakin Institute of Bioorganic Chemistry,
Moscow, USSR



PERGAMON PRESS

OXFORD · NEW YORK · TORONTO · SYDNEY · PARIS · FRANKFURT

U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
U.S.A.	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
CANADA	Pergamon of Canada, Suite 104, 150 Consumers Road, Willowdale, Ontario M2J 1P9, Canada
AUSTRALIA	Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W. 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, 6242 Kronberg-Taunus, Pferdstasse 1, Federal Republic of Germany

Copyright © 1980 International Union of Pure and
Applied Chemistry

All Rights Reserved. No part of this publication may
be reproduced, stored in a retrieval system or
transmitted in any form or by any means: electronic,
electrostatic, magnetic tape, mechanical, photocopy-
ing, recording or otherwise, without permission in
writing from the copyright holders.

First published 1980

British Library Cataloguing in Publication Data

International Symposium on Frontiers of Bioorganic
Chemistry and Molecular Biology, Moscow and
Tashkent, 1978

Frontiers of bioorganic chemistry and molecular
biology. - (International Union of Pure and
Applied Chemistry. IUPAC symposium series).

1. Biological chemistry - Congresses
2. Chemical reactions - Congresses

I. Title II. Ananchenko, S N III. Series
574.1'9283 QP514.2 79-41671

ISBN 0-08-023967-6

*In order to make this volume available as economical-
ly and as rapidly as possible the author's typescript
has been reproduced in its original form. This method
has its typographical limitations but it is hoped that
they in no way distract the reader.*



Printed in Great Britain by A. Wheaton & Co., Ltd., Exeter

RECOGNITION OF PEPTIDE HORMONES AND KININS: MOLECULAR ASPECTS OF THE PROBLEM

G. Chipens, F. Mutulis and S. Galaktionov

*Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga,
USSR*

Abstract - Some aspects of space structure complementarity of oligopeptide hormones and receptor sites and the possible contribution of intramolecular ionic-type interactions to the stability of the "biological" conformation of peptide bioregulator molecules have been discussed. The theoretical conformational analysis of the bradykinin molecule performed earlier revealed close proximity of the C-terminal carboxyl group and the guanino group in the arginine residue; a cyclic analogue of bradykinin has been synthesized, in which the close location of the two groups was stabilized by covalent bonding. The CD spectra of the synthesized compound were identical with those of bradykinin, and the new compound was active in eliciting a strong and prolonged depressor effect in rats.

Interaction of molecules or complex systems thereof underlies most regulatory processes operative in the living cells and organisms. According to our present knowledge, interaction of a low-molecular components - "effector" (hormones, antigens, various modulators, substrates or inhibitors of enzymatic reactions, etc.) with high-molecular components - "receptor system" (cell membrane receptors, antibodies, enzymes, etc.) results in a mutually-induced alteration of the stereo-electronic structure of the components and, consequently, in altered functional properties of the resultant complex. Regulatory processes are known to be characterized by high specificity, owing to the correspondence of geometrical forms and the appropriate spacing of functional groups in the effector-receptor pair. As evidenced by X-ray analysis, specific recognition and binding occurring in the course of protein-protein interaction is provided by a small area on their surface characterized by rigid structure with loops or ledges on the effector molecule and hollows - "pockets" or "slots" on the receptor (Refs.1-4). The active centres of protein effectors (enzyme inhibitors, antibodies, etc.) comprise, on the average, 6-8 amino acid residues (Refs. 1-5). Geometry of these "recognition and binding" centres is spatially stabilized by means of loops or β -bends and is "cemented" by the overall space structure of the protein globule. The ends of the "active" fragments (especially in the case of mini-proteins) are frequently immobilized by means of disulphide bonds (Refs.6 & 7).

The concept of biochemical universality makes it conceivable to expect that the process of "recognition" and binding of peptide hormones and kinins to receptors located on the cell membrane would be equally determined by similar "active" regions (containing 6-8 amino acid residues) on the peptide molecules. However, in contrast with the proteins, the space structure of peptide effectors in solution is not so well-defined: it is most likely that there exists an equilibrium of several equally stable conformers in solution, the best suited of them being "selected" by the receptor. The existence of such a limited set of conformers, characterized by relatively rigid space structures seems to be a prerequisite for the purposeful transfer of information to occur at the molecular level and for the effector-receptor interaction involving peptide effectors to be specific (Ref.8).

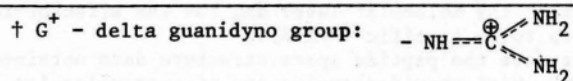
Extremely interesting, in this respect, are the peptide space structure data obtained using semi-empirical conformational analysis, which provides evaluation of intramolecular conformational energy for each possible molecular conformation as a measure of its stability (Refs.9-10). Having acquired similar data for a number of peptide molecules, it may be possible to establish the common principles shared by the space organization of the peptides. In fact, our studies along this line have led us to the establishment of a number of characteristic features inherent in the structural organization of low-molecular peptides which are well-correlated with our earlier findings on their functional organization (Ref.8). Thus, the conformational calculations performed in our laboratory for the molecules of biologically active peptides - bradykinin, angiotensin, Met-enkephalin, tuftsin, reveal the presence in all cases of a more or less limited set of stable conformers characterized by compact structures with N- and C-terminal parts located in close proximity, hence, quasicyclic structure being a prominent feature of their space organization (Refs.11-14). In each case, the observed structure of the molecule is determined by the overall system of intramolecular interactions as a whole; one can discern, however, several structural elements, each of them contri-

buting to the stability of the quasicyclic molecular structures. The first to be mentioned in this connection are the glycine and proline residues, distinguishable from the rest of the natural amino acid residues present in the amino acid sequence of the above peptides, owing to the characteristic steric conditions of their backbone (Ref.15). The presence of proline in the peptide chain is known to substantially limit conformational lability of the peptide backbone, the effect not being confined to the position occupied by proline itself, but being also present in the preceding position (ref.16); consequently, proline plays the role of a conformationally rigid element in the peptide chain. Conversely, the glycine residue exhibits enhanced conformational lability and is a kind of "conformational joint-hinge" providing close spacing of N- and C-terminal parts of the peptide chain. This fact is in good agreement with the results of calculations performed on the glycine-containing peptides bradykinin and Met-enkephalin, as well as the recent results with luliberin (Ref.17): the most stable backbone conformations in all these peptides are characterized by the glycine residue conformation which is sterically inconsistent for any other type of amino acid residues. The specific role of glycine and proline residues in peptide molecules is also indicated by their increased relative content (~12%), as compared with proteins, in the total amino acid composition of short (up to 30 amino acid residues) peptides (Ref.18).

The compact quasicyclic structures of peptides mentioned are stabilized predominantly by non-bonded interactions; an additional factor contributory to their stability is present in all the cases described above (Table 1), viz. strong electrostatic interaction between the functional groups carrying alternative charges - guanidyl group of arginine residue, ϵ -amino group of lysine residue or α -amino group and C-terminal carboxyl group. Such interaction occurs between ionogenic groups in the molecule on their close spacing, the process being accompanied by the appropriate hydrogen bonding. It is notable that in aqueous solution the above interactions are weakened by hydration, whereas in non-polar medium, i.e. in the course of effector-receptor complex formation they are significantly enhanced leading to increased relative content of quasicyclic conformers of the peptide effector molecule. The aforesaid gains indirect substantiation from the protein-protein interaction kinetics studies on enzymes binding to their specific inhibitors. The results of these studies demonstrated the occurrence of desolvation of the interacting surfaces and charged side groups in amino acids during the early stages of interaction (Ref.1). Furthermore, according to the results of our quantum chemical calculations carried out in vacuo, the energy of interaction between guanidyl and carboxyl ions was estimated to be about -55 kcal/mole, the value comparable with the dissociation energy of covalent disulphide bond.

TABLE 1. Quasi-cyclic structures of oligopeptides, as characterized by the data of semi-empirical conformational analysis

Peptide	Type of interaction	Size of quasi-cycle	Ref.
Bradykinin ArgProProGlyPheSerProPheArg	Arg ¹ δ -G ⁺ ... ⁻ OOC(Arg ⁹)	1-9	(11)
Angiotensin AsnArgValTyrValHisProPhe	Arg ² δ -G ⁺ ... ⁻ OOC(Phe ⁸)	2-8	(12)
Met-Enkephaline TyrGlyGlyPheMet	α -NH ₃ ⁺ ... ⁻ OOC(Met ⁵)	1-5	(13)
Tuftsins ThrLysProArg	Lys ² ϵ -NH ₃ ⁺ ... ⁻ OOC(Arg ⁴)	2-4	(14)
BPP pGlyTryProArgProGluIleProPro	Arg ⁴ δ -G ⁺ ... ⁻ OOC(Pro ⁹)	4-9	(23)



Thus, the results obtained using semi-empirical conformational analysis suggest important implication of ionogenic groups in the formation and maintenance of the space structure in the peptide molecules, especially during the process of their interaction with receptors. It is appropriate to recall, in this connection, that we had postulated previously the presence of typical elements in amino acid sequences in the molecules of biologically active low-molecular peptides. These sequences contained an ionogenic basic amino acid incorporated between proline and valine residues, on the one side, and acidic amino acid and glycine, on the other side (Refs.8,20,21). These fragments were also shown to exhibit a rather wide range of non-specific biological action, but their conjugation with the "shortest" peptide fragments induced a significant rise in the specific activity of these fragments (Ref.22). However, there is no clear-cut evidence, at present, demonstrating direct implication of these fragments in the activation of receptor during effector-receptor interaction. At the same time, the finding concerning the space organization of the peptide effectors described above serve, in our

opinion, to emphasize the structural role of the detected fragments in the oligopeptide molecules.

The localization of ionogenic side chains, which participate in the interactions responsible for the closure of quasicyclic structures in non-cyclic peptides is somewhat similar to the localization of disulphide bonds, responsible for the closure of ring structures in cyclic peptides (see hypothesis on the equifunctionality of ionic and disulphide bonds (Refs.8,27)). This can be viewed as another indication of the important role reserved for quasicyclic (or cyclic) structures in the molecules of biologically active peptides comparable size values (approximately, 6-8 amino acid residues) of functionally active disulphide cycles and tentative quasicycles in the molecules of some peptides (Refs.8,27).

The above consideration lead to assume that high selectivity and specificity observed during the processes of mutual "recognition" and peptide effector binding to the receptor are due to the interaction of conformationally rigid quasi-cyclic structures of the effectors with the receptor "pockets", providing large interacting areas and multiple contact sites. It can be expected, therefore, that covalent fixation of the stable quasi-cyclic structures of the effector molecule "selected" by the receptor will result in increased interaction efficiency. The hypothesis was put to trial using bradykinin, a peptide endowed with high specific activity, exhibiting a variety of biological effects. The stable conformations calculated for this compound demonstrated, in agreement with spectroscopy data (ref.24), considerable prevalence of 4 types of quasi-cyclic structures² for the peptide backbone (2 of them are depicted in Fig.1) in which guanidyl group of Arg⁷ is located close to C-terminal carboxyl group.

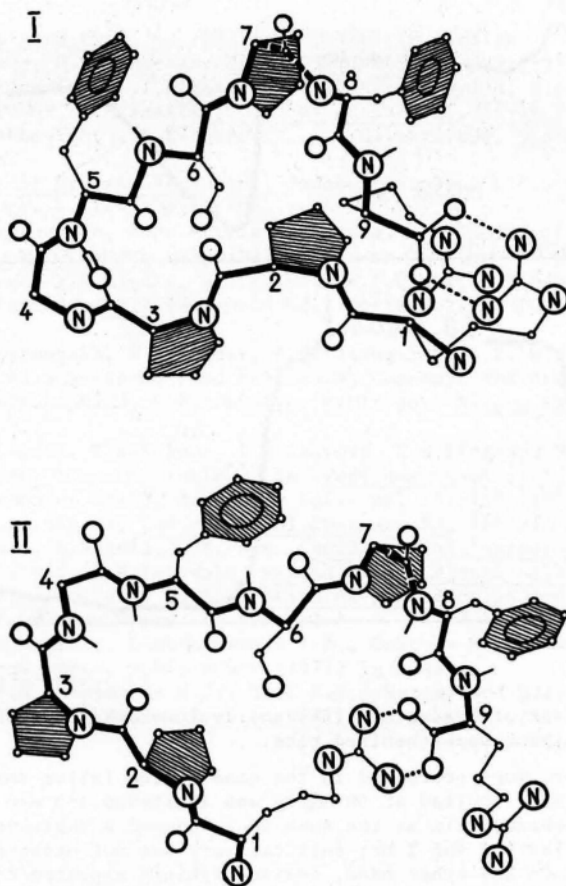


Fig. 1. Two of the four types of the most stable quasi-cyclic bradykinin structures (Ref.24).

According to calculations, these structures show highest stability also in the absence of electrostatic interaction of the above mentioned ionogenic groups. Bearing these results in mind, we undertook an attempt to synthesize bradykinin analogue in which mutual location of N- and C-terminal groups, as predicted by the calculations, was stabilized by covalent bon-

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.