Biochimica et Biophysica Acta, 596 (1980) 137—140

BBA Report

BBA 71425

POLY(D Trp-L Leu), POLY(D Phe-L leu) AND POLY(D Val-L Val-D Val-L Ala) AS MODEL POLYMERS FOR THE ELUCIDATION OF THE GRAMICIDIN A CONFORMATION*

FREDERIC HEITZ[a], BERNARD LOTZ[b], GILLES DETRICHE[a] and GERARD SPACH[a]

[a]*Centre de Biophysique Moléculaire, C.N.R.S., 1A, avenue de la Recherche Scientifique, 45045 Orléans (France) and* [b]*Centre de Recherches sur les Macromolécules, 6 rue Boussingault, 67083 Strasbourg (France)*

(Received October 29th, 1979)

Key words: Model polymer; Gramicidin A conformation; Poly(dipeptide)

Summary

From infrared observations it is concluded that the conformation of poly(D Trp-L Leu), poly(D Leu-L Trp), poly(D Phe-L Leu) and poly(D Val-L Val-D Val-L Ala) wich are model polymers of Gramicidin A is the same as one species of the antibiotic. Diffraction investigations indicate that poly-(D Phe-L Leu) and poly(D Val-L Val-D Val-L Ala) have a helical conformation which is most probably the π^{8}_{DL} helix.

The properties of ion transport across lipid bilayer membrane of the antibiotic peptide Gramicidin A, HCO-L Val-Gly-L Ala-D Leu-L Ala-D Val-L Val-D Val-L Trp-(D Leu-L Trp)$_3$-NH-CH$_2$-CH$_2$OH [1], are well known. Despite numerous investigations, including examination of model polypeptides and oligopeptides and crystallographic studies of the antibiotic itself [2], its active conformation, supposed to be a channel structure, has not been established so far. In particular, it is not yet known whether this conformation is based on the end to end pairing of two single stranded helices [3], or alternatively, on the side-by-side association of two Gramicidin molecules leading to a double stranded helix [4].

In the last years, investigations by conventional fibre science techniques on a high molecular weight stereochemical model of Gramicidin, the alter-

*Supplementary data to this article are deposited with, and can be obtained from, Elsevier/North-Holland Biomedical Press B.V., BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/128/71425/596 (1980) 137—140. The supplementary information includes a scheme of synthesis and tables of characteristics of the intermediate products.

nating poly(γ-benzyl-D-L-glutamate) [5, 6] have shown that both single and double stranded structures are stable, and that their occurrence primarily depends on the physicochemical treatment applied to this polypeptide.

In a continuation of the latter investigations, a number of poly(D-L-peptides) with various combinations of amino acids have been synthesized. We report here recent results obtained with poly(D Trp-L Leu), poly(D Leu-L Trp), poly(D Phe-L Leu) and also poly(D Val-L Val-D Val-L Ala)* the chemical structures of which closely mimic the C and N terminal moieties of the Gramicidin molecule respectively. Experimental difficulties encountered in orienting these samples do not allow unambiguous conclusions about their structure to be drawn. Results obtained so far point however to the possible existence of a structure seldom considered among possible models for Gramicidin A, and therefore justify, in our opinion, publication of the present report.

The solid state infrared spectra of the listed polypeptides are very similar to that described for 'species 3' of Gramicidin [4] (Amide 1 band at 1630 cm^{-1} with shoulders up to 1690 cm^{-1} and broad Amide II band centered around 1540 cm^{-1}) suggesting that the conformation of the model polypeptides are closely related to that of the antibiotic (Fig. 1).

Indications on the dichroism of the infrared bands have only been obtained for the phenylalanine and valine containing samples which were oriented by stroking to dryness from dioxane and hexafluoroisopropanol solutions respectively. While the spectra clearly indicate a parallel dichroism for Amide I band (Fig. 2) the situation appears more complicated for Amide II band. Indeed, for poly(D Val-L Val-D Val-L Ala) this band can clearly be de-

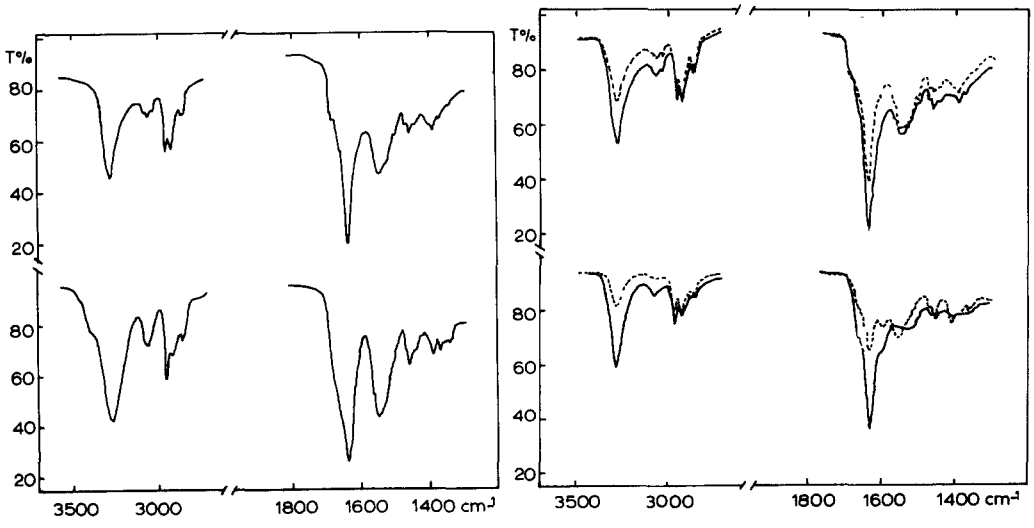

Fig. 1. Infrared spectra of poly(DPhe-LLeu) upper part, Gramicidin A: species 3 lower part.

Fig. 2. Polarized infrared spectra of poly(DPhe-LLeu) upper part, poly(DVal-LVal-DVal-LAla) lower part. —, electric vector parallel to the direction of stroking; - - - -, electric vector perpendicular to the direction of stroking.

*See Data Bank for the scheme of synthesis and the tables of characteristics of intermediate products.

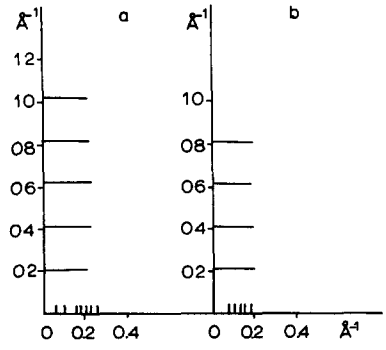

Fig. 3. Schematic representations of the electron diffraction patterns of oriented films of (a) poly(DPhe-LLeu) and (b) poly(DVal-LVal-DVal-LAla).

composed into two components with opposite dichroism. On the other hand, for poly(D Phe-L Leu) this band has no significant dichroism which is a situation already observed with poly(γ-benzyl-D-L-glutamate) in its $\pi^{4.4}_{DL}$ helical structure [5].

X rays and electron diffraction investigations show for poly(D Phe-L Leu) that all the equatorial reflections can be indexed on the basis of a hexagonal unit cell (a = 1.93 nm) and that the non-equatorial reflections are located on layer lines whose spacings are 0.49, 0.245, 0.163 and 0.122 nm (Fig. 3a). However, owing to the poor degree of orientation, we are not able to specify on which layer line is located the first meridional reflection and thus determine unambiguously the exact periodicity of the structure. For poly(D Val-L Val-D Val-L Ala), an orthorhombic lattice with parameters a = 1.55 and b = 1.31 nm was determined by X ray diffraction. Electron diffraction patterns obtained on an oriented film display two off-meridional reflections on a layer line at Z^{-1} = 0.486 nm and one apparently meridional of spacing 0.239 nm (Fig. 3b).

Observation of a hexagonal lattice for poly(D Phe-L Leu) strongly suggests the existence of a helical rather than any kind of sheet structure. Further, the existence of a layer line at 0.490 nm which would then correspond to the pitch of the helix eliminates all double helices, whose (half-) pitches are larger than 0.5 nm [6]. Within the family of single stranded π_{DL} helices, the existence of a member of which is suggested by infrared data, we select the π^{8}_{DL} helix. Indeed a pitch as low as 0.490 nm is consistent with this structure [7]. Moreover, the calculated density is 1.09 cm^{-3} for 8 residues per turn, to compare with 1.20 $g \cdot cm^{-3}$ calculated from the specific volume of the residues [8]. For poly(D Val-L Val-D Val-L Ala), combining the layer lines and meridional periodicities would lead to an irrational helix with 2.03 repeat unit per helical turn. Again, this could be a π^{8}_{DL} helix with 8.12 residues per turn (calculated density 1.26 $g \cdot cm^{-3}$, in good agreement with the measured one: 1.19$\cdot cm^{-3}$, but somewhat higher than that estimated from the specific volume of the residues: 1.21 $g \cdot cm^{-3}$ [6]).

Clearly, more confidence in the above conclusions would be desirable, but is subject to obtaining better orientation of the samples. The reasons for this poor ability to orient are unknown, but we may note that similar diffi-

culties have already been encountered for polypeptides bearing bulky side chains [9].

The possible existence of π^{s}_{DL} helical structures is rather unexpected, as previous energy analyses have demonstrated this structure to be less stable than π_{DL} helices with fewer residues per turn [7] or double stranded $\pi\pi_{DL}$ helices [10]. These calculations, however, have neglected possible stabilizing interactions involving the side-chains. Further, part of the steric conflicts arising in the backbone of the π^{s}_{DL} helices could be reduced, or even removed, by tilting one peptide group out of two in order to bring its CO group closer to the helical axis (Colonna-Cesari, F. and Lotz, B., unpublished data). In addition, stabilization may also be introduced by interactions of CO group with solvent molecules trapped within the wider helical core. Although an exact balance of all involved interactions is difficult to evaluate a priori, our contention is that π^{s}_{DL} helices, possibly with specific modifications of the geometry first considered, represents a valid proposal for the model polypeptides examined in this paper.

The experimental finding of new structures for model polypeptides closely linked to the Gramicidin sequence raises the question of their applicability to the antibiotic itself, especially since recent measurements [11], although disputed [12], of the water content of the Gramicidin channel have led to the proposal of a π^{s}_{DL} helix.

Clearly, the shorter rise per repeat unit in such helices would yield, for two Gramicidin molecules attached head to head, to channels whose length is shorter than the membrane thickness. It does not seem, however, that such a match is as mandatory as previously thought [3]; indeed, channel conductance has been observed in artificial layer membranes with a fairly wide range of thickness [13]. This observation suggests that rearrangements of the membrane structure are likely in the vicinity of the peptide, thus weakening the length matching requirement [14].

Our thanks are due to Mrs. J. Verhaeghe, Mrs. A. Caille and Miss M.A. Boudeville for skilful technical assistance.

References

1 Sarges, R. and Witkop, B. (1965) Biochemistry 4, 2491—2498
2 Koeppe, R.E., Hodgson, K.O. and Stryer, L. (1978) J. Mol. Biol. 121, 41—54
3 Urry, D.W. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 1610—1614
4 Veatch, W.R., Fossel, E.T. and Blout, E.R. (1974) Biochemistry 13, 5249—5256
5 Heitz, F., Lotz, B. and Spach, G. (1975) J. Mol. Biol. 92, 1—13
6 Lotz, B., Colonna-Cesari, F., Heitz, F. and Spach, G. (1976) J. Mol. Biol. 106, 915—942
7 Ramachandran, G.N. and Chandrasekaran, R. (1972) Ind. J. Biochem. Biophys. 9, 1—11
8 Cohn, E.J. and Edsall, J.T. (1943) in Proteins, Amino Acids and Peptides as Ions and Dipolar Ions, p. 375, Hafner, New York, NY
9 Peggion, E., Cosani, A., Verdini, A.S., Del Pra, A. and Mammi, M. (1968) Biopolymers 6, 1477—1486
10 Colonna-Cesari, F., Prémilat, S., Heitz, F., Spach, G. and Lotz, B. (1977) Macromolecules 10, 1284—1288
11 Levitt, D.G., Elias, S.R. and Hautman, J.M. (1978) Biochim. Biophys. Acta 512, 436—451
12 Rosenberg, P.A. and Finkelstein, A. (1978) J. Gen. Physiol. 72, 327—340
13 Hladky, S.B. and Haydon, D.A. (1972) Biochim. Biophys. Acta 274, 294—312
14 Haydon, D.A. (1975) Ann. N.Y. Acad. Sci. 2—15