JOURNAL OF
PEPTIDE SCIENCE

Cell-physiological Effects of Elastin Derived (VGVAPG)$_n$ Oligomers in a Unicellular Model System

LÁSZLÓ KŐHIDAI,[a]* **LÍDIA KUN,**[a] **PÁLLINGER ÉVA,**[b] **GYÖRGY CSABA,**[a] **NIKOLETT MIHALA,**[c] **ZSUZSA MAJER**[d] and **HELGA SÜLI-VARGHA**[c]

[a] Department of Genetics, Cell- and Immunobiology, Semmelweis University Budapest, H-1089 Budapest, Nagyvárad tér 4. Hungary
[b] Molecular Immunological Research Group of the Hungarian Academy of Sciences, Budapest, H-1089 Budapest Nagyvárad tér 4. Hungary
[c] Research Group for Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Lorand University, H-1518 Budapest 112, PO Box 32, Hungary
[d] Institute of Organic Chemistry, Eötvös Lorand University, H-1518 Budapest 112, PO Box 32, Hungary

Abstract: Elastin is one of the most significant components of the extracellular matrix, which supports the stretchiness of the blood vessels via its helical structure and cross-links. Enzymatic decomposition of this protein could induce chemotactic responses of cell populations in the surrounding tissues by several peptide sequences, e.g. XGXXPG. In our present work the VGVAPG variant and its oligomers were studied. The objective of the experiments was to learn (i) whether the chemotactic effect of these peptides is general in different levels of phylogeny; (ii) whether increasing the number of monomer units influences the chemotactic behaviour of the cell? The trimer had the strongest chemoattractant effect in a wide concentration range (10^{-12}–10^{-7} M), while the monomer and the pentamer were chemorepellent. All tri-, tetra-, penta- and hexamers could chemotactically select subpopulations with a high chemotactic responsiveness to the identical peptide, in the long term. With regard to its repellent effect, the pentamer had a negative effect on phagocytosis. All six oligomers had a growth-promoter effect in *Tetrahymena*. The characteristic cell-physiological effects of VGVAPG oligomers signal that molecules of the extracellular matrix can induce identical responses even in lower levels of phylogeny, e.g. in the Ciliates. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: elastin; VGVAPG; chemotaxis; growth; selection; *Tetrahymena*

INTRODUCTION

Elastin is one of the main components of the extracellular matrix (ECM). Its insoluble polypeptide chains are randomly cross-linked [1] and the plasticity of the rubber-like, elastic fibres provides high tensile strength and a 'mechanical-buffer' function

to the network formed by elastin molecules. The soluble precursor of elastin is tropoelastin [2] a well-conserved protein of phylogeny, the estimated difference of amino acid composition being 1% during 5.8 million years [3]. In their hydrophobic regions both tropoelastin and elastin possess unique, hydrophobic repeating sequences. These clusters of VGVAPG (VGVPG, VGAPG) and derivatives have high structural and functional importance. These sequences are significant determinants of the folded (type VIII β turn) conformation of the protein, and it is presumed that an optimal relation of the 3–6

VGVAPG clusters is required for interactions with the appropriate binding sites of the surface membrane [4]. For this reason VGVAPG and VAPG are considered as quantitative markers of elastin [5].

The biological activity of elastin is based on the presence of the 67 kD, peripheral elastin binding peptide (EBP) [6], its signal transduction is induced effectively by the VGVAPG sequence [6], however, another ECM protein, laminin is also a ligand of the receptor [7]. Induction of the elastin receptor is mediated by the G protein–phospholipase C–protein kinase C cascade [8] and results in several cell physiological responses. Besides the induction of cell differentiation or the suppression of proliferation [9] one of the most significant responses is the VGVAPG mediated upregulation of matrix–metalloproteinases (MMP1, MMP2 and MMP3) [8,10]. Production of MMPs is essential in the removal of ECM components and remodelling of tissues, and there is growing evidence that fragments of the ECM broken down by the MMPs work as chemoattractants in the ECM–cell–ECM feedback mechanism. The VGVAPG induced 'decryptase' activity of MMPs, including stimulation of pro-MMP1, contributes to the invasion of tumour cells [11], while disturbance of the VGVAPG–receptor interaction (e.g. alterations in size of the receptor [12] or enhanced expression of the effective sequence or its derivatives) is responsible for the development of clinical syndromes such as prolapsus uteri [12], chronic abdominal aortic aneurysm [13] or elastosis perforans serpiginosa [9].

Chemotaxis is considered as one of the most significant cellular mechanisms triggered in the above mentioned pathophysiological conditions. Monocytes [14] and fibroblasts [15] are two characteristic chemotactic responder cell types induced by elastin and its derivatives, however, other cells, keratinocytes [14] or vascular smooth muscle cells [16] also detect these chemoattractant molecules. On the basis of the above mentioned and well-conserved phylogenetic background of elastin, besides the vertebrate target cells, unicellular models, i.e. *Dyctiostelium* [17] are also accepted as chemotactic responders of components of ECM components. As the chemotactic responsiveness of these relatively simple organisms is one of the most fundamental cell physiological activities and their test systems provide an easy and reliable evaluation of chemotactic ligand–receptor interactions, they are dedicated models for investigating complex problems, too.

In the present study, the characterization of the chemotactic ability of oligopeptides composed of varying numbers of VGVAPG motifs was also supported by the theoretical considerations mentioned above.

Our reference object was the eukaryotic ciliate, *Tetrahymena pyriformis* GL, a frequently applied model of cell biology and physiology [18]. Its membrane receptors [19], second messenger systems [20], metabolic pathways [18] and the response provoked by a hormone are mostly homologous to the higher ranked, vertebrate cells [18]. Among others, amino acids [21], oligopeptides [22], chemokines [23], lectins [24] or volatile oils [25] are detected with a high chemotactic sensitivity by these unicellulars, and slight molecular differences in for example, dipeptides [26] or chemical conditions of the ligands [27] are detectable in the chemotactic behaviour of these cells.

In the present work our main objectives were focused on the cell physiological responsiveness induced by VGVAPG oligomers and on the possible relationship between the conformational change and the biological activity of the ligands. The questions were:

(i) whether the VGVAPG sequence or its oligomers possess chemotactic ability on model cells representing free living, unicellular organisms?

(ii) is there any difference between the effect of the monomer and that of the oligomers, i.e. has the multiplying of the sequence any influence on the chemotaxis or does it cause another type of response?

(iii) is there any correlation between the chemoattractant/repellent potential of the peptides and their conformation characterized by CD spectroscopy?

(iv) whether the receptors/binding sites responsible for chemotactic responsiveness belong to the short- or the long-term expressed type of chemotaxis receptors?

(v) what other basic cell physiological activities are modulated by this characteristic motif of elastin? Whether the physiological reactions of *Tetrahymena* to elastin oligomers are similar to those of the mammalian cells (known in the literature) or not?

MATERIALS AND METHODS

General

The protected amino acids were purchased from Reanal (Budapest, Hungary) the Boc-Gly-PAM resin

(0.2 mmol/g 200–400 mesh, polystyrene cross-linked with 1% divinylbenzene) was obtained from Bachem Feinchemikalien AG (Bubendorf, Switzerland) DIC, HOBt, HODhbt, TFA, were from Chem-Impex International (Wood Dale, IL), chloroform and phenol were from Fluka.

Analytical Methods

Analytical RP-HPLC was run on a Knauer instrument with a Phenomenex Jupiter C18 5 μm, 300 Å, (150 × 4.6 mm) column, using a linear gradient (10 → 60, 35 min); A: 0.1% TFA in water, B: 0.08% TFA in acetonitrile, flow rate 1 ml/min. ESI-MS spectra were obtained on a PE SCIEX API-2000 spectrometer.

Peptide Synthesis

H-VAPG-OH. Boc–VAPG-OH was prepared stepwise, with the Fmoc technique [28] on 2-chlorotrityl chloride resin with the aid of HBTU. The Boc-protected peptide was cleaved from the resin by treatment with chloroform–methanol–acetic acid 8:1:1 for 2 h at room temperature and purified with HPLC. The calculated M ($C_{20}H_{34}N_4O_7$) 442.2, measured MH$^+$ 443.3. Boc–VAPG-OH was treated with TFA for 15 min at room temperature, then the solution was concentrated *in vacuo*, the residue was dissolved in water and lyophilized to give the title compound. Calculated M ($C_{15}H_{26}N_4O_5$) 342.2, measured MH$^+$ 343.3.

H-VGVAPG-OH, Boc-VGVAPG-OH and Boc-VGVAP-OH were prepared in the same way as H-VAPG-OH and Boc-VAPG-OH, respectively (see above).

H-(VGVAPG)$_{2-6}$-OH. Boc-VGVAPG-PAM (which was prepared by coupling Boc-VGVAP-OH to H-Gly-PAM (0.2 mmol/g) was treated with 35% TFA/DCM

for 25 min, washed with DCM (3×), neutralized with 5% DIEA/DMF (3×), washed with DMF (3×) and with chloroform–phenol 3:1 mixture. Fragment condensation was carried out as described earlier [29]. Briefly, the coupling steps (1–5) were carried out with 1.5 eq Boc-peptide fragment and 2.5 eq HODhbt, 0.5 eq TBA∗ODhbt which were dissolved in chloroform–phenol (3:1), then added to the resin and 3 min later the DIC was added in solution, and then coupling was allowed to proceed for 5 h. After each coupling step, a proportion of the peptide-resin was taken out and the appropriate oligomer (2–6) was cleaved from the resin by the usual liquid HF procedure, purified by HPLC and identified by MS (see Table 1).

Circular dichroism (CD) spectra. These were recorded on a Jobin-Yvon Mark VI dichrograph (calibrated with epiandrosterone) at room temperature in 0.02 cm cells. Water and TFE (Aldrich, NMR grade) were used as solvents, the sample concentrations were in the range 3.2–4.7 m$_M$ (c ∼0.4 mg/cm^3). The spectra were smoothed by the Savitzky–Golay algorithm [30], the CD values are given in mean residue ellipticity $[\theta]_{MR}$, in deg cm^2 dmol^{-1} using a mean residue weight of 83 (Figure 1a and 1b).

Cells and culturing. Populations of *Tetrahymena pyriformis* GL in the logarithmic phase of growth were cultured at room temperature, in 1% Tryptone medium (Difco, Michigan, U.S.A.) containing 0.1% yeast extract.

Chemotaxis Assay and Chemotactic Selection

Chemotaxis assay. The two-chamber capillary chemotaxis assay of Leick and Helle [31], modified by us [25] was applied. In this assay a multi-channel micropipette was used, in which the tips of the pipette filled with test substances (VGAPG

Table 1   Analytical Data of the Oligomers

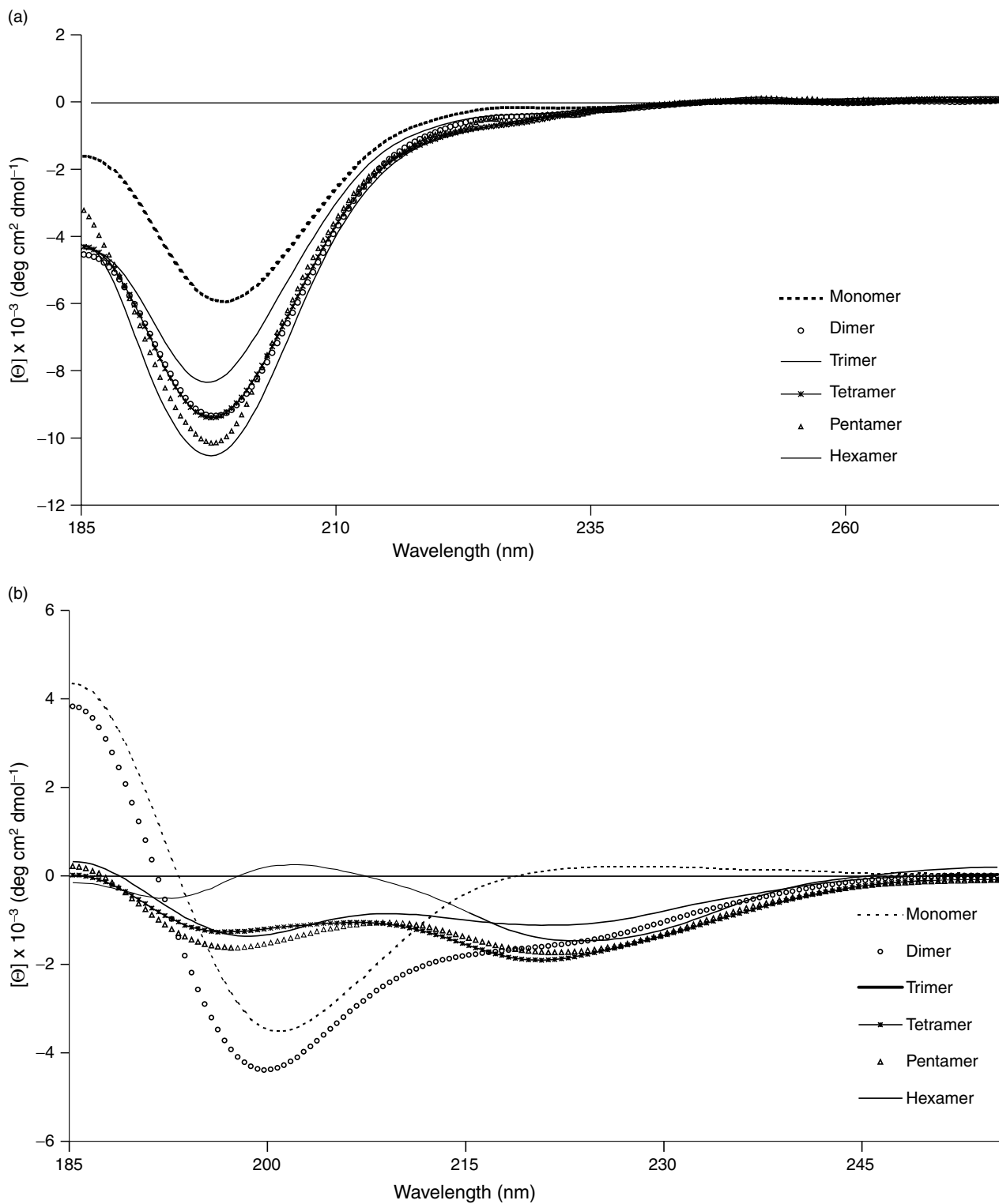| Peptide	Monoisotopic mass		HPLC
	Measured [M + H]$^+$	Calculated	t_R/min
H-VGVAPG-OH ($C_{22}H_{38}N_6O_7$)	499.4	498.3	7.2
H-(VGVAPG)$_2$-OH ($C_{44}H_{74}N_{12}O_{13}$)	979.6	978.5	9.4
H-(VGVAPG)$_3$-OH ($C_{66}H_{110}N_{18}O_{19}$)	1460.0	1458.8	13.1
H-(VGVAPG)$_4$-OH ($C_{88}H_{146}N_{24}O_{25}$)	1940.2	1939.1	14.6
H-(VGVAPG)$_5$-OH ($C_{110}H_{182}N_{30}O_{31}$)	2421.6	2419.3	16.2
H-(VGVAPG)$_6$-OH ($C_{132}H_{218}N_{36}O_{37}$)	2901.7	2899.6	17.3

(a)

(b)

Figure 1   CD spectra of the elastin derived VGVAPG oligomers in water (a) and in trifluoroethanol (b).

or VGVAPG oligomers) served as inner chambers, while 96-well microtitre plates filled with *Tetrahymena* cultures (cell density 10^4 cell/ml) served as outer chambers. The incubation time was 20 min. According to our pilot experiments this was the optimal incubation time when the concentration gradient required for chemotaxis was still present in the chamber. The shorter times did not provide enough cells in the sample, while at times longer than 20 min chemotactic-responder cells could not be distinguished from chemokinetic responder cells. In the concentration course assays the chemotactic responsiveness of the cells was tested in the range 10^{-12}–10^{-7} M. Then the samples were fixed in 4% formaldehyde containing phosphate-buffered saline (PBS). The number of responder cells was counted oculometrically in a Neubauer cytometer by light microscopy. Following the relevant findings in the literature, derivatives were determined (in narrow and wide ranges alike) as chemoattractants (or chemorepellents) when their chemotactic ability was significantly higher (or lower) than the corresponding controls.

Chemotactic selection. The chemotaxis assay described above was used to select chemotactically high previously identified responder subpopulations from cultures in the logarithmic phase of growth [23]. Chemoattractant and chemorepellent VGVAPG oligomers were tested alike in this part of the study. Fresh culture medium was used as a negative control. After the chemotaxis assay with optimal concentrations of the substances, the positive responder cells were transferred into fresh culture medium and these cultures were consecutively transferred every 48 h for 7 days. After this, the cultures were tested in an identical chemotaxis assay. The theoretical groups of samples were: VGVAPG/VGVAPG: cells selected with the VGVAPG oligomer in the first run and assayed for the identical VGVAPG oligomer in the second run; VGVAPG/C: cells selected with the VGVAPG oligomer in the first run and assayed for the control substance in the second run; C/VGVAPG: cells selected with the control substance in the first run and assayed for the VGVAPG oligomer in the second run; and C/C: cells were selected with the control substance in the first run and assayed for the control substance in the second run.

Phagocytosis assay. The phagocytotic activity of the cells treated with 10^{-12}, 10^{-10} and 10^{-8} M VGVAPG oligomers was evaluated with FITC-labelled *E. coli* particles (Phagotest; Orpegen Pharma) [32].

Bacteria (20 µl) or *Tetrahymena* cells (100 µl) with different concentrations of VGVAPGs were incubated for 10 min. Then the samples were fixed with 4% formaldehyde in PBS. The extracellular fluorescent activity was neutralized with quenching solution. The samples were washed with PBS thrice. The number of fluorescent particles taken up by cells was measured with fluorescent-activated cell sorter (FACS-Calibur, Becton-Dickinson). The number of evaluated cells was 10 000/sample.

Cell-proliferation assay. Low-density cultures of *Tetrahymena* (10^1 cell/ml) were treated with 10^{-7}–10^{-12} M VGVAPG monomer for 20 min. Then the cultures were transferred into microtitre plates and were obtained for 18, 24, 30 and 48 h. The density of the control and VGVAPG-treated samples was counted with the MTT assay described above. Each data set of the experiment represented the average of counts of 10 individual parallels.

Statistical analysis. All experiments were repeated five times. Data were evaluated by SigmaPlot 4.0 and Origin 2.8, using Student's *t*-test.

RESULTS AND DISCUSSION

Chemotaxis

Chemotactic responsiveness elicited by VGVAPG and its derivatives was tested in different combinations. The VGVAPG hexapeptide is repeated seven times in the 505–547 sequence of human elastin. In certain pathological conditions, an elevated level of elastolysis occurs resulting in different smaller and biologically active elastin fragments. One of them is the chemotactic VGVAPG sequence generated by the hydrolysis of the G-V peptide bond. However, it is not known whether this is a final product of elastin digestion or not. Since this segment contains a further G-V peptide bond, it seems reasonable to test the chemotactic behaviour of the VAPG tetrapeptide as well. At first the significance of the *N*-terminal part of the monomer VGVAPG form was tested in a comparative study with VAPG tetrapeptide (Figure 2).

Our data show that VAPG has a wide range of neutral chemotactic properties, with a weak chemoattractant peak at 10^{-10} M. In contrast to VAPG tetrapeptide, elongation of the *N*-terminal part of the molecule with the Val-Gly residue results a wide range (10^{-12}–10^{-7} M) chemorepellent moiety. Divergency in chemotactic responses to these two
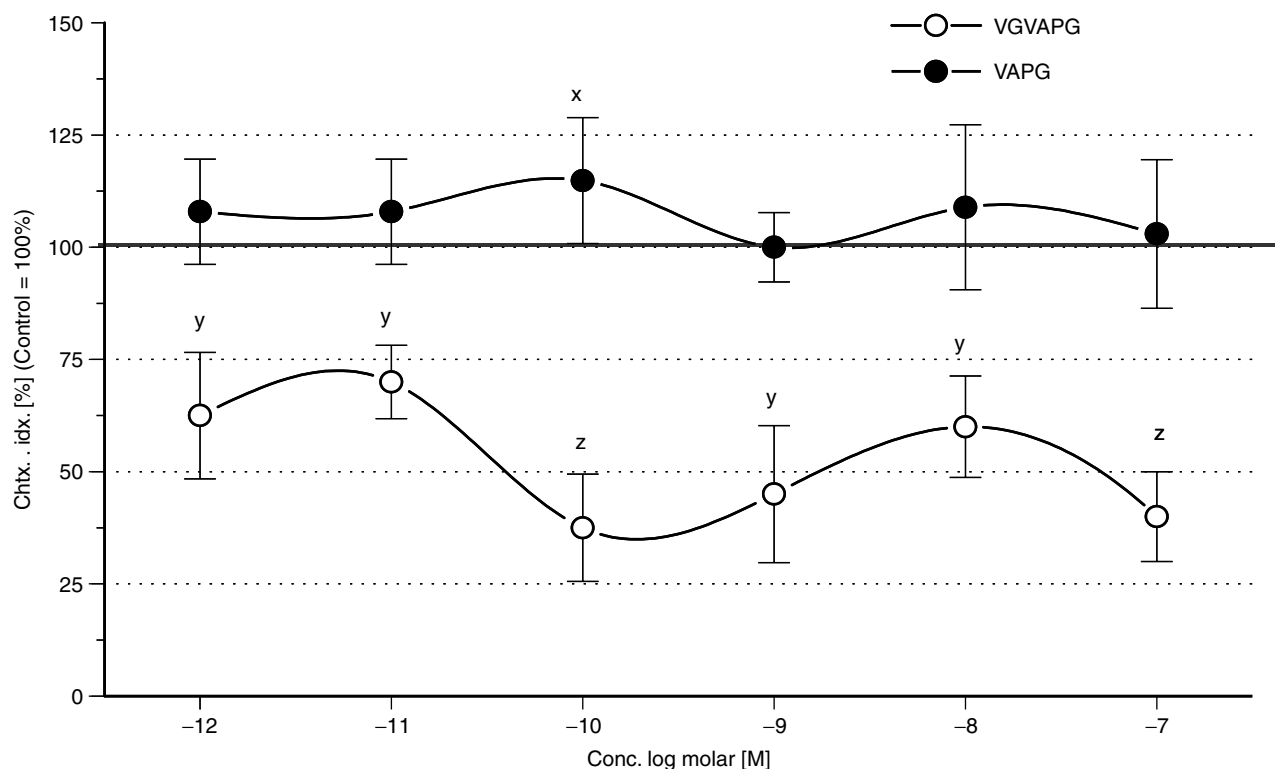
Figure 2   Chemotactic responsiveness of *Tetrahymena pyriformis* GL induced with VGVAPG and VAPG elastin peptides (x: $p < 0.05$; y: $p < 0.01$; z: $p < 0.001$).

relatively short elastin sequences underlines the suitability of *Tetrahymena* as a responsive model in the study of these peptides and shows that the hexapeptide is required for inducing chemotaxis.

The results of the concentration course study of VGVAPG oligomers demonstrate that by changing the number of VGVAPG units there are characteristic alterations of the chemotactic ability of the ligand (Figure 3). In the case of the mono- and the pentamer a concentration dependent, chemorepellent activity was detected, both elastin peptides could elicit the most repellent effect at the highest, 10^{-7} M, concentration. Dimer, trimer, tetramer and hexamer peptides had chemoattractant properties, however, the concentration ranges varied. The dimer was attractant at the lowest (10^{-12} M) concentration tested, while in the higher concentrations it was neutral. A unique, wide range (10^{-12}–10^{-7} M) chemoattractant character was found in the VGVAPG trimer, with the maximal effect at 10^{-8} M, while the hexamer's chemoattractant effects were elicited only in the two highest (10^{-8}–10^{-7} M) concentrations. The only biphasic profile was found in the case of the VGVAPG tetramer, with a chemoattractant

(10^{-10} M) and chemorepellent (10^{-8} M) moiety, as well.

Chemotactic Selection

Chemotactic selection was used to test whether in the background of the chemotactic responses elicited by VGVAPG peptides there were short- or long-term signalling mechanisms (Figure 4).

First, the chemotactic responsiveness of subpopulations selected with the culture medium, which was different from the results of the concentration course study and depressed in four cases (VGVAPG-monomer, -dimer, -pentamer and -hexamer) should be considered. The possible explanation for this effect of the control medium is that this reference substance also contains organic components (tryptone and yeast extract). Inorganic media, such as Losina-Losinsky solution [33] can be used as they lack organic molecules that might interfere with the chemotactic selection. However, the inorganic media also provide a non-physiological condition for chemotaxis, which is undesirable and therefore fresh medium was chosen as the control.
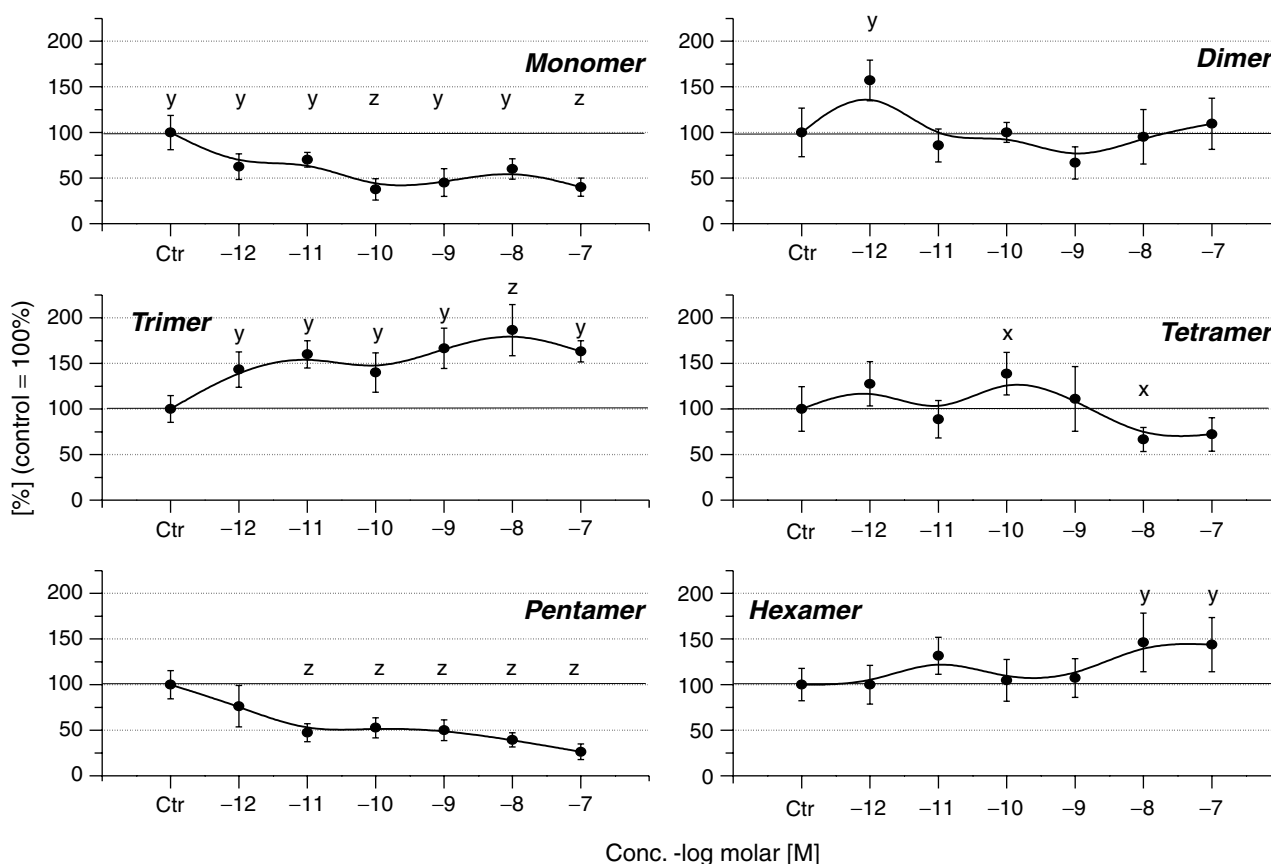
Figure 3 Concentration course study of chemotaxis induced by elastin peptides composed of repeating VGVAPG sequence (x: $p < 0.05$; y: $p < 0.01$; z: $p < 0.001$).

Data gained by repeated inductions of chemotactically selected subpopulations with the elastin peptides show good correlation with the concentration course results. An unambiguously enhanced chemotactic responsiveness was detected in subpopulations selected with the two chemoattractants, VGVAPG trimer and tetramer, which points to these two ligands inducing chemotactic responses via constant, long-term expressed components of the membrane. The chemoattractant VGVAPG-hexamer could also select subpopulations possessing a moderate chemoresponsive character to the selector ligand, nevertheless signalling of these subpopulations proved to be highly specific as these cells distinguished the plain culture medium as a negative signal. The intensity of the chemotactic responsiveness of the subpopulations selected with the chemoattractant VGVAPG dimer was moderate. In contrast to its chemoattractant character in a short-term concentration course study, it proved to be chemorepellent in the identical subpopulations, pointing to *Tetrahymena* having some short-term chemotaxis

receptors inducible by this ligand, however, these components are not constitutive components of the membrane.

In the case of the two chemorepellent ligands opposite effects were detected. A selection study of the VGVAPG-monomer verified that the strong chemorepellent effect of this ligand detected in the mixed cultures is a general, dominant character in long-term aspects, too. The data of the VGVAPG-pentamer call attention to the fact that there is the possibility to select small, but positive responder subpopulations also with a chemorepellent ligand. This phenomenon is not unique, as endothelin-2 [34] and hydrocortisone [35] are also chemorepellent in general in the mixed cultures, however, they have the ability to act positively on small groups of cells in long-term studies.

Phagocytosis

Phagocytosis is one of the most significant target reactions of chemotaxis. Degraded components of
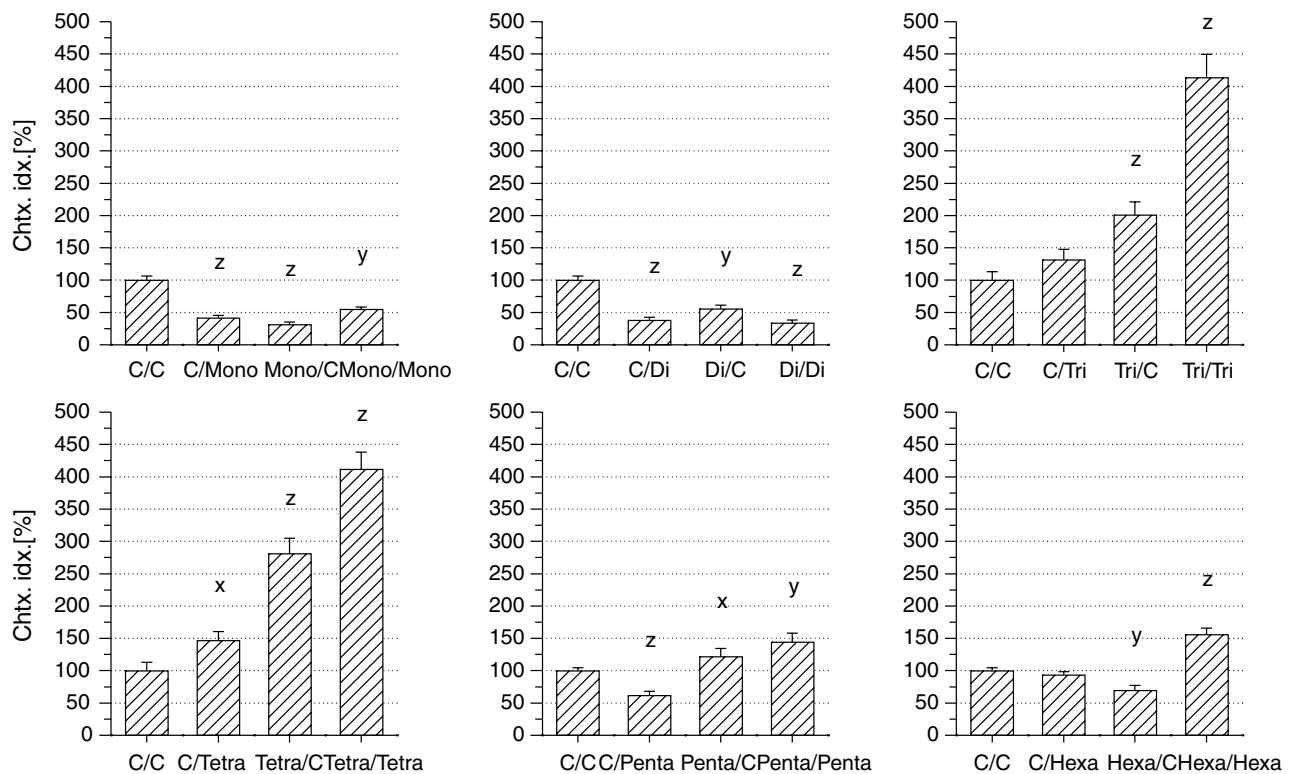
Figure 4 Chemotactic selection with elastin derived VGVAPG oligopeptides in *Tetrahymena* (x: $p < 0.05$; y: $p < 0.01$; z: $p < 0.001$).

ECM are strong inducers of macrophage phagocytosis in an age-dependent manner [36,37], nevertheless the literature data are poor on the effects elicited by the elastin sequences.

Our data indicate that, contrary to chemotaxis, phagocytotic activity is not highly vulnerable to VGVAPG oligomers in *Tetrahymena* (Figure 5), which result is unexpected as both proteolytic enzymes and elastin have a deep phylogenetic background.

The four shorter derivatives (mono-, di-, tri-, tetramer) had no effect on phagocytosis, while the two longer ones (penta and hexamers) had a slight phagocytosis inhibitor effect at 10^{-10} M. A study of the reverse side of this 'activity' — evaluation of the ratios of non-responder (non-phagocytotic) cells — points to the fact that there is a significant divergence of the peptides, as 10^{-12} M monomer proved to be the most neutral (225%) versus the 10^{-10} M trimer (95.5%). In respect of the dose-response curves describing positive or negative phagocytotic responsiveness, the 10^{-10} M concentration proved to be critical in the case of the elastin sequences studied. Dominance of neutral effects of VGVAPGs on this cell physiological activity

underlines the possibility that, however closely chemotaxis and phagocytosis are related, their signalling mechanisms are not definitely the same or are working in synergism.

Proliferation

In the higher levels of phylogeny, interactions of the elastin–receptor complex can influence the proliferation of cells [38]. This relation has a special feature in vascular smooth muscle, where mechanisensitive responses elicited by elastin are accompanied by a marked inhibition of proliferation, while the VGVAPG sequence of elastin could restore the normal physiological responsiveness of the cells [7].

In our model, proliferation is also considered as a basic cell physiological property.

The growth curves (Figure 6) demonstrate that treatment with VGVAPG peptides slightly stimulated the multiplication of cells, however, this activity was manifested only around the 20th generation after treatment. Theoretically, signal-sequences of elastin and their derivatives could also be consumed as nourishment by these cells, nevertheless the
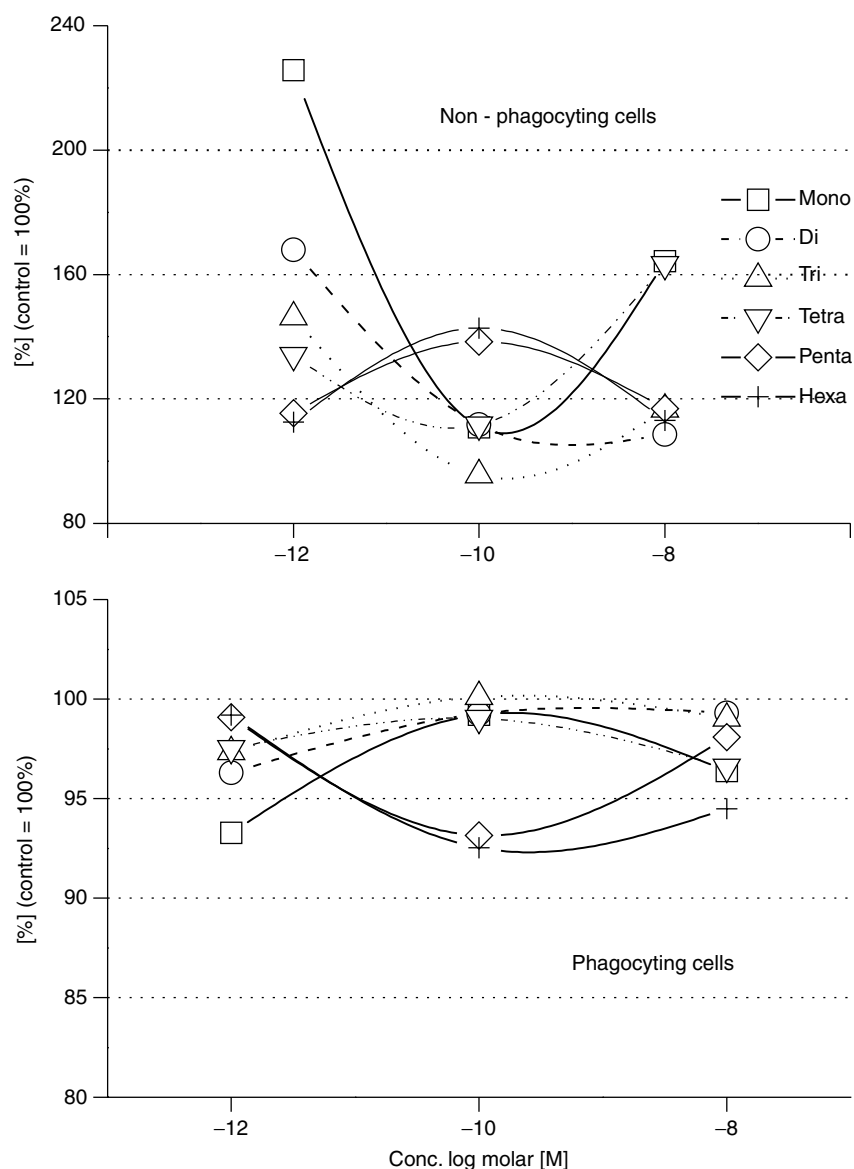
Figure 5   Effect of treatment with VGVAPG oligopeptides on the phagocytotic activity of *Tetrahymena*.

overlapping profiles of the growth-curves obtained from cultures treated with high (10^{-7} M) and low (10^{-12} M) concentrations and homology to the effects in the vertebrate systems suggest that the registered shift of the curves is due to the inducer effect of the signal molecule and not a plain substance in food.

Correlations of CD Spectra and Biological Activity

The CD spectra of the (VGVAPG)$_n$ oligomers ($n = 1$–6) in water (Figure 1a) reflect the presence of a conformation-mixture in the case of both the monomer and the oligomer peptides. In TFE (Figure 1b) the spectrum of the monomer, characterized by a dominant band at $\lambda \sim 200$ nm, still shows the presence of a mixture of unordered, extended conformers, in the case of the dimer a faint sign of the appearance of conformers having a more ordered, likely turn structure (negative band, 220–230 nm) was seen. A definite increase of turn population can be observed in the spectra of the tri-, tetra-, penta- and hexamer, which is due to the presence of the Pro-Gly unit in all repeating elastin sequences, and to hydrophobic interactions within the peptides.
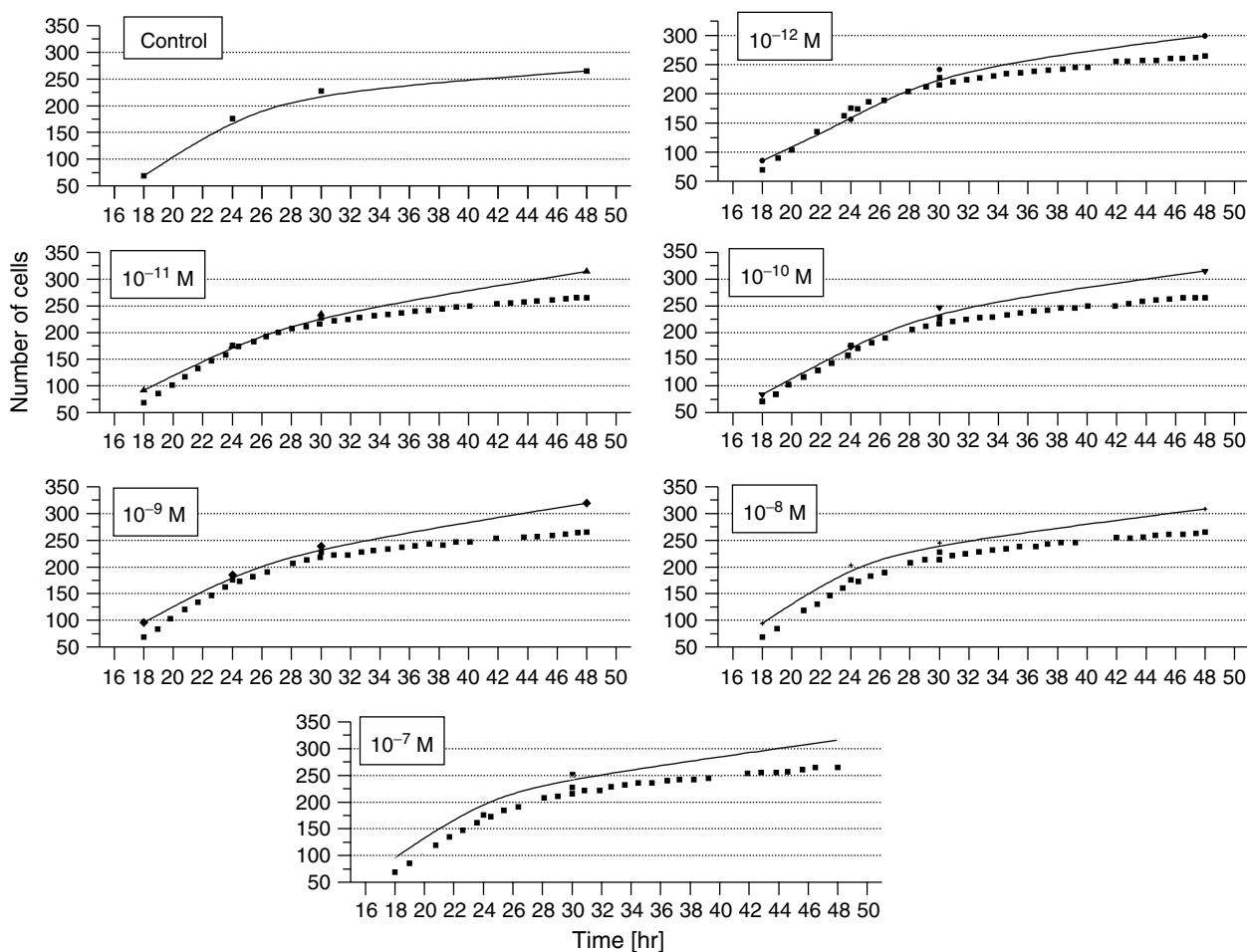
Figure 6 Representative growth curves on growth promoting character of VGVAPG-oligopeptides — VGVAPG-monomer in the figure — in *Tetrahymena*. Dotted line represents the control.

Although the formation of more ordered structures parallels the increasing number of the monomer units, no direct correlation was found between the repetition of the VGVAPG sequence and the chemotactic behaviour of the cells mediated by the elastin receptor.

The CD measurements of the VGVAPG oligomers exclude neither the β-pleated sheet structure, nor a 'super structure', meaning the repeat of β-sheet and β-turn structures, and it cannot be excluded that some fine differences in the conformation of the ligand are required by the receptor for exerting different biological effects. (Therefore a more detailed spectroscopic study is necessary to complete the understanding of the role and significance of the repeating peptide sequences in the elastin molecule.)

According to the literature data a similar tendency has been found in the case of the VPGG tetrapeptide [39] and VPGVG pentapeptide [40]; namely the monomers form a type II β-turn and the polymers form a β-spiral with type II β-turns.

CONCLUSIONS

Comparing the results with those gained in mammalian cell cultures it can be concluded that in some cases *Tetrahymena* reacts to the elastin oligomers in a similar manner, however, in other cases there are differences. This means that *Tetrahymena* selects between the oligomers according to its special recognition capacity which is not closely correlated with the specific (ECM) receptors of mammalian cells. This is understandable considering that *Tetrahymena* is a free-living organism, which can make a selection between the different molecules present around it, however, in contrast to mammalian cells, ECM receptors are not needed for it. However,

considering the selection-ability of *Tetrahymena* between the elastin oligomers according to their length and concentration, it seems likely that the elastin receptors of higher animals can be deduced from the sensitivity to elastin oligomers at the unicellular level, as happened in some other cases, e.g. insulin [41], opioids [42], etc, where the presence of the receptor-like molecule was demonstrated, as well as the function provoked by the hormone. Similar to the integrin receptors, elastin receptors at higher level have a double function: (1) the main function is the fixation of the cell and, (2) as a consequence of (1), the transduction of the signal given by elastin. Theoretically, these should be studied in the case of *Tetrahymena*. Although, fixation could not be expected, signal transduction — if it does happen indeed — can be observed. Experiments to clear this problem are in progress.

In conclusion we can summarize our observations as follows:

Ligands composed by VGVAPG oligomers have the potency to modulate chemotaxis or other cell-physiological responses in the early eukaryotic model, *Tetrahymena.*

The effect of oligomers was diverse: the chemoattractant potency of the trimer and the chemorepellent effects of the mono- and pentamers had no correlation with the CD spectra of the molecules, while in chemotactic selection there was significant overlapping between the selector potency and the CD spectra (e.g. tri- and tetramers). The chemorepellent and phagocytosis blocker effect of VGVAPG pentamers was concordant. The proliferation promoting effect of VGVAPG was present in a wide concentration range.

Acknowledgements

REFERENCES

1. Welsh ER, Tirrell DA. Engineering the extracellular matrix: a novel approach to polymeric biomaterials. I. Control of the physical properties of artificial protein matrices designed to support adhesion of vascular endothelial cells. *Biomacromolecules* 2000; **1**: 23–30.

2. Smith DW, Weissman N, Carnes WH. Cardiovascular studies on copper deficient swine. XII. Partial purification of a soluble protein resembling elastin. *Biochem. Biophys. Res. Commun.* 1968; **31**: 309–315.

3. Boyd CD, Christiano AM, Pierce RA, Stolle CA, Deak SB. Mammalian tropoelastin: multiple domains of the protein define an evolutionarily divergent amino acid sequence. *Matrix* 1991; **11**: 235–241.

4. Alix AJ. A turning point in the knowledge of the structure-function-activity relations of elastin. *J. Soc. Biol.* 2001; **195**: 181–193.

5. Price LS, Roos PJ, Shively VP, Sandberg LB. Valyl-alanyl-prolyl-glycine (VAPG) serves as a quantitative marker for human elastins. *Matrix* 1993; **13**: 307–311.

6. Mecham RP, Hinek A, Entwistle R, Wrenn DS, Griffin GL, Senior RM. Elastin binds to a multifunctional 67-kilodalton peripheral membrane protein. *Biochemistry* 1989; **28**: 3716–3722.

7. Spofford CM, Chilian WM. The elastin-laminin receptor functions as a mechanotransducer in vascular smooth muscle. *Am. J. Physiol. Heart Circ. Physiol.* 2001; **280**: H1354–H1360.

8. Brassart B, Fuchs P, Huet E, Alix AJ, Wallach J, Tamburro AM, Delacoux F, Haye B, Emonard H, Hornebeck W, Debelle L. Conformational dependence of collagenase (matrix metalloproteinase-1) up-regulation by elastin peptides in cultured fibroblasts. *J. Biol. Chem.* 2001; **276**: 5222–5227.

9. Fujimoto N, Tajima S, Ishibashi A. Elastin peptides induce migration and terminal differentiation of cultured keratinocytes via 67 kDa elastin receptor *in vitro*: 67 kDa elastin receptor is expressed in the keratinocytes eliminating elastic materials in elastosis perforans serpiginosa. *J. Invest. Dermatol.* 2000; **115**: 633–639.

10. Brassart B, Randoux A, Hornebeck W, Emonard H. Regulation of matrix metalloproteinase-2 (gelatinase A, MMP-2), membrane-type matrix metalloproteinase-1 (MT1-MMP) and tissue inhibitor of metalloproteinases-2 (TIMP-2) expression by elastin-derived peptides in human HT-1080 fibrosarcoma cell line. *Clin. Exp. Metastasis.* 1998; **16**: 489–500.

11. Hornebeck W, Emonard H, Monboisse JC, Bellon G. Matrix-directed regulation of pericellular proteolysis and tumor progression. *Semin. Cancer Biol.* 2002; **12**: 231–241.

12. Yamamoto M, Akazawa K, Aoyagi M, Yamamoto N, Yamamoto K. Changes in elastin-binding protein in fibroblasts derived from cardinal ligaments of patients with prolapsus uteri. *Cell Biol. Int.* 2002; **26**: 441–449.

13. Hance KA, Tataria M, Ziporin SJ, Lee JK, Thompson RW. Monocyte chemotactic activity in human abdominal aortic aneurysms: role of elastin degradation peptides and the 67-kD cell surface elastin receptor. *J. Vasc. Surg.* 2002; **35**: 254–261.

14. Bisaccia F, Morelli MA, De Biasi M, Traniello S, Spisani S, Tamburro AM. Migration of monocytes in the presence of elastolytic fragments of elastin and

in synthetic derivates. Structure-activity relationships. *Int. J. Pept. Protein Res.* 1994; **44**: 332–341.

15. Kamoun A, Landeau JM, Godeau G, Wallach J, Duchesnay A, Pellat B, Hornebeck W. Growth stimulation of human skin fibroblasts by elastin-derived peptides. *Cell Adhes. Commun.* 1995; **3**: 273–281.

16. Wachi H, Seyama Y, Yamashita S, Suganami H, Uemura Y, Okamoto K, Yamada H, Tajima S. Stimulation of cell proliferation and autoregulation of elastin expression by elastin peptide VPGVG in cultured chick vascular smooth muscle cells. *FEBS Lett.* 1995; **368**: 215–219.

17. Condeelis J, Jones J, Segall JE. Chemotaxis of metastatic tumor cells: clues to mechanisms from the *Dictyostelium paradigm. Cancer Metastasis Rev.* 1992; **11**: 55–68.

18. Csaba G. The unicellular *Tetrahymena* as a model cell for receptor research. *Int. Rev. Cytol.* 1985; **95**: 327–377.

19. Christensen ST, Guerra CF, Awan A, Wheatley DN, Satir P. Insulin receptor-like proteins in *Tetrahymena thermophila* ciliary membranes. *Curr. Biol.* 2003; **13**: R50–R52.

20. Kovács P, Csaba G. Involvement of the phosphoinositol (PI) system in the mechanism of hormonal imprinting. *Biochem. Biophys. Res. Commun.* 1990; **170**: 119–126.

21. Köhidai L. Chemotaxis: The proper physiological response to evaluate phylogeny of signal molecules. *Acta Biol. Hung.* 1999; **50**: 375–394.

22. Köhidai L, Kovács P, Csaba G. Chemotaxis of the unicellular green alga *Dunaliella salina* and the ciliated *Tetrahymena pyriformis* — Effects of glycine, lysine, and alanine, and their oligopeptides. *Biosci. Rep.* 1996; **16**: 467–476.

23. Köhidai L, Csaba G. Chemotaxis and chemotactic selection induced with cytokines (IL-8, RANTES and TNFα) in the unicellular *Tetrahymena pyriformis. Cytokine* 1998; **10**: 481–486.

24. Köhidai L, Csaba G. Different and selective chemotactic responses of *Tetrahymena pyriformis* to two families of signal molecules: lectins and peptide hormones. *Acta Microbiol. Immunol. Hung.* 1996; **43**: 83–91.

25. Köhidai L, Lemberkovits É, Csaba G. Molecule dependent chemotactic responses of *Tetrahymena pyriformis* elicited by volatile oils. *Acta Protozool.* 1995; **34**: 181–185.

26. Köhidai L, Soós P, Csaba G. Effects of dipeptides containing the amino acid, proline on the chemotaxis of *Tetrahymena pyriformis.* Evolutionary conclusions on the formation of hormone receptors and hormones. *Cell Biol. Internat.* 1997; **21**: 341–345.

27. Csaba G, Kovács P, Köhidai L. *Tetrahymena* cells distinguish insulins according to their amorphous and crystalline condition or their bovine and porcine origin. Study of imprinting in aspects of hormone binding and chemotaxis. *Microbios* 1994; **80**: 215–221.

28. Fields GB, Noble RL. Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Peptide Protein Res.* 1990; **35**: 161–214.

29. Mihala N, Bodi J, Gömöry A, Süli-Vargha H. An alternative solid phase peptide fragment condensation protocol with improved efficiency. *J. Peptide Sci.* 2001; **7**: 565–568.

30. Savitzky A, Golay MJE. Smoothing and differentiation of data by simplified least square procedures. *Anal. Chem.* 1964; **36**: 1627.

31. Leick V, Helle J. A quantitative assay for ciliate chemotaxis. *Anal. Biochem.* 1983; **135**: 466–469.

32. Bassone C-F. Flow-cytometric studies on phagocyte function in bacterial infections. *Acta Path. Microbiol. Immunol. Scand.* 1984; **92**: 167–170.

33. Losina-Losinsky LK. Zur Ernärungsphysiologie der Infusorien. Untersuchungen über die Nahrungsmittel und Vermehrung bei Paramecium caudatum. *Arch. Protistenk.* 1931; **74**: 18–20.

34. Köhidai L, Tóth K, Ruskoaho H, Csaba G. Effect of vasoactive peptides in *Tetrahymena* — Chemotactic properties of endothelins (ET-1, ET-2, ET-3, fragment 11–21 of ET-1 and big endothelin) — a short-term inducible signaling mechanism of chemotaxis. *Cell. Biol. Internat.* 2001; **25**: 1173–1177.

35. Köhidai L, Katona J, Csaba G. Effects of steroid hormones on five functional parameters of *Tetrahymena*: evolutionary conclusions. *Cell Biochem. Funct.* 2003; **21**: 19–26.

36. Chapman HA Jr, Stone OL. Comparison of live human neutrophil and alveolar macrophage elastolytic activity *in vitro*. Relative resistance of macrophage elastolytic activity to serum and alveolar proteinase inhibitors. *J. Clin. Invest.* 1984; **74**: 1693–1700.

37. Varga Z, Jacob MP, Robert L, Csongor J, Fulop T Jr. Age-dependent changes of K-elastin stimulated effector functions of human phagocytic cells: relevance for atherogenesis. *Exp. Gerontol.* 1997; **32**: 653–662.

38. Li DY, Brooke B, Davis EC, Mecham RP, Sorensen LK, Boak BB, Eichwald E, Keating MT. Elastin is an essential determinant of arterial morphogenesis. *Nature* 1998; **393**: 276–280.

39. Urry DW, Harris RD, Long MM, Prasad KU. Polytetrapeptide of elastin. *Int. J. Peptide Res.* 1986; **28**: 649–660.

40. Castiglione Morelli MA, Bisaccia F, Spisani S, De Biasi M, Traniello S, Tamburro M. Structure-activity relationships for some elastin-derived peptide chemoattractants. *J. Peptide Res.* 1997; **49**: 492–499.

41. Christensen ST, Guerra CF, Awan A, Wheatley DN, Satir P. Insulin receptor-like proteins in *Tetrahymena thermophila* ciliary membranes. *Curr. Biol.* 2003; **13**: R50–R52.

42. O'Neill JB, Pert CB, Ruff MR, Smith CC, Higgins WJ, Zipser B. Identification and characterization of the opiate receptor in the ciliated protozoan, *Tetrahymena. Brain Res.* 1988; **450**: 303–315.