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Possible Involvement of Aqueous Medium in Distant Signal Transmission from Immunoactive Dipeptides

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Possible involvement of aqueous medium in distant signal transmission to target cells through solitons without formation of the ligand—receptor complexes is discussed. Temperature dependence of IR spectra for dipeptide and amino acid solutions in the far and near IR regions are presented, which prove principal possibility of such processes.

Key Words: *signal transmission; peptides; solitons; receptors; IR spectroscopy*

Biological effects of many substances, in particular peptides, cannot be comprehensively explained from the viewpoint of the theory of ligand—receptor interactions. It remains unclear how compounds with different primary and secondary structures act as agonists or antagonists of the same receptors [8]. The fact that biological effects can often be recorded at ligand concentrations of 10^{-15}-10^{-19} M and even lower makes unclear how ligand—receptor complex forms: at a concentration of 10^{-15} M less than 10 ligand molecules falls within 1 target cell [1], and at a concentration of 10^{-19} M there is a possibility that experimental volume (1 ml) contains no ligand molecules [7]. There is no reliable explanation for the fact that exogenous preparations produce appreciable effects in the presence of the same endogenous compound in concentrations higher by several orders of magnitude in the organism [4]. The hypotheses based on the classical theory do not explain all these principal problems [7,8]. For understanding of the effects of signal molecules on target cells we should take into consideration the possibility of other mechanisms of development, transmission, and registration of the signal on the basis of field and wave interactions, where water acts as a medium and as a participant. Several hypotheses are now put forward: the resonance hypothesis of ligand—receptor interactions [2], signal transmission through aqueous medium to the receptor with the help of ligand molecules present in the intercellular matrix [10], water "memory" [9,11], *etc.* Unfortunately, none of these publications specifies more or less concrete mechanisms of the studied processes, or offer well-grounded physical explanations of the role of water, or present experimental data confirming these hypotheses.

We describe a possible element of distant transmission of a signal carrying information on the presence and type of ligand molecules to target cells in aqueous medium. We also present experimental data which can confirm principle possibility of such processes.

MATERIALS AND METHODS

Synthetic dipeptides L-Glu-L-Trp (I) Thymogen, L-Lys-L-Glu (II) Vilon, L-Glu-L-Lys (III), and individual amino acids of these peptides were used. The dipeptides were synthesized at St. Petersburg Institute of Bioregulation and Gerontology; their purity was at least 99% and their structure was confirmed by amino acid analysis and UV and proton magnetic resonance spectroscopy. Conformation was studied in MM2 field (water medium, CSChem3DPro). Immunoactivity was evaluated by the effects on the expression of receptors

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on guinea pig thymocytes treated with trypsin (Ea-RFC) and by their effects on subpopulations of human blood T cells ($CD4^+$, $CD8^+$). Temperature relationships of the parameters of IR spectra for water solutions in the near (5180 cm^{-1}) and far (180 cm^{-1}) bands were evaluated at 5-45°C. Deionized water (specific resistance≥17 MΩ/cm) was used.

RESULTS

The immunoactive characteristics of the studied synthetic dipeptides were described for different *in vitro* models [5,14]. The maximum difference between these compounds by Ea-RFC is no more than 12% at relative error of 7-10%; Vilon is more active towards $CD4^+$ receptors, and none of the three dipeptides affects $CD8^+$ receptors. The results indicate that the dipeptides are similar by immunoactive characteristics in these tests, despite differences in their primary structure. However, these differences are essential. Peptide I has a hydrophobic (due to its indole ring) C-terminal tryptophan, while peptides II and III have hydrophilic N- and C-terminals lysine with positively charged ε-amino group. According to the conformation analysis, peptide II has a pseudocyclic energetically beneficial conformation due to approximation of ionized ε-amino groups of lysine and α-carboxylic group of glutamic acid. This cyclic conformations are not realized in two other peptides (Fig. 1). Hence, dipeptides I, II, and III differ essentially by their primary structure and conformation, but show almost the same biological activity in the same tests. These facts contradict the main principle of the ligand—receptor interactions, *i .e.* complimentarity of the ligand to the receptor binding site: in our case dipeptides I, II, and III cannot be complimentary the same binding site.

It can be hypothesized that dipeptides interact with different receptors (which is hardly possible because of their universal biological effect) or that the receptor or other membrane elements do not recognize structural and spatial differences between ligands, but are tuned to other parameters, which are similar in these dipeptides. This latter phenomenon is possible, if the classical mechanism of the ligand—receptor interactions is paralleled by another type of reception, when the conformation signal about the presence and type of the ligand (peptide) is transmitted to the cell through aqueous medium acting as a transmitter. We believe that the soliton theory, actively developed during recent decades [6,12], best of all explains the mechanism of distant signal transmission to receptors in aqueous medium. Stability of solitons, their dual nature (wave and corpuscle), and capability of energy transmission make them possible carrier of information about the presence and structure of the ligand (formation and translation of a signal) to the receptor. The data on scattering of slow neutrons confirm the presence of solitons in water [15]; study of temperature curves of aqueous IR spectra in the near (5180 cm^{-1}) and far (180 cm^{-1}) regions indirectly confirms their presence in water [3,13]. Nonmonotonic wave-like fluctuations of water IR spectra (shift of absorption maximum, variation of intensity) depend on the nature of dissolved substance. We believe that the information about the presence and nature of dissolved ligand can thus be reflected in individual temperature curves of far and near IR spectra of water. The dissolved substance via oscillatory interactions with the medium modulates the type of solitons forming in the water, thus choosing between potentialities in a nonlinear medium. Therefore, along with manifestation of individual features, relationships similar by shape and

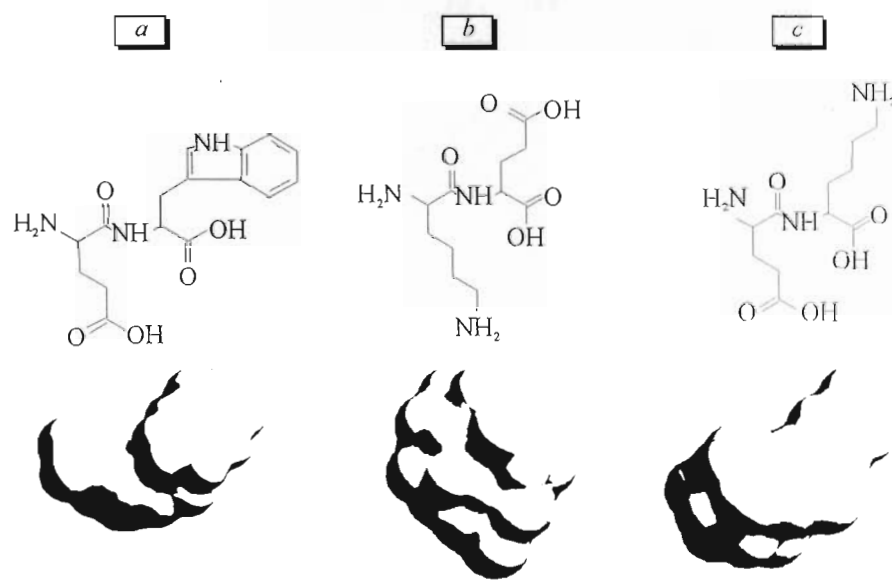

Fig. 1. Structural formulaes and energetically beneficial conformations (in CSChem3DPro surface format available for water) of H-Glu-Trp-OH (*a*), H-Lys-Glu-OH (*b*), and H-Glu-Lys-OH (*c*) dipeptides.

intensity can form even for chemically different structures, which can explain similarity of their biological effects.

We studied the temperature curves of water IR spectra for synthesized dipeptides and individual amino acids (Fig. 2). The presence of nonmonotonicities in these temperature curves specific for each compound can be interpreted as modification of water solitons, forming as a result of interactions with added molecules (peptides and amino acids). Temperature curves of water IR spectra for Thymogen and Vilon water solutions are similar and at the same time individual for each compound (Fig. 2). It can be hypothesized that similar signals are translated through water to the receptor, which results in the realization of similar biological effects. Temperature curves of water spectrum for individual amino acids are absolutely different and contain no obvious nonmonotonicities. However some experiments showed differences in the effects of Vilon and Thymogen (culturing of thymic and splenic explants from rats of different age, stimulation macrophages and neutrophils) [5]. These and other differences in biological effects of Vilon and Thymogen can be explained by not only their specific primary structure during biochemical interactions, but also their different effect on the dynamic characteristics of water in some frequency bands (Fig. 3), which can lead to differences in the parameters of soliton signals transmitted to the receptors.

Hence, we proposed a new mechanism of signal transmission to receptor (target cell) in aqueous medium (blood, lymph, matrix). This mechanism is based on changes in the wave parameters of water solitons depending on the ligand nature. This hypothesis explains the absence of specific interactions with the receptors (cell) for various agonists (formation of a uniform soliton signal) and efficiency of extremely low doses. The location of the ligand molecules in immediate proximity to the receptor becomes unnecessary; soliton signals modified by solitary ligand molecules diffuse simultaneously in all directions and reach many receptors (signal amplification). It is also

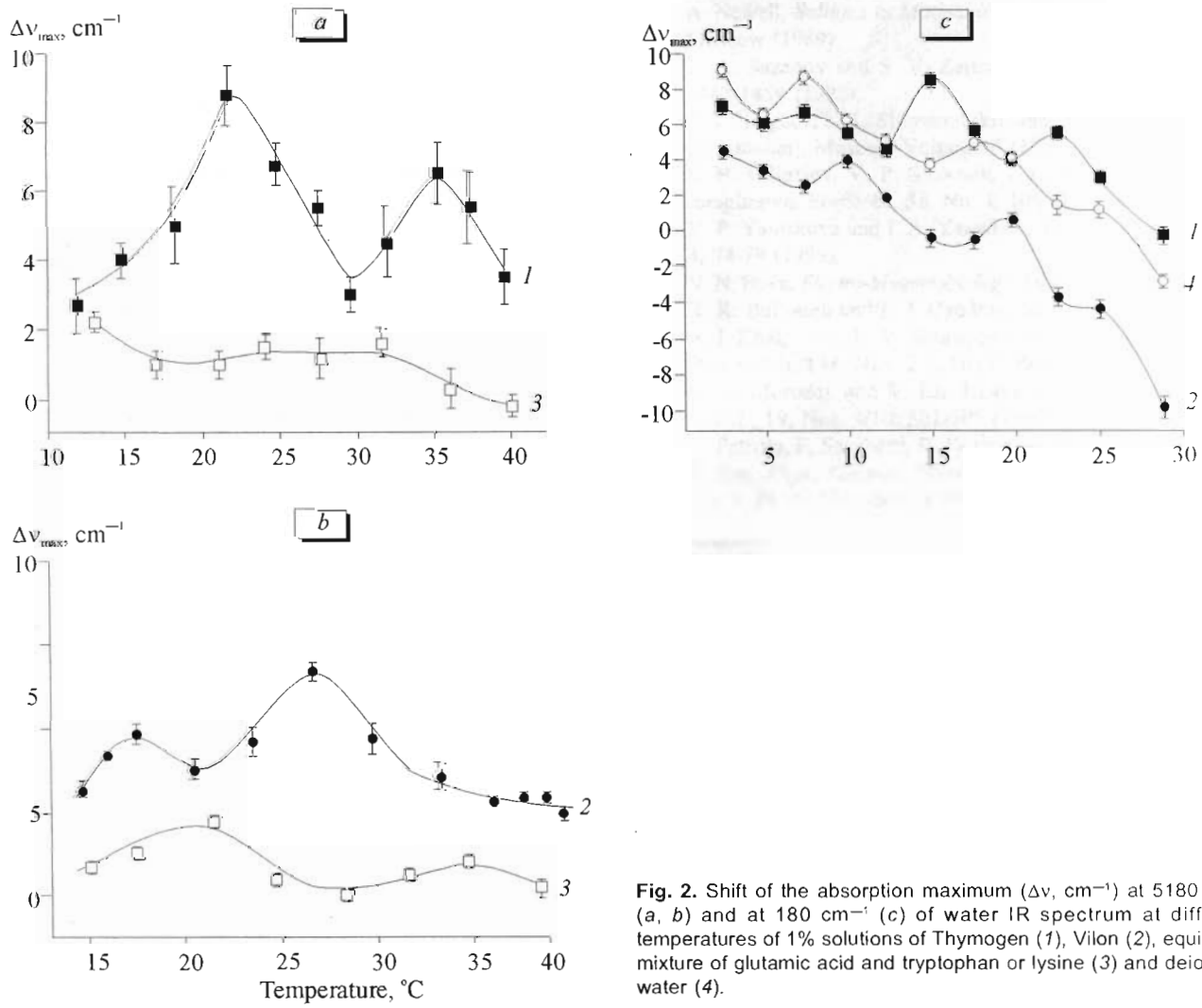

Fig. 2. Shift of the absorption maximum ($\Delta\nu$, cm^{-1}) at 5180 cm^{-1} (*a*, *b*) and at 180 cm^{-1} (*c*) of water IR spectrum at different temperatures of 1% solutions of Thymogen (*1*), Vilon (*2*), equimolar mixture of glutamic acid and tryptophan or lysine (*3*) and deionized water (*4*).

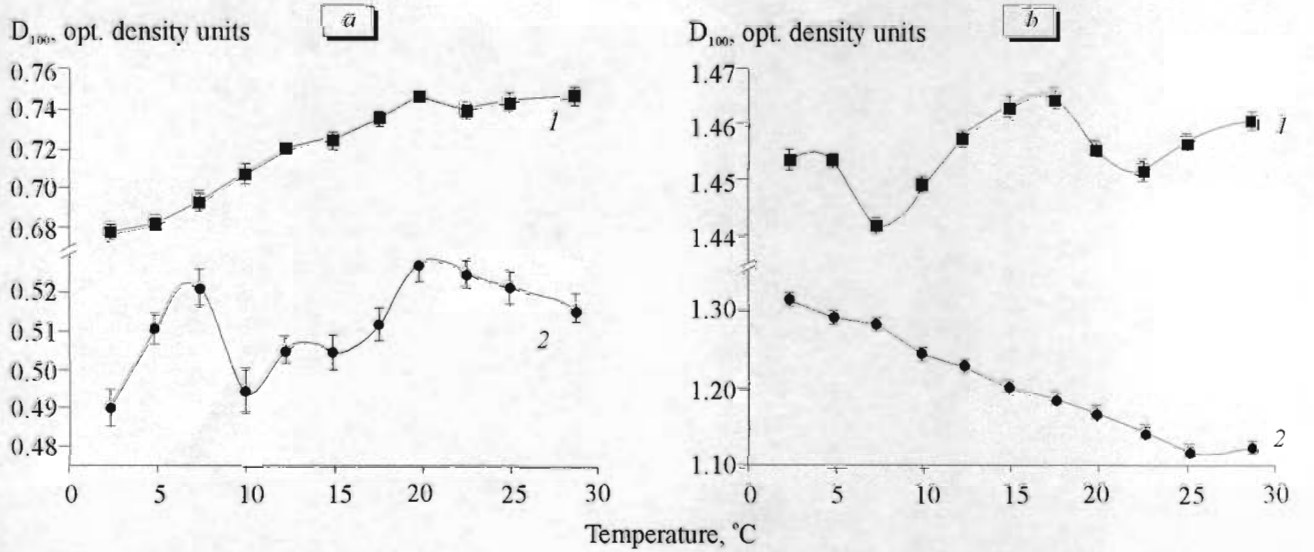

Fig. 3. Temperature curves of optical density of aqueous IR spectra at 100 cm^{-1} (*a*) and 230 cm^{-1} (*b*) for 1% Vilon (*1*) and Thymogen .

possible that the presence of an endogenous ligand, like the presence of other dissolved substances and the microenvironment in general, modulates the formation of the total wave, field homeostasis in the local area of the extracellular medium in a certain type of tissues (dynamic equilibrium), while the receptor records only its changes, caused by the molecules of newly entering bioactive substance in certain frequency and amplitude bands.

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