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ORIGINAL ARTICLE

Effect of the external electric field on selected tripeptides

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Abstract The effect of external electric field (EEF) of 5.14, 25.70, and 51.40 MV/cm upon Cys-Asn-Ser, Glu-Arg-Leu, Glu-Cys-Glc, Ser-Asp-Leu, Ser-Glu-Met tripeptide inner salts was simulated involving HyperChem 8.0 software together with the AM1 method for optimization of the molecules' conformation. The reaction to EEF is diverse and specific to particular peptides. EEF stimulated an increase in the positive charge density on the hydrogen atoms of the $N^{+}H_3$, peptide bond NH, NH_2, and COOH groups as well decrease in the negative charge density on the oxygen atoms of the peptide bond carbonyl groups. Thus, EEF could control behavior and action of tripeptides, such as an increase in their catalytic activity.

Keywords Cys-Asn-Ser · Glu-Arg-Leu · Glu-Cys-Glc · Ser-Asp-Leu · Ser-Glu-Met

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Introduction

Living organisms are steadily exposed to the external electric (EEF) and electromagnetic (EMF) fields. Some parts of these fields originate from natural sources and some result from the human activity. Regardless of the origin of these emanations they interact with the bioelectric and biomagnetic fields of the living systems in either beneficial or harmful manner (US Congress Office of Technology Assessment 1989; Malmivuo and Plonsey 1995; Ponne and Bartels 1995; Armstrong and Wilson 2000). Laboratory studies (Nechitailo and Gordeev 2001) and observations of plants exposed to the electromagnetic field from the low, medium and high voltage power lines (Nechitailo and Gordeev 2001; Aksoy et al. 2010; Maziah et al. 2012) showed a stimulating effect of low and medium frequency EEF upon plant growth. Stimulation of microorganisms by EEF could be utilized during various fermentative processes such as ethanol production by *Saccharomyces cerevisiae* (Nakanishi et al. 1988; Bauer et al. 1986), nourseothricin antibiotic synthesis by *Streptomyces noursei* (Grosse et al. 1988) and preparation of citric acid by *Aspergillus niger* (Fiedurek 1999). Pulsed EEF was considered as the factor controlling the growth and activity of *Escherichia coli* and *Listeria innocua* in liquid food products (Dutreux et al. 2000).

There are documented effects of EEF upon animal and human cells. Thus, cAMP synthesis in embryotic cells (Jones 1984), DNA synthesis in bone cells (Liboff et al. 1984), and membrane transport into those cells (Sisken et al. 1984; Murgida and Hildebrandt 2004, 2005) were reported. EEF also stimulates DNA synthesis in human fibroplasts (Delport et al. 1985). Pulsed EEF stimulated synthesis of ADNP in respiration inhibited submitochondrial particles (Tiessie et al. 1981), protein synthesis

of proteins in hamster (Goodman et al. 1985) and rat (Colazzio and Pilla 1985) increase in the rate of growth of chicken ganglia (Smith 1986) and induced apoptosis in oral mucosa cancer cells by NADPH oxidase-derived reactive oxygen species (Wartenberg et al. 2008).

Effects of electromagnetic fields in terms of public health were recently discussed in WHO document (WHO 2005). A regulatory effect of EEF on metabolism of alcohol was suggested (Crabb et al. 1987; Ambroziak and Pietruszko 1993; Berry et al. 1993) Interactions of extremely low frequency electric field with humans were studied by Tenforde and Kaune (1987). Electric field of 25 kV/cm appeared to be fatal for microorganisms (Sale and Hamilton 1967).

Several mechanisms can be involved in the observed phenomena. The EEF influences the active transport of ions through the ionic channels of the membranes (Tsong and Astumray 1986). It might affect functioning of membranes (Clarke 2001), change the conformation of enzymes (Harrison et al. 1997; Todorovic et al. 2005, 2006) and, hence, switch enzymatic reactions in micelles (Harada and Kataoka 2003) and their transport (Murgida and Hildebrandt 2004, 2005). Likely, these phenomena could be associated with the influence of EEF upon molecules of water and the macromolecular character of water as well as its effect upon particular molecules building the living matter. Thus, the knowledge of the changes caused by EEF on the molecular level is crucial. Based on such knowledge some biosensors can be constructed (Willner and Katz 2000; Katz and Willner 2004).

In cases where such kind information is non-accessible for experiment computer simulations has been proposed (Bikiel et al. 2006). They provided information on the pure, direct influence of EEF on the molecules under study. Since the computations were performed in the computer vacuum, environmental effects such as intermolecular interactions, and solvent effects could be eliminated. Following this approach we presented the papers devoted to the effect of EEF on nitrogen, oxygen, water, carbon dioxide, and ammonia (Mazurkiewicz and Tomasik 2010). Numerical simulations showed that these molecules in the EEF of enhanced strength might be involved in the observed phenomena as carriers of energy and even reagents. In subsequent papers effect of EEF upon monosaccharides (Mazurkiewicz and Tomasik 2012a), alkanols (Mazurkiewicz and Tomasik 2012b), porphyrin and metalloporhyrins (De Biase et al. 2007; Mazurkiewicz and Tomasik 2013a), proteogenic amino acids (Mazurkiewicz and Tomasik 2013b) and selected dipeptides (Mazurkiewicz and Tomasik 2014) was simulated.

This paper presents simulations of the changes of molecular energy, dipole moments, conformations, bond lengths, and charge density distribution caused by EEF of the strength varying from 0.00 through 5.14, 25.70 to 51.40 MV/cm in Cys-Asn-Ser, Ser-Glu-Met, Ser-Asp-Leu, and Glu-Arg-Leu tripeptides. The selection of tripeptides for these considerations was rationalized in terms of their role in stress reactions. The pentapeptide (Met-Gly-Cys-Asn-Ser) with anti-inflammatory activity is synthetised by parasite *Entomoeba histolytica* that is the most prevalent intestinal pathogen worldwide. The pentapeptide is thermostable and in vivo inhibits mononuclear phagocyte locomotion. The influence of electron donor and acceptor groups on electronic structure of the pentapeptide proved that the Cys-Asn-Ser is responsible for the anti-inflammatory properties (Soriano-Correa et al. 2010). It is hypothesized that the increase in the relative acidity of hydrogen atom (H2) of the serine amide group with the electron withdrawing groups S37–H39 and the further atomic and group charges analysis showed that the tripeptide possess important interaction sites, e.g., H2 and H39 atoms which are exposed to nucleophilic attacks and the N1–H2 bond being weaker than the S37–H39 bond. In such a situation that amide group is more acidic compared to the absence of such exposed atoms. Together with capability of forming hydrogen bonds between H2…O25C the structural stability increase and in consequences also the anti-inflammatory activity is higher.

Revealing the subcellular localization of unknown proteins is of major importance for inferring protein function and the tripeptides (Ser-Asp-Leu, Glu-Arg-Leu, Ser-Glu-Met) play an important role as a peroxisome targeting signal for *Arabidopsis thaliana* proteins (Chowdhary et al. 2012). The peroxisome proteins are imported from cytosol and the cytosol proteins are targeting at the peroxisome matrix by a conserved targeting signals protein type 1 and 2 (PTS1/2). These proteins include C-terminal tripeptides either non-canonical or canonical ones. The large number of plant proteins carrying one and the same non-canonical tripeptide is ineffective in terms of peroxisome targeting, only a small subset is peroxisome-targeted. The reason is that non-canonical, in contrary to the canonical tripeptides e.g., (Ser-Lys-Leu), require auxiliary targeting-enhancing patterns, e.g., basic residues located upstream of the tripeptides.

Computations

HyperChem 8.0 software was used together with the AM1 method for optimization of the conformation of the molecules under study. Optimization was performed for molecules out of the field as well as with the field. Then, charge distribution, potential and dipole moment for molecules placed in the external electric field of 5.14, 25.70, and 51.40 MV/cm were calculated. The molecules were

situated along the *x*-axis. The *y*- and *z*-axes were perpendicular in plane and perpendicular to plane containing this structure, respectively.

Results and discussion

Molecular energy computed for particular tripeptides out of EEF decreased in the order: Cys-Asn-Ser > Ser-Asp-Leu > Ser-Glu-Met > Glu-Arg-Leu that is according to the number of atoms in the molecules being 37, 39, 46, 46, 48, and 62, respectively (Table 1).

The sensitivity of molecular energy of particular tripeptides was independent of the number of atoms. Dipole moments (DM) of the tripeptides under consideration increased with the EEF strength applied. The EEF of 5.14 MV/cm had a minor effect upon DM but the stronger EEF caused already its remarkable increases. The effect of 51.40 MV/cm EEF upon DM of Glu-Arg-Leu was particularly considerable.

Generally, changes of molecular energy, DM, and peptide bond lengths were non-linear against EEF strength applied. Molecular energy of all tripeptides decreased against increase in the EEF strength but changes of the bond length of Ist and IInd peptide bonds were specific for particular tripeptides and sometimes their change was irregular.

In Cys-Asn-Ser and Ser-Asp-Leu the IInd peptide bond was longer and remained longer on application of EEF whereas in Glu-Cys-Glc and Ser-Glu-Met the IInd peptide bond was shorter and remained shorter on application of EEF. In Glu-Arg-Leu the IInd peptide bond was shorter without field and after application of 5.14 MV/cm EEF. At stronger EEF the length of the IInd peptide bond increased whereas the length of Ist peptide bond decreased. In such manner at 25.70 and 51.40 MV/cm EEF the IInd peptide bond turned longer than the Ist peptide bond. Irregularities appeared in the lengths of the Ist peptide bond in Ser-Glu-Met as well as in the lengths of the IInd peptide bond of Cys-Asn-Ser and Ser-Asp-Leu. Moreover, in case

Table 1 Effect of EEF upon energy, dipole moment and length of the C–N peptide bond in tripeptides

Tripeptide	EEF strength (MV/cm)	Energy[a] (kcal/mole)	Dipole moment (D)	Peptide C–N bond length[b] (Å)	
				I	II
Cys-Asn-Ser	0.00	−93635.4	8.455	1.4182	1.4368
	5.14	−1.8	9.103	1.4198	***1.4366***
	25.70	−16.6	15.25	1.4208	1.4417
	51.40	−37.2	18.60	1.4244	1.4441
Glu-Cys-Glc	0.00	−89533.3	9.96	1.4356	***1.4316***
	5.14	−2.3	10.00	1.4371	***1.4312***
	25.70	−15.3	14.92	1.4446	***1.4310***
	51.40	−45.1	20.25	***1.4222***	***1.4188***
Ser-Glu-Met	0.00	−103975	6.989	1.4474	1.4011
	5.14	−2	10.71	1.4479	1.4041
	25.70	−16	16.00	***1.4475***	1.4062
	51.40	−44	23.41	***1.4429***	1.4088
Ser-Asp-Leu I	0.00	102366	6.783	1.4005	1.4190
	5.14	−1	7.366	1.4019	***1.4119***
	25.70	−13	11.29	1.4078	***1.4144***
	51.40	−37	16.86	1.4217	1.4222
Ser-Asp-Leu II	0.00	−102345	18.55	***1.4288***	1.4628
	5.14	−5	19.51	***1.4272***	1.4637
	25.70	−26	23.10	***1.4268***	1.4660
	51.40	−57	27.35	1.4313	1.4678
Glu-Arg-Leu	0.00	−118267	10.33	***1.4253***	1.4185
	5.14	−3	11.25	***1.4238***	1.4203
	25.70	−92	17.36	***1.4168***	1.4308
	51.40	−561	59.32	***1.4186***	1.4406

[a] Given as initial energy for the molecules out of EEF and decrease in it caused by application of EEF of a given strength

[b] Decreasing of the bond length is denoted in bold italics

of Glu-Cys-Glc and Glu-Arg-Leu, the 51.40 MV/cm EEF reversion of the tendency in the increase and decrease, respectively, in the length of the Ist peptide bond against EEF was encountered. For irregular changes of the peptide bond lengths under the influence of the weaker EEF one could charge either an involvement of the keto-enol equilibrium [see paper (Mazurkiewicz and Tomasik 2014)], taking conformations favoring intramolecular interactions or polarization of the atoms and bonds. However, intervention of the keto-enol tautomerism might be rejected as the bond length of the enolized peptide bond of the Ala–Ala dipeptide inner salt out of EEF was 1.2975 and increased to 1.3007 Å at 51.40 MV/cm EEF whereas for the keto-form the corresponding values were 1.3743 and 1.3809 Å, respectively (Mazurkiewicz and Tomasik 2014). Thus, the polarization of bonds and atoms seemed to be the most likely reason for the effects observed.

There were three types of EEF caused behavior of the charge density at particular atoms of tripeptides. Type I included relative low sensitivity of the density to 51.40 MV/cm EEF. Type II includes densities which are insensitive to EEF up to 25.70 MV/cm and considerably change in 51.40 MV/cm EEF. Densities varying considerably with increase in the whole range of changes of the EEF strength constitute Type III of behavior. Within this type the charge distribution changed with EEF either irregularly decreasing or increasing.

In the vast majority of cases, the increase in the strength of the EEF applied produced increase in the positive charge density at hydrogen atoms and decrease in the negative charge density at the oxygen and nitrogen atoms. Irregularities caused by increasing EEF strength could be observed in the trends in the changes of the charge density on selected atoms in the tripeptides under consideration. These changes were individual properties of particular tripeptides. Some moieties of the tripeptides appeared fairly insensitive to the increase in the EEF strength. As a rule, the application of the 5.14 MV/cm EEF produced negligible (if any) changes in the charge distributions.

It seemed likely that deviations from the tendency mentioned, that is, observed with increase in the EEF strength an decrease in the positive charge density and negative charge densities could result from the formation of intramolecular hydrogen bonds influencing geometry of peptides. Geometry of particular tripeptides and its change with increase in the EEF strength is presented in Fig. 1.

The charge density distribution at selected atoms of Cys-Asn-Ser is presented in Table 2.

Charge densities at all atoms of the Ist peptide bond and the hydrogen atom of the IInd peptide bond belonged to Type I. The oxygen atoms of the ionized carboxylic group as well as the amido group nitrogen atom, and the oxygen atom of the IInd peptide bond could be accounted to Type II.

Behavior of the charge densities at remaining atoms qualified them to Type III. Truly sensitive to increase in the EEF strength were charge distributions at both atoms of the thiol group. Computations for the S-atom showed that the susceptibility of the thiol group to oxidation increased with increase in the EEF strength applied. The molecule was stabilized by the hydrogen bond between the hydrogen atom of the $N^{+}H_3$ group and the negative charge bearing oxygen atom of the COO^{-} group. The length of the bond was 1. 675 Å. Increase in the EEF strength did not destroy that bond. It solely became longer reaching 1.681, 1.704, and 1.738 Å at the 5.14, 25.70, and 51.40 MV/cm field, respectively. The increase in dipole moment (Table 1) and the length of the hydrogen bond can be responsible for improving the antiflammatory activity of the peptide following suggestion of Catalina Soriano-Correa (2010). EEF declined the positive charge density at one of the hydrogen atoms of the amide group at each strength of EEF and at the hydrogen atom of the Ist peptide bond at 25.70 MV/cm decreased. Simultaneously, the negative charge density turned less negative at the negatively charged oxygen atom of the ionized carboxylic group at all strength of the EEF, at the carbonyl oxygen atom of that group at 5.14 and 51.40 MV/cm and the carbonyl oxygen atom of the Ist peptide bond at 25.70 and 51.40 MV/cm.

EEF induced changes in behavior of charge densities at particular atoms of Glu-Cys-Glc differed from these observed in the former tripeptide (Table 3).

Only the charge density at the carbon atom of the IInd peptide bond belongs to Type I. The charge densities at the hydrogen atoms of the thiol and non-ionized carboxylic group, protonated nitrogen atom of the Cys amino group, the nitrogen atom of the IInd peptide bond, and the oxygen atom of the Ist peptide bond belong to Type II. The remaining atoms exhibited behavior specific to Type III. The S-atom of the Cys thiol group took negative charge increasing with EEF. Like the COOH group the protonated amino group became less ionized as the strength of EEF increased. Simultaneously, the negative charge in the ionized carboxylic group shifted from the negatively charged oxygen to the carbonyl oxygen atom. Increase in the EEF strength is accompanied by increase in the positive charge at all carbon and hydrogen atoms of the Ist peptide bond and hydrogen atoms of both peptide bonds. The opposite trend in the charge density under the influence of elevating EEF strength was observed for the oxygen atoms of both peptide bonds and the nitrogen atom of the Ist peptide bond.

EEF decreased the positive charge density at the hydrogen atoms of the thiol group at 5.14 and 25.70 MV/cm, at

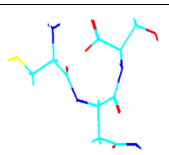
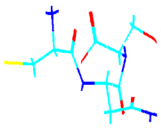
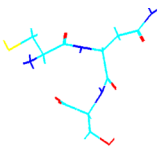
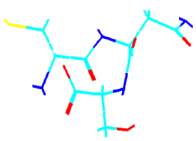
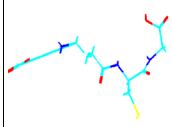
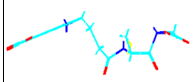
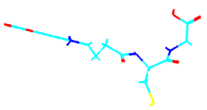
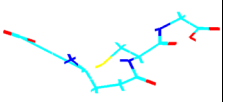
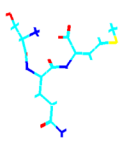
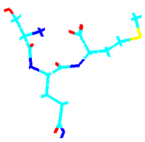
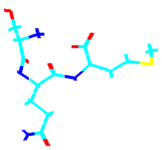
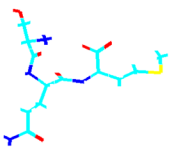
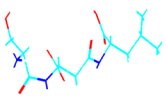
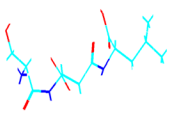
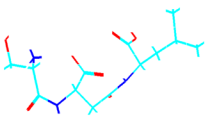
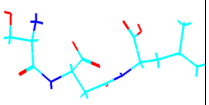
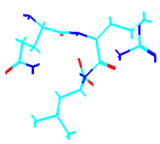
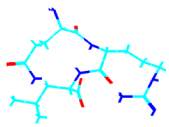
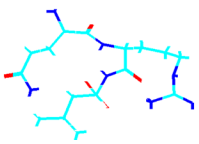
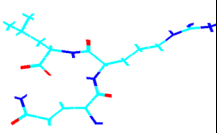
Tripeptide	EEF [MV/cm]			
	0.00	5.14	25.70	51.40
Cys-Asn-Ser				
Glu-Cys-Glc				
Ser-Glu-Met				
Ser-Asp-Leu				
Glu-Arg-Leu				

Fig. 1 Conformations of tripeptides under consideration computed out of field as well as in the 5.14, 25.70, and 51.40 MV/cm EEF, respectively. *Red* the oxygen atoms, *blue* the nitrogen atoms, and *yellow* the sulfur atoms (color figure online)

the hydrogen atom of the non-ionized carboxylic group at 51.40 MV/cm and hydrogen atoms of the N^+H_3 group at 25.70 and 51.40 MV/cm. Because the negative charge density at the sp^3 oxygen atoms of the ionized and non-ionized carboxylic groups simultaneously declined the hydrogen bond between these partners could be likely. Insight in Fig. 1 did not confirm that assumption. Thus, EEF did not favor the formation of the hydrogen bonds. Computations showed that there was no hydrogen bond in the tripeptide out of EEF.

In Ser-Glu-Met, EEF induced charge density at the carbon atoms of both peptide bonds and the hydrogen atom of the IInd peptide bond belonged to Type I and charge densities at oxygen and nitrogen atoms of the Ist peptide bond as well both atoms of the ionized carboxylic groups belonged to Type II. In spite of insensitivity of the carbon atom of the methyl group to an increase in the EEF strength, the negative charge at the sulfur atom considerably increased. A considerable increase in the positive charge density and decrease in the negative charge accompanying increase in the EEF strength was noticed at the d nitrogen atoms of the protonated Ser amino group, amido group, and Ist peptide bond. Charge density at sulfur atom as well as oxygen and nitrogen atoms of the IInd peptide bond showed opposite effect to an increase in the EEF strength (Table 4).

Table 2 Charge density on selected atoms of the Cys-Asn-Ser tripeptide inner salt

Field (MV/cm)	Atoms														
	H–S		COO^-		N^+H_3	NH_2			Peptide bond I				Peptide bond II		
	H	S	$-O^-$	=O	N^+	N	H1	H2	C	O	N	H	C	O	N	H
0.00	0.014	0.012	−0.597	−0.610	0.642	0.000	0.064	0.060	0.247	−0.351	−0.010	0.098	0.211	−0.315	−0.046	0.075
5.14	0.018	0.016	***−0.595***	***−0.609***	0.647	0.004	***0.061***	0.061	0.248	−0.351	−0.013	0.099	0.213	−0.320	−0.048	0.075
25.70	0.042	0.003	***−0.593***	−0.611	0.661	0.003	***0.055***	0.062	0.246	***−0.346***	−0.015	***0.097***	0.231	−0.324	−0.060	0.079
51.40	0.070	−0.003	***−0.589***	−0.604	0.682	0.029	***0.072***	0.065	0.248	***−0.344***	−0.017	0.098	0.237	−0.339	−0.076	0.083

Decreasing of the bond length is denoted in bold italics

Only direction of the changes of the positive charge density at the hydrogen atoms of the amino group at 25.70 MV/cm, Ist peptide bond at 5.14 MV/cm and the IInd peptide bond at 25.70 and 51.40 MV/cm as well as simultaneously at the oxygen atom of the IInd peptide bond at 25.70 and 51.40 MV/cm points to them as the partners of eventual hydrogen bonds. Examination of the structures (Fig. 1) showed that no hydrogen bond could be formed. In the tripeptide out of EEF there was the hydrogen bond of 1.836 Å between the N^+H_3 group and the oxygen atom of the IInd peptide bond. As the EEF strength increased the length of that bond varied irregularly. It was 1.836, 1.842, and 1.839 Å at 5.14, 25.70, and 51.40 MV/cm, respectively.

There were two carboxylic groups in non-ionized Ser-Asp-Leu. Insight into the structure of the tripeptide, strongly suggested that the Asp carboxylic group should participate in the formation of inner salt. The Leu carboxylic group should be less acidic because of the positive inductive effect (Taft 1953) of the isopropyl moiety in the vicinal position to it. Computation of the molecular energy for inner salts resulting from the ionization of each of two carboxylic groups confirmed that the Asp carboxylic group was involved (Table 1). Molecular energy for that form was slightly less negative than that computed for the second isomeric inner salt.

In this tripeptide EEF induced changes of charge density at the hydrogen atoms of Ist peptide bond, the protonated nitrogen atom of the Ser amino group and carbon atoms of both peptide bonds belonged to Type I. The non-ionized Asp carboxylic group turned more acidic just in the 51.40 MV/cm EEF field, thus the charge density at the hydrogen atom of that group was of Type II. The charge densities at all other atoms were of Type III. In the ionized Leu carboxylic group the negative charge at the O^- atom decreased with EEF whereas the negative charge of the carbonyl oxygen atom increased. The increase in the EEF strength polarized both peptide bonds in the opposite direction. The negative charge at the oxygen atom and the positive charge at the hydrogen atom of the Ist peptide bond decreased with EEF whereas the charges on such atom in the IInd peptide bond changed in the opposite direction. Only the positive charge at nitrogen atom of the Ist peptide bond and the negative charge at the nitrogen atom of the IInd peptide bond decreased with the increase in the EEF strength (Table 5). The change in the charge distribution at the negatively charged oxygen atom of the ionized carboxylic group and the hydrogen atom of the non-ionized carboxylic group on exposure to 0.01 a.u. EEF could support the assumption on the proton transfer of the proton between both moieties.

There was no hydrogen bond in that tripeptide out of EEF.

In Glu-Arg-Leu the formation of the inner salt involved the Leu carboxylic group and the amino group of the Arg

Table 3 Charge density on selected atoms of the Glu-Cys-Glc tripeptide inner salt

Field (MV/cm)	Atoms															
	H–S		COO^-		COOH			N^+H_3	Peptide bond I				Peptide bond II			
	H	S	$-O^-$	=O	H	$-O^-$	=O	N^+	C	O	N	H	C	O	N	H
0.00	0.009	0.002	−0.230	−0.311	0.235	−0.320	−0.381	1.199	0.253	−0.367	−0.059	0.076	0.248	−0.335	−0.029	0.076
5.14	***0.006***	0.002	***−0.225***	−0.317	0.235	***−0.315***	−0.385	1.200	0.255	−0.368	−0.064	0.088	0.249	−0.342	−0.029	0.080
25.70	***0.004***	−0.018	***−0.184***	−0.359	0.235	***−0.296***	−0.409	***1.190***	0.265	−0.374	−0.081	0.087	0.256	−0.366	−0.027	0.093
51.40	0.061	−0.075	***−0.127***	−0.420	***0.226***	***−0.275***	−0.448	***1.036***	0.284	−0.449	−0.237	0.093	0.254	−0.432	−0.014	0.114

Decreasing of the bond length is denoted in bold italics

Table 4 Charge density on selected atoms of the Ser-Glu-Met tripeptide inner salt

Field (MV/cm)	Atoms															
	S-CH_3		COO^-		N^+H_3	NH_2			Peptide bond I				Peptide bond II			
	C	S	$-O^-$	=O	N^+	N	H1	H2	C	O	N	H	C	O	N	H
0.00	−0.199	−0.037	−0.671	−0.526	0.569	−0.006	0.046	0.065	0.223	−0.253	−0.041	0.076	0.220	−0.449	0.042	0.077
5.14	−0.200	−0.043	−0.671	−0.526	0.569	0.047	0.047	0.065	0.233	−0.251	−0.042	***0.075***	0.219	−0.452	0.046	0.078
25.70	−0.197	−0.083	−0.673	−0.533	0.596	0.099	0.054	0.070	0.234	−0.254	−0.039	0.080	0.227	***−0.437***	0.028	***0.076***
51.40	−0.196	−0.137	***−0.648***	−0.579	0.658	0.046	0.072	0.071	0.230	−0.265	−0.027	0.088	0.230	***−0.433***	0.029	***0.074***

Decreasing of the bond length is denoted in bold italics

Table 5 Charge density on selected atoms of the Ser-Asp-Leu tripeptide inner salt

Field (MV/cm)	Atoms													
	COO^-		COOH			N^+H_3	Peptide bond I				Peptide bond II			
	$-O^-$	=O	H	$-O^-$	=O	N^+	C	O	N	H	C	O	N	H
0.00	−0.669	−0.477	0.227	−0.299	−0.377	0.705	0.225	−0.389	0.026	0.097	0.252	−0.377	−0.037	0.111
5.14	***−0.668***	***−0.472***	***0.224***	−0.301	***−0.373***	***0.701***	0.223	***−0.387***	0.024	0.099	0.254	−0.382	−0.037	0.114
25.70	***−0.656***	−0.526	***0.223***	−0.321	***−0.370***	***0.701***	0.230	***−0.365***	0.002	***0.091***	0.262	−0.413	−0.113	0.180
51.40	***−0.603***	−0.574	0.235	−0.323	***−0.353***	***0.697***	0.223	***−0.311***	−0.016	***0.088***	0.263	−0.400	−0.148	0.189

Decreasing of the bond length is denoted in bold italics

guanidyl moiety. The EEF induced charge densities at the carbonyl oxygen atom of the ionized carboxylic group, and the hydrogen atom of the IInd peptide bond belonged to Type I whereas these nitrogen atom of the C–NH–C moiety and amido nitrogen atom as well as the carbon atoms of both peptide bonds could be accounted for Type II. The increase in the strength of EEF considerably increased negative charge density at the nitrogen atom of the amino group and decreased the negative charge density at the nitrogen and oxygen atoms of both peptide bonds. Simultaneously, the positive charge density in the remained guanidyl nitrogen atoms also increased. Fully insensitive to the EEF were the carbonyl oxygen atom of the ionized carboxylic group and hydrogen atom of the IInd peptide bond (Table 6).

In the tripeptide out of field the biforcate hydrogen bond was found involving the negatively charged oxygen atom of the carboxylato group and hydrogen atoms of the amido group (2.607 Å) and the Ist peptide bond (1.816 Å). The first branch of that bond decreased to 2.601, 2.461, and 1.783 Å at 5.14, 25.70, and 51.40 MV/cm field, respectively. The length of the second branch of that bond changed irregularly into 1.815, 1.805, and 1.846 Å, respectively. Moreover, at 25.70 MV/cm, additional hydrogen bond appeared between the amino group of the guanidyl moiety and the oxygen atom of the IInd peptide bond. Its length was 1.797 Å.

Changes of the charge distribution, particularly on the polar groups such as carboxylic, amino, and peptide bond NH as well as CO groups influence the intermolecular interactions of peptides, that is, their catalytic activity. Stimulated by EEF increase in the positive charge density on the hydrogen atoms of the N^+H_3, peptide bond NH, NH_2, and COOH groups as well as decrease in the negative charge density on the oxygen atoms of the peptide bond carbonyl groups as well as these atoms constituting either ionized or non-ionized carboxylic groups seemed potentially increase the catalytic activity of the peptides. The presented data demonstrated diverse, specific to particular tripeptide reaction to EEF. Therefore, neither the positive nor negative effect of EEF on the biological functions of tripeptides can be a priori assumed.

As mentioned above the upstream basic position in the non-canonical tripeptides functioned as an enhancing elements for proteins transported into peroxisome. The fact that not only basic amino acids, but hydroxylated (Ser, Thr), hydrophobic (Ala, Val), and also acidic residues are able to enhance peroxisome targeting seemed to be of great interest. The influence of the enhancer residues on C-terminal tripeptides could be related to charge distribution and, in consequences, could be responsible for the strong signal directing the protein to peroxisomes. The EEF effect on protein import from cytosol into organelle requires further studies and it revealed the import mechanisms.

In this study, the weakest EEF (5.14 MV/cm) taken under consideration was by 4 orders stronger than EEF reported (Sale and Hamilton 1967) as harmful for microorganisms under realistic conditions. The computations performed for tripeptides in the 5.14 MV/cm EEF revealed relatively subtle influence of that field upon the charge distribution and bond lengths in tripeptides but conformational changes under such conditions (Fig. 1; Table 1) might be essential for their biological functions. The latter could considerably influence tripeptide hydration conditions and, hence, the macrostructure of solutions. This assumption received a strong backing from theoretical computations by Kaminsky and Jensen (2007). One should also take into account the effect of EEF on water molecules estimated in our recent paper (Mazurkiewicz and Tomasik 2010). Thus, the harmful effects of EEF under realistic conditions might result from generated conformational changes and response of water molecules to EEF rather than changing charge density at particular atoms and bond lengths.

Conclusions

1. Since EEF stimulates an increase in the positive charge density on the hydrogen atoms of the N^+H_3, peptide bond NH, NH_2, and COOH moieties and decreases

Table 6 Charge density on selected atoms of the Glu-Arg-Leu tripeptide inner salt

Field (MV/cm)	Atoms																			
	COO^-		Amide NH_2			Amino NH_2			$NH^1C(=NH^2)N^+H_3$					Peptide bond I				Peptide bond II		
	O^-	=O	N	H1	H2	N	H1	H2	H1	-N	H2	=N	N^+	C	O	N	H	C	O	N	H
0.00	−0.670	−0.601	−0.122	0.079	0.063	−0.029	0.032	0.045	0.071	−0.018	0.100	0.006	0.697	0.258	−0.371	−0.131	0.167	0.263	−0.437	−0.072	0.066
5.14	***−0.669***	***−0.599***	−0.120	0.081	***0.062***	−0.036	***0.027***	***0.043***	0.086	−0.018	***0.099***	0.014	0.705	0.265	−0.389	−0.127	0.168	0.265	***−0.434***	−0.072	***0.062***
25.70	***−0.652***	***−0.599***	−0.120	0.101	***0.060***	−0.061	***0.018***	***0.041***	0.094	−0.021	0.104	0.058	0.736	0.255	−0.397	−0.106	0.173	0.267	***−0.404***	−0.037	0.066
51.40	***−0.665***	***−0.599***	−0.066	0.181	***0.059***	−0.065	0.036	***0.044***	0.082	−0.007	0.129	0.070	0.824	0.216	***−0.330***	−0.075	***0.097***	0.233	***−0.330***	−0.004	***0.061***

Decreasing of the bond length is denoted in bold italics

the negative charge density on the oxygen atoms of the peptide bond carbonyl groups and either ionized or non-ionized carboxylic groups it potentially increases the catalytic activity of the peptides.

2. Charge distribution and bond length changes generated by the weakest EEF considered in this paper are accompanied by conformational changes which might be essential for the biological effects of tripeptides.
3. Biological effects of EEF observed under realistic conditions may involve the effect of EEF on the solvent.

Conflict of interest The authors of the submitted text (Effect of external electric field on selected tripeptides) know nothing about potential conflict of interest between us and known to us researchers involved in the peptide study.

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