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Effect of External Electric Field Upon Selected Model Dipeptides

Józef Mazurkiewicz[a]; Piotr Tomasik[b],*

[a]Department of Chemistry and Physics, University of Agriculture, Cracow, Poland.
[b]Cracow College of Health Promotion, Cracow, Poland.
*Corresponding author.

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Abstract
Effect of external electric field (EEF) of 0.001, 0.005 and 0.01 a.u. upon molecular energy, charge distribution and dipole moments of non-dissociated and inner salt forms of selected dipeptides formed from alanine (Ala), glutamic acid (GluA), and ornithine (Orn) that is, Ala-Ala, Ala-GluA, GluA-Ala, Ala-Orn, Orn-Ala involving α-amino group of Orn and Orn-Ala involving δ-amino group of Orn. For that purpose HyperChem 8.0 software was used together with the AM1 method for optimization of the conformation of the molecules in a computer vacuum.

Key words: Alanylalanine; Carboxyglutamylalanine; Alanylglutamic acid; Alanylornithine; α–ornithylalanine; δ-ornithylalanine

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INTRODUCTION
In our former papers attention was paid to the effect of external electric field (EEF) upon biologically important molecules. Thus, the effect of EEF of the strength increasing from 0.001 to 0.05 a.u. was recognized in case of simple di- and tri-atomic molecules (Mazurkiewicz & Tomasik, 2010), selected monosaccharides (Mazurkiewicz & Tomasik, 2012a), alkanols (Mazurkiewicz & Tomasik, 2012b), porphine and selected metalloporphyrins (Mazurkiewicz & Tomasik, 2013a) and 19 proteogenic amino acids in their non-dissociated and inner salt structures (Mazurkiewicz & Tomasik, 2013b). These studies based on numerical simulations of the charge distribution and structural changes induced by EEF shed some light upon changes in reactivity, physical properties, and biological functions of the compounds under consideration.

Pulsed electric field is willingly used in synthesis, manufacturing and processing inorganic materials such as glasses and ceramic materials (Munir et al., 2006). Electric field-assisted levitation-jet aerosol synthesis of Ni/NiO nanoparticles was recently published by Morozov et al. (2012). There is a number of reports documenting effect of EEF upon the course of functioning of enzymes (Tiessie, Knox, Tsong & Wehrle, J, 1981; Tsong & Astumray, 1986; Harada & Kataoka, 2003) living cells such as, *Escherichia coli* and *Listeria innocua* (Dutreux *et al.,* 2000) and *Saccharomyces cerevisiae* (Harrison, Barbosa-Canovas, & Swanson, 1997), microbial synthesis of ethanol (Grosse, Bauer, & Berg, 1988; Nakanishi, Tokuda, Soga, Yoshinaga, & Takeda, 1988) and its metabolism (Ambroziak & Pietruszko, 1993; Berry, Grivel, & Phillips, 1993; Crabb, Bosron, & Li, 2005), synthesis of citric acid using *Aspergillus niger* (Fiedurek, 1999). Nechitailo and Gordeev (2001) showed how EEF influenced the plant growth under microgravity conditions.

In this paper effect of EEF on some selected dipeptides is presented. Peptides constitute an abundant group of natural and synthetic biocatalysts (Howl, 2005; Sewald & Jakubke, 2008; Jensen, Tofteng & Pedersen, 2013). Regardless their structure and role their catalytic action involves formation of intermediary active complexes with a substrate. In this stage of reaction the charge distribution in the peptide and their geometry are essential. Therefore, it is essential whether and to what extent EEF affects these peptide parameters. EEF can either promote or inhibit the peptide catalysis.

Simulations were performed for model dipeptides composed of alanine (Ala), glutamic acid (Gln) and ornithine (Orn) representing monoamino monoic, monoamino dioic and diamino monoic acids, respectively. They were organized in dipeptides as follows: Ala-Ala, Ala-Gln, Gln-Ala, Ala-Orn, Orn-Ala with involvement of the α-amino group in Orn, and Orn-Ala with involvement of the δ-amino group in Orn.

In every case computations were performed for non-ionized and inner salt structures in their keto-forms. Enol form was taken under consideration only for alanylalanine (Ala-Ala) because according to Kamiya *et al.,* (2006) enol forms are less stable than the keto-forms.

1. COMPUTATIONS

HyperChem 8.0 software was used together with the AM1 method for optimization of the conformation of the molecules of amino acids under study. Then, charge distribution, potential and dipole moment for molecules placed in the external electric field of 0.000, 0.001, and 0.01 a.u. 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.

2. RESULTS AND DISCUSSION

Values of molecular energy of dipeptides in the non-ionized forms (Table 1) slightly decreased with the strength of EEF and that increase is comparable with that noted for particular amino acids (Mazurkiewicz & Tomasik, 2013b). Particularly considerable increase produced the 0.01 a.u. EEF. A small irregularity in the increase in the molecular energy with the increase in the EEF strength applied was noted in case of the enol form of the Ala-Ala and GluA-Ala dipeptide. Its energy in the 0.005 and 0.001 a.u., respectively, slightly decreased in order to increase again at higher EEF strength. In case of the GluA-Ala dipeptide it could be rationalized in terms of the conformation taken at 0.001 a.u. by that peptide. It favored through space intramolecular interaction of the carbonyl group of the α-carboxylic group with the hydrogen atom at the β−carbon atom in the GluA moiety. Some irregularities in the charge densities at these atoms support that rationalization (see Supplementary material).

Increase in the EEF strength caused changes in the distribution of the charges of particular atoms, conformation of dipeptides, hence, also in their dipole moments (DM) and bond lengths (see supplementary material for details).

Generally, dipole moments of dipeptides increased with increase in the EEF strength (Table 1) but irregularities could be noted for the Ala-Ala and GluA-Ala dipeptides in the 0.001 a.u. EEF.

Table 1
Effect of EEF Upon Energy Dipole Moment and Length of the C-N Peptide Bond in Dipeptides[a]

Dipeptide	EEF strength[c] [a.u.]	Energy [kcal/mole]	Dipole moment [D]	Peptide C-N bond length
Ala-Ala[b]	0.000	**-48494.9**	**3.523**	**1.4279**
		-48433.8	20.56	1.3743
	0.001	**-48495.8**	**3.852**	**1.4271**
		-48439.0	21.30	1.3744
	0.005	**-48500.5**	**5.383**	**1.4302**
		-48462.7	24.54	1.3706
	0.01	**-48508.1**	**6.818**	**1.4326**
		-48493.5	26.91	1,3809
Ala-Ala[c]	0.000	**-48485.9**	**7.568**	**1.2969**
		-48411.6	13.45	1.2976
	0.001	**-48487.8**	**7.181**	**1.2974**
		-48448.2	11.88	1.2994
	0.005	**-48486.7**	**6.255**	**1.2987**
		-48460.7	13.88	1.2999
	0.01	**-48495.3**	**7.723**	**1.3031**
		-48478.3	15.18	1.3007
Ala-GluA	0.000	**-68230.9**	**3.912**	**1.4276**
		-68199.9	8.714	1.4033
	0.001	**-68231.8**	**4.551**	**1.4263**
		-68202.1	9.442	1.4051
	0.005	**-68237.4**	**6.532**	**1.4227**
		-68212.6	11.80	1.4119
	0.01	**-68246.7**	**8.371**	**1.4184**
		-68229.6	15.24	1.4213
GluA-Ala	0.000	**-68229.9**	**1.742**	**1.4278**
		-68179.4	16.58	1.3932
	0.001	**-68229.6**	**1.454**	**1.4289**
		-68183.6	17.32	1.3947
	0.005	**-68234.0**	**4.592**	**1.4099**
		-68202.6	12.79	1.4044
	0.01	**-68244.7**	**8.215**	**1.4029**
		-68225.0	16.49	1.4746
Ala-Orn	0.000	**-59485.4**	**3.976**	**1.4267**
		-59461.8	6.622	1.4610
	0.001	**-59486.4**	**4.638**	**1.4253**
		-59463.4	6.986	1.4592
	0.005	**-59491.9**	**6.373**	**1.4215**
		-59471.1	8.495	1.4539
	0.01	**-59501.0**	**8.265**	**1.4156**
		-59484.1	10.94	1.4152
α-Orn-Ala	0.000	**-59482.2**	**3.938**	**1.4259**
		-59442.2	15.60	1.3735
	0.001	**-59483.2**	**4.384**	**1.4250**
		-59446.1	16.16	1.3740
	0.005	**-59488.4**	**6.140**	**1.4211**
		-59463.1	18.29	1.3787
	0.01	**-59497.4**	**8.422**	**1.4132**
		-59489.7	23.90	1.3773
δ-Orn-Ala[d]	0.000	**-59487.1**	**0.477**	**1.4281**
		-59439.1	16.04	1.4372
		-59437.6	*15.07*	*1.4126*
	0.001	**-59487.2**	**1.194**	**1.4302**
		-59418.3	28.99	1.4362
		-59441.4	*15.76*	*.1,4125*
	0.005	**-59493.3**	**6.525**	**1.4259**
		-59448.3	31.52	1.4287
		-59451.9	*44.55*	1.8689
	0.01	**-59502.4**	**8.201**	**1.4235**
		-59486.7	33.78	1.4282
		-59508.5	*47.10*	*1.3878*

Note. [a]Upper data in bold characters are for non-ionized forms and lower data in normal font are for inner salt forms. Values of the molecular energy, dipole moment and peptide bond lengths which change

irregularly with the increase in the EEF strength are underlined.
[b]The keto form.
[c]The enol form.
[d]Values in bold characters are these for non-ionized peptide, lower values in normal characters are these for inner salt bearing H^+ at the Orn amino group and lower values in italics are these for inner salt with H^+ at the Ala amino group.

More irregularities in reaction of the dipeptide structure to EEF could be noted in case of the peptide bond length. Generally, the peptide bond length in the keto and enol forms of the Ala-Ala dipeptide increased with EEF whereas it decreased with EEF in case of remaining dipeptides (Table 1). However, the changes in that bond length have irregular impact to the charge densities on the atoms constituting the peptide bond moiety, that is, at the N, H, carbonyl both C and O atoms. General tendencies in either increase or decrease charge densities at these atoms frequently but not solely broke at the strength of 0.01 a.u. In the Ala-Ala dipeptide positive charge density at the H and C atoms as well as negative charge density at the O atom increased with the EEF strength indicating increasing polarity of the carbonyl group as a result of the intramolecular α-C-H… O=C< interactions. A relatively small increase in the dipole moment supported this assumption. The negative charge density at the N atom increased at 0.001 a.u. in order to decline at higher field strength. At 0.01 a.u. the acidity of that dipeptide in terms of the charge density at the carboxylic hydrogen atom increased and then decreased at higher field strength. Change in the basicity of that dipeptide considered in terms of the charge density at the amino group nitrogen atom decreased at 0.001 a.u. in order to increase at the higher field strength (Table 2).

Table 2
Charge Density at Selected Atoms of Ala-Ala Dipeptide in Its Keto-Form[a]

Field [a.u.]	Atoms					
	Carboxyl H	Amino N	Peptide bond			
			N	H	C	O
0.000	0.229	-0.040	-0.042	0.087	0.251	-0.356
	-0.004	0.834	0.002	0.165	0.228	-0.454
0.001	0.239	-0.038	-0.041	0.089	0.252	-0.362
	-0.001	0.836	0.000	0.166	0.226	-0.454
0.005	0.225	-0.043	-0.046	0.100	0.258	-0.378
	0.010	0.838	0.013	0.168	0.208	-0.451
0.01	0.218	-0.049	-0.052	0.109	0.266	-0.401
	0.026	0.841	0.004	0.160	0.211	-0.429

Note. [a]Upper data in bold characters are for non-ionized forms and lower data in normal font are for inner salt forms.

Table 3
Charge Density at Selected Atoms of Ala-Ala Dipeptide in Its Enol-Form[a]

Field [a.u.]	Atoms					
	Carboxyl H	Amino N	Peptide bond			
			N	H	C	O
0.000	0.210	-0.046	-0.242	0.238	0.121	-0.271
	0.189	0.511	-0.118	0.198	-0.004	-0.220
0.001	0.214	-0.050	-0.242	0.229	0.125	-0.250
	0.177	0.545	-0.172	0.226	0.039	-0.246
0.005	0.212	-0.153	-0.216	0.216	0.120	-0.232
	0.163	0.580	-0.181	0.219	0.051	-0.239
0.01	0.212	-0.156	-0.250	0.231	0.150	-0.241
	0.145	0.615	-0.149	0.211	0.068	-0.230

Note. [a]Upper data in bold characters are for non-ionized forms and lower data in normal font are for inner salt forms.

In the peptide bond in Ala-GluA positive charge at the H atom and negative charge at the N atom declined regularly and so increased positive charge at the C atom and negative charge at the O atom of the carbonyl group. Regardless its strength, EEF increased acidity in the Ala moiety and it had no influence on the acidity in the GluA moiety. Simultaneously, EEF decreased the basicity at the amino group (Table 3).

In the GluA-Ala dipeptide having two carboxylic groups the changes were irregular (Table 4) for all atoms but the H atom. At the latter, positive charge rose with the field strength. In the case of other atoms of the peptide

bond irregularity took place at 0.001 a.u. strength. Above that strength, the positive charge density at the C atom and negative charge density at the O atom rose and negative charge density at the N atom decreased. Acidity of the α-carboxylic group increased with the field strength whereas that of the δ−carboxylic group varied irregularly. Basicity of the dipeptide consequently decreased up to 0.005 a.u. and increased at 0.01 a.u..

Table 4
Charge Density at Selected Atoms of Ala-Glua Dipeptide[a]

Field [A.U.]	Atoms						
	Carboxylic H		Amino N	Peptide Bond			
	Ala	Glua		N	H	C	O
0.000	**0.230**	**0.229**	**-0.033**	**-0.044**	**0.103**	**0.241**	**-0.349**
	0.020	0.233	0.553	0.021	0.108	0.200	-0.364
0.001	**0.232**	**0.228**	**-0.031**	**-0.043**	**0.102**	**0.243**	**-0.356**
	0.021	0.236	0.550	0.017	0.106	0.203	-0.360
0.005	**0.233**	**0.229**	**-0.029**	**-0.038**	**0.096**	**0.250**	**-0.383**
	0.024	0.247	0.581	0.004	0.096	0.210	-0.349
0.01	**0.237**	**0.229**	**-0.030**	**-0.033**	**0.088**	**0.260**	**-0.420**
	0.030	0.257	0.627	-0.013	0.083	0.223	-0.337

Note. [a]Upper data in bold characters are for non-ionized forms and lower data in normal font are for inner salt forms.

Table 5
Charge Density at Selected Atoms of GluA-Ala Dipeptide[a]

Field [a.u.]	Atoms						
	Carboxyl H		Amino N	Peptide bond			
	α-side	δ-side		N	H	C	O
0.000	**0.235**	**0.230**	**-0.033**	**-0.045**	**0.102**	**0.204**	**-0.355**
	0.006	0.228	0.833	0.077	0.104	0.220	-0.432
0.001	**0.235**	**0.229**	**-0.031**	**-0.045**	**0.104**	**0.203**	**-0.348**
	0.007	0.229	0.836	0.073	0.103	0.220	-0.425
0.005	**0.243**	**0.239**	**-0.027**	**-0.021**	**0.106**	**0.209**	**-0.404**
	0.015	0.230	0.852	0.033	0.094	0.224	-0.378
0.01	**0.244**	**0.235**	**-0.039**	**-0.002**	**0.105**	**0.213**	**-0.437**
	0.021	0.228	0.649	-0.119	0.114	0.260	-0.253

Note. [a]Upper data in bold characters are for non-ionized forms and lower data in normal font are for inner salt forms.

The 0.001 a.u. field strength was critical for the regularity of the changes in the charge densities at the atoms of the peptide bonds of the Ala-Orn dipeptide (Table 5). The field strength above 0.001 a.u. increased the acidity and the basicity of both aminio groups of that dipeptide varied irregularly.

In the Orn-Ala dipeptide formed involving α-amino group of Orn (α−Orn-Ala) only positive and negative charges at the C and O atoms, respectively of the carbonyl group increased regularly regardless the applied strength of EEF. Above 0.001 a.u. positive charge at the hydrogen atom and negative charge at the N atom declined with the field strength. Above that field strength increased the acidity as well as basicity of both amino groups of that dipeptide (Table 6).

Table 6
Charge Density at Selected Atoms of Ala-Orn Dipeptide[a]

Field [a.u.]	Atoms						
	Carboxyl H	Amino N		Peptide bond			
		α-side	δ-side	N	H	C	O
0.000	**0.227**	**-0.039**	**-0.041**	**-0.032**	**0.095**	**0.255**	**-0.387**
	0.004	-0.110	0.517	-0.092	0.071	0.226	-0.340
0.001	**0.222**	**-0.044**	**-0.050**	**-0.027**	**0.089**	**0.261**	**-0.405**
	0.006	-0.109	0.524	-0.090	0.068	0.227	-0.348
0.005	**0.232**	**-0.038**	**-0.035**	**-0.038**	**0.089**	**0.249**	**-0.359**
	0.014	-0.101	0.556	-0.085	0.056	0.230	-0.372
0.01	**0.232**	**-0.022**	**-0.036**	**-0.037**	**0.106**	**0.250**	**-0.364**
	0.027	-0.105	0.593	-0.040	0.064	0.222	-0.445

Note. [a]Upper data in bold characters are for non-ionized forms and lower data in normal font are for inner salt forms.

Table 7
Charge Density at Selected Atoms of α-Orn-Ala Dipeptide[a]

Field [a.u.]	Atoms						
	Carboxyl H	Amino N		Peptide bond			
		Ala	Orn	N	H	C	O
0.000	**0.229**	**-0.022**	**-0.032**	**-0.041**	**0.091**	**0.252**	**-0.364**
	0.016	-0.032	0.664	0.009	0.179	0.259	-0.549
0.001	**0.228**	**-0.023**	**-0.034**	**-0.042**	**0.091**	**0.254**	**-0.370**
	0.015	-0.032	0.668	0.008	0.177	0.260	-0.545
0.005	**0.229**	**-0.023**	**-0.042**	**-0.039**	**0.087**	**0.263**	**-0.398**
	0.012	-0.034	0.689	-0.006	0.168	0.264	-0.527
0.01	**0.235**	**-0.026**	**-0.053**	**-0.025**	**0.067**	**0.274**	**-0.439**
	0.027	-0.037	0.742	-0.009	0.156	0.263	-0.530

Note. [a]Upper data in bold characters are for non-ionized forms and lower data in normal font are for inner salt forms.

Table 8
Charge Density at Selected Atoms of δ – Orn-Ala Dipeptide[a]

Field [a.u.]	Atoms						
	Carboxyl H	Amino N		Peptide bond			
		Ala	Orn	N	H	C	O
0.000	**0.228**	**-0.044**	**-0.029**	**-0.047**	**0.084**	**0.241**	**-0.365**
	0.023	0.529	-0.018	0.011	0.078	0.224	-0.354
	0.017	*-0.046*	*0.874*	*-0.045*	*0.072*	*0.241*	*-0.353*
0.001	**0.226**	**-0.043**	**-0.029**	**-0.051**	**0.083**	**0.241**	**-0.358**
	0.023	0.538	-0.064	0.013	0.077	0.223	-0.351
	0.019	*-0.039*	*0.779*	*-0.014*	*0.089*	*0.278*	*-0.450*
0.005	**0.219**	**-0.039**	**-0.033**	**-0.052**	**0.078**	**0.252**	**-0.392**
	0.028	0.743	-0.071	0.100	0.090	0.182	-0.418
	0.018	*-0.044*	*0.761*	*-0.012*	*0.096*	*0.287*	*-0.472*
0.01	**0.209**	**-0.041**	**-0.042**	**-0.053**	**0.067**	**0.263**	**-0.424**
	0.036	0.771	-0.059	0.056	0.087	0.202	-0.395
	0.016	*-0.052*	*0.739*	*-0.010*	*0.109*	*0.298*	*-0.500*

Note. [a]Upper values in bold are these for nonionized dipeptide, lower values in normal font are these for ionized dipeptide protonated at the Ala amino group, and bottom values in italics are these for ionized dipeptide protonated at the Orn amino group.

The charge densities at relevant atoms of the peptide bond of the Orn-Ala dipeptide formed involving δ-amino group reacted differently to increase in the EEF strength. Positive charges at the H atom and negative charge at the N atom declined regularly with the increase in the field strength. Above 0.001 a.u. the positive charge at the C atom and negative charge at the O atom increased. The acidity of the dipeptide decreased against the field strength. The basicity of the amino group in the Ala moiety had a tendency to decrease with the field strength and, simultaneously, the basicity of the amino group in the Orn moiety showed the opposite tendency.

In the Ala-Ala dipeptide in the enol form its total energy is slightly less negative than that for the corresponding keto form (Table 1) and it decreased as EEF increased. EEF of the strength up to 0.005 a.u. decreased the dipole moment of the molecule and the 0.01 a.u. field again increased it. The peptide bond length in the enol form is, obviously, lower than in the keto form and it increased with the EEF strength applied.

Acidity of the enol form in therms of the charge density at the hydrogen atom of the carboxylic group is lower than that of the keto form and it is only subtly dependent on the EEF strength (Table 3). Simultaneously, basicity of the amino group significantly increased as the negative charge at its nitrogen atom declined. Variation of the charges at the atoms at the peptide bond are given in Table 3. The 0.01 a.u. EEF introduced remarkable perturbations in the changes of the charges with the EEF.

Generating inner salt shifted the proton from the carboxylic group to the amino group. It resulted in a small increase in the energy of the system. sensitivity of to increasing EEF strength is higher compared to that of non-ionized forms. Simultaneously, dipole moments remarkably

increased stimulating their globular form. All computations were performed for inner salts in their keto forms.

Total energy of the inner salts was always slightly less negative than that of corresponding non-ionized molecule exposed to EEF of a given strength. However, in some selected cases the change of the energy is not proportional to EEF applied. Such irregularities in changes of dipole moment and the peptide bond length were also randomly observed mainly at higher EEF strength. Dipole moments were usually at least twice as high as these for non-ionized molecules, but sometimes that increase was even higher by six times.

The construction of the GluA-Ala dipeptide involved the terminal carboxylic acid group of the dioic acid which was situated closer to the amino group. The inductive effect of this group made the carboxylic acid group at the opposite end of the molecule more dissociated (Neuberger, 1936).

Two amino groups of ornithine distinguished in their basicity. Because of the inductive effect of the carboxylic group its δ-amino group as more basic was engaged in the construction of the Ala-Orn dipeptide and, hence, in the inner salt the amino group of the Ala moiety accepted the proton from the carboxylic group. The Orn-Ala dipeptide was constructed in two possible ways, that is engaging either α– or δ–amino group of Orn. In the latter case the dipeptide could form its inner salt by shifting the proton of the carboxylic group to each of two amino groups. The total energy of the inner salts involving the amino group of the Ala moiety were more negative in EEF but out of the field the energy of dipeptide with positive charge at the Orn amino group was slightly more negative.

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