J. At. Mol. Sci.
doi: 10.4208/jams.010410.012210a

Vol. **1**, No. 1, pp. 68-77
February 2010

Quantum chemistry PM3 calculations of sixteen mEGF molecules

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Received 4 January 2010; Accepted (in revised version) 22 January 2010;
Available online 15 February 2010

Abstract. Murine epidermal growth factor (mEGF) is a single polypeptide of fifty-three amino acid residues containing three intramolecular disulfide bonds, widely found in body fluids like milk. We performed semi-empirical quantum chemistry calculations on sixteen mEGF molecules at level of PM3 to investigate the electronic properties of these molecules. The equilibrium structure, heats of formation, molecular orbitals, polarizability, and dipole moment were computed in vacuum and in solvents (carbon tetrachloride, ethanol, and water). Electronic structure calculations in vacuum show that the highest occupied molecular orbital (HOMO) of all mEGF molecules distributes on five C-terminal residues (Tyr49/Tyr50 and Arg48/Arg53 residues), while the lowest unoccupied molecular orbital (LUMO) locates on the disulfide bonds, indicating that the disulfide bridges and C-terminus are important for the biological activity of EGF. The locations of LUMO and HOMO show almost no solvent effects. In the solvent of relative larger dielectric constant, the heat of formation and mean polarizability of the mEGF molecules become lower while the dipole moment is enhanced. Examination of the locations of hydrogen bonds demonstrates that the B loop (residues 14-31) is crucial for the conformational stability and biological activity of EGF. All these theoretical results are in general agreement with experiments. We suspect that the N- and C-terminal residues play some roles in stabilizing the molecular conformation of EGF, probably associated with the orientation of EGF binding to EGFR (epidermal growth factor receptor).

PACS: 82.20.Wt, 03.67.Lx, 87.15.R-, 31.15.-p, 87.18.Nq, 87.15.-v

Key words: epidermal growth factor (EGF), PM3, solvent, HOMO, LUMO

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1 Introduction

EGF (53 amino acid residues) was discovered by Stanley Cohen in 1962 who won the Nobel Prize in 1986. EGF is a kind of micro-molecule polypeptide widely found in human and other animals in vivo. Human EGF gene is located on chromosome 4 [1]. The presently known experimental structures of murine EGF (mEGF) have essentially the same folding as human EGF [2,3]. EGF adopts a well-defined 3D structure and comprises three distinct loops (as shown in Fig. 1) determined by three disulfide bridges [2–5]; these disulfide bridges are essential for structural stability and biological activity. The conformation of EGF, like many other small disulfide proteins, is determined essentially by its amino acid sequence alone [6,7].

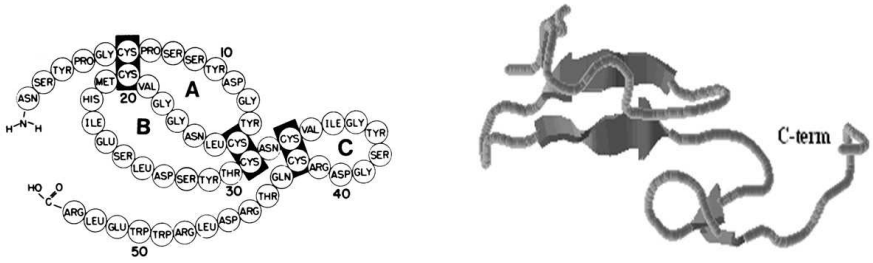

Figure 1: Molecular structure of mEGF: (a) proximity relation of residues in folding peptide skeleton, the black bands denote the disulfide bonds [30]; (b) cartoon rendering for NO.5 mEGF molecule.

EGF regulates cell proliferation and differentiation by binding to its receptor (EGFR) extra-cellular domains [4,6,8,9]. The EGF receptor is a transmembrane protein tyrosine kinase. Binding of EGF to the extracellular region of EGFR induces receptor dimerization [10–12], which is supposed to bring the two cytoplasmic tyrosine kinase domains of the receptors close enough for autophosphorylation and thereby to activate the intrinsic tyrosine kinase activity [13–17]. The EGF receptor tyrosine kinase triggers numerous downstream signaling pathways. Thus, EGF plays an important role in embryonal development, tissue repair, wound healing [18], carcinogenesis, blood coagulation, fibrinolysis, neural development, and cell adhesion [19–21]. Besides, EGF is mitogenic for a number of cell types and demonstrates a variety of actions in vitro and in vivo, including stimulation of metabolite transport, activation of glycolysis, stimulation of production of RNA, protein, and DNA, enhancement of cell proliferation, alteration of cell morphology, and inhibition of gastric acid secretion [6,8,19,21–23].

Due to its biological effect, EGF has many potential applications in clinical medicine and cosmetics. A variety of N-/C-terminus truncated and residues mutated forms have been used in experiments to locate the biologically active sites of EGF molecules and to investigate their functions. However, there have been some debates on the function of C-terminal residues. Deletion of the C-terminal five or six residues results in a marked reduction in both receptor affinity and mitogenic activity in vitro [3,18,24–26]. On the

contrary, it was proposed that the C-terminal amino acids are not necessary for biological activity [12, 27]. Komoriya *et al*. showed that the primary functional roles of the N- and C-terminal regions of the EGF are to provide conformational stability for the middle region [28]. It was also argued that the N-terminal A loop formed via connection of Cys6 and Cys20 is essential for the biological activity of EGF [29]. Komoriya and co-workers found that the linear and cyclic forms of the B-loop peptide (residues 20-31) are active in both receptor binding and biological stimulation in cell culture, while the other loops are inactive [28,30]. C loop is capable of receptor binding but has no function on stimulation of mitogenesis [9,30].

Protein structures are usually stabilized by a variety of weak non-bond interactions, such as hydrogen bonds, hydrophobic, electrostatic and dipole-dipole interactions. Covalent cross-links also contribute in stability and activity of many proteins. Among them, disulfide bridges are considered to be most important. The stabilization effect of the disulfide bridges in proteins was traditionally explained by their ability to reduce the conformational degrees of freedom of the unfolded polypeptide chain [31–33]. Besides, hydrogen bonds and hydrophobicity are considered as the major interactions between EGF and EGFR. The Tyr13, Leu15, Met21, Ile23, Leu26 and Leu47 residues of EGF are involved in hydrophobic interactions with EGFR [1, 34, 35], whereas the Asn32, Gln43 and Arg45 residues of EGF are responsible for hydrogen-bonding interactions between EGF and EGFR. In previous mutagenesis and biochemical studies, it was shown that Arg41 is crucial for the EGFR-binding [32, 36]. Meanwhile, Asn32 located between two Cys residues may function as a hinge between the two loops constituting EGF [37]. The relative orientation between the two loops in the present receptor bound structure is essentially the same as that in the solution structure [26,38]. To elucidate the function of N- and C-terminus and to investigate the correlation between active sites and molecular orbitals, quantum calculations at the atomistic level are essential to provide useful insights that are not accessible from experiments. In this paper, we investigated the mEGF molecules using semi-empirical quantum chemistry PM3 method [39,40].The solvent effects are discussed by comparing the molecules in different environments like vacuum, carbon tetrachloride, ethanol, and water. The electronic properties of the mEGF molecules, including the energy and locations of HOMO and LUMO, dipole moment, mean polarizability, spatial distribution of hydrogen bonds, will be discussed.

2 Computational methods

Due to the large number of atoms in the mEGF molecules ($C_{257}O_{83}N_{73}S_7H_{375}$, totally 795 atoms), exact *ab initio* calculations of these molecules are computationally prohibitive. Instead, sixteen mEGF (PDB ID: 3egf, and there are 16 molecules in this PDB code) molecules were studied using the self-consistent field Hartree-Fock (SCF-HF) method within the semi-empirical PM3 approximation [39, 40], as implemented in the MOPAC program [41]. The molecules were first fully optimized without any symmetry con-

straints. Then, single-point energy calculations were performed to analyze the electronic properties of the molecules in the equilibrium configurations. In addition to the calculations in vacuum, solvent effect was modeled by the COSMO method [42]. We chose three solvents with distinct dielectric constant, that is, carbon tetrachloride ($\varepsilon = 2.238$), ethanol ($\varepsilon = 24.3$) and water ($\varepsilon = 78.54$).

3 Results and discussion

The initial configurations of the sixteen isolated mEGF molecules were taken from the PDB database [43], which were measured from the EGF-EGFR complexes. After relaxation, there is only little change from the initial conformation. Moreover, solvent effect approximated by the COSMO calculations has almost no effect on the geometry structures of the molecules. As representatives, the optimized molecular configurations for NO.1 and NO.5molecules in vacuum are displayed in Fig. 2.

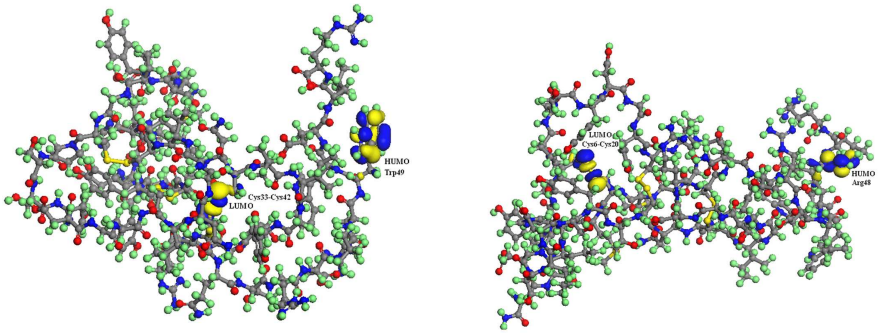

Figure 2: (color online) Spatial distributions for the wavefunction of HUMO and LUMO: (a) NO.1 mEGF molecule in vacuum; (b) NO.5 mEGF molecule in vacuum. C: gray, O: red, N: blue, H: green.

3.1 Heat of formation

Table 1 lists the heat of formation (H_f) computed for the sixteen mEGF molecules in vacuum. One can see that Hf ranges between -3288.187 kcal/mol and -3224.469 kcal/mol, with the average value of -3260.489 kcal/mol. The negative heats of formation mean that the formation of all the sixteen mEGF molecules is exothermic. The narrow range of Hf values implies that the stabilities of all these sixteen molecules are comparable. To discuss the solvent effect, we chose mEGF molecules of NO.1, NO.8, and NO.11 as representatives and computed these molecules in different solvents. The heats of formation for those molecules in carbon tetrachloride, ethanol and water are compared in Table 2. One can clearly see that the larger dielectric constant, the lower heat of formation. This implies that the solvent environment may enhance the conformational stability of the mEGF molecules.

Table 1: Heats of formation, dipole moment, mean polarizability, HOMO energy (E_{HOMO}), HOMO-LUMO gap Δ, locations of HOMO and LUMO of the sixteen mEGF molecules in vacuum

No.	Heat of formation (kcal/mol)	Dipole moment (Debye)	Mean polarizability (Å^3)	E_{HOMO} (eV)	Δ (eV)	HOMO location	LUMO location
1	-3261.77	26.282	585.159	-8.548	5.886	Trp49	Cys33-Cys42
2	-3261.013	18.617	586.332	-8.538	5.541	Trp49	Cys14-Cys31, Cys33-Cys42
3	-3234.036	31.997	584.902	-8.751	6.091	Trp50	Cys33-Cys42
4	-3268.756	26.088	585.055	-8.450	5.983	Trp50	Cys33-Cys42
5	-3236.851	23.983	585.225	-8.335	5.642	**Arg48**	Cys6-Cys20
6	-3278.238	18.197	585.289	-8.331	5.754	Trp49	Cys33-Cys42
7	-3265.064	10.354	587.396	-8.409	5.173	Trp49	Cys14-Cys31, Cys33-Cys42
8	-3270.886	14.353	584.828	-8.404	5.575	Trp50	Cys6-Cys20
9	-3265.910	19.070	584.987	-8.572	5.807	Trp50	Cys33-Cys42
10	-3259.581	13.652	585.109	-8.404	5.761	Trp49	Cys33-Cys42
11	-3265.392	43.551	585.059	-8.345	5.377	**Arg53**	Cys14-Cys31
12	-3224.469	23.191	587.883	-8.836	6.149	**Arg48**	Cys33-Cys42 Cys14-Cys31
13	-3278.872	11.055	585.742	-8.589	5.839	Trp49	Cys33-Cys42
14	-3288.187	25.073	585.386	-8.440	5.768	Trp49,Trp50	Cys33-Cys42
15	-3248.368	25.184	584.942	-8.344	5.482	Trp49,Trp50	Cys6-Cys20, Cys14-Cys31
16	-3278.238	17.994	585.807	-8.096	5.362	Trp49	Cys6-Cys20 Cys14-Cys31

3.2 HOMOs and LUMOs

The HOMO and LUMO of a molecule are usually important characteristics for its chemical reactivity and probably biological activity [44–47]. The HOMO energies for all these sixteen molecules are rather close to each other, i.e., ranging between -8.096 eV to -8.836 eV. Similarly, the HOMO-LUMO energy separations are found to uniformly distribute from 5.173 eV for NO.7 molecule to 6.149 eV for NO.12 molecule. The fairly large HOMO-LUMO gaps imply that these molecules should be relatively inert during many chemical and biological processes.

In vacuum, the HOMOs for most of the molecules distribute on Trp49 and Trp50 residues, while there are two molecules with HOMO on Arg48 and one on Arg53 (bold in Table 2 and Fig. 2. On the other hand, the LUMOs for all the molecules locate on the disulfide bonds. Therefore, we conjecture that the six residues at C terminal and the three disulfide bonds are closely related to the chemical and biological activity of mEGF molecules. It is worthy to point out that in most cases LUMO are on the hydrophobic Trp49 and Trp50 residues. Moreover, the coherent distribution of HOMO and LUMO in all molecules suggests that the EGF molecules interact with EGF receptor in some particular way associated with the electronic and hydrophobic interactions [35].

For all solvents considered, the locations of HOMO and LUMO are nearly the same as the vacuum situation. One exceptional case is the NO.11 molecule, for which the HOMO in all three solvents moves to Trp50 instead of Arg53 in vacuum. On the other hand,

Table 2: Heats of formation, dipole moment, mean polarizability, HOMO-LUMO gap Δ, and locations of HOMO and LUMO for NO.1, NO.8 and NO.11 mEGF molecules in selected solvents.

No.	Solvent Name	dielectric constant	Heat of formation (kcal/mol)	Dipole moment (Debye)	Mean polarizability ($Å^3$)	Δ (eV)	HOMO location	LUMO location
1	Vacuum	1.000	-3261.711	26.282	585.159	5.886	Trp49	Cys33-Cys42
	Carbon Tetra-chloride	2.238	-3416.315	31.453	583.861	5.842	Trp49	Cys33-Cys42
	Ethanol	24.300	-3613.602	39.151	582.155	5.833	Trp49	Cys33-Cys42 Cys14-Cys31
	Water	78.540	-3632.730	40.066	582.002	5.821	Trp49	Cys33-Cys42 Cys14-Cys31
8	Vacuum	1.000	-3270.886	14.353	584.828	5.575	Trp50	Cys6-Cys20
	Carbon Tetra-chloride	2.238	-3427.487	18.040	583.600	5.560	Trp50	Cy6-Cys20
	Ethanol	24.300	-3616.242	20.120	581.775	5.690	Trp50	Cy6-Cys20
	Water	78.540	-3646.528	22.988	581.766	5.641	Trp49 Trp50	Cy6-Cys20
11	Vacuum	1.000	-3265.392	43.551	585.059	5.377	**Arg53**	Cys14-Cys31
	Carbon Tetra-chloride	2.238	-3421.231	50.182	583.759	5.523	Trp50	Cys14-Cys31
	Ethanol	24.300	-3622.380	59.409	582.013	5.596	Trp50	Cys14-Cys31
	Water	78.540	-3642.965	60.928	581.882	5.589	Trp50	**Cy6-Cys20**

LUMO still occupies disulfide-bridge, but changes to the Cys_6-Cys_{20} in water with the large dielectric constant. Therefore, solvent may have moderate influence on the distribution of HOMO and LUMO. It is noteworthy that the solvent effect is approximated here by an effective dielectric constant. In the realistic situations, the solvent molecules may interact with the EGF molecule to a certain extent and the true solvent effect would be more complicated.

3.3 Hydrogen bonds

In this work we focus on the hydrogen bonds between nonadjacent residues, which play some major roles in the molecular conformation. The cutoff distance for a hydrogen bond was chosen as 2.5 Å. We found that the locations of hydrogen bonds are exactly the same in vacuum, and in carbon tetrachloride, ethanol, and water. In other words, solvent seems to have no effect on the spatial distributions of the hydrogen bonds.

In Table 3, we count the times of appearance for hydrogen bonds at different locations in the sixteen molecules. Among them, hydrogen bonds at Tyr3-His22 [29] and Val19-Asn32 [18] are most frequently observed, and some mEGF molecules even form double hydrogen bonds between Tyr3 and His22, Val19 and Asn32 residues, respectively. The appearance numbers of Asn1-Glu24, Ile23-Ser28, Met21-Thr30, Val34-Tyr37, and Ser38-Leu44 [18] hydrogen bonds are comparable, that is, around five to seven. Previously, NMR measurements have revealed the hydrogen bonds at locations of Tyr3-His22 [29] Val19-Asn32, Ile23-Ser28, Met21-Thr30, Val34-Tyr37, and Ser38-Leu44 [18], while hydro-

Table 3: Spatial distribution of hydrogen bonds counted for the sixteen mEGF molecules.

Hydrogen bonds between nonadjacent residues	Times of appearance in sixteen mEGF molecules
Asn1-Glu24	7
Tyr3-His22	11
Tyr13-Arg41	1
Val19-Asn32	11
Met21-Thr30	5
Ile23-Ser28	7
Tyr29-Asp40	4
Val34-Tyr37	5
Cys33-Glu51	2
Gly36-Asp46	5

gen bond at Asn1-Glu24 has not been reported yet. Despite there are some controversy on PM3 describing hydrogen bonding [48,49], our computational results agree well with the experimental measurements. As shown in Table 3, most hydrogen bonds are found in B loop (residues 20-31), while C loop (residues 33-42) has fewer and A loop (residues 6-19) the least. The distribution of hydrogen bonds indicates that N-terminus is functional to the conformational stability of EGF. Asn32 residue located between the two Cys residues may play a special role between the two component loops of EGF. Indeed, the B loop is mainly stabilized by the large number of backbone hydrogen bonds as well as the side-chain to side-chain interactions. Hence, the B loop is crucial for the conformation stability and biological activity of the molecules, as proposed by Komoriya *et al* [28].

Among the sixteen mEGF molecules, the NO.12 molecule possesses the largest number of hydrogen bonds. Interestingly, its HOMO-LUMO gap of 6.149 eV is highest among all molecules studies. This implies that the amount of hydrogen bonds have some correlation with the electronic properties of the mEGF molecules.

Some other hydrogen bonds between Val34 and Tyr37, Tyr13 and Arg41, Gly36 and Leu47 residues were observed, but with less population. These residues are capable of accepting or donating proton, and consequently are functional for binding to the EGFR. In particular, Tyr37 and Arg41 residues are significant in the affinity of the receptor [50]. Rege *et al*. underscore the cardinal role played by hydrogen bonds in biological electron transfer processes [51].

3.4 Polarizability

Molecular polarity is a key feature of a molecule that reflects its bond polarity and molecular structure [52], which is resulted from the uneven partial charge distribution between various atoms in a molecule. In a polar covalent bond, valence electrons are unequally shared between the two bonded atoms, which leads in partial positive and negative

charges and creates a dipole. Normally, greater polarity corresponds to stronger attractions among the molecules. The polarizability is defined as the linear coefficient between an applied electric field and the induced dipole moment. As shown in Table 1, the computed mean polarizability for all molecules are very close, i.e., 584.828-587.883 $Å^3$. Inside solvents, the mean polarizability reduces with increasing dielectric constant (Table 3), indicating that the biological activity and ability of EGFR-binding vary in different solvents.

3.5 Dipole moment

It is known that many drugs and biological molecules carry certain dipole moments due to intramolecular charge transfer. The dipole moment is related to its geometrical and electrical structures. The amplitude of dipole moments may affect the intermolecular interaction, biomolecular solvation as well as the biochemical activities [53]. Thus, it would be interesting to explore the dipole moments of the EGF molecules with different configurations, and effected by different solvents. As shown in Table 1, the computed dipole moments of the sixteen mEGF molecules in vacuum fall in a rather wide range (from 10.354 Debye to 43.551 Debye), which is obviously related to distinctly different conformations of the molecules. For a given mEGF molecule, its dipole moment increases with dielectric constant of the solvent (Table 2), which may result in stronger biological activities.

4 Conclusions

To summarized, using PM3 method we computed the geometry structures and electronic properties of sixteen mEGF molecules, both in vacuum and in a few selected solvents. The locations of the HOMO and LUMO were examined to discuss the relations between the molecular orbitals and activity sites. We found that all HOMO distributes on Tyr49/Tyr50 and Arg48/Arg53 residues, while LUMO locates on the disulfide bonds, and the locations of these frontier orbitals are nearly the same in different solvents. From the analysis of HOMO and LUMO, we suggest that: (1) the three disulfide bonds in the molecule are critical for the conformational stability and biological activity of mEGF; (2) the six C-terminal residues may be important for the EGFR-binding; (3) the hydrophilic Arg and the hydrophobic Trp residues may play some roles in the activity of mEGF molecules.

The spatial locations of hydrogen bonds imply that the A and B loops are especially responsible to the conformational stability of mEGF molecules. Hydrogen bonds located on other sites indicate that Met21, Tyr29 and Tyr37 residues may be also the active sites. The orientation of EGF within EGF-EGFR complex might be affected by the dipole moment, as well as the C- and N-terminus. Solvent effect was discussed via choosing three solvents with distinctly different dielectric constants using COSMO approach. In the solvent with larger dielectric constant, the heat of formation and mean polarizability of

the mEGF molecules are reduced with regard to the vacuum values, whereas the dipole moment is enhanced. The present theoretical results generally agree with the available experimental observations, suggesting that the semi-empirical quantum chemistry PM3 method is accessible to investigate the biological and chemical activity of small proteins via discussing the electronic properties.

Acknowledgements. This work was supported by Program for New Century Excellent Talents in Universities of China under Grant No. NCET-06-0281. We thank Prof. Tian-Ying Yan, Dr. Chun-Li Yan, Dr. Tao Liu, and Mr. Ting Wei for helpful discussions.

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