ELECTROSTATICS OF HORSE HEART CYTOCHROME *C* AND MONTMORILLONITE MONOLAMELLAR PLATE

Sv. Hristova[1], A. Zhivkov[1] and B. Atanasov[2]
Institute of Physical Chemistry „Rostislav Kaishev", Bulgarian Academy of Sciences, 1113-Sofia, Bulgaria [1]
Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113-Sofia, Bulgaria [2]
Correspondence to: Sv. Hristova
E-mail: Svetlana_83_hristova@abv.bg

ABSTRACT

Monomolecular forms of the protein Cytochrome C (CytC) and the mineral Montmorillonite (MM) were studied theoretically by pH-dependent approach of home maid program „PHEI package" with aim to find optimal conditions (pH and ionic strength) of CytC adsorption on MM. The main calculated characteristics were: net-charge titration [Z(pH)], electrostatic term and total free energy (stability) [ΔG_{el}(pH) and ΔG_{tot}(pH)], proton affinities of individual ionic groups [$pK_{a,i}$(pH)], Coulomb interaction of each site [E_{el}(pH)] and electric moments (vectors and scalars) [μ_e(pH) and μ_s(pH)]. CytC was calculated with and without reaction field effects (Born corrections) to understand the role of the water in the charge-charge interactions. Abnormal properties were obtained for many of the surface ionic sites on CytC (Lys 87, Lys 86, Lys 13, Lys 27, Lys 25, etc). In agreement with the experiments the reduced protein is more stable with -7.5 kcal/mol and both forms have isoelectric point (pI) above 10. In pH interval 5-8 all pH-dependent properties are practically non-changed. The electric moments are large and have similar orientation (parallel to the hem plane). The 3D-structure of 35x35Å MM plate was designed. The theoretical and the experimental [Z(pH)] titration curves of MM were compared at acidic and alkaline (close to pI) pH with and without counter-ions. The montmorillonite plate (pMM) undergoes strong ionic interaction between allumo-hydroxyls fixed as a monolayer under Na^+ ions. Their intrinsic pK_a = 9 increased in 10-25 orders of magnitude and are distributed in nine "levels". They produce a big electric moment directed normally to the plate. In the range of pH 5-10 no considerable changes in the effective charge were found.

Keywords: Cytochrome C, Montmorillonite, pH-dependent properties

Introduction

The montmorillonite (MM) is the most often studied swelling clay mineral. It is of a 2:1 layered structure, containing a single layer of aluminum octahedral sheet sandwiched between two layers of silicon tetrahedral sheets (5). The surface isoelectric point (IEP) of the MM varies from pH 6.8 to 9.5 depending on the isomorphically substituted cations (10). It is well known that the montmorillonite particles carry two kinds of electric charges: a variable (pH dependent) charge resulting from proton adsorption/desorption reactions on the surface of hydroxyl groups and a structural negative charge resulting from isomorphous substitutions within the clay structure. As a consequence of this negative potential, MM shows up cation exchange properties in the structure and cation adsorption on the surface.

Cytochrome *c* (Cyt. *c*) is a typical hemoprotein and functions as an electron carrier. Cyt. *c* contains 104 residues and 1 *c*-type heme group (Fe-protoporphyrin) (8). It has an acidic-denatured state at pH 2 and a basic-denatured state at pH 12 (2). No structural changes take place between pH 3 and pH 12 (3). The potentiometric titration curves of oxidized and reduced horse heart Cyt. *c* display the isoelectric points at pH 10.2 and 10.4 respectively (6). The heme edge is surrounded by a number of surface lysine residues, which create a positive electrostatic potential functional to the recognition of the complementary domain in the electron-transport partner.

The interaction between the positively charged surface of Cyt. *c* and the negatively charged surface of MM is predominantly electrostatic. The species-dependent orientation of the protein toward the negatively charged Self-Assembled Monolayers (SAM) is influenced by the electrostatic interactions (1). So, this is the charge-driven

mechanism of protein orientation. The lysine residues contribute most significantly to the orientation. The dipole moment of Cyt. *c* is an important factor determining the orientation of the protein on negatively charged surfaces. The heme group is almost perpendicular to the negatively charged surface. The direction of the Cyt. *c* dipole is determined by the lysine residues near the surface, and glutamic acid residues far away from the surface. Lysine residues Lys 25, Lys 27, Lys 72, and Lys 79 are responsible for strong electrostatic interactions with surfaces (11). The binding of Cyt. *c* to negatively charged surface of liposomes become by lysine residues: Lys 72 and Lys 73 (7).

In the present work we aim to study pH-dependent properties of isolated forms of Cyt. *c* and MM in order to define optimal conditions for absorption of Cyt. *c* on to MM particles. The electrostatic properties of Cyt. *c* and MM monolayer were studied theoretically by home maid program „PHEI package" approach at pH-dependent mode (4).

Materials and methods

Electrostatic calculations

X-ray structure of Cyt. *c* is received of Protein data bank www.pdb.org (pdb code: **1HRC**) and X-ray structure of montmorilonite is received from www.webmineral.com. One lamellar plate of MM is calculated by the program "Mercury". The plate contains 1068 atoms and its size is 33 x 35Å. The electrostatic properties in aqueous solution are computed by a program of the serer PHEPS (http:/pheps.orgchm.bas.bg) (4). It is generally accepted that a model for protein electrostatics can be built on the assumption for continuum medium description, fixed atom approximation, protein–solvent boundary numerically described by atomic static accessibilities, solvent accessibility (SA) and two types of charges: (i) permanent (pH-independent) partial charges and (ii) proton-binding sites with pH-dependent titrable charges. The model accepts experimentally measured pK_a of model compounds (e.g. *N*-acetyl amides of each *i*-th ionogenic amino acids) (p$K_{mod,i}$) and evaluates work for charge transfer from highly polar water solvent (ε_w = 80) to protein macromolecule (4 < $\varepsilon_{p,i}$ < 40). The exposure to the solvent is evaluated by SA_i in absence of other ionic groups. The Born term, which is proportional to [1 – ($\varepsilon_{p,i}/\varepsilon_w$)], is roughly estimated as (1 – SA_i). Partial charges assume values from AMBER and PARSE parameterization sets. Since the ratio of the number of ionic AAR (N_{ion}) and the total number of AAR (N_{tot}) R_{el} = N_{ion}/N_{tot} is relative high for protein particles with small radii (R_p), the pairwise interaction between any *i*-th and *j*-th ionic groups counts contributions from charge–charge, charge–dipole and dipole–dipole interactions, which can be simulated by an empirical three exponential curve:

$$\mathbf{W}_{ij}(r, a_k) = \sum_k \left(\frac{a_k}{r_{ij}^k}\right), \qquad (1)$$

where k = 1 for long-range (Columbic) interactions; k = 2 for mid-range, charge–dipole interactions; and k = 3 for short-range dipole–dipole interactions. The a_k were estimated by a non-linear procedure by minimizing the functional $F(a_1,a_2,a_3)$

$$F(a_1,a_2,a_3) = \sum_{\mathrm{pH}_*} \left\{ \left[\frac{\partial Z^{\exp}(\mathrm{pH}_*)}{\partial \mathrm{pH}}\right] - \left[\frac{\partial Z^{\mathrm{th}}(\mathrm{pH}_*, a_1, a_2, a_3)}{\partial \mathrm{pH}}\right] \right\}^2 \qquad (2)$$

where the values of Z^{exp} are taken from experimental data and Z^{th} are the calculated values of the protein net charge as a function of pH. The initial values of the coefficients a_k are obtained by numerical approximation of $\mathbf{W}(r_{ij})$. Through extensive testing, using large dataset of structures, it was found that a_1, a_2 and a_3 values are practically constants for a great number of proteins. The pH-dependence of the electrostatic potential $\Phi_{el,i}$ (pH) at the *i*-th proton binding site in PHEI was evaluated according to the following equation :

$$\Phi_{el,i}(\mathrm{pH}) = 2.3RT \sum_{j \neq i} \left\{ \boldsymbol{Q}_j(\mathrm{pH}) \boldsymbol{W}_{ij} \left[1 - \left(\frac{\mathbf{SA}_i + \mathbf{SA}_j}{2} \right) \right] \right\}, \qquad (3)$$

Here $\boldsymbol{Q}_j$(pH) is defined by the degree of dissociation or statistical mechanical proton population of given H^+-binding site; $\boldsymbol{Q}_j$ (pH) = (1 – $\langle \mathbf{s}_j \rangle$) and $\boldsymbol{Q}_j$ (pH) = –$\langle \mathbf{s}_j \rangle$ for basic and acidic groups respectively, where:

$$\langle s_j \rangle = \frac{10^{(\mathrm{pH} - \mathrm{p}K_j)}}{\left[1 + 10^{(\mathrm{pH} - \mathrm{p}K_j)}\right]}. \qquad (4)$$

Thus using partial titration of each *j*-th group we can find the pH-dependent net-charge of the whole molecule, Z(pH), i.e. potentiometric titration curve:

$$\boldsymbol{Z}(\mathrm{pH}) = \sum_j \boldsymbol{Q}_j(\mathrm{pH}). \qquad (5)$$

By definition if Z = 0 than pH = pI, i.e. the isoelectric point (the only pH at which the dipole moment of a protein molecule can be evaluated). Thus starting with p$K_{int,i}$ = p$K_{mod,i}$ + Δp$K_{Born,i}$ + Δp$K_{par,i}$, where p$K_{mod,i}$ is the pK_a of the *i*-th site according to the model compounds – see set of p$K_{mod,i}$ in; Δp$K_{Born,i}$ is the Born self-energy of the *i*-th site buried within the 'uncharged' protein, and Δp$K_{par,i}$ is the contribution of the

i-th site interacting with the set of partial (permanent, fixed) atomic charges (see above).

$$\begin{aligned} & \mathrm{p}K_{a,i}(\mathrm{pH}) = \mathrm{p}K_{\mathrm{int},i} + \mathrm{p}K_{\mathrm{tit},i} \\ & = \mathrm{p}K_{\mathrm{int},i} + \left(\frac{1}{2.3RT}\right) \\ & \times \sum_{j \neq i} \left\{ Q_j(\mathrm{pH})\left(W_{ij} - C\right)\left[1 - \left(\frac{SA_i + SA_j}{2}\right)\right] \right\}, \end{aligned} \qquad (6)$$

where ***C*** is the Debye–Hückel term for ionic strength (Is). The term $pK_{tit,i}$ is the pK_a shift of the *i*-th site caused by interactions with all other proton-binding groups and is evaluated according to efficient self-consistent iterative procedure. Coming to self consisted pH-dependent ionization the free energy term G_{el}(pH) is calculated as follows.

$$\Delta G_{\mathrm{el}} = \sum_{j \neq i} \boldsymbol{Q}_i \boldsymbol{Q}_j(\mathrm{pH}) \mathbf{W}_{ij} \left[1 - \left(\frac{\mathbf{SA}_i + \mathbf{SA}_j}{2} \right) \right], \qquad (7)$$

as well as pH-dependent Coulomb energy of each *i*-th ionic group with whole charge multipole:

$$\mathbf{E}_{el,i}(\mathrm{pH}) = \boldsymbol{Q}_i \left\{ \sum_{j \neq i} \boldsymbol{Q}_j(\mathrm{pH}) \boldsymbol{W}_{ij} \left[\frac{1 - (\mathbf{SA}_i + \mathbf{SA}_j)}{2} \right] \right\}. \qquad (8)$$

Results and Discussion

In **Fig. 1** is displayed a term pH-dependent electrostatic free energy: $\Delta G_{t,min}$ is –18.3 kcal/mol and $\Delta G_{el,min}$ is –5.8 kcal/mol. The contribution of hydrophobic interaction to the total charge stability is –12.5kcal/mol. Cyt. *c* is stable in wide pH diapason. In pH interval 5–8 the protein has negative charge.

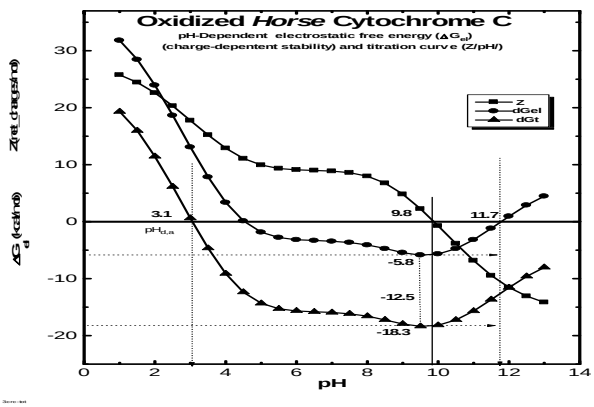


Fig. 1. pH-dependant electrostatic free energy (charge-dependant stability) and titration curve

The theoretically calculated value of the isoelectric point (pI) is 9.8. Cyt. *c* has acidic-denatured point (where $\Delta G_t = 0$) at pH 3.1. The evaluated theoretical data are in good agreement with the literature experimental data (2,6).

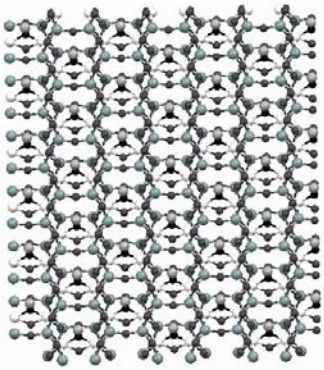
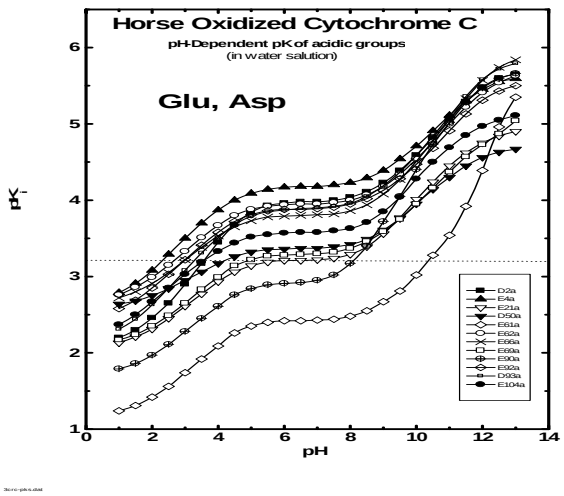


Fig. 2. Model of MM plate: ● titrable allumo-OH groups; ● O-atoms; ○ Al-atoms ● Si-atoms; ○ Ca^{2+};

Our model of MM monolayer presumes that the only titrable groups are allumo-OH and their dissociation determines the negative charge of MM surface. The electrostatic calculations show that pK_a values of OH-groups are increased abnormally. That is because the OH groups are sandwiched between two layers of silicon tetrahedral sheets and therefore their accessibility for the solvent is decreased. These groups are organized at 9 subgroups at different values of pK_a. The MM plate in our model shows up a dipole moment, perpendicularly oriented to its surface; that result is in agreement with the experimental results obtained electro-optically (9).

In **Fig. 3a** and **3b** are displayed curves of pH-dependent values of pK_a of the ionizable groups in oxidized Cyt. *c*. The pK_a values of some residues are decreased (Lys 87, Lys 86, Lys 13, Lys 27, Lys 25 and Glu 61), but pK_a of others are increased (Lys 38, Lys 99, Lys 5, Glu 4, Glu 62, Glu 93, Glu 66, Asp 2, Asp 104) in comparison with pK_{int}. The curves of the acidic groups are shifted significantly; therefore they are situated in negative field with its center around a cluster of glutamic acid residues. The strong positive potential is localized around a number of surface lysine residues surrounded by one of the heme edges. The electrostatic field distribution determines a dipole-like structure of Cyt. *c*. The dipole moment is an important factor determining the orientation of Cyt. *c* on charged surfaces (11). Lysine residues Lys 25, Lys 27, Lys 72, Lys 87, Lys 86 and Lys13 are responsible for the strong electrostatic interactions of Cyt. *c* with the surfaces of MM plate. The heme group is almost perpendicular to MM plate at such orientation of the adsorbed Cyt. *c* (See **Fig.4**).

A



B

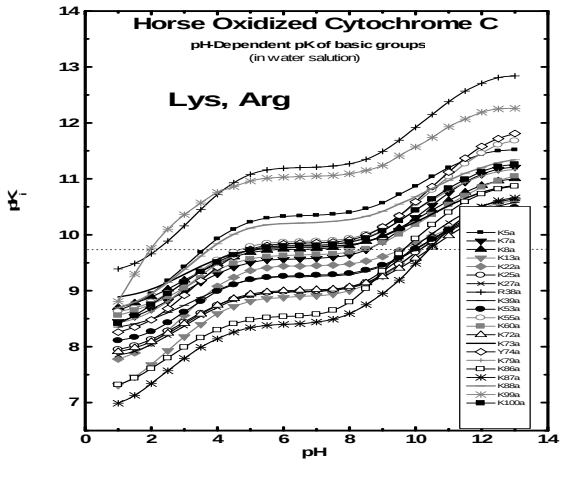


Fig. 3. pH-dependant pK_a of acidic (**3a**) and basic (**3b**) groups at oxidized horse heart Cyt. *c* The pK_a values of residues Lys 87, Lys 86, Lys 13, Lys 27, Lys 25 and Glu 61 are decreased. The pK_a values of residues Lys 38, Lys 99, Lys 5, Glu 4, Glu 62, Glu 93, Glu 66, Asp 2, Asp 104 are increased in comparison with pK_{int}.

The electrostatic interactions are of great importance for the protein structure and its adsorption on charged surface. The present work shows the role of the local electrostatic potential for the adsorption of Cyt. *c* on negatively charged MM plate. The interactions are determined by the pH-dependent dipole-like structure of the protein. The MM model suggested by us showed abnormally values of pK_a because OH^- groups are not accessible for the solvent. The dipole moment and the lysine residues play the main role for the oriented adsorption of the protein on the surface of MM particles.

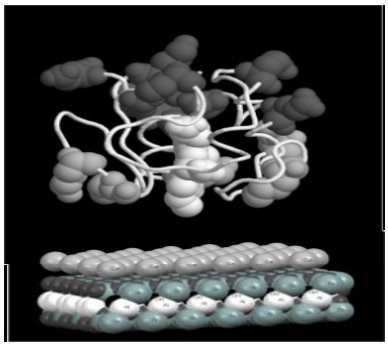


Fig. 4.. Model of electrostatic interactions between Cyt. *c* and MM monolayer plate. The acidic residues determining the local negative electrostatic potential are shown in dark grey; the lysine residues in the region of local positive electrostatic potential are shown in bright grey. The heme is colored in white.

REFERENCES

1. **Battistuzzi G., Borsar M., Bortolotti C., Rocco G., Ranier A., Sola M.** (2007). J. Phys. Chem., **111**, 10281-10287.
2. **Boffi F., Bonincontro A., Cinelli S., Congiu Castellano A., De Francesco A., Della Longa S., Girasole M., Onori G.** (2001). Biophys. J., **80**, 1473-1479.
3. **Bos M., Kleijn M.** (1995). Biophys. J., **68**, 2573-2579.
4. **Kantardjiev A., Atanasov B.** (2006). Nucleic Acids Res., **34**, 43-47.
5. **Lagaly G., Ziesmer S**. (2003). Adv. Colloid Interface Sci., **100/102**, 105-128.
6. **Marini M., Marti G., Berger R., Martin C**., (2004). Biopolymers, **19,** 885-898.
7. **Rytomaa M., Kinnunen P..** (1995). J. Biol. Chem., **270,** 197-322.
8. **Sato F., Yoshihino T., Era M., Kashiwag H**. (2001). Chem. Phys. Lett., **34**1, 645-651.
9. **Stoylov S.** (1991). Colloid Electro-optics – theory, techniques, applications, Colloid Science - Series of Monographs 129, Academic Press Ltd, p. 120
10. **Tombacz E., Szekeres M.** (2004). Appl. Clay Sci., **27,** 75– 94.
11. **Zhou J., Zheng J., Jiang S.,** (2004). J. Phys. Chem., **108,** 17418-17424.