

ELECTRONIC-CONFORMATIONAL INTERACTIONS IN BIOPOLYMERS

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SUMMARY

Specific features of biopolymers which determine their functionality, in particular the catalytic activity of enzymes, are discussed. Electronic-conformational interactions (ECI) are responsible for the most important properties of biopolymers. A survey of theoretical work is given, devoted to ECI, electron and atom transfer in enzymatic reactions. The qualitative methods of quantum chemistry are described, allowing to establish the advantageous conformations of molecular complexes corresponding to their electronic states. The conformations and the ways of their transformations are treated using limiting molecular orbitals. The results of experimental investigations of ECI in hemoproteins are presented, using methods based on the Faraday effect (dispersion of magnetic rotation and magnetic circular dichroism), and on the electronic paramagnetic resonance of the spin-labeled proteins.

1. INTRODUCTION

This paper is devoted to the specificity of biopolymers. Which are the physical mechanisms responsible for biological functionality of proteins and nucleic acids? Which features of the structure of biopolymers determine these mechanisms?

These problems have been already investigated during many years. It is appropriate now to sum up the various ideas and concepts used in their solution.

Biopolymers consist of macromolecules. It is necessary to establish the universal, general properties of macromolecules independent on their special structure. Two decades ago the group of theoretical physicists which I headed in Leningrad, developed the statistical physics of macromolecules. Because of the internal rotations around the single bonds in a polymeric chain, the free macromolecules in solution form loose statistical coils (Fig. 1). These internal rotations change continuously the conformations of the monomeric links and of the polymeric chain as a whole. Every link can exist in the states of several conformers or rotamers. The rotations in the neighbouring links

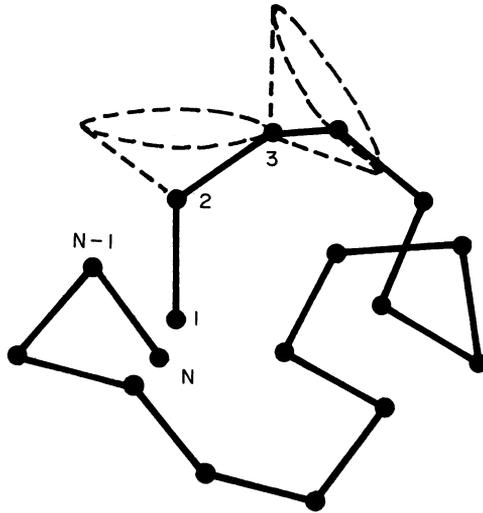


Figure 1

are not independent, and the macromolecule is a cooperative system. On the basis of these ideas, it is possible to evaluate the dimensions of the macromolecular coils, their dipole moments, anisotropic polarizabilities, optical activity, etc. in good agreement with experiment. The stretching of rubber and the entropic nature of its high elasticity is found to be due to the rotamerization of the links, *i.e.* to their conformational transformations. The solution of these problems is performed with the help of the contemporary methods of theoretical physics, by averaging the necessary parameters over all conformations taking into account the cooperativity. This theory has been presented in two monographs [1,2] (cf. also [3]). Later the statistical theory of macromolecules has been largely perfected and supplemented in the outstanding book of Flory [4].

If there exists some mutual attraction of the links of the chain, defined by their properties or by the properties of the solvent, the polymeric coil condenses into a globule. The corresponding theory has been developed by Lifshitz and his coworkers [5-8]. The macromolecule possesses "linear memory" - the monomers are linked in the chain and localized sequentially. Therefore the links are distinguishable, every one of them possesses a definite number in the chain and mutual replacements of links require the breakage of the chemical bonds. Therefore the chain is not in an equilibrium state. These features determine the macroscopic character of the fluctuations of the coil. Forming a globule, the macromolecule turns into a condensed state and obtains a specific structure. The globule is found to be formed by a solid crystal-like core, surrounded by the motile "liquid" surface layer (Fig. 2). In the papers [6-8] different phase states of the globule have been investigated.

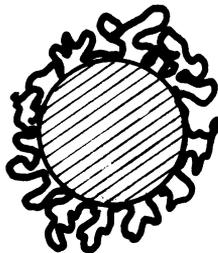


Figure 2

Thus the general geometrical and physical properties of the macromolecules are determined mainly by the conformational motility of the links of the chain. The change of conformation requires a much smaller energy than the stretching of the valence bonds or the deformation of the valence angles. This energy is of the same order of magnitude as the thermal one and at conventional temperatures the macromolecule undergoes conformational transitions. The second feature is the important role of the entropic factor in the behavior of the macromolecules. Thus, the changes of the enthalpy in the process of stretching of rubber are small, and the changes of entropy are great.

These general properties of macromolecules must be inherent to biopolymers also, at least under some definite conditions. But the biopolymers possess important features which are absent in synthetic polymers. These features are (cf. [4]) :

a. Biopolymers are heterogeneous systems with definite sequences of links. They are informational macromolecules, forming "texts" written by 4- or 20-letter alphabets.

b. Side by side with the weak (*i.e.* non-chemical) interactions responsible for the potential energy of internal rotation, in the chains of biopolymers exist weak interactions (hydrogen bonds etc), which stabilize a definite secondary structure of the protein or nucleic acid (Fig. 3). Hence, the native biopolymer is not a statistical coil which can be formed only by denaturation, by transitions helix-coil, globule-coil.

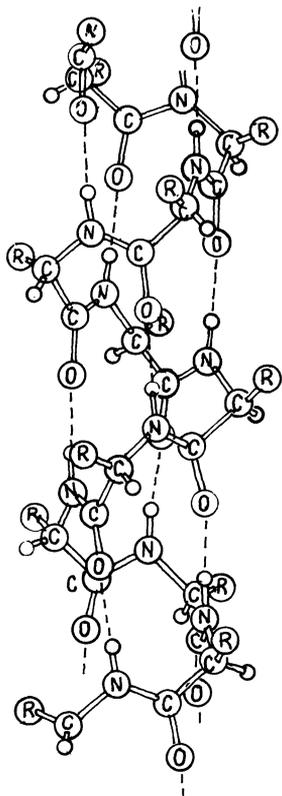


Figure 3a :
Secondary structure
of a protein

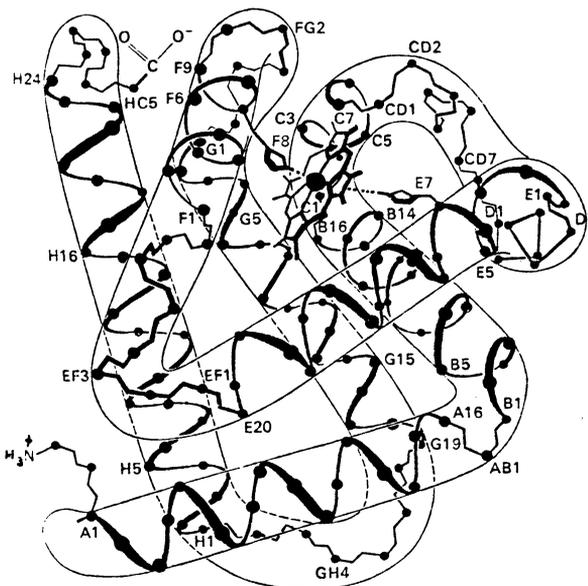


Figure 3b :
Tertiary structure
of myoglobin

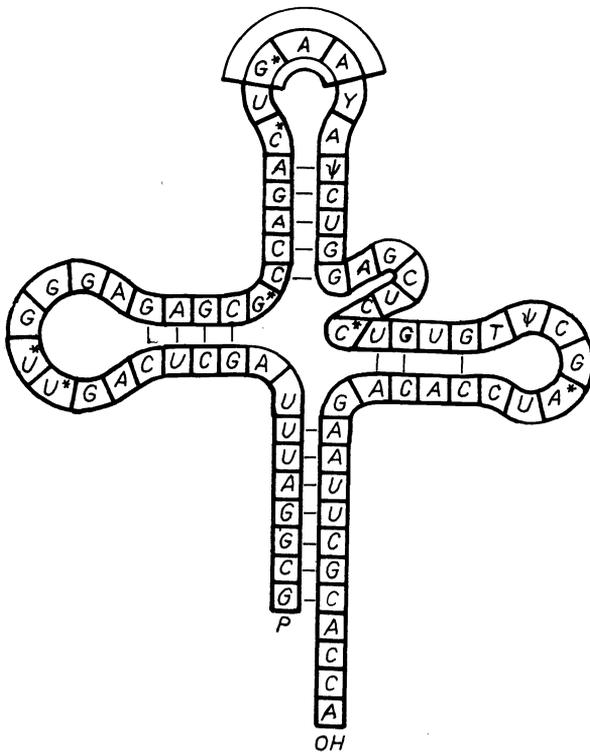


Figure 3c :
Secondary structure
of Phe-tRNA

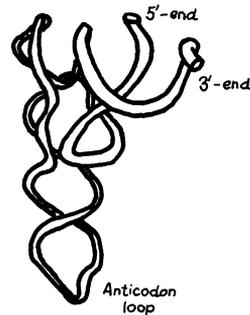


Figure 3d :
Tertiary structure
of a t-RNA

c. In many cases (globular proteins, tRNA) the weak interactions of links form tertiary and quaternary native structures. The globule is an aperiodic crystal, possessing limited conformational motility.

Synthetic polymers are used for their valuable mechanical, dielectric, ionic-exchange properties. Biological functionality of nucleic acids and proteins is connected mainly with their chemical properties. DNA and mRNA serve as templates for biosynthesis ; the most important function of proteins is their participation as enzymes in homogeneous catalysis. In both cases the initial stage of the chemical process occurring at the template or at the globular catalyst is the mutual recognition of the interacting molecules, *i.e.* their multipoint interaction realized by the weak forces.

Therefore the understanding of the behavior of biopolymers must be based on the connection of the conformational properties inherent to the macromolecules, and of the special chemical properties of informational macromolecules which possess some fixed secondary, tertiary and sometimes quaternary structure. The investigation of biopolymers is directly connected with the study of the electronic-conformational interactions (ECI), *i.e.* of the interactions of these two kinds of degrees of freedom (cf. [9-11]). ECI is the subject of this paper.

The important difference between the protein globule and the polymeric coil is the dynamic organization of the globule and statistical character of the coil. The protein globule is a dynamic system, whose elements (*i.e.* amino-acidic residues) possess definite positions and perform definite functions consistent with the behavior of other elements. The protein molecule is

therefore similar to a machine. Evidently these dynamic properties arose in the course of evolution, both pre-biological and biological. The machine-like, dynamic behavior of the globule is the cause of great difficulties of its theoretical treatment. These difficulties bear on fundamental principles as they touch upon the most general problems of theoretical physics - the problems of relation of dynamics and statistics.

2. PHYSICAL MODELS OF ENZYMATIC PROCESSES

The electronic-conformational interactions must be considered as the basic mechanisms of enzymatic activity. We have to distinguish between the oxidative-reductive enzymes which serve directly for the electron transfer (cytochromes, etc), and enzymes, acting as catalysts of chemical transformation of some substrates, *i.e.* the transfer of atoms. In the first case direct electron transfer occurs, changing for example, the charge of the iron atom of the heme group. In the second case the electron transfer does not occur but as the substrate molecule bound at the active site of enzyme produces a perturbing action at the atoms of this site, changes of their electronic states occur. It is actually a perturbation because the substrate is bound by weak interactions. As the same weak interactions are responsible for the potential surfaces of internal rotations, the change of interactions during the formation of the enzyme-substrate complex (ESC) produces a change of conformation of the enzyme or of the substrate or of both of them. These ideas have been formulated and argued by Koshland [12,13]. Hence, the conformational transitions in ESC provoke a change of the electronic density, which in its turn determine new changes of conformational states. The idea of ECI becomes quite obvious.

There exists a series of attempts to interpret ECI in a qualitative way. The concept of "rack" suggested by Lumry [14,12] is based on ECI. The "rack" means the stress of the substrate molecule in ESC. A stressed, entatic state of the active sites of a series of metalloenzymes has been suggested by Vallee [15]. The ECI can be treated using the conformon - a tentative quasi-particle similar to polaron in some respects [16]. In the physics of solids, the polaron is an electron which moves in a crystal together with the polarized state of the surrounding ions or atoms. The conformon is a cluster of electronic density which is transferred in a biopolymer together with a conformational deformation of the neighboring groups. In contrast to the polaron, the conformon cannot migrate over great distances, as its energy dissipates. The concept of ECI and of the resulting limitation of the rate of a multi-stage enzymatic reaction by the relatively slow conformational transitions are suggested also in the works of Blumenfeld [17-19].

Together with ECI other physical mechanisms of enzymatic activity were also proposed, the mechanisms of transfer of electrons and atoms. In the case of electron-transfer enzymes the tunnelling of electrons is possible through the barrier. This effect is especially important at low temperatures [18,20,21]. The consideration of tunnelling together with multi-phonon electronic transitions in a condensed medium allowed to develop a quantitative theory of the

dependence of the rate of oxidation-reduction of cytochrome-C on temperature [22].

The mechanism of the multi-phonon electronic transitions has been studied in details in the references [23-26].

The macromolecule of a biopolymer fluctuates between different conformational states. These transitions can be characterized by very long times. The nature of conformational transitions is different from usual low-frequency vibrations. The conformational transitions are more similar to chemical conversion but they are determined by weak interatomic interactions. The shift of the electronic density or the excitation of the electron can change a given conformational state and change the relative energies of different conformational states. The solution of the corresponding quantum-mechanical problem, *i.e.* the determination of the probability of the final transition substrate-product requires the knowledge of the potential surfaces of electronic states and their dependence on positions of atomic nuclei. For the non-adiabatic reactions of electron and atom transfer, *i.e.* for reactions which are accompanied by conformational shifts of nuclei, the key role is played by the Franck-Condon principle. As the motion of nuclei is much slower than that of the electrons, the probability of reaction differs from zero only if the electronic levels of the initial and final states become equal because of conformational motion. The corresponding scheme is shown in Fig. 4. Q is the conformational coordinate.

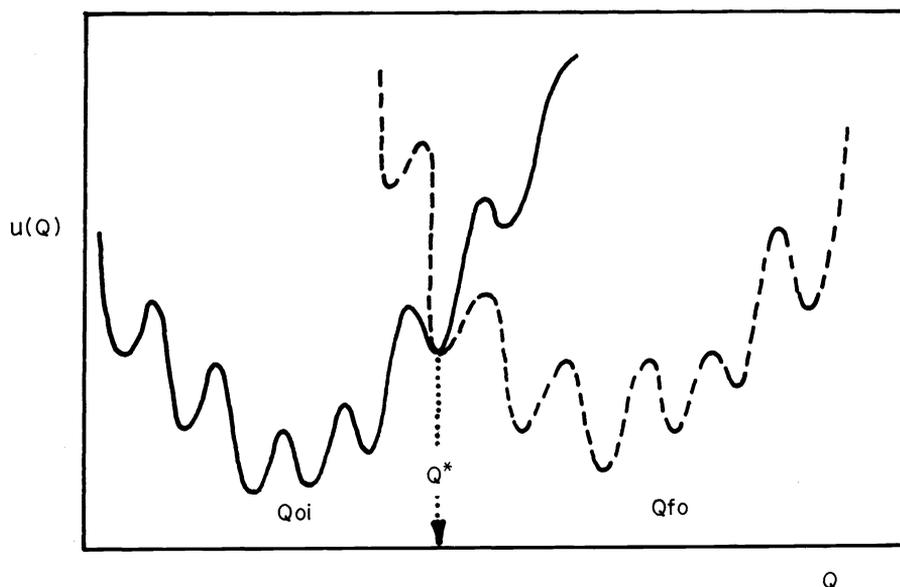


Figure 4

The electronic reorganization is preceded by the complicated motion from Q_{oi} to Q^* on the surface of potential energy. After electronic reorganization in the region of intercept of the surfaces, the system is transferred into a conformationally excited state, which differs strongly from the equilibrium state. Then occurs the relaxation of the system to the new equilibrium state, $Q^* \rightarrow Q_{of}$. The kinetics of the process is determined by ECI.

The quantitative theory of the process introduces physical parameters - the components of electric field, etc. - which are the functions of spatial coordinates. Thus, the interaction can be determined by the effective electric field due to the whole system of nuclei surrounding the active site. The biopolymeric system can be considered as linear; the changes of its internal parameters are suggested to be proportional to comparatively weak perturbations. The parameters can be represented by the sum of harmonic nuclear oscillations. The energy of the nuclear, i.e. conformational; subsystem is found in its initial and final states. The total energy of the system contains also elements representing the energy of the active site, and of the surrounding solvent whose state is also represented by a set of oscillators. The probability of transition from the initial to the final state in the unit time is found with the help of the quantum-mechanical perturbation theory. The fundamental role is played by slow oscillators, corresponding to conformational motions, and to the most part of molecular motions of the solvent. The energy of these slow oscillators is much less than the thermal one

$$\hbar\omega \ll k.T$$

These are the classical oscillators. The expression of probability of transition obtained in the papers [26,27] depends exponentially on the sum of the free energy of reaction and of the energy of reorganization of conformational subsystem and solvent. The sense of the energy of conformational reorganization is shown on Fig. 5. Q_{oi} and Q_{of} are classical

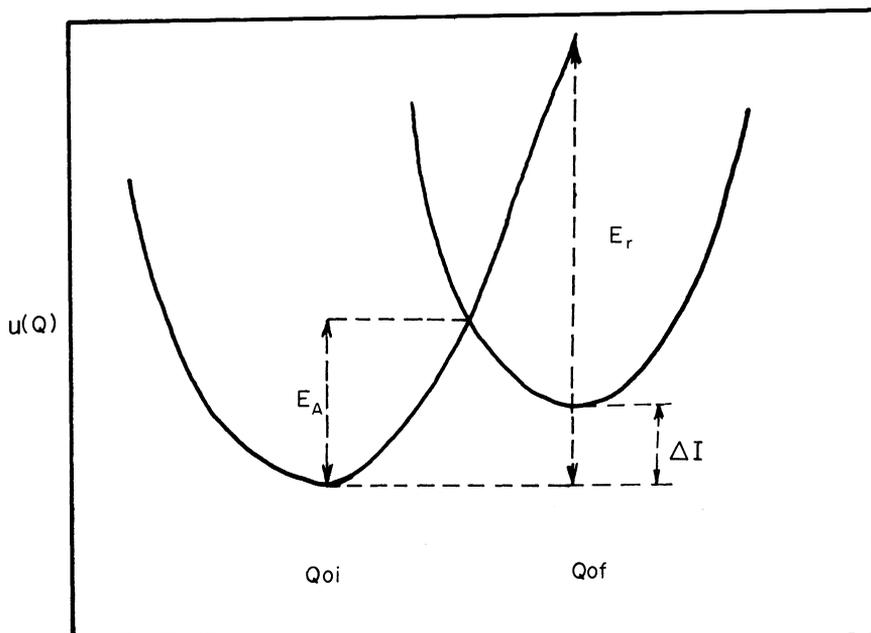


Figure 5

Q

equilibrium coordinates of the initial and final states, E_A is the activation energy, E_r - the energy of reorganization of classical modes, I - the heat of reaction.

Thus, the system moves along a series of classical coordinates and comes to the region of intercept of potential surfaces with definite probability. The expression of probability contains a factor, which has the form of Arrhenius potential barrier. The time of existence of the system in the transition region is determined by the effective frequency of motion, and by the reorganization energy. During this time the electron is transferred from the initial state into the final one with a certain probability. Similar suggestions allow to treat the dynamics of complex, multistage enzymatic processes.

The theory of Dogonadze *et al.* presented here in a short form, has a clear and rigorous physical meaning. However the calculations based on this theory are rather complicated and can be performed only in the simplest cases.

Physical enzymology now requires some well founded qualitative models, which allow to understand the sense of observed phenomena. Quantitative estimates are also necessary, in order to get the orders of magnitude of the characteristic parameters, such as the activation energy. It is important, however, to remember that the expenses for such estimations can be quite high. Therefore, the significance of the rigorous quantum-mechanical theory is not the possibility of quantitative calculations *ab initio*, but the elucidation of the physical regularities.

3. THE MODELING OF ECI BY POTENTIAL BOX

Gray and Gonda suggested a simple model of interaction of electrons and nuclei [28]. In their work, an attempt is made to use this model for interpretation of the muscular contraction. This attempt is based on some arbitrary assumptions and does not give a convincing explanation of muscular contraction. At the same time the model of Gray and Gonda illustrates well the ECI.

Let us represent the atomic nuclei of the molecule or of its functional part by potential box with infinite high walls (Fig. 6). $2n$ electrons occupy n levels in the box. The values of electronic energy inside the box

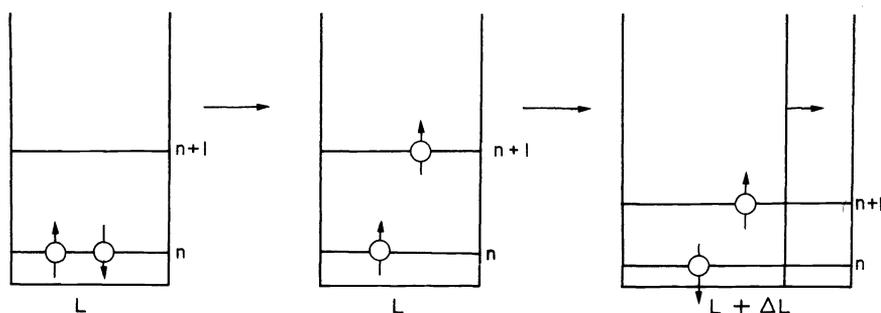


Figure 6

are calculated using the standing de Broglie waves with the knots at the walls. Let us perform some illustrative calculations. The energy of the

n-th level is

$$E_n = \pi^2 n^2 \hbar^2 / 2 m \mathcal{L}^2 \equiv a n^2 / \mathcal{L}^2 \quad (1)$$

where \mathcal{L} is the length of the box, $\hbar = 2\pi\hbar$ - Planck constant, m - the mass of electron, $n = 1, 2, \dots$ is the quantum number. The electron exercises a pressure at the walls with the force (cf. [29])

$$|f| = 2a \frac{n^2}{\mathcal{L}^3} \quad (2)$$

At equilibrium these forces are compensated by the external ones. The change of equilibrium occurs either as the result of excitation of electrons, or because of the addition of electrons. In both cases, the pressure is increased. The walls of the box are shifted into a new equilibrium position at the distance $L + \Delta L$. In other words, work is performed, and according to the formula (1) the electronic energy is lowered. The work is done by the shift of nuclei, in particular by the conformational changes.

The change of energy resulting from excitation of an electron (Fig. 6) is

$$E = E_{n+1} - E_n = \frac{a}{\mathcal{L}^2} (2n+1) \quad (3)$$

The initial (equilibrated) force produced by all $2n$ electrons is

$$f_i = \frac{4a}{\mathcal{L}^3} \sum_{j=1}^n j^2 = \frac{2}{3} \frac{a}{\mathcal{L}^3} (2n^3 + 3n^2 + n) \quad (4)$$

If an electron becomes excited and the walls are shifted by x , the force gets the value

$$f = \frac{4a}{(\mathcal{L}+x)^3} \sum_{j=1}^n j^2 + \frac{2a}{(\mathcal{L}+x)^3} \cdot (2n+1) \quad (5)$$

The work produced is

$$\begin{aligned} W &= - \int_0^{\Delta \mathcal{L}} (f - f_i) \cdot dx = \\ &= -2a \left\{ \left[2 \sum_{j=1}^n j^2 + (2n+1) \right] \cdot \int_0^{\Delta \mathcal{L}} \frac{dx}{(\mathcal{L}+x)^3} - \frac{2}{\mathcal{L}^3} \sum_{j=1}^n j^2 \Delta \mathcal{L} \right\} \end{aligned} \quad (6)$$

The efficiency of this device - of the molecular machine - is

$$\eta = \frac{W}{E} = \frac{1}{3} \gamma \cdot \left[(n^2 + n + 3) \cdot \frac{2+\gamma}{(1+\gamma)^2} + 2n(n+1) \right] \quad (7)$$

where

$$\gamma = \Delta \mathcal{L} / \mathcal{L}$$

Let us calculate now the efficiency of the machine working because of addition of an electron (Fig. 7). We get

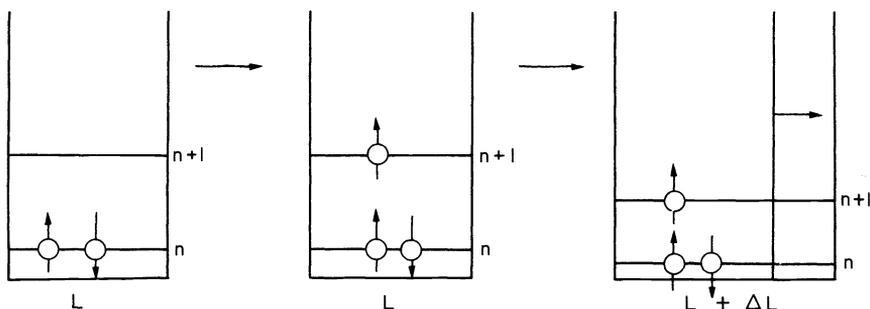


Figure 7

$$E' = E_{n+1} = \frac{a}{L^2} \cdot (n+1)^2 \quad (8)$$

$$f_i = \frac{4a}{L^3} \cdot \sum_{j=1}^n j^2 = \frac{2}{3} \cdot \frac{a}{L^3} \cdot (2n^3 + 3n^2 + n) \quad (9)$$

$$f = \frac{4a}{(L+x)^3} \cdot \sum_{j=1}^n j^2 + \frac{2a}{(L+x)^3} \cdot (n+1)^2 \quad (10)$$

and

$$\eta' = \frac{W'}{E'} = \frac{1}{3} \gamma \left[\left(2n - \frac{n}{n+1} + 3 \right) \frac{2+\gamma}{(1+\gamma)^2} + 2n \left(2 - \frac{1}{n+1} \right) \right] \quad (11)$$

The first machine is more efficient than the second one. The excitation of the electron can perform larger conformational changes than the addition of the electron.

This result agrees with the fact that the conformational changes can be observed in many cases of the ESC formation, but not in the case of the change of charge of heme in cytochrome C. The X-ray studies do not show any difference between the oxidized and reduced cytochrome-C [30].

This calculation is of course purely illustrative. The model of the potential box is not realistic because e.g. the levels in the box diverge with increasing n , and in atoms and molecules they converge. The model allows however to connect the positions of the electronic energy levels with the geometry of nuclei.

4. THE STUDIES OF ECI BY MEANS OF THE QUALITATIVE METHODS OF QUANTUM CHEMISTRY

It follows that the appearance of the stressed state of the substrate in ESC (entatic state, "rack") is determined by ECI. Which is the origin of energy of electronic excitation, converted into conformational work which lowers the activation barrier ?

Independently on the model of the process, the necessary free energy is liberated in the act of sorption of substrate by enzyme, in the act of formation of ASC. The sorption is a multi-point binding involving due mainly to weak interactions. The construction of the molecular machine provides the possibility of transformation of the energy of these weak interactions into the energy of strong chemical interactions, *i.e.* the energy of electronic excitation. In turn, the electronic excitation produces conformational work because of the change of weak interactions. The final result is a new alteration of the strong interactions due to the decrease of the activation energy of the catalyzed chemical reaction. This sequence of events is shown schematically on Fig. 8. The free energy of sorption ΔG is partly liberated

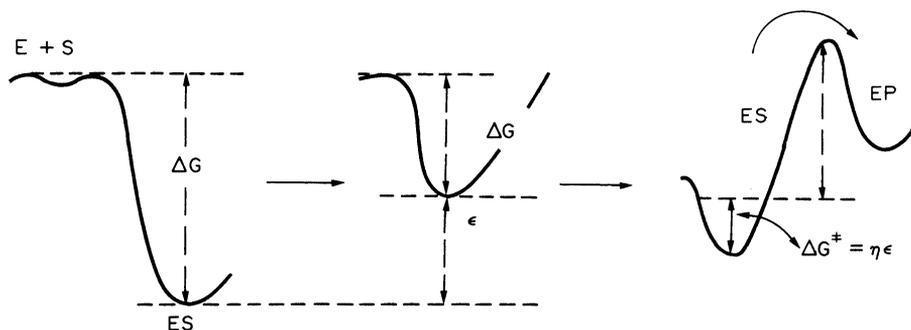


Figure 8

in the form of the observed heat of sorption $\Delta G'$, partly transformed into energy of electronic excitation E

$$\Delta G = \Delta G' + E \quad (13)$$

E is then transformed into conformational work W . A considerable part of this work is used for the effective decrease of the free energy of activation

$$\Delta G^\ddagger = \alpha \cdot W = \alpha \beta \cdot E = \alpha \cdot \beta (\Delta G - \Delta G') \quad (14)$$

$$\alpha, \beta < 1$$

The rigorous theory of these dynamic processes is, as it was said already, the theory of the multi-phonon electronic transitions. A useful simplification can be however obtained in another way.

Valuable information about ECI can be obtained by means of the qualitative methods of quantum chemistry. These methods allow to obtain an understanding of ECI, of the connection between the changes of electronic states and those of conformational states. Of course quantum chemistry gives us only static picture. However this picture explains the important features of enzymatic processes. We have the possibility to visualize the most advantageous conformations of molecules, structures of complexes, and the pathways of reactions. The qualitative methods of quantum chemistry can be used for

description of the structure of the active site of the enzyme before and after the ESC formation, for the elucidation of the mechanism of substrate activation etc. This work has been developed in the Institute of Biological Physics Acad. Sci. USSR (cf. [31-36]).

We use the molecular orbitals (MO). The MO of ESC can be represented by linear combinations of MO describing the fragments of the complex. The division of ESC into fragments is made on the basis of qualitative physical suggestions. Let us consider the simplest case - ESC contains two fragments or subunits - the active site (a) and the rest of ESC (b). The MO's are

$$\psi_i = \sum_k C_{ik}^{(a)} \cdot \psi_k^{(a)} + \sum_l C_{il}^{(b)} \cdot \psi_l^{(b)} \quad (15)$$

The alteration of energy due to ESC formation is

$$\Delta E = \Delta E_a + \Delta E_b + \Delta E_{ab} \quad (16)$$

Here ΔE_a is the change of energy at the active site calculated by means of quantum chemistry. We shall call the corresponding subsystem the electronic one, as a considerable redistribution of electronic density occurs therein. ΔE_b is the change of energy in the conformational subsystem b, where only the conformational changes occur but not the breaking or formation of chemical bonds. The energy ΔE_b required for the conformational transition of the subsystem is calculated by the methods of conformational analysis. These methods consist in the semiempirical investigations of weak interactions responsible for the differences of the energy of conformers. Finally, ΔE_{ab} expresses the energy of interaction of the electronic and conformational subsystems, i.e. the ECI energy. ΔE_{ab} contains two contributions

$$\Delta E_{ab} = E_{ab}^{orb.} + E_{ab}^{coul.} \quad (17)$$

The first contribution is the orbital one, determined by the overlapping of the electronic clouds a and b. The quantum-mechanical calculation of E_{ab}^{orb} is complicated; therefore, it is performed usually in a semiempirical way with the help of conformational analysis. The second contribution is the Coulombic, electrostatic interaction of the charges of atoms of the fragments a and b

$$E_{ab}^{coul.} = \sum_j^a \sum_k^b \frac{q_j \cdot q_k}{\epsilon \tau_{jk}} \quad (18)$$

q_j, q_k - the charges, τ_{jk} - the distance between them, ϵ - the dielectric constant.

It follows from formula (15) that in PO's of ESC the orbitals of the ground and excited states of the fragments are mixed. This fact can be used for the representation of the changes occurring in the molecules during complex formation. Let us consider a complex containing two molecules A and B. The

The intermolecular orbitals (IMO) will be mixtures of the ground and excited states of both molecules A and B. The most important role will be played by the interactions of MO's near the limiting ones, i.e. the highest occupied (HOMO), and the lowest unoccupied (LUMO) MO's [32]. For the qualitative treatment we can take into account only these MO's of the free molecules. As the result of mixing of the ground and excited states of every molecule, alteration of electronic distribution occurs. In "weak" complexes this change is small, but in the "strong" complexes, where the admixture of the excited state can be great, all the characteristics of the molecules can approach those in their excited states. At the new electronic distribution the initial nuclear configuration becomes a non-equilibrium one and the system will be transferred into a new equilibrium position. This description agrees with the model of potential box with mobile walls (part 3). Geometrical, electrical etc properties of the molecules will be changes. The corresponding states of the simple molecules CO_2 , C_2H_2 are shown on Fig. 9.

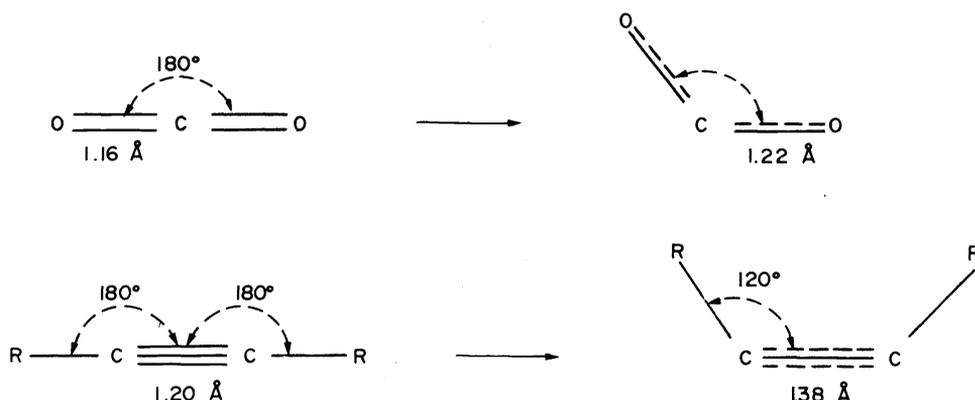


Figure 9

The fragments (molecules) can possess different mutual orientation in various complexes. For instance, in the complexes $A\dots B$ and $A\dots B\dots C$

$$E_{A\dots B} = E_A + E_B + E_{AB} \quad (19)$$

$$E_{A\dots B\dots C} = E_A + E_B + E_C + E_{AB} + E_{AC} + E_{BC}$$

the situation is possible when the contributions E_{AC} and E_{BC} are greater than E_{AB} . The mutual orientation of A and B will be determined by their interactions with the third component C. The deviation from the complex $A\dots B$ can be great. The action of the active groups of the enzyme at the substrate is concerted and this is one of the causes of the high catalytic efficiency of enzymes.

In the approximation of the limiting MO's the value E_{ab}^{orb} in expression (17) can be written as

$$E_{ab}^{\text{orb}} = C_1 \langle \text{HOMO}_a | \text{LUMO}_b \rangle^2 + C_2 \langle \text{HOMO}_b | \text{LUMO}_a \rangle^2 \quad (20)$$

HOMO_a and HOMO_b - the highest occupied MO's of subsystems a and b, LUMO_a and LUMO_b - the lowest non-occupied MO's of these subsystems, $\langle 1 \rangle$ - the superposition integrals, C_1, C_2 - coefficients.

Let us explain the use of this qualitative method by the simplest examples of the systems H₂, HeH⁺, He₂, containing 2, 2, and 4 electrons. On Fig. 10, the levels of the divided atoms, of the "complexes" and the corresponding

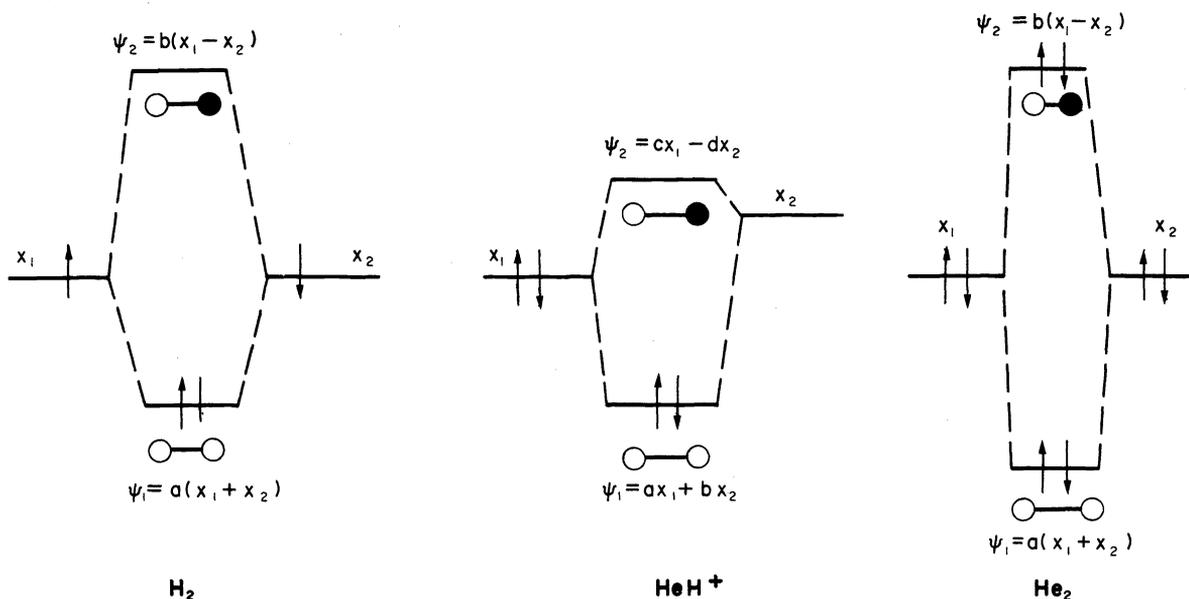


Figure 10

orbitals are shown. The interaction of two levels with two electrons stabilizes the system, and with four electrons - destabilizes the system.

The determination of the favorable nuclear configuration depending on mutual orientation of non-spherical molecules A and B implies is the evaluation of the configuration which corresponds to the minimal energy E^{orb} and (or) E^{Coul} . Some simple examples are shown at Fig. 11. We obtain the following conclusions.

- 1) The formation of a complex provides the activation of the molecules. The MO of degenerated states become mixed and the strengths of the bonds become changed.
- 2) The molecules in the complex orient in such a way that the maximal overlapping of MO occurs, i.e. the integrals $\langle \text{HOMO}_A | \text{LUMO}_B \rangle$ and $\langle \text{LUMO}_A | \text{HOMO}_B \rangle$ become maximal.
- 3) In complicated complexes which contain several components reactions forbidden for single components can become allowed.

Evidently similar suggestions can be used in the search of optimal conformations of free molecules. For example the ethane molecule is described by the set of MO's localized at the bonds CH and CC (LMO). Interaction of LMO's of these bonds does not depend on mutual orientation of CH₃-groups. However the interactions of LMO of CH-bonds of different C-atoms depend on the orientation of CH₃-groups. The LMO's of CH-bonds can be represented approximately by

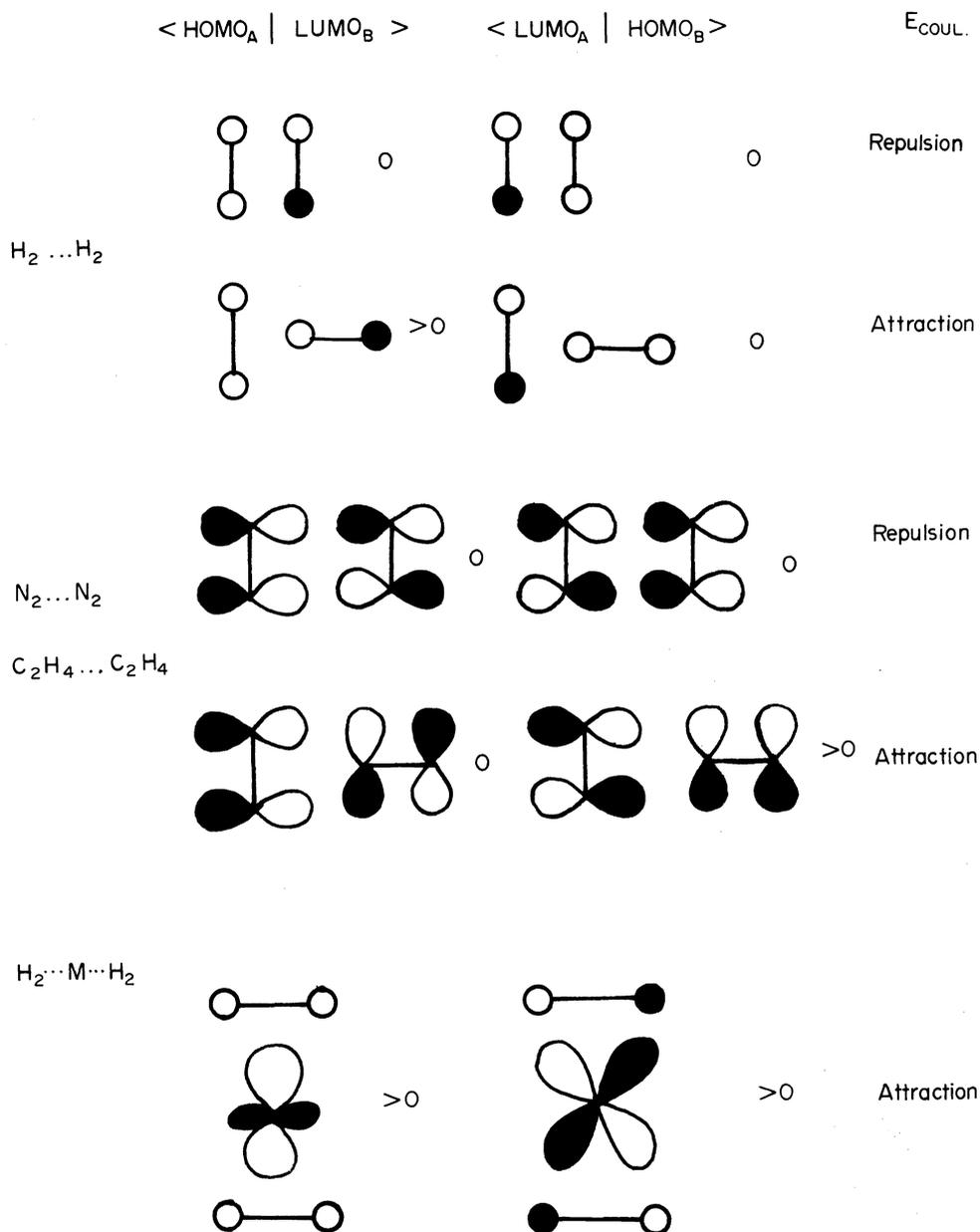


Figure 11

linear combinations of the hybrid orbitals of atom C and 1s orbital of atom H. LMO's are therefore similar to MO's in H_2 molecule (Fig. 12). Hence the most favorable localization of the bonds $\text{C}^{(1)}\text{-H}$ and $\text{C}^{(2)}\text{-H}$ is perpendicular. In other words the trans-conformation of ethane is favorable. We come to the same conclusion taking into account the interaction of the double occupied MO's of the groups CH_3 .

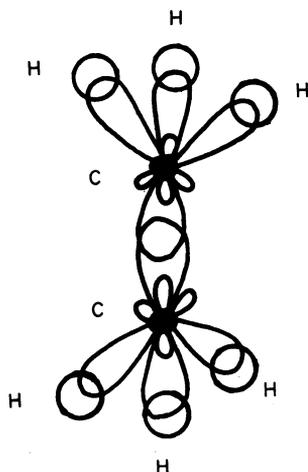


Figure 12

5. ENZYME-SUBSTRATE COMPLEXES

The problem of substrate activation in ESC can be formulated as the problem of the behavior of this molecule in the field of several ligands. The influence of perturbations produces the mixing of MO's of the ground and excited states of substrate and every ligand. The degree of mixing depends on mutual orientation of substrate and ligands. Let us consider the model of the active site of nitrogenase (NG). This enzyme catalyzes the reduction of N_2 into NH_3 . The active site of NG contains two Mo-atoms. The interaction with these atoms activates the N_2 molecule, altering the length and strength of the bond NN.

On Fig. 13 are shown the levels of energy of the higher occupied and lower non-occupied MO's of N_2 and the atom of transition metal M. The scheme

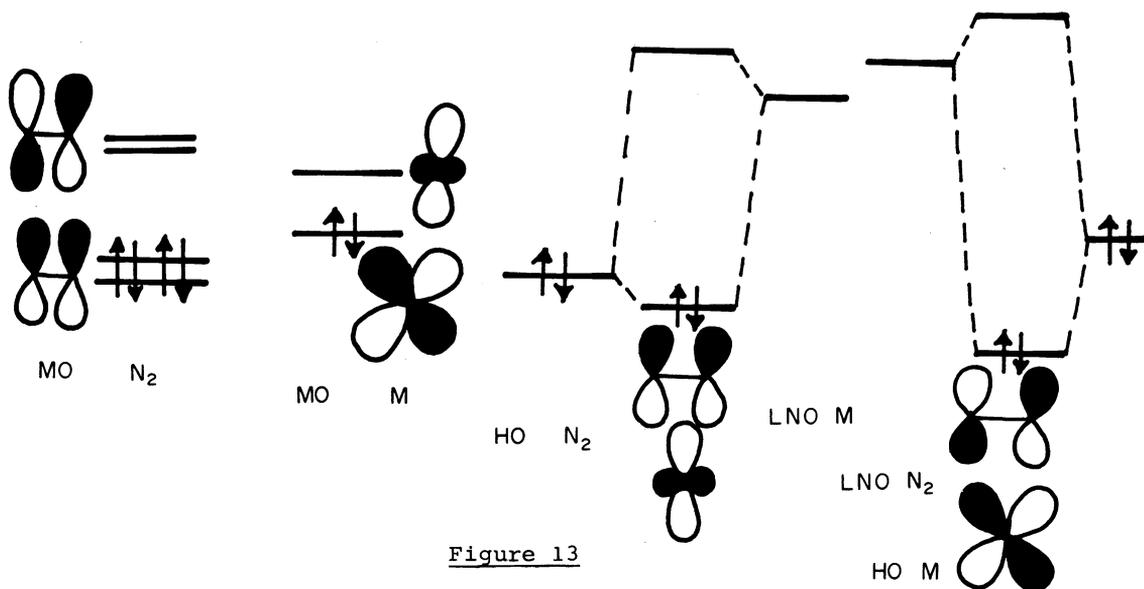
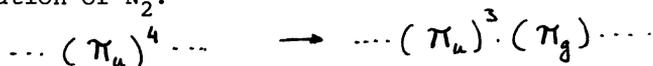


Figure 13

of interaction of the orbitals happens to be similar with that shown at Fig. 11. Two higher occupied MO's of N_2 σ_g^- and π_u have the energies very near one to another. Therefore both MO's take part in the formation of bonds with the LNO MO of atom M. The interaction of HO MO N_2 with LNO MO M decreases the population of the bonding MO N_2 , the interaction of LNO MO N_2 with HO MO M increases the population of the anti-bonding MO N_2 . This is equivalent to the excitation of N_2 .



The triple bond NN becomes transformed into the double bond, and the molecule N_2 becomes activated. The second π -bond in N_2 is also weakened if N_2 interact with two M atoms (Fig. 14). Interaction of N_2 with two M atoms is equivalent to double excitation:

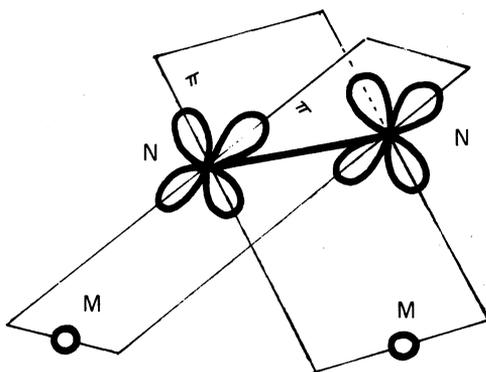
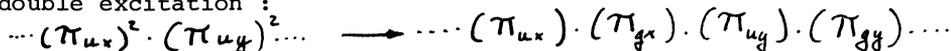


Figure 14

The bond NN becomes single. The X-ray investigations of the complexes of this type show that the bond length NN becomes 1.37 Å (for triple bond 1.10 Å, double bond 1.24 Å, single bond \sim 1.44 Å) [38].

A crude model of the active site of NG following these suggestions is shown on Fig. 15. Atoms M are bound together by the electronegative atoms X

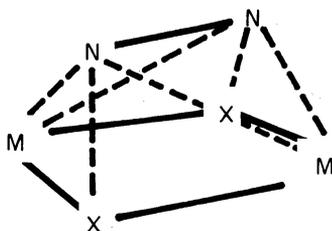


Figure 15

(oxygen or sulfur) providing also the transfer of protons to N_2 .

This model explains many experimental facts. Other molecules such as H_2C_2 , N_3^- , CH_3NC , etc are also substrates of NG. Being different, these molecules have topologically identical limiting MO's and their activation must proceed in a similar way. The reduction of acetylene into dideutero-ethylene in D_2O with help of NG gives always cis- $C_2H_2D_2$. The model actually shows that the geometry of acetylene in its excited state corresponds to cis-configuration (Fig. 16).

The calculations for the model N_2 complexes by the CNDO/2 method corroborate these qualitative pictures. The degree of MO mixing depends on the

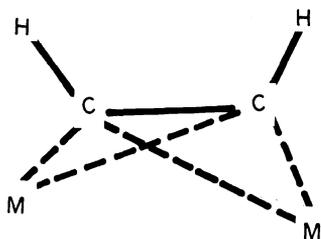


Figure 16

number of ligands, their orientations and electronic state. This determines the concerted action of the atomic groups of the active site. We obtain the possibility of determining in principle the active conformation of the active site.

As a second example, let us consider the proton transfer in enzymic reactions in which the systems with hydrogen bonds (H-bonds) are formed.

The section of the potential energy surface along the line of hydroxyl and H-bonds O-H...X is shown schematically on Fig. 17. Curve 1 corresponds

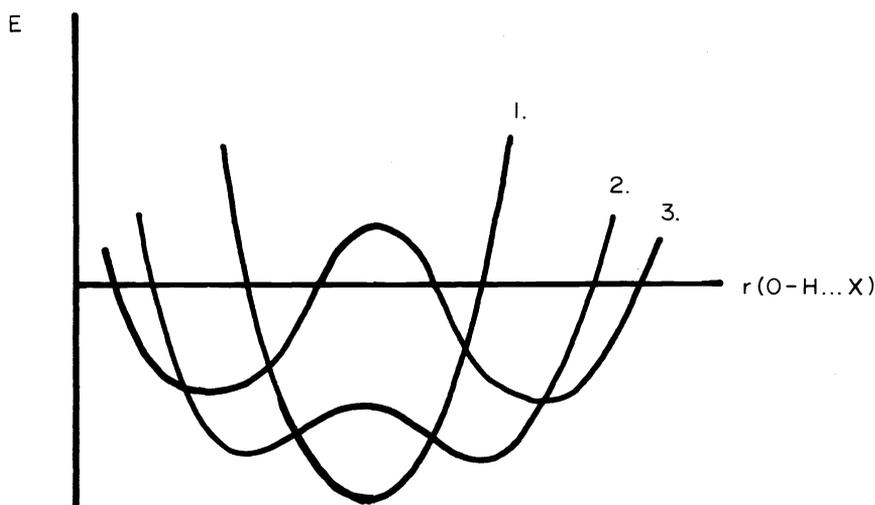


Figure 17

to the short H-bond (small O-X distance) ; the proton is localized nearly in the middle of the line O-X. Curve 2 shows the case of larger distance O-X, and we have here two minima divided by the moderate barrier. On curve 3, the distance O-X is still larger and the barrier higher. The energy of the system is minimal in the case of the short H-bond. However in this case the proton is far from the X-atom and the transfer is not realized. For the H-transfer, it is necessary to transfer the system into the conformationally excited state corresponding to curve 2. The energy of H-bond and hence the potential curves of Fig. 17 are determined mainly by the Coulombic interactions of H and X. We meet here the charge-controlled reactions.

This example shows that the transfer of the system into the conformationally excited state can actually render the potential curve smoother and accelerate the reaction. The energy required for the conformational transition can be provided by the act of sorption or can be transduced from another subsystem. The system as a whole works as a machine. The corresponding examples of proton-transfer in reactions with participation of chymotrypsin are described in paper [39] and in paper [34], devoted to hydrolysis of acetals.

The investigations based on the qualitative methods of quantum chemistry have been performed in other cases also, in particular for ESC of lysozyme with oligosaccharide [36].

6. EXPERIMENTAL INVESTIGATIONS OF ECI. THE FARADAY EFFECT

Let us consider now the experimental investigations of ECI. The studies of the complexes of apo-aspartate-aminotransferase with various coenzymes have been presented earlier [10,40]. The change of ligand implies the alteration of the electronic state of the complex. This alteration influences directly its conformational properties, and this is readily expressed in denaturation [10] and proteolysis [40].

In this paper we shall present some work in which the information concerning ECI has been obtained by methods based on the Faraday Effect and on the electronic paramagnetic resonance. The Faraday investigations have been performed in the Institute of Molecular Biology Acad. Sci. USSR, the EPR studies - in the Institute of Biophysics Acad. Sci. USSR.

The Faraday Effect - namely, the dispersion of magnetic rotation of the plane of polarization of light and the magnetic circular dichroism (MRD and MCD) - happens to be an extremely susceptible method for the studies of electronic structure and ECI in the case of heme-containing proteins. Such are the important enzymes (cytochromes, peroxydase etc), and the "honorary enzymes" - myoglobins, hemoglobins, etc. which model the formation of ESC. MRD and MCD characterize the electronic state of the system and its change under the influence of ECI much better than the electronic absorption spectra.

In the long wave length visual spectral region, the heme group possesses a band α with wave-length from 560 to 590 nm. This band corresponds to the $\pi \rightarrow \pi^*$ transition of the porphyrine ring. In a series of heme-complexes, the β -band is also observed at wave lengths 520-560 nm, which is the vibronic satellite of the α -band.

There is a direct correlation between the fine structure of the MRD and MCD spectra of heme and its protein surrounding ([41], cf. also [3]). On Fig. 10, MRD and absorption spectra of the native and modified cytochromes C are shown. In modification I, the acetate residue is linked to the S-atom of Met-80, which is the sixth ligand of Fe in the native protein, in modification II - the acetamide residue. These modifications do not influence practically the absorption spectra but in the MRD spectra clear alterations appear, which can be explained theoretically. The fine structure in band which is especially pronounced on curve 1 of Fig. 18, is hardly observable

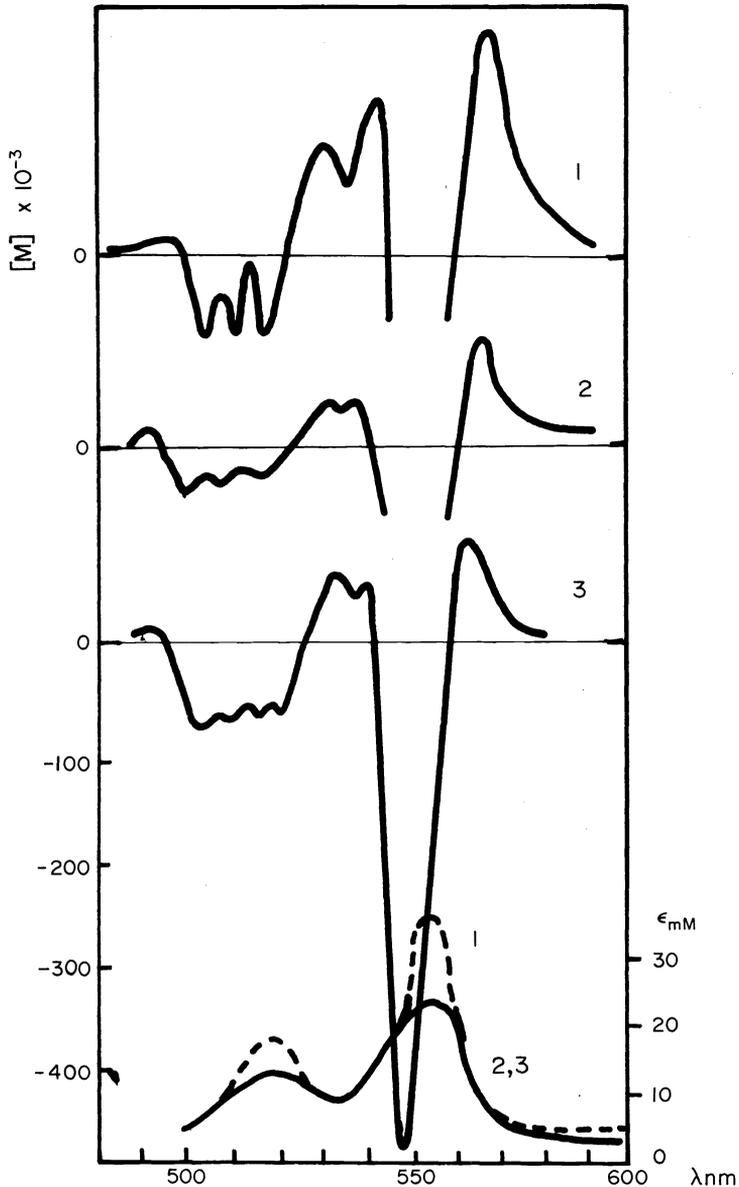


Figure 18

in the case of the heme complex with imidazole being the fifth and the sixth ligand. The investigations have been made of heme-pentapeptide and heme-nonapeptide - the segments of the cytochrome C chain with heme linked in a covalent way (Fig. 19). In the pentapeptide the fifth coordination valence of heme is occupied by imidazole of His-18, and the sixth valence is free, in the nonapeptide both these valences are occupied. On Fig. 20, MRD absorption spectra of heme (3), heme-nonapeptide (1), and heme-pentapeptide (2) are shown. The differences are considerable. The fine structure observed in the heme-nonapeptide is determined by the contacts of non-polar amino-acidic residues with the heme plane. In the case of the pentapeptide these residues are replaced by water.

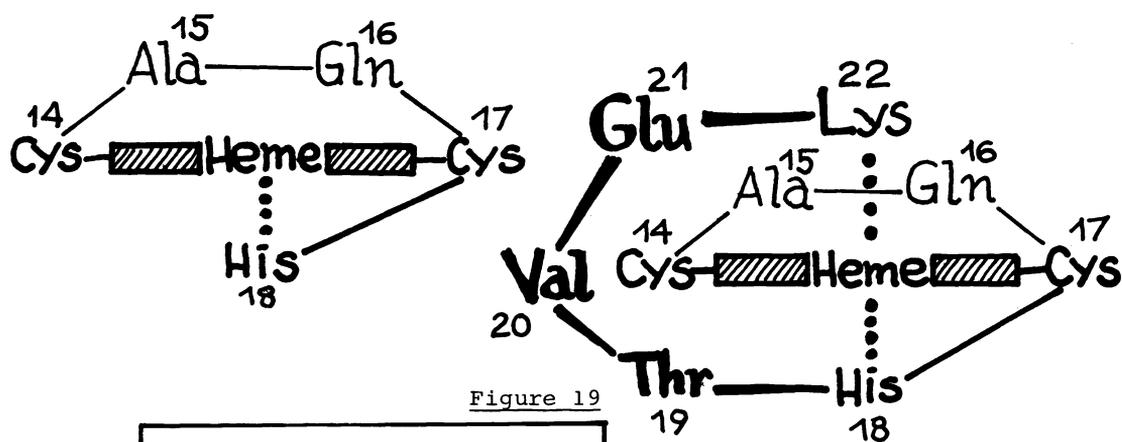


Figure 19

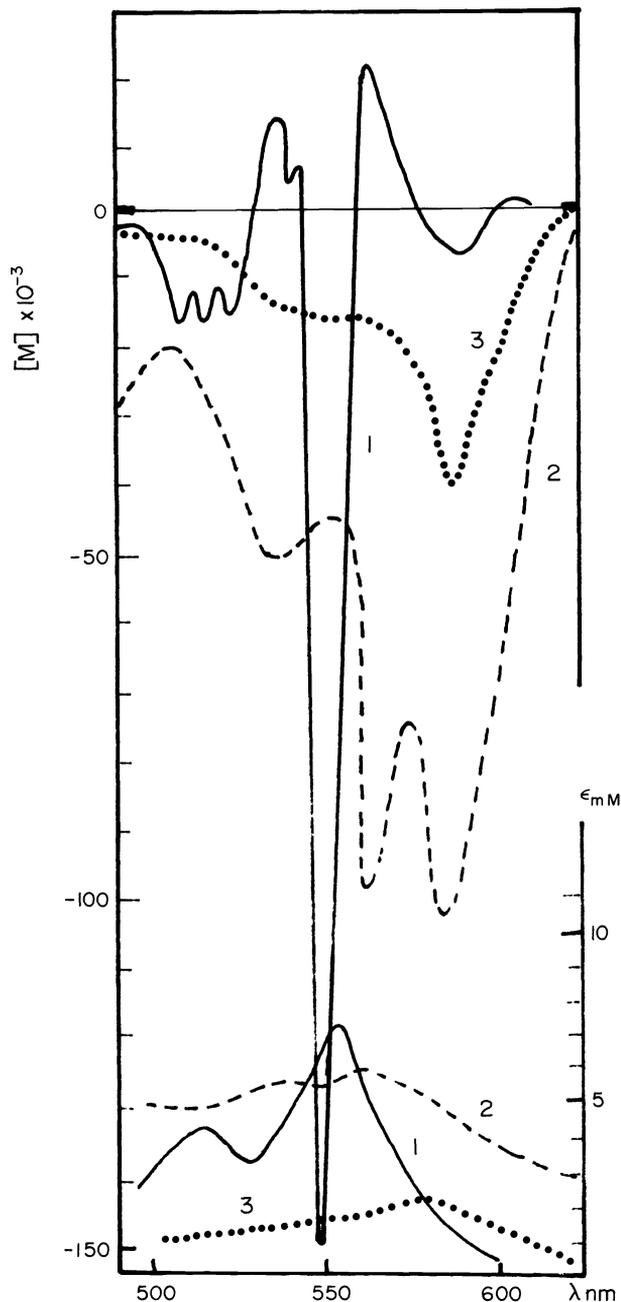


Figure 20

The curves of MRD and absorption spectra of ferro-cytochrome C and products of its modification are typical for the low-spin complexes of heme with strong axial ligands - such as imidazole. In the native cytochrome C the sixth valence of iron is occupied by the S-atom of residue Met-80, but in the modified proteins it is impossible and evidently the Met-80 is replaced by Lys-79. In the stressed, "entatic" [15] state of heme corresponding to the heme-pentapeptide with free sixth valence of iron, the MRD-curve possesses two equal minima (Fig. 20, curve 2). Similar MRD-spectrum is observed in deoxymyoglobin (Fig. 21). On the same Fig. 21, the MRD curve of

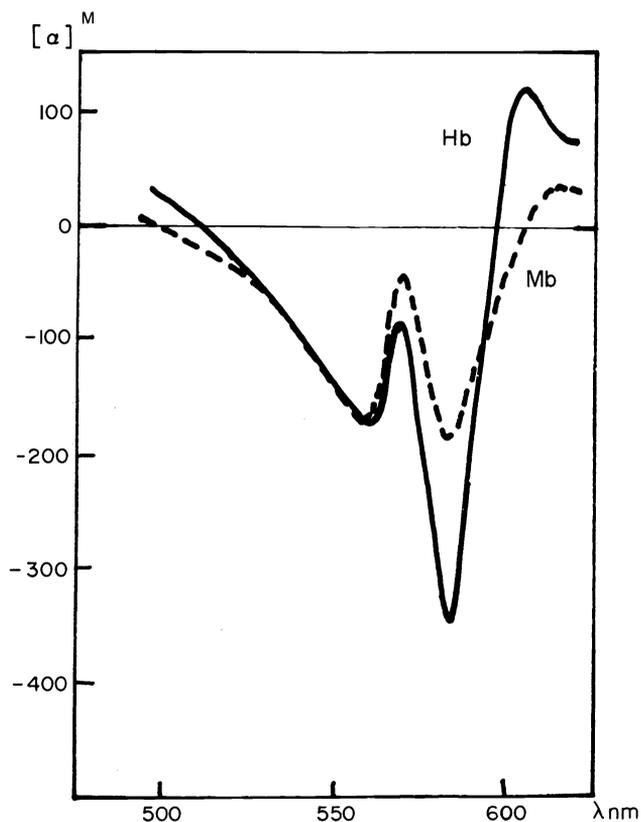


Figure 21

deoxyhemoglobin is shown (cf. [3]). The existence of the quaternary structure in the case of hemoglobin (Hb) changes the ratio of intensities of α and β MRD-bands from 1:1, typical for myoglobin, to 1:2.

Thus, the MRD in the region of the α and β absorption bands of heme is very susceptible to interaction of heme with its protein surroundings, to the quaternary structure of protein. As these interactions are determined by conformational positions of atomic groups, MRD (and MCD) of heme in hemo-proteins expresses ECI directly.

In the work of our laboratory together with Atanasov (from the Institute of Organic Chemistry of Bulgarian Academy of Sciences) the dependence

of MRD and MCD of deoxy- and methemoglobin on quaternary structure has been studied in details [42]. According to Perutz's model [43] the change of affinity of Hb towards oxygen is produced only by alterations of its quaternary structure. The cooperative properties of Hb can be described by means of the theory of indirect cooperativity of Monod, Changeux and Wyman (cf. [3]). Both Hb and HbO₂ possess two quaternary structures: T with low affinity and R with high affinity toward O₂. The equilibrium of these two structures in Hb is shifted to T, after ligand binding it is shifted to the R-state.

The following table presents the characteristics of MRD of α - and β -bands of deoxy-hemoproteins obtained in the paper [42].

MRD of hemoproteins

Protein	λ nm of β -band	λ nm of α -band	Ratio ρ of MRD intensities of α - and β -bands
Myoglobin	557	586	0.90
Leghemoglobin	556	584	0.87
Fraction III of Hb of <u>Chironomus</u>	560	578	1.00
α -chain Hb	559	582	1.10
β -chain Hb	559	585	1.05
Fraction Y of lamprey Hb	556	585	1.35
Tetrameric hemoglobins	556	586	1.85-2.00
Bis(N-maleimido- methyl)-ester-Hb	558	587	1.85
Des-Arg-N-ethyl succinimide-Hb	560	585	1.30
The same with inosi- tol hexaphosphate	559	587	1.75

Values of ρ near unity characterize a non-cooperative system with high affinity, i.e. the R-conformation. In the V-fraction of Hb of lamprey, dimers are present and the equilibrium is partly shifted towards T-form. In tetrameric Hb's ρ is near to 2.

MRD (and MCD) allow to observe different conformations of protein and their changes due to the ligand action. Higher susceptibility of these methods in comparison with, e.g. Raman-spectroscopy and Mössbauer-effect, can be explained. The last methods characterize only the ground electronic state but the differences between single chains and tetrameric Hb are expressed in the excited electronic state responsible for the Faraday Effect.

In the next paper [44] the R-T transition in Hb of carp induced by alteration of pH has been studied by means of MRD. The parameters of cooperativity could be determined from these data.

A detailed study of the MCD of high-spin ferro-derivatives of myoglobin, of tetrameric, dimeric, and monomeric hemoglobins and peroxidase has been made in our laboratory [45]. The MCD has been measured at room temperature and at the temperature of liquid nitrogen. The effects produced by the heme surroundings, by its stressed conformational state determined by the tertiary and quaternary structures of protein, have been observed. These effects are especially strong at low temperatures. On Fig. 22 the

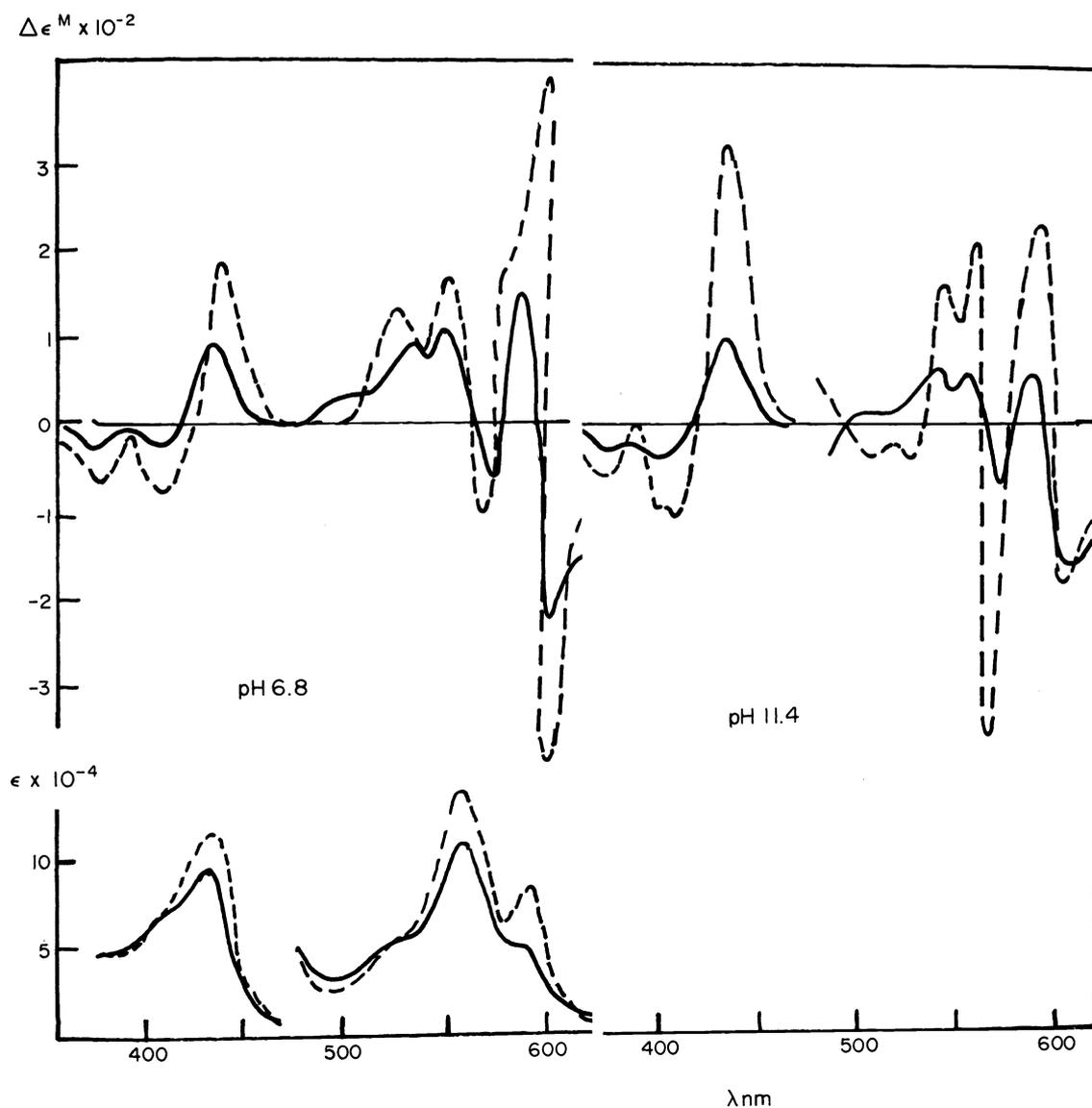


Figure 22

MCD spectra of reduced peroxidase are shown at pH 6.8 and 11.4. Two pH-dependent forms of ferropoxidase have been discovered, the neutral peroxidase with the "hemoglobin-like" MCD and the alkaline peroxidase with the "myoglobin-like" MCD spectrum.

Recently in work made together with Blumenfeld (Institute of Chemical Physics Acad. Sci. USSR), the MCD spectra of non-equilibrium states of Hb and its derivatives formed by reduction of oxidized forms of hemoproteins by thermal electrons have been studied at 77°K [46]. Mixtures of low spin and high spin ferro-forms for non-equilibrium states of these proteins have been observed as also the temperature relaxation of these systems.

The Faraday Effect being a direct expression of electronic state of heme in a hemoprotein is especially susceptible to conformational changes. Thus, MRD and MCD are good methods of ECI investigations. The increase of magnetic field will allow to solve similar problems for other, non heme-containing biopolymers.

7. EXPERIMENTAL INVESTIGATIONS OF ECI. THE USE OF SPIN-LABELS

Hemoproteins have been studied also with the help of the spin-label and EPR spectra. In work made together with B. Atanasov (Bulgarian Acad. Sci.), myoglobin has been investigated [47]. The selective modification of His A10 by spin-label on the base of bromoacetate has been performed. The EPR spectra show that the spin-label at His A10 of metmyoglobin expresses the pH-induced conformational transitions with pK 7.75 and 9.9 produced by ionization of the α -amino-group of N-end Val and of the ϵ -amino-group of Lys H9 which participate in formation of the N-end salt cluster together with the carboxyl group of Glu A4.

In the case of the low-spin cyanide complex of myoglobin the pK's of observed transitions are 7.4 and 9.4. The alteration of pK shows that the replacement of ligand connected with the change of the electronic state of heme changes the conformation in the region of the N-end salt cluster.

On Fig. 23, the pH dependence of mobility of the spin-label at His A10 is shown, representing the observed conformational transitions.

Similar conformational changes have been observed in the monomer fraction of Hb from larvae of Chironomus with the help of selective modification of His G19 by the same label.

In paper [48] results are presented of the study of the effects of conformational changes at the N-end of Mb produced by chemical modification of α -amino-group by methylisocyanate and fluorescein-isothiocyanate.

The absorption spectra of myoglobin derivatives modified by isothiocyanate reagents have shown a moderate shift of equilibrium of metmyoglobin conformers towards the low spin complex. The spectrophotometric titration of the modified Mb derivatives in the Soret band at pH 6.5-11.0 has shown a shift of pK of ionization of water bound in the sixth position to 8.5 in the case of methylisocyanate derivative and to 8.3 in the case of phenylisothiocyanate derivative of Mb in comparison with 8.9 for the original protein.

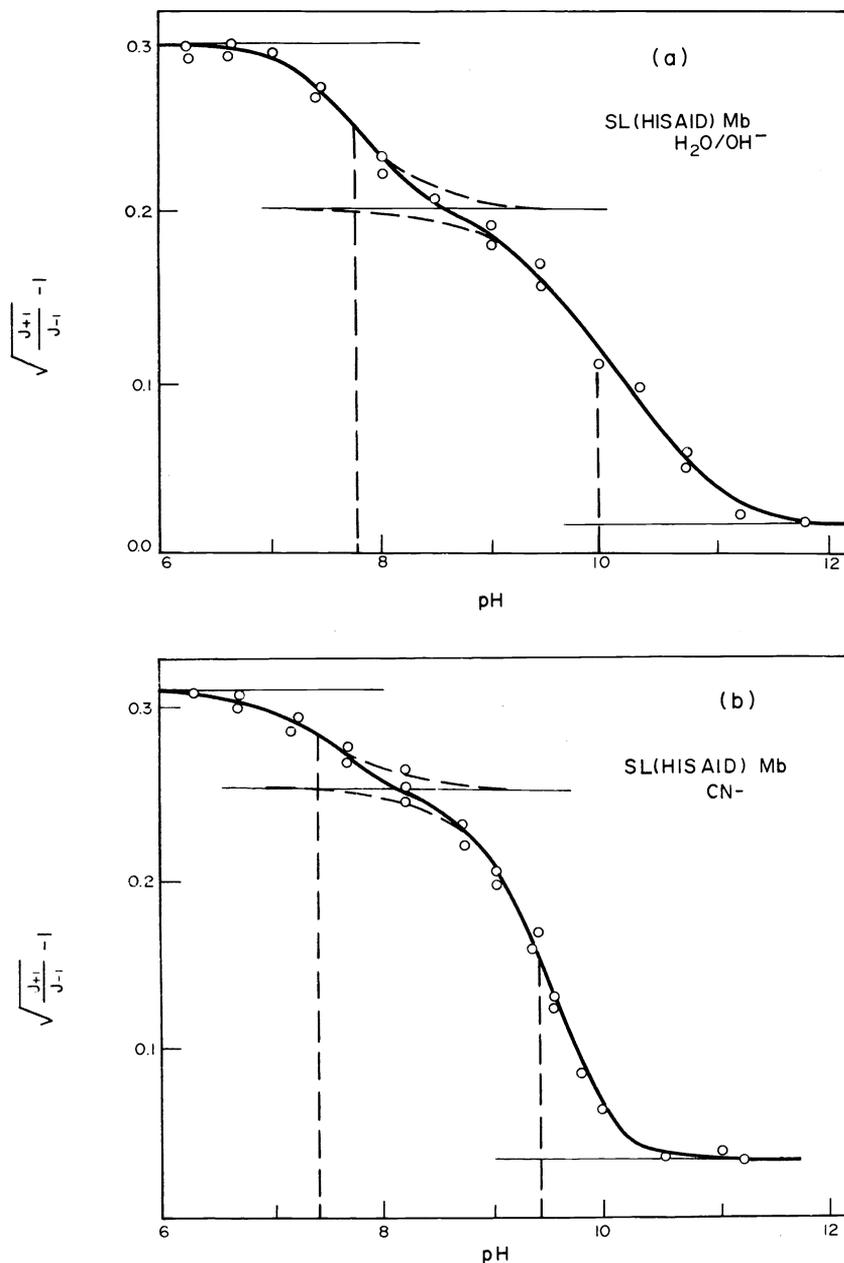


Figure 23

The modified Mb derivatives in deoxy-form have not shown any differences of spectral properties from the original protein. Hence, the modification of α -amino-group does not disturb the orientation and the contacts of the heme group.

All these data show that the change of conformation at the N-end of Mb, influences the surroundings of the sixth ligand of Fe and therefore the spin-state of heme. The change of this state determines the conformational transition at the N-end of the molecule. A directional ECI in Mb has been observed.

The comparison of these data with the recent data of X-ray studies of met- and deoxy-Mb [49] suggests that the transmission of the influence of change of the heme electronic state at the conformation in the region of the N-end and vice-versa is due to the shift of the ABCDE-fragment of Mb as a whole, relative to the GH-fragment.

This paper shows that contemporary biochemistry and biophysics possess many theoretical and experimental methods of investigation of ECI. These interactions are of fundamental importance for enzymatic activity and other properties of biopolymers. The beginning of the studies of ECI seems to be promising, it means a new approach towards understanding of behavior of proteins and nucleic acids.

At the end of this paper, I want to emphasize the importance of ECI studies in two fields of knowledge.

ECI must play a prominent role in any macromolecules, not only in biopolymers. ECI can determine the connection of the chemical reactivity of an atomic group of a polymer with the conformational lability of the site of the macromolecule containing this group. Such connection has been really established in the works of Anufrieva, Krakoviak et al. performed at the Institute of High-molecular compounds Acad. Sci. USSR in Leningrad (cf. [50-56]). The kinetic conformational mobility is studied by means of polarized luminiscence of corresponding labels. The reactivity can be expressed particularly as the percentage of the chemically transformed groups. High reactivity correlates with high conformational mobility. Further investigations in the field of macromolecular chemistry based on the notions of ECI are very important.

Especially interesting are the studies of the complexes of biopolymers with metals, particularly with the transient metals. Metals are the cofactors of a multitude of enzymes. Transition metals are very convenient and sensitive labels for ECI studies as they possess specific spectral properties.

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