ELECTRONIC–CONFORMATIONAL INTERACTIONS IN PROTEINS

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ABSTRACT

The functionality of biopolymers and of the supermolecular structures in biology depends strongly on the interactions between electronic excitations and the shifts of the electronic density with the conformational changes in these systems. The study of the electronic–conformational interactions can help to form the physical theory of enzymatic activity and of the other biomolecular phenomena. The concept of the conformon can be introduced for the theoretical description of the electronic–conformational interactions, describing a kind of the quasi-particle containing the shift of the electronic density and the conformational changes. The direct experimental investigations of the electronic–conformational interactions were performed with the systems apo-aspartate-amino transferase with a series of different coenzymes. The denaturation of such systems (i.e. their conformational properties) depends strongly on the kind of the electronic interactions at the active site.

INTRODUCTION

The living organism and its functional systems including the molecules of proteins and nucleic acids are complex chemical machines characterized by the self-consistent regular and regulated behaviour of their elements. The main functions of biological systems are chemical, i.e. participation—primarily the catalytic one—in electronic transformations, and the realization of the electronic and ionic motions. In this sense the theoretical physical treatment of biomolecular systems and particularly of proteins has to be based on quantum mechanics.

The motion of the electrons in a molecule is coupled with the nuclear motion. The nuclear movements in biopolymers are specific—the lowest energy is required for the rotations of the atomic groups around single bonds, i.e. for conformational changes. The peculiarities of the structure and properties of the protein molecules are determined mainly by their conformational motility. The conformational and electronic motions of a protein molecule can be separated as the shifts of the nuclei are much slower than the electronic transitions. The cause of this separability is the same as in the theory of vibrational molecular spectra—the Born–Oppenheimer theorem. Therefore the physical theory of biopolymers, the theory of proteins, has to be based on investigation of the electronic–conformational interactions (ECI)[1]. The analysis of ECI must lead to an explanation of the main features of enzyme catalysis. On the other hand the behaviour of contractile

proteins responsible for biological mechanochemical processes such as muscular contraction, follows also from transconformations produced by some electrochemical phenomena. ECI build the fundamentals of mechanochemistry.

In this paper we shall deal mainly with enzyme catalysis.

Koshland's theory, which has played a great role in the development of the modern theoretical treatment of enzymes, supports the great importance of transconformations for the electronic (i.e. chemical) processes catalysed by enzymes. However, this theory considers only the static pattern of the induced structural fit of enzyme and substrate but not the dynamical phenomena which determine the formation and further fate of the enzyme–substrate complex.

How can we approach the treatment of these dynamical phenomena on the basis of ECI?

Five years ago Perutz wrote that the enzyme acts not only as a reagent but also as a peculiar medium of reaction[2]. Undoubtedly, this is sound reasoning. The study of ECI needs the treatment of dynamical properties of such a medium possessing conformational motility. The theory of enzyme catalysis must be developed as a theory of chemical reactions occurring in specific condensed media characterized by conformational dynamics.

It is clear that the 'gaseous' theory of Eyring which takes into account the properties of a medium only via its macroscopic constant—the dielectric constant—is not sufficient for the dynamical treatment of ECI. In the works of Marcus[3] and in later more accurate studies of the problem[4, 5], the theory of an electronic exchange between ions in a condensed polar medium was developed. It is shown that all kinds of motions of particles taking part in the reaction have to be divided into classical (with small vibration frequencies $\omega \ll kT/\hbar$) and quantum-mechanical (with $\omega \gg kT/\hbar$) systems. The activation energy of the process is defined by the height of the barrier for the classical subsystem and the pre-exponential factor in the rate expression—by the tunnelling motion of the quantum subsystem below the barrier. The dynamical behaviour of the non-polar proteinic medium can be described in a harmonic approximation in terms of a set of acoustical vibrations, of the phonons. The probability of an elementary reaction act will be the sum of the products of the probability for the system to exist in a definite electronic and conformational state and the probability of transition of the system into another state. The consideration of both kinds of motions results in the change of the energetic map of the reaction compared with the purely electronic process. Here we have to look for the theoretical explanation of the complementarity of chemical and conformational energy suggested by Lumry and Biltonen[6].

The essential features of a non-polar protein medium are, first, the high entropy changes in the course of reaction. These are determined just by the pronounced acoustical nuclear motions. The second feature is that because of the small values of these acoustical frequencies the electronic subsystem follows the nuclear one adiabatically and the transmission coefficient is equal to unity[7].

We need of course not only the phenomenological general theory but also the theory of definite enzymatic processes taking into account the real

transconformations and corresponding relaxation times (i.e. frequencies). These times can be determined with the help of the relaxational methods of chemical kinetics. The model of ECI which describes satisfactorily a lot of facts was suggested by Perutz for haemoglobin oxygenation[8].

On the basis of the general phenomenological description of ECI, a theory for the multicentrum catalysis was developed using the bridge mechanism[9]. The models of oxidative phosphorylation which are based on the same principles were proposed in other papers[10–12]. The quantum mechanical calculations of the electronic exchange in cytochrome, taking into account the dynamics of conformational motions, were recently performed by Madumarov[13].

The physical meaning of ECI is that the electronic transformation in the course of an enzymatic process determines the conformational changes in the macromolecule. Such a situation can be described in terms of modern solid state physics. The shift of an electron or of the electronic density in the macromolecule produces the deformation of the 'lattice', i.e. the conformational changes. It can be treated as the excitation of the long-wave phonons and the system electron plus conformational deformation of the macromolecule becomes like a polaron. Let us call such a system 'the conformon'[14].

The conformon is different from the polaron because the biopolymeric molecule lacks periodicity and homogeneity. Therefore the conformon is not a real quasi-particle. which can move far—its energy rapidly dissipates. However, for the realization of an enzymatic process the conformational change, i.e. the phonon excitation in the range of some peptidic bonds, is quite sufficient. The further transconformation is determined not by ECI but by the cooperative conformational movement of the nuclei in the macromolecule.

It seems natural to use the notion of the conformon for the study of the semi-conductive properties of biopolymers. However, the existence of such properties and their biological importance cannot be considered to be firmly established. Theory and experiment contradict the existence of semiconduction in proteins and nucleic acids. The observed conduction can be determined by some ionic contamination. It is difficult to imagine that noncoloured substances such as proteins and nucleic acids are electronic semiconductors.

On the other hand, ionic transport on biomembranes has to be studied, using the notion of the conformon. The physical theory of membranes can be built up with the help of the ideas from the modern physics of solids.

Let us now discuss the experimental study of ECI in the enzymatic processes.

EXPERIMENTAL STUDY OF ECI

One possible way to study ECI is the systematic investigation of changes in the chemical (electronic) properties of the protein produced by the change in the ligand or cofactor and of the simultaneous changes in the conformational behaviour of the macromolecule as a whole. The latter changes can express themselves in the course of denaturation and proteolysis. Such effects have been observed before in myoglobin[15]. The results obtained in

this work suggest that the changes in conformational properties of the protein as a whole can be produced by events occurring locally, at the active site. With the aim of studying ECI in detail we have investigated aspartate-amino transferase (AAT) with different modifications at the active site. This enzyme consists of two subunits with the molecular weight 47 000 each. The active site contains coenzyme—pyridoxal phosphate. The chemical transformations occurring on the active site of AAT during the action of enzyme were studied in detail by Braunstein and Shemyakin[16] and by Snell and co-workers[17]. Ivanov, Okina and other co-workers of our laboratory examined the denaturation in the urea solutions (pH 7.1, 0.05 M phosphate buffer) of the apoenzyme, normal holoenzyme, and of the complexes of the apoenzyme with modified coenzymes. Seven systems were studied as shown in *Figure 1*,

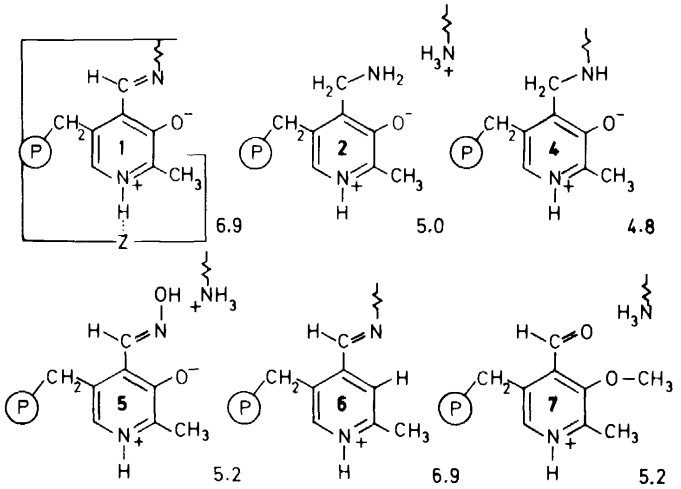

Figure 1. Modifications of the AAT active site.

where **1** is a holoenzyme with coenzyme in the aldimine form; **2** is a holoenzyme with coenzyme in the amino form (without covalent bond with ε-amino group of lysyl on the active site); **3** (not shown) is an apoenzyme, **4** is a holoenzyme treated with sodium borohydride ($NaBH_4$), i.e. with a reduced C=N bond; **5** is a holoenzyme treated with hydroxylamine (NH_2OH), i.e. with a broken bond of the coenzyme with lysyl; **6** and **7** are complexes with modified coenzymes. *Figure 1* shows the values C_m^* of the urea concentration in moles, corresponding to the middle point of the denaturation isotherm. Denaturation was observed with the help of the CD band at 222 nm which characterizes the secondary structure. The equilibrium isotherms are shown in *Figures 2–4*. The seven systems studied correspond to two kinds of curves with C_m^* values near to 5.0 and 7.0. The systems whose electronic structures are very much alike, containing correspondingly the

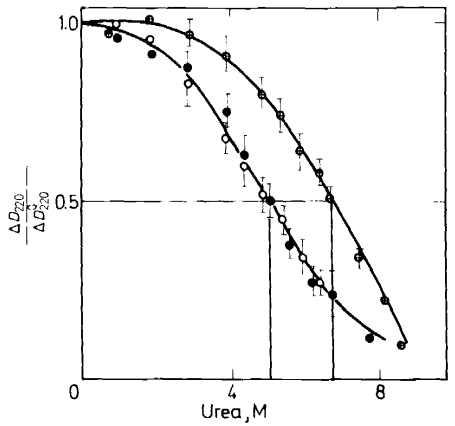

Figure 2. Denaturation curves for holo-AAR (⊕), apo-AAT (○) and holo-AAT + NH_2OH (●).

C—O—CH_3 and C—H groups, give different curves. It has been shown earlier that the —O—CH_3 group hinders the formation of the aldimine bond with lysyl[18]. The curves for the amino form and for the holoenzyme treated with $NaBH_4$ are practically the same (*Figure 4*), notwithstanding that in one case (amino form) the aldimine bond does not exist and in another case this bond is very strong.

These results show directly that the electronic changes on the active site produce a strong effect on the conformational stability of the protein.

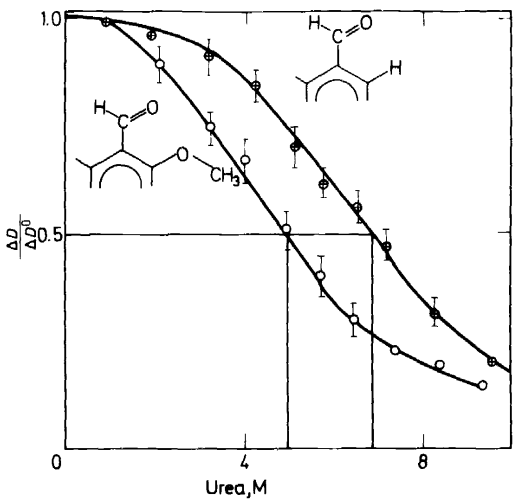

Figure 3. Denaturation isotherms for complexes of AAT with modified coenzymes.

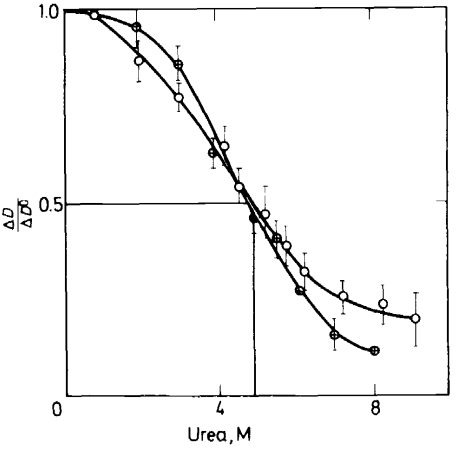

Figure 4. Denaturation isotherms for AAT treated with $NaBH_4$ (⊕) and for AAT with coenzyme in amino form (○).

The choice of AAT as the object for investigations of ECI was determined also by the multistage character of the transamination reaction catalysed by AAT. The stages of the reaction and their mechanisms have been described in detail[16, 17]. They are shown schematically in *Figure 5*. The numbers

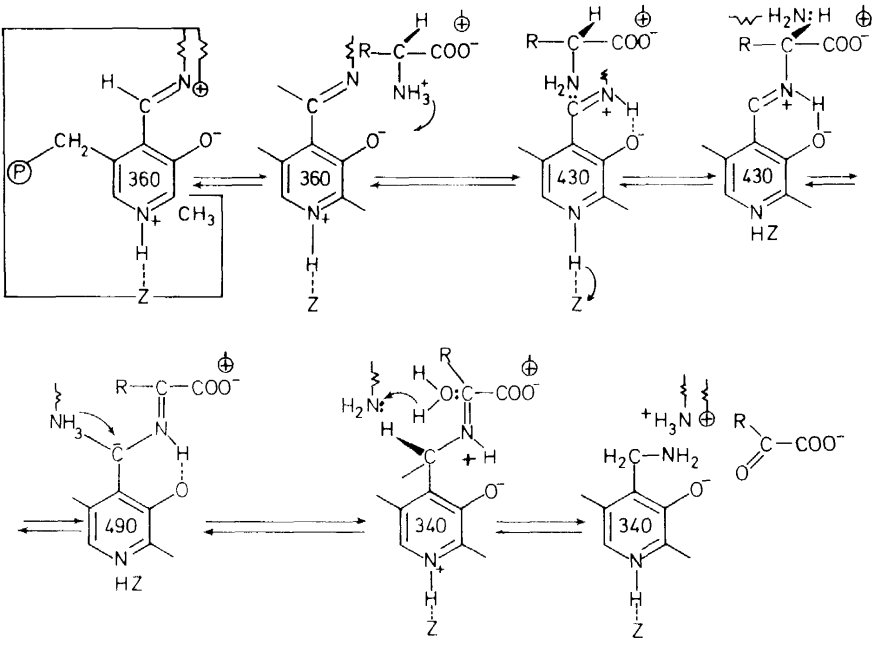

Figure 5. Enzyme-substrate intermediates of AAT.

in the rings give the wavelengths corresponding to the maxima of the absorption bands of these intermediate compounds.

An analogous reaction can occur in a congruent model system, containing a substrate, a free coenzyme and low molecular weight catalysts of proton separation and addition (i.e. imidazole). The reaction rate on such a system is lower by a factor $\sim 10^4$ than in an enzymatic one although the same intermediate chemical compounds were observed in the model system. In the model system the dominant forms correspond to absorption at 340 nm. This means that their free energy is lowest. In the enzymatic system all other forms are observed, including the form at 490 nm corresponding to the highest free energy. This means that the energetical levels of different forms do not differ much in the enzymatic system —they remain in the range of several kcal/mol. We see that the enzyme equalizes the free energy levels of different intermediate forms. Therefore the activation barriers between these forms become lower (Broensted's rule). According to the suggestion of Lumry and Biltonen[6] this equalization arises because of the complementarity of the curves of the chemical and conformational energy. It can be suggested that the changes of the conformational stability of the protein as a whole entity correlate with the free energy of the multistage process. If this is really so, then the conformational stability of different forms arising in the multistage reaction has to be approximately complementary to the chemical free energies. We used the complexes of enzyme with inhibitors which stop the reaction at different stages as models of the intermediate forms. The conformational stability of these complexes was investigated. According to Tanford[19] from the values $C_m^{\star}$ the free energy of denaturation ΔF can be derived. *Figure 6* shows as

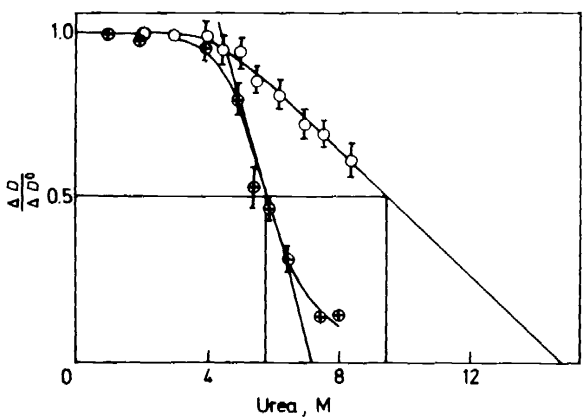

Figure 6. Denaturation isotherms of AAT + D,L-Asp (⊗) and of AAT + glutarate (○).

examples the denaturation isotherms for the complex of AAT with the substrate (corresponding to the equilibrium mixture of all intermediate forms) and for the complex of AAT with an inhibitor-glutarate modelling the sorption of the substrate. The curves differ considerably. In *Figure 7*, the

values of ΔF are shown calculated from such curves according to Tanford for different complexes as models of the intermediate forms of the multistage reaction. We see that the conformational stability is different for different stages. The forms at 340 nm possessing the lowest chemical energy in the

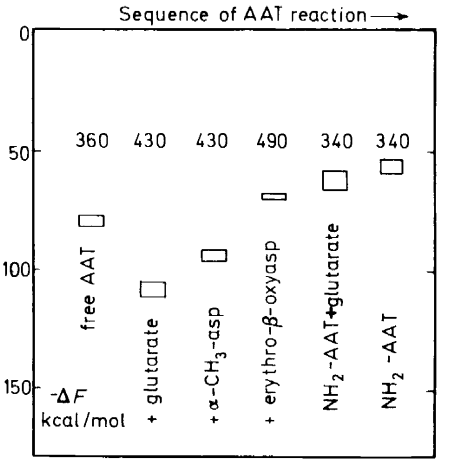

Figure 7. The free energies of denaturation of different AAT complexes.

congruent system have the highest conformational energy in correlation with the complementarity mentioned above. The exact complementarity is of course not observed as the complexes studied do not reproduce exactly the intermediate complexes of the reaction.

Let us summarize in *Table 1* all quantitative characteristics of denaturation —the $C^{\star}_{m}$(M) values, the free energy changes and tan α values for the slope of the tangent of the denaturation curve at the point $C^{\star}_{m}$.

Table 1. Denaturation in urea solution

System	C(M)	F (kcal/mol)	tan α (M^{-1})
1. Holo-AAT	6.9 ± 0.2	79 ± 2	−1.8 ± 0.4
2. Aminoform of AAT	5.0 ± 0.3	58 ± 3	− 1.3 ± 0.3
3. Apo-AAT	5.2 ± 0.3	60 ± 3	− 1.6 ± 0.4
4. AAT reduced by $NaBH_4$	4.8 ± 0.4	54 ± 4	− 1.8 ± 0.4
5. AAT + NH_2OH	5.2 ± 0.3	60 ± 3	− 2.0 ± 0.9
6. Apo-AAT + 3-deoxy-PLP	6.9 ± 0.3	80 ± 3	− 1.3 ± 0.3
7. Apo-AAT + 3-methoxy-PLP	5.1 ± 0.2	59 ± 2	− 1.4 ± 0.4
8. AAT + D,L-aspartate	5.8 ± 0.3	67 ± 3	− 3.5 ± 1.1
9. AAT + α-methyl-aspartate	8.1 ± 0.2	94 ± 2	− 1.9 ± 0.5
10. AAT + erythro-β-oxyaspartate	6.1 ± 0.1	70 ± 1	− 2.8 ± 1.0
11. AAT + glutarate	9.4 ± 0.3	108 ± 3	− 1.0 ± 0.2
12. Amino-AAT + glutarate	5.6 ± 0.4	64 ± 4	− 2.0 ± 0.6

Similar information concerning ECI can be obtained from the studies of proteolysis. Kinetics and thermodynamics of the proteolytic splitting of a protein correlates with its denaturation behaviour and characterizes the conformational properties of the whole molecule. We have obtained some preliminary results corresponding to this suggestion.

In this way we obtained some early results in our theoretical and experimental investigations of ECI. These phenomena are extremely important as they determine the physicochemical and therefore the biological properties of biopolymers.

REFERENCES

[1] M. Volkenstein. *Izvest. Akad. Nauk. SSSR, Ser. Biol.* No. 6, 805 (1971).
[2] M. Perutz. *Proc. Roy. Soc. B*, **167**, 448 (1967).
[3] R. Marcus. *J. Chem. Phys.* **24**, 966 (1956); **26**, 867 (1957).
[4] R. Dogonadze and A. Kusnetzov. *Elektrochimia*, **3**, 1324 (1967); R. Dogonadze and A. Kusnetzov. *Itogi Nauki. Elektrochimia* 1967. VINITI: Moscow (1969).
[5] R. Dogonadze, A. Kusnetzov and V. Levitch. *Dokl. Akad. Nauk SSSR*, **188**, 383 (1969).
[6] R. Lumry and R. Biltonen in *Structure and Stability of Biological Macromolecules.* Marcel Dekker: New York (1969).
[7] M. Volkenstein, R. Dogonadze, A. Madumarov, Z. Urushadze and J. Harkatz. *Molec. Biol.* **6**, 431 (1972).
[8] M. Perutz. *Nature, London*, **228**, 726 (1970).
[9] A. Madumarov. *Thesis.* Institute of Chemical Physics of the Academy of Sciences of the USSR.
[10] L. Blumenfeld and V. Koltover. *Molec. Biol.* **6**, 161 (1972).
[11] N. Chernavskaja, D. Chernavsky and Grigorov in *Vibrational Processes in Biological and Chemical Systems*, Vol. II, p 72 Pushtshino: Nauka (1971) (in Russian).
[12] D. Green. *Proc. Nat. Acad. Sci., Wash.* **67**, 544 (1970).
[13] A. Madumarov. *Molec. Biol.* in press.
[14] M. Volkenstein. *J. Theor. Biol.* **34**, 193 (1972).
[15] B. Atanasov, J. Sharonov, A. Shemelin and M. Volkenstein. *Molec. Biol.* **1**, 477 (1967).
[16] A. Braunstein and M. Shemyakin. *Biochimia*, **18**, 393 (1953).
[17] D. Metzler, M. Ikawa and E. Snell. *J. Amer. Chem. Soc.* **26**, 648 (1954).
[18] S. Mora, A. Bocharov, V. Ivanov, M. Karpeisky, O. Mamaeva and N. Stambolieva. *Molec. Biol.* **6**, 119 (1972).
[19] C. Tanford. *J. Amer. Chem. Soc.* **86**, 2050 (1964).