A DISCUSSION OF THE POSSIBILITY OF BANDS OF ENERGY LEVELS IN PROTEINS

ELECTRONIC INTERACTION IN NON BONDED SYSTEMS

by

M. G. EVANS AND J. GERGELY*

Department of Chemistry, University of Leeds (England)

INTRODUCTION

Several recent publications have pointed to the need for a mechanism of protein action which involves not only the action of specific groups and bonds but also the co-operative action of the whole structure.

SZENT-GYÖRGYI[1, 2] thinks of a protein as a metallic like structure or a semi-conductor and he and his collaborators have devised experiments on luminescence and photoconductivity to test this idea. WIRTZ[4] in discussing the transfer of energy and the action of protons on a protein structure invokes bond structures which are characteristic of the protein assembly and not of any particular protein group or chain.

Before discussing possible structures which would confer such a co-operative behaviour on a protein macromolecule, we will review some of the salient facts which seem to us to point to the necessity for a new approach to this question.

BIOLOGICAL AND OTHER EVIDENCE

The three iron containing proteins cytochrome a, b and c play an important role in the oxidation processes in the animal cell. The cytochromes are bound to the structure of the cell so that they are in effect in an oxidized or reduced state having a tri- or divalent iron atom respectively. The overall reaction can be represented as follows:

$$\begin{aligned}
\mathrm{Fe^{+2}C_a} + \mathrm{O_2} &\rightarrow \mathrm{Fe^{+3}C_a} + \mathrm{O_2^-}\\
\mathrm{Fe^{+3}C_a} + \mathrm{Fe^{+2}C_c} &\rightarrow \mathrm{Fe^{+2}C_a} + \mathrm{Fe^{+3}C_c}\\
\mathrm{Fe^{+3}C_c} + \mathrm{Fe^{+2}C_b} &\rightarrow \mathrm{Fe^{+2}C_c} + \mathrm{Fe^{+3}C_b}\\
\mathrm{Fe^{+3}C_c} + \mathrm{HR} &\rightarrow \mathrm{Fe^{+2}C_b} + \mathrm{H^+} + \mathrm{R}
\end{aligned}$$

Cytochrome a can be oxidized aerobically, and the oxidizing power thus conferred on cytochrome a is transferred through the cytochromes c and b, and the oxidized cytochrome b can then oxidize a reduced compound HR.

It has been assumed[3] that the cytochromes are localized in space at distances very much greater than those normally necessary for intermolecular reaction, which assumption would rule out the possibility of mutual interaction of the cytochromes and leads

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* British Council Scholar from the Department of Biochemistry, University of Budapest.

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one to seek some agents which can transmit the oxidizing power. There seems to be no evidence for a mechanism based on the action of free radicals or a soluble carrier and indeed the very specific character ot the reactions of this system rather precludes such a mechanism.

Another example of the co-operative action of prosthetic groups attached to a common protein appears to be furnished by an observation of P. GEORGE[5] on the reactivity of catalase. This enzyme contains four haem groups (probably arranged in the configuration found by PERUTZ for Haemoglobin). If one of the haems is "blocked" or poisoned by cyanide or azide there is a change in the catalytic reactivity of the remaining haem groups, and it seems therefore, that the activity of the unimpaired enzyme cannot be considered as being made up of the additive reactivity of four individual haems but that there is a special reactivity associated with the assembly of four heams.

The above suggests that there is some special interaction between the prosthetic group and the protein assembly. This interaction is revealed by the change in the oxidation-reduction potential of haemoglobin when the globin is denatured (BARRON[6]).

SPEAKMAN AND ELLIOTT[7] showed that the combination of wool protein with acid dyes is stoichiometric, *i.e.*, the combining power is the same as for simple acids. ASTBURY AND DAWSON and MCARTHUR found that X-ray examination of the dyed proteins showed very little distortion of the protein structure. The setting power of the fibres was, however, greatly impaired showing that the hydrogen ions had, as was indicated by the stoichiometry, penetrated and combined with the protein. These authors concluded that in spite of the inaccessibility of the crystalline protein to the large dye anions, the hydrogen ion penetrates.

The separation of charges envisaged by this mechanism would lead to extremely unstable systems. In the discussion the authors referred to the possibility of electron mobility in proteins as envisaged by HUGGINS[8] and by DENBIGH[9] as a possible solution of the difficulty.

BUCHNER AND KASPERS[10] have shown that when the complex of myoglobin and carbon monoxide is irradiated with light of wavelength of 280 mμ, carbon monoxide is liberated, and the ratio of quanta absorbed to moles of carbon monoxide evolved is approximately unity. Myoglobin is a haemprotein in which the haem absorbs at a wave length of 313 to 400 mμ whereas light of wave length 280 mμ is absorbed only by the tyrosine and tryptophane residues of the protein moiety. Nevertheless the absorption of wave length of 280 mμ by the protein moiety liberates carbon monoxide from the complex with the same quantum efficiency of unity as does the absorption of wave length 313–400 mμ by the haem carbon-monoxide complex, suggesting that the energy absorbed by specific groups in the protein moiety can be transmitted to the reaction bond, *viz.*, the Haem-Fe–Co bond, without a loss of efficiency.

In discussing the action of metal ions and of A. T. P. on the reaction of the protein in muscle, SZENT-GYÖRGYI[11] has pointed out that in effect these reactions appear to occur on a mole fraction basis, that is one or two moles of ions are able to modify the whole behaviour of a mole of protein in spite of the enormous disparity in molal volume. Experience with the reactions of polymers in solution would indicate a volume fraction for the basis of such changes and the only conclusion that can be drawn from SZENT-GYÖRGYI's results is that the protein polymer in these cases is not behaving as an assembly of individual units, that the action is not localized to the site of attack but affects the behaviour throughout the structure.

This weight of suggestive but not conclusive experimental evidence has led us to an examination of the possibility of some co-operative valency behaviour in a protein structure which could give rise to the effects described above.

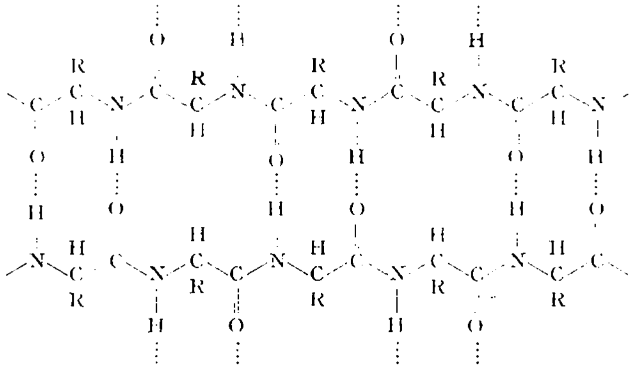
Certain models suggest themselves. Thus in the case of the coupled oxidation reduction of the cytochrome, the protein moiety seems to be behaving as a metal connecting the independent systems as in a multiple oxidation reduction cell.

In the decomposition of the haemprotein -CO complex it appeared that a photon could be transferred through the protein moiety to the site of reaction of the Haem-CO bond. Here the protein appeared to behave as a phosphorescent solid containing colour centres.

Finally the influence of denaturation on the oxidation-reduction potential of a prosthetic group seems to indicate a coupled resonance between the natural protein and the group, a coupling which is modified in the denaturation process.

These points of similarity of common behaviour have led us to seek the possible existence of molecular energy levels in the protein similar perhaps in kind to those existing in a metal or in a polyconjugated structure. The changes in the activity of the prosthetic groups accompanying denaturation would indicate that these molecular energy levels are connected with the particular regular structure in the natural protein.

We ascribe, as has been done before[12, 13, 14] the regular protein structure to the hydrogen bonds between the polypeptide chains thus:



This possibility exists both in the extended β form and in the folded α form of the chains. Hydrogen bonds can be formed between opposing CO and HN groups of two different polypeptide chains or between these groups in the same folded chain.

We assume therefore that the hydrogen bonding is a characteristic of the structure and enquire what effect this will have on the electron distribution and behaviour of the C, O and N atoms.

The hydrogen bond of the type:

$$>N-H\ldots\ldots O=C<$$

in which the centres are colinear and coplanar, trigonal symmetry has been conferred on the bonds of the nitrogen centre leading to a distortion from the usual pyrimidal symmetry of the nitrogen centre. This change in bond symmetry can arise from a change in the hybridization of the nitrogen. The nitrogen in the hydrogen-bonded structure seems to be entering into bonding not as the structure:

N $(1s)^2\ (2s)^2\ 2p\ 2p\ 2p$ but rather as:
N $(1s)^2\ (2s\ 2p\ 2p)^3(2p)^2$ in which there is sp^2 hybridization.

Such as electronic structure would lead to the formation of three equivalent electron distributions set at an angle of 120° to each other. The $(2p)^2$ electron density distribution will be then normal to the plane of the $> N — H \ldots O = C <$ structure and in the same plane as the π electron distribution of the O and C centres of the O = C group.

Fig. 1 attempts to represent this electron density distribution.

A similar state of affairs arises if we consider a fully ionic N-H bond in this structure. In this case the electronic configuration of the N^- would be $(1s)^2\ (2s)^2\ (2p)^2\ 2p\ 2p$ and Fig. 2 represents the electron density on the separate centres.

The state represented in diagram 1, probably represents a resonating state between the fully ionic state and that in which the nitrogen has pyrimidal symmetry of electron distribution.

In both these cases we seek the energy levels of the molecular orbitals (MO) arising from the separate distributions on the individual centres and these molecular orbitals are occupied by 4 electrons for each unit of $> NH \ldots O = C <$.

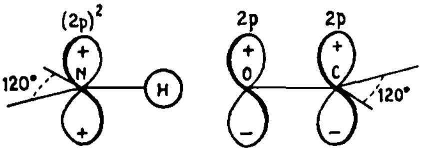


Fig. 1

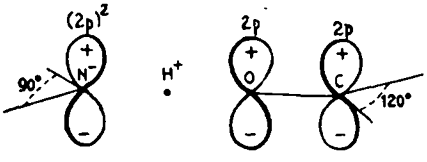
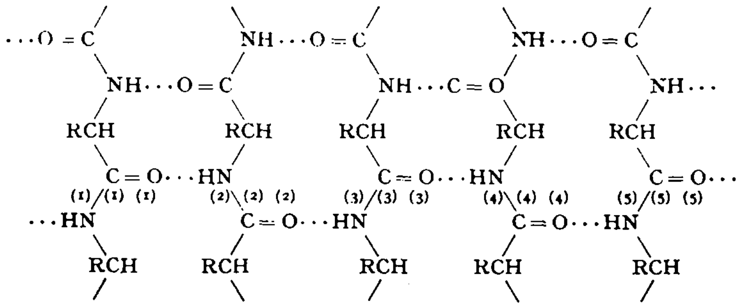


Fig. 2

The importance of the hydrogen bonds is twofold: (a) they give rise to the type of hybridization discussed above and (b) they confer a definite skeleton structure on the configuration, bringing the N and O atoms of neighbouring chains into juxtaposition at a separation of about 2.65 Å and in the same plane. This means that the π electron distributions of the N, O and C atoms are parallel throughout the whole structure.

These are just the conditions for the formation of molecular orbitals from the individual atomic π functions[15] and we propose therefore to explore the system from this point of view.



At each of the centres marked in the above diagram there is an atomic orbital directed normal to the plane of the structure. The molecular orbitals for the whole linear system will be given by:

$$\psi = \sum_i (a_{N_i}\,\psi_{N_i} + a_{C_i}\,\psi_{C_i} + a_{O_i}\,\psi_{O_i})$$

The secular determinant arising from these ψ functions will be

$$\left|\begin{array}{ccccccccc} q_N-E & \beta_{NC} & 0 & & & & & & \\ \beta_{NC} & q_C-E & \beta_{CO} & & & & & & \\ 0 & \beta_{CO} & q_O-E & \beta_{ON} & & & & & \\ & & \beta_{ON} & q_N-E & \beta_{NC} & 0 & & & \\ & & & \beta_{NC} & q_C-E & \beta_{CO} & & & \\ & & & 0 & \beta_{CO} & q_O-E & & & \\ & & & & & \beta_{ON} & q_N-E & \cdots & \\ & & & & & & \vdots & & \end{array}\right| = 0$$

In the equation q_i represents the integral $\int \psi_i\, H\, \psi_i\, d\tau$ for the centre i, and β the integral $\int \psi_i\, H\, \psi_j\, d\tau$; β is zero when i and j are not neighbouring centres except in the special case that we have discussed of the centres N – H – – – O in which case β is given a value a value appropriate to the N – O distance of 2.65 Å.

For all practical purposes we may consider the determinant an infinite one in which case the solutions are identical with those given by a cyclic structure. Mathematically it is easier to deal with the cyclic determinant, and H. D. URSELL[16] has developed a method to deal with this problem. We have used his method in this work.

We have taken a mean value q_m as a standard for the integral $\int \psi_i\, H\, \psi_i\, d\tau$ and expressed individual integrals as $q_i = q_m + \delta_i$.

The mean value of q_m has been obtained from the electron affinities of the individual centres NH, O = and C = and $q_m = -13.24$ e.V.

The values of δ are shown in Table I. The value of β_{ij} has been taken, as is usual, equal to one half the $\pi\pi$ bond energy in the C = O, N = C and N = O bonds at the appropriate nuclear separation[17] and these values together with the relevant distances are given in Table I.

TABLE I

$\delta_N = -0.58$	$\delta_O = -1.49$	$\delta_C = 2.07$ eV
$\beta_{NO} = -0.20$	$\beta_{CO} = -2.30$	$\beta_{CN} = -1.50$ eV
$\gamma_{NO} = 2.65$	$\gamma_{CO} = 1.25$	$\gamma_{CN} = 1.33$ Å

Using these values the solution of the secular equation leads to the result that for such a structure the electron levels are arranged in three bands as shown in Fig. 3; the details are given in Table II.

In the lowest energy state the 4n electrons (where n is the number of repeat units (NH . . . O = C–) fully occupy the two lowest bands, each level in each band being occupied by two electrons with opposing spins (See Fig. 3).

It is inherent in the MO method that given a proper orientation of orbitals, there will be a certain overlap between adjacent orbitals and hence a corresponding lowering in total energy and increase in the mobility of electrons.

TABLE II

RANGE OF ENERGY BANDS IN eV RELATIVE TO LOWEST FILLED LEVEL

	a	b	c
Band 1	0–0.20	0–0.13	0–0.26 doubly filled
Band 2	2.04–2.34	3.17–3.43	2.50–2.89 doubly filled
Band 3	6.57–6.67	6.48–6.60	7.65–7.79 unfilled

a) N pyramidal
b) N trigonal
c) NH isoelectronic with O

If the distance between neighbouring centres is too large or if the orientation of the orbitals does not favour overlap we may put $\beta = 0$. Let us therefore consider what changes would occur in the properties of the above structure if we put $\beta_{ON} = 0$. We should get a set of sharply defined levels, not bands, lying at about the same energy values as the means of the bands in the case discussed above. Each set of 3 levels would correspond to one NH . . O = C group in the whole structure. The energy difference between the average energy of one group in the non-localized structure and the energy of the same group of electron orbitals in the localized structure is approximately 0.5–1.0 kcal in the lowest state, in the sense that the non localized is the more stable. For a structure in which there are many such units linked through the H-bonds the total energy of stabilization might be quite considerable and thus an important contribution to the behaviour of such a structure.

The parameters used in the above treatment have been varied; thus an alternative value for the ionization potential of the centre N, 12.24 eV, has been taken corresponding to the change in hybridization[18].

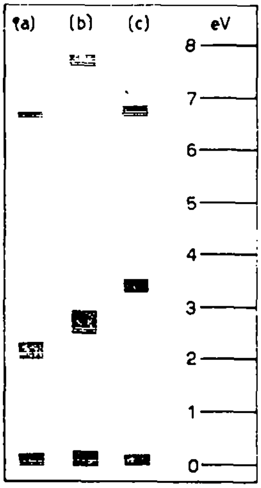


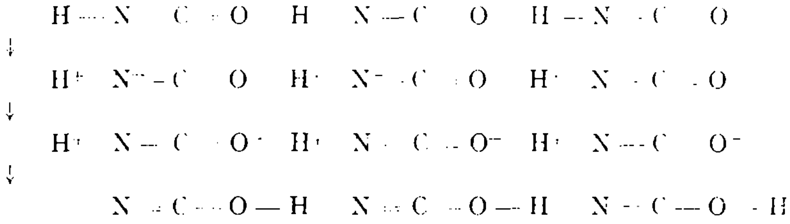
Fig. 3. Position of energy bands in proteins. For details see Table II.

Another approach is to treat the NH group as isoelectronic with an oxygen centre, and to put $\beta_{CN} = \beta_{CO}$. The results based on these alternative values of the parameters are shown in Fig. 3 and Table II. The general results discussed above *viz.*, that the electronic levels are arranged in three bands, is unaltered. The width of the bands and their relative positions are only slightly changed.

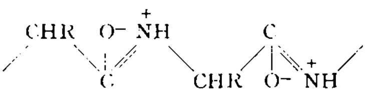
If on the basis of an assembly of separate entities $C = O \cdot H — N$ the idea of co-operative effect between these structural units is not to be given up entirely, the only way to establish a connection between distant parts of the structure is by means of proton migration reaction, this migration being initiated by some ionogenic reaction, involving the step

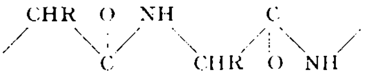
$$C = O + H^+ \rightarrow COH^+ \text{ or } NH \rightarrow N^- + H^+,$$

the charge left on the structure after the removal of the H facilitating the migration of the protons. This has been discussed by WIRTZ[4].



This mechanism does not appear to cover all the biological instances referred to in the introduction, *e.g.*, coupled oxidation reduction, and photon energy transfer. DENBIGH[9] on the other hand does not assume a connection between parallel polypeptide chains but rather a 'conjugation' along a chain which he represents as a resonance between the structures:





DENBIGH made no attempt at a quantitative approach nor does he make it clear that in order to obtain conjugation through a > CHR group one must take into account hyperconjugation involving this group.

It may be of some interest to consider the differences and similarities between metals and such a system as discussed here, particularly because the analogy with metals is being used in biological connection.

1. A protein structure is seen to be non-conducting in the ground state, and can assume conducting properties only on excitation.

2. Judging from the width of the energy bands in a protein structure the electrons are much more localized than in a metal or a graphite structure. The narrowness of these bands suggests that in order to transfer electrons to or from empty or full level of another system the energy levels of the structures must be closely matched. This may indeed have a bearing on specificity of reactions between coupled resonating systems.

3. It follows from (1) that the proposed structure possesses 'semiconductive' properties, but these could never be realized by thermal excitation. The peculiar feature is that the energy gap separating the highest occupied from the lowest unoccupied level is very large compared with kT and is comparable with bond energies, so that thermal excitation cannot bring about the required electronic transition. In fact the gap seems to be of the order of the energy of the photons which are absorbed in the UV by biological structures.

The optical properties of such an array of coupled polypeptide units should be similar to those of the isolated units making up the structure but this work would suggest that the 'coupled' array should manifest slight photoconductivity.

It is worth while to enquire whether other evidence can be adduced to support the principle advanced in this discussion. We have postulated here that there may be interaction between π orbitals belonging to centres which are not connected by a σ bond. These π orbitals may belong to centres in different molecules and the only condition is that the molecules should be oriented by intermolecular forces in such a way that the orbitals on different centres all lie normal to the molecular plane. In the case we have here discussed in detail the H-bond is effective in bringing about the required orientation, and indeed one might expect that in other cases H-bonds would play a major part.

We feel that the observations of Scheibe[19, 20, 21, 22] on the absorption spectra of associated cyanine dyes can be interpreted in terms of the above discussion. Scheibe found that such aggregates, if irradiated with light polarized in the plane of the molecules, emitted depolarized light. He, therefore, assumed that the absorbed photon was transmitted in the polymer to such points where the curvature of the elongated molecules corresponded to a different direction than that of the polarized light. He further observed that in dried polymers there was a widening of the absorption band with a consequent shift towards the red. Scheibe himself suggested some sort of interaction between orbitals of neighbouring molecules. In terms of the method here developed, we can perhaps give a more definite picture of this effect. In Fig. 4 we show how on the basis of the above method the position and the width of the bands would change with increasing interaction between adjacent molecules and the results reveal qualitatively the shift to the longer wave lengths and the widening of the absorption band which Schiebe observed. This then is an extension to an oriented but non-bonded array of molecules of the well-known principle in truly conjugated molecules that increasing conjugation increases the wave length and the width of the absorption.

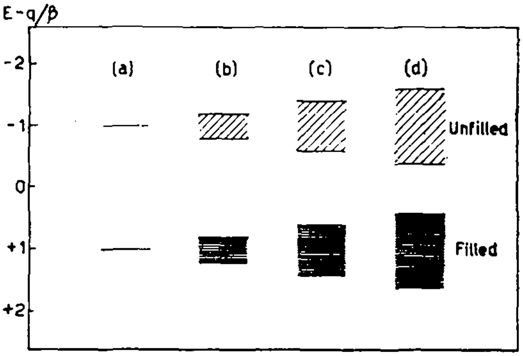
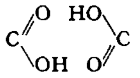


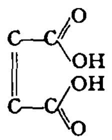
Fig. 4. The influence of the magnitude of the exchange integral β' between adjacent molecules on the spacing and width of the energy bands. The model upon which this diagram is based is that of a linear array of ethylene molecules in which β is the exchange integral between the bonded centres. a) $\beta' = 0$: b) $\beta' = 0.2\,\beta$: c) $\beta' = 0.4\,\beta$: d) $\beta' = 0.6\,\beta$

Diamagnetic anisotropy has been observed in molecules in which there is no conjugated ring structure in the accepted sense, as well as in truly conjugated rings[23, 24]. London's[25] treatment of conjugated ring structures such as benzene naphthalene etc. provides an entirely adequate explanation, based on the quantum mechanical equivalent of a circular current in the plane of the molecules, of the experimental facts for such cases.

If, as we have done here, an interaction between the orbitals of non-bonded but suitably oriented centres is postulated, then a similar treatment could be given for the diamagnetic anisotropy of such molecules as:



the dimer of an aliphatic acid and



In Fig. 5 we show the arrangement of π orbitals which would lead to diamagnetic anistropy.

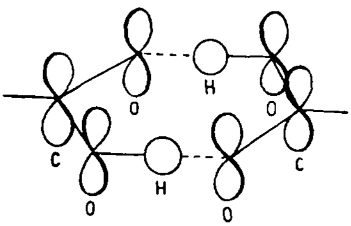


Fig. 5

In some simplified cases we have calculated, using LONDON's method, very approximate values of the diamagnetic anisotropy arising from the π-π interaction between adjacent molecules consisting of identical centres, and with a reasonable choice of parameters have obtained the correct order of magnitude for this effect. We have not given here detailed results because the absolute magnitudes are so approximate. If this concept of the electron behaviour in a protein is correct we should expect an oriented protein structure to show diamagnetic anisotropy, the susceptibility being larger normal to the planes of the hydrogen bonds.

In the case of the protein structure in which the conjugated repeat unit is only of three centres, the separation of the highest filled and the lowest unfilled band is so large that there would be no observable thermal semiconductivity since the requirement for semiconductivity is that this separation should be of the order of 1 eV. We enquire what size of conjugated repeat unit would confer thermal semiconductivity on the structure. Consider a model consisting of an infinite linear array of units each containing m identical centres connected by σ bounds and having one π orbital orthogonal to the plane of the structure on every centre. Assume that the exchange integral between the unconnected atoms is 1/5 to 1/10 of that between σ-linked atoms. Calculations show that with $m \sim 12$ the energy separation between the filled and unfilled band is of the required order of magnitude. If the exchange integral between unlinked atoms is bigger than above, the necessary size of the unit for semiconductance is accordingly smaller.

SUMMARY

The possibility of conjugation through the orbitals in a protein structure is considered and an attempt has been made to calculate the magnitude of the possible effects.

The results indicate the possibility of a banded electronic structure which confers a small extra stability on the system. The importance of such a model is however that it leads to the possibility of electron 'mobility' and suggests a mechanism for the transfer of elections, excitation and chemical action through the protein structure.

The model also suggests that an examination of the photoconductivity and diamagnetic anisotropy should reveal the correctness or otherwise of the assumptions on which this work is based.

RÉSUMÉ

La possibilité d'une conjugaison par les orbitales dans la structure d'une protéine est envisagée, et une tentative est faite pour calculer l'ordre de grandeur des effets possibles de cette conjugaison.

Les résultats montrent la possibilité de liaisons électroniques conférant une légère stabilité supplémentaire au système. L'importance d'un tel modèle consiste en la possibilité d'une "mobilité" des électrons et suggère un mécanisme pour le transport d'électrons, leur excitation et une action chimique à travers la structure de la protéine. Le modèle suggère également que l'étude de la photoconductivité et de l'anisotropie diamagnétique peuvent permettre d'établir expérimentalement la validité des hypothèses qui sont à la base du présent travail.

ZUSAMMENFASSUNG

Die Möglichkeit einer Konjugation durch die Elektronenbahnen in einer Eiweisstruktur wird betrachtet und der Versuch wurde unternommen, die Grösse der möglichen Effekte zu berechnen.

Die Resultate zeigen die Möglichkeit einer Elektronenbandenstruktur an, die dem System eine

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geringe zusätzliche Stabilität verleiht. Die Bedeutung eines solchen Modells liegt jedoch darin, dass es zu der Möglichkeit der "Beweglichkeit" von Elektronen führt und einen Mechanismus für Elektronenübertragung, Exzitation und chemische Wirkung durch die Eiweisstruktur vorschlägt.

Das Modell führt auch zu der Folgerung, dass eine Untersuchung der Photoleitfähigkeit und der diamagnetischen Anisotropie die Richtigkeit der Annahmen, auf denen das Modell beruht, anzeigen sollte.

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