TRANSFER OF ELECTRONIC EXCITATION ENERGY BETWEEN TRYPTOPHANS AT THE ACTIVE SITE OF LYSOZYME

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35—40% of the total fluorescence of lysozyme in aqueous solution is known to originate from the intensities I_A+I_B of fluorescence of tryptophan-62 and tryptophan-63 (TRP-62 and TRP-63). On account of their proximity I_A and I_B could not be determined separately with the chemical methods applied to date.

A kinetical model was developed which permits determination of I_A/I_B and $I_{AB}/(I_A+I_B)$, where I_{AB} is the intensity of fluorescence arising from the transfer of electronic excitation energy between TRP-62 and TRP-63. From this model $I_A/I_B=0{,}53$ and $I_{AB}/(I_A+I_B)=0{,}25$, in other words, the total intensity of the fluorescence of the *pair* originates from energy transfer, this is 9—10% of the total fluorescence of *lysozyme*, one third of the fluorescence intensity of the pair arising from TRP-63, and two thirds from TRP-62.

From the depolarization of fluorescence, using literature data, we obtained 8% of the total fluorescence of lysozyme for the intensity of the fluorescence from energy transfer within the pair, in good accordance with the model, and therefore the separation of fluorescences I_A and I_B is verified.

For the evaluation of the literature data on degrees of fluorescence polarization, the relative positions of the two indole rings of TRP-63 and TRP-62 were calculated from crystallographical data. For the calculations with the model, the total interaction energy (1,75 eV) and the transfer frequency ($3{,}7\cdot10^{12}$ sec^{-1}) were determined.

Introduction

The proteins containing luminescent amino acids can also be studied by luminescence analysis in addition to the known biochemical and *X*-ray crystallographical methods. Lysozyme is a good choice for luminescence analysis because, among others, it contains 3 phenylalanines (PHE), 3 tyrosines (TYR) and 6 tryptophans (TRP). All these are flurorescent, but only three of the TRP-s contribute to the totel flurorescence, the fluorescence of the other amino acid residues being quenched [1]. These three are TRP-62, TRP-63 and TRP-108, located at sites 62, 63 and 108 in the sequence of amino acids. These TRP-s are found at active sites of the enzyme, and therefore their fluorescence (and other properties) are very sensitive to environmental effects. This is a special reason for the extended fluorescence studies carried out with lysozyme in many laboratories.

Several authors have used chemical methods to investigate the individual contributions of the TRP-s to the total fluorescence of the enzyme. TAKAHASHI [2] pointed out that only TRP-62 and TRP-63 are oxidized by low-concentration *N*-bromosuccinimide (NBS); at higher concentrations TRP-108 is oxidized, too, as are the

other TRP-s with further increase of the concentration. This process is reflected in the fluorescence: at low NBS concentrations the fluorescences of TRP-62 and TRP—63 are quenched, as is that of TRP-108 at higher NBS concentrations. LEHRER [3] and TEICHBERG [4] studied the fluorescence quenching with iodide ion. The final conclusion of their investigations was the finding that 56% of the total fluorescence originates from TRP-108, 35—40% from the TRP-62, TRP-63 pair, and 5—10% from the other TRP-s. On account of the small distance between TRP-62 and TRP-63 chemical methods were unable to differentiate between the fluorescences of TRP-62 and TRP-63.

The differentiation of the fluorescence within this TRP-pair was attempted by using the transfer of excitation energy between the two. Since the structure of lysozyme is known with high precision from *X*-ray crystallographical investigations [1], the relative positions of the indole-rings determining the fluorescence of the TRP are also well known. The degree of polarization of the fluorescence appearing after energy transfer is influenced by the relative positions of the indole-rings. From analysis of the polarized fluorescence of lysozyme, therefore, information can be expected on the individual fluorescences of TRP-62 and TRP-63.

The possibility of energy transfer between TRP-62 and TRP-63 was suggested by LONGWORTH [5] without quantitative studies. In general, the quantitative study of energy transfer is based upon the inductive resonance transfer theory elaborated by FÖRSTER [6]. In our case this theory cannot be applied because, due to the proximity of TRP-62 and TRP-63, the interaction cannot be reduced to dipole-dipole interaction, as was stated already by WEBER [7]. Because of the small overlap integral, the Förster-type critical distance for TRP→TRP homotransfer was found to be $R_0=6$ Å [8]. This is a distance commensurable with the linear dimensions of TRP, and therefore the dipole-dipole approximation is not correct and the total interaction energy should be determined. From the total interaction energy the transfer frequency can be calculated and, using the geometrical and fluorescence data, the contributions of two amino acid residues to the total fluorescence of lysozyme can be established.

1. *The frequency of excitation transfer between TRP-62 and TRP-63*

The general relation for the frequency of energy transfer, obtained *via* time-dependent perturbation calculations [9], is:

$$k = \frac{2\pi}{\hbar} |U|^2 \int \varrho_A(E) \varrho_B(E)\, dE. \tag{1}$$

Here $\varrho_A(E)dE$ and $\varrho_B(E)dE$ are probability density functions identical with the normalized fluorescence spectrum $f_A(E)dE$ of the donor molecule A and with the normalized absorption spectrum $\varepsilon_B(E)dE$ of the acceptor molecule B, i.e. they are measurable quantities. U is the interaction energy. ($\varrho_A(E)dE=\int f_A(E)dE$, $\int f_A(E)dE=1$ and $\varrho_B(E)dE=\varepsilon_B(E)dE$, $\int \varepsilon_B(E)dE=1$.) U and the overlap integral should be given for the determination of k.

a) *The interaction energy for the pair TRP-62 and TRP-63*

The total interaction energy is the sum of the different possible interaction energies. The following interaction energies exist between two atoms with centers a and b situated on the two indole-rings.

α) Electrostatic repulsive potential between the positive nuclei at a distance R is $F_0^c = e^2/R^2$. To this an exchange potential $F_0^A = e^2 S/R$, should be added where $S = \int \chi_a \chi_b dV$ is the overlap integral of the electron clouds localized on centres a and b.

β) Transition attractive potential appears between the charge cloud and the opposite nucleus. Its coulombic term is

$$F_1 = q_a^2 e^2 \int \chi_a \frac{1}{r_b} \chi_a dV + q_b^2 e^2 \int \chi_b \frac{1}{r_a} \chi_b dV$$

and its exchange term

$$F_3 = q_a q_b e^2 S \int \chi_a \frac{1}{r_a} \chi_b dV + q_a q_b e^2 S \int \chi_b \frac{1}{r_b} \chi_a dV;$$

q_a and q_b denote the charge densities of π-electrons on the centres a and b, and χ_a and χ_b are the atomic orbits of atoms with centres a and b.

γ) The interaction between electrons has a coulombic term

$$F_2 = q_a^2 q_b^2 e^2 \iint \chi_a(1) \chi_b(2) \frac{1}{r_{12}} \chi_a(1) \chi_b(2) dV_1 dV_2$$

and an exchange term

$$F_4 = q_a^2 q_b^2 e^2 \iint \chi_a(1) \chi_b(2) \frac{1}{r_{12}} \chi_a(2) \chi_b(1) dV_1 dV_2,$$

where r_{12} is the distance between the electrons, and the functions $\chi_a(i)$ and $\chi_b(i)$ refer to the i-th electrons belonging to the atoms with centres a and b, respectively.

The *total interaction energy* is the sum of the above interaction energies:

$$U = F_0^c - F_1 + F_2 + F_0^A - F_3 + F_4.$$

For calculation, use was made of the transformed form of the atomic wave functions (given in the Appendix) depending on the relative orientations of the two indole-planes. For the next two pairs of atoms of TRP-63 and TRP-62 (*i.e.* for the pairs C_2, C_8 and C_3, C_7 in Fig. 3.) the results are included in Table I. The distances and energies are given in atomic units and 10^{-12} erg, respectively. The total interaction energy can be considered to be the sum of interaction energies of the two next-situated pairs of atoms in TRP-62 and TRP-63 and therefore

$$U = -2.81 \cdot 10^{-12} \text{ erg} = -1.75 \text{ eV}.$$

b) *The overlap integral.* For the calculation of the transfer frequency from (1), in addition to the interaction energy U the overlap integral $\left(\int \varrho_A(E) \varrho_B(E) dE = \int f_A(E) \varepsilon_B(E) dE\right)$ must also be determined. This integral is not identical with

Table I

Quantities for the calculation of the interaction energy between tryptophan-63 and tryptophan-62 in lysozyme

	C_2-C_8	C_3-C_7
R	3.4	4.4
q_a	0.78	0.96
q_b	1.01	1.02
S	− 0.18	− 0.10
F_0^e	12.8	9.8
F_0^A	0.414	0.098
F_1	22.8	18.9
F_2	8.64	7.80
F_3	1.33	0.44
F_4	0.677	0.230
C	− 1.36	− 1.10
A	− 0.24	− 0.11

the Förster overlap integral (see p. 85 in [6]), here both spectra are normalized and the dimension of the integral is energy^{-1}. From the spectra in Fig. 2. the overlap integral is of the order of 10^{-4} eV^{-1}, which is extremely small. Since, in (1) the total interaction energy must be considered; however, not only the term from dipole-dipole interaction, but also the transfer frequency between the two TRP-s becomes appreciable:

$$k = \frac{2\pi}{\hbar} U^2 \int f_A(E)\varepsilon_B(E)\,dE =$$

$$= \frac{4\pi^2}{6{,}6 \cdot 10^{-27}} (-1{,}75)^2\, 10^{-4}\,\mathrm{sec}^{-1} =$$

$$= 3{,}7 \cdot 10^{12}\,\mathrm{sec}^{-1}.$$

2. *The ratio of the fluorescence intensities from molecules in energy transfer relationship*

Let us consider molecules A and B with (radiative and radiationless) deactivation frequencies k_A and k_B, the transfer frequencies for transfer from A to B and from B to A being k_{AB} and k_{BA} (Fig. 1.). $W_A(t)$ and $W_B(t)$ are the probabilities of finding the molecules A and B in excited states at time t. Since the molecules are subject to spontaneous deactivation and energy-transfer processes, these probabilities are time-dependent according to the equations

$$\frac{dW_A(t)}{dt} = -(k_{AB}+k_A)W_A(t)+k_{BA}W_B(t), \tag{2}$$

$$\frac{dW_B(t)}{dt} = k_{AB}W_A(t)-(k_{BA}+k_B)W_B(t). \tag{3}$$

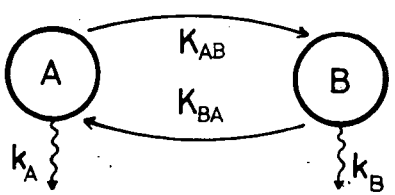

Fig. 1. Kinetics of radiationless and radiative deactivation of fluorescent molecules A and B

Taking probabilities p_A and p_B for having excited A and B molecules at time $t=0$, the solution of the system of equations is the following:

$$W_A(t) = \frac{[p_B k_{BA} - p_A(k_A+\lambda_B)]\exp\lambda_A t + [p_A(k_A+\lambda_A) - p_B k_{BA}]\exp\lambda_B t}{\lambda_A - \lambda_B}, \quad (4)$$

$$W_B(t) = \frac{[p_B(k_A+\lambda_A) + p_A k_{AB}]\exp\lambda_A t - [p_A k_{AB} + p_B(k_A+\lambda_B)]\exp\lambda_B t}{\lambda_A - \lambda_B}. \quad (5)$$

For instantaneous excitation the probabilities $W_A(t)$ and $W_B(t)$ decay biexponentially, and λ_A and λ_B are negative constants.

Within time dt after the t-th point of time the number of spontaneous deactivations of molecules A is proportional to $k_A W_A(t)dt$, and the number of radiative deactivation processes is proportional to $\eta_A k_A W_A(t)dt$, where η_A denotes the absolute quantum yield of fluorescence of molecules A. The total intensity of fluorescence $I_A = \eta_A k_A \int_0^\infty W_A(t)dt$. From the probabilities given in (4) and (5), the ratio of the intensities of fluorescence of molecules A and B is:

$$\frac{I_A}{I_B} = \frac{\eta_A k_A}{\eta_B k_B} \frac{\int_0^\infty W_A(t)\,dt}{\int_0^\infty W_B(t)\,dt} = \frac{\eta_A k_A}{\eta_B k_B} \frac{\left(1+\frac{p_A}{p_B}\right)k_{BA} + \frac{p_A}{p_B}k_B}{\left(1+\frac{p_A}{p_B}\right)k_{AB} + k_A}. \quad (6)$$

The part I_{AB} of the fluorescence of molecule B which originates from exciting energy obtained from molecule A through energy transfer can be calculated from relations (2)—(3):

$$I_{AB} = \eta_B \int_0^\infty (k_{AB} W_A(t) - k_{BA} W_B(t))\,dt = \left(\frac{p_A}{p_B}k_{AB} - \frac{k_A}{k_B}k_{BA}\right)\eta_B p_B k_B. \quad (7)$$

A useful expression is $I_{AB}/(I_A+I_B)$, the ratio of the intensity of fluorescence due to energy transfer to the total intensity of fluorescence. This expression gives information about the contributions of the members of the molecule pair to the total fluorescence of the pair.

Relations (6)—(7) can be simplified if they refer to the pair TRP-63 and TRP-62 in the lysozyme. The transfer frequency is $3.7 \cdot 10^{12}\ \mathrm{sec}^{-12}$ (see section 1), and the frequency of radiative deactivation from measurement of the decay of fluorescence is of the order of $10^9\ \mathrm{sec}^{-1}$ [11]. The terms with k_A and k_B can therefore be neglected in comparison to k_{AB} and k_{BA}. The simplified relations are:

$$\frac{I_A}{I_B} = \frac{\eta_A k_A k_{BA}}{\eta_B k_B k_{AB}}, \quad (8)$$

$$\frac{I_{AB}}{I_A+I_B} = \frac{\frac{p_A}{p_B} - \frac{k_A}{k_B}\cdot\frac{k_{BA}}{k_{AB}}}{\left(1+\frac{p_A}{p_B}\right)\left(1+\frac{\eta_A k_A k_{BA}}{\eta_B k_B k_{AB}}\right)}. \quad (9)$$

3. *The contributions of TRP-62 and TRP-63 to their total fluorescence*

a) *The ratio of the intensity of fluorescence from energy transfer to the total intensity of fluorescence.* The observed emission anisotropy of lysozyme, r, is the sum of the emission anisotropies r_A, r_B and r_C relating to the fluorescences of TRP-62 TRP-63 and TRP-108, denoted below as A, B and C. According to [7]:

$$r = \frac{r_A I_A + r_B I_B + (r_{AB} - r_B) I_{AB} + r_C I_C}{I_A + I_B + I_C}.$$

From this relation:

$$\frac{I_{AB}}{I_A + I_B} = \frac{r_A - r}{r_A - r_{AB}}. \tag{10}$$

According to the measurements of WEBER [7] the degree of polarization of fluorescence of TRP in neutral water solution excited with unpolarized light of 290 nm wavelength is $p=0.10$; consequently, $r_A = 2p/(3+p) = 0.065$. The depolarization of fluorescence appears since the absorption and emission oscillators of TRP are not parallel but form an angle ω. This angle can be calculated from the depolarization factor given in [12]: $\omega = \pm 43°$ or $\pm 137°$. Under the same conditions as above for TRP [13] the degree of polarization of the fluorescence of lysozyme, $p=0.085$ and therefore $r=0.055$. According to [13] the degree of polarization is temperature-independent in the range 0 to 60 °C, and thus the depolarization found in addition to that already obtained in TRP cannot be ascribed to Brown-rotation, but only to the energy transfer between TRP-62 and TRP-63. From the transfer depolarization factor, r_{AB} can be determined: $r_{AB} = 0.2 \left(\frac{3}{2} \langle \cos^2 \Theta_{AB} \rangle - \frac{1}{2} \right)$. Here Θ_{AB} is the angle formed by the absorption oscillator of A and the emission oscillator of B. Θ_{AB} can be calculated *via* simple geometrical relations by means of the transformation matrix T^{63-62} (see in the Appendix) and the angle ω given above. The physically meaningful single value is $r_{AB} = -0.058$.

If the values $r_A = 0.065$, $r = 0.055$ and $r_{AB} = -0.058$ are introduced into (10), $I_{AB}/(I_A + I_B) = 0.08$ is obtained. Accordingly 8% of the total fluorescence originates from indirect excitation via energy transfer.

The ratio $I_{AB}/(I_A + I_B)$ can also be determined from equation (9). Unfortunately, the exact values of the quantities in (9) cannot be given, because the environmental effects on the absorption and fluorescence of TRP-62 and TRP-63 within lysozyme are not known. LASKOWSKI [14] and HERSKOVITS [15] determined the probabilities of perturbation of different TRP-s by solvent molecules. According to these investigations TRP-62 undergoes perturbation as if it were completely surrounded by solvent molecules. Consequently, TRP-62 in water solution can be modellized with TRP in water solution. Based upon the data in [14]—[15], TRP-63 can be modelled with TRP in methanol solution. The absorption and fluorescence spectra (corrected for reabsorption) of TRP were therefore determined in water and in methanol with a Spectrophotometer Optica Milano CF—4 and with a Perkin—Elmer MPF4 Spectrofluorimeter (excitation and observation perpendicular, concentration $5 \cdot 10^{-5}$ M, exciting wavelength 290 nm). p_A/p_B was considered to be indentical with the

ratio of the absorptions in methanol and water at the exciting wavelength. This gives $p_A/p_B=1.46$. k_A/k_B was determined with the method given by STRICKLER and BERG [16]. This method has been successfully applied in the case of complex molecules [17]. Assuming that the degrees of degeneration of the ground and excited states in water and methanol are the same, $k_A/k_B=1.32$, the ratio of the transfer frequencies

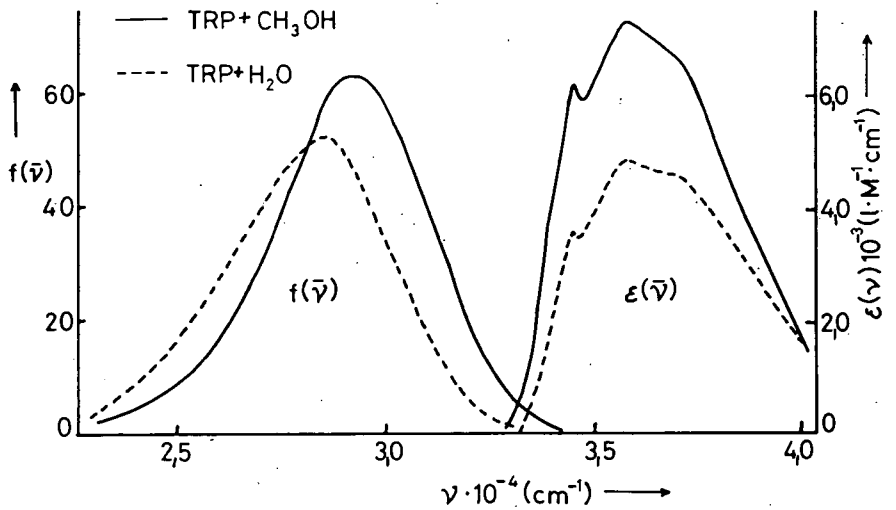

Fig. 2. Absorption and fluorescence spectra of tryptophan in methanol and water

between TRP-63 and TRP-62 was considered to be identical with the ratio of the overlap integrals: $k_{AB}/k_{BA}=2.5$. The absolute quantum yields of fluorescence from TRP-63 and TRP-62 can be taken as equal ($\eta_A=\eta_B$), because neither involves H-bonding, neither is in van der Waals interaction with any group in the pH region studied and they have approximately identical invironments [1].

If the values $p_A/p_B=1.46$, $k_A/k_B=1.32$, $k_{AB}/k_{BA}=2.5$ and $\eta_A=\eta_B$ are substituted into equation (9), we obtain $I_{AB}/(I_A+I_B)=0.25$. As mentioned earlier, the sum of the fluorescence intensities from TRP-63 and TRP-62 is 35—40% of the total fluorescence intensity of lysozyme in aqueous solution. The obtained ratio of 0.25 tells us that $35\cdot0.25$ to $40\cdot0.25=9$—10% is the intensity of fluorescence (I_{AB}) arising from energy transfer of the total fluorescence of lysozyme. This is in good aggreement with the value of 8% obtained by means of fluorescence polarization, and therefore the model outlined in section 2 can be used to distinguish between the fluorescences from TRP-63 and TRP-62. With the numerical values substituted earlier into (9), equation (8) yields $I_A/I_B=I_{TRP\text{-}62}/I_{TRP\text{-}63}=0.53$. Consequently, the total fluorescence intensity of this TRP pair in an aqueous solution of lysozyme is composed of one third weight TRP-63 and two thirds weight TRP-62 fluorescence.

Appendix

1) *The relative orientations of the indole rings of TRP-63 and TRP-62*

The scheme of the indole ring of TRP is shown in Fig. 3., together with the coordinate system (X, Y, Z) attached to the ring. The angles formed by the X and Z axes of any two TRP-s in lysozyme are known from crystallographical measurements. The relative orientations (Fig. 3.) of the indole-planes of two TRP-s have not been unambiguously determined. For an unambiguous determination the angles of all three axes should be known. However, if *three* TRP pairs are chosen, the relative orientations of *one* pair are sufficiently determined from the $3\times2=6$ angles.

Table II

Angles between axes X, X′ (upper numbers) and Z, Z′ (lower numbers) of the three indole rings of triptophans (62, 63 and 108) in lysozyme

	TRP-62	TRP-63	TRP-108
TRP-62	———	41.5 50.0	139.0 118.6
TRP-63	41.5 50.0	———	179.1 162.9
TRP-108	139.0 118.6	179.1 162.9	———

For TRP-63 and TRP-62 it is advantageous to chose TRP-108 because the angle relations promote the calculations. Let us take two coordinate systems X, Y, Z and X', Y', Z' attached to two different indole-rings with common origin. The angle relations according to [1] are shown in Table II. The upper and lower numbers denote the angles between X, X' and Z, Z', respectively. The angles (or, more conveniently, their cosines) can be obtained from the matrix equation

$$T^{62-63}\cdot T^{63-108}=T^{62-108}$$

$$\begin{vmatrix} 0.75 & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & 0.64 \end{vmatrix} \begin{vmatrix} -1 & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & -0.96 \end{vmatrix} = \begin{vmatrix} -0.75 & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & -0.48 \end{vmatrix}.$$

The matrix elements to be determined are denoted by dots. Due to the normalization condition the elements to be determined in the first row, and similarly all elements of the first column of matrix T^{63-108}, disappear. The missing elements of the second and third rows are determined by the normalization condition, too (apart from their signs.). Therefore

$$T^{63-108}=\begin{vmatrix} -1 & 0 & 0 \\ 0 & \pm0.96 & \pm0.29 \\ 0 & \pm0.29 & \pm0.96 \end{vmatrix}.$$

The third element in the third row of T^{62-108} is obtained by multiplying the third row of T^{62-63} and third column of T^{63-108}. This multiplication gives a linear equation from which the middle element of the third row of T^{62-63}, and then — by using this element — all other matrix elements can be calculated. The signs of the matrix elements can be found from the atomic distances of the characteristic atoms of the two indole-rings, given in [1]. In this way T^{62-63} can be determined. Since we need T^{63-62}, T^{62-63} should be inverted (in this case, since the rotation is an orthonormalized transformation, with transposition). The final result is

$$T^{62-63}=\begin{vmatrix} 0.75 & 0.61 & 0.28 \\ -0.26 & 0.65 & -0.72 \\ -0.61 & 0.46 & 0.64 \end{vmatrix}; \quad T^{63-62}=\begin{vmatrix} 0.75 & -0.26 & -0.61 \\ 0.61 & 0.65 & 0.46 \\ 0.28 & -0.72 & 0.64 \end{vmatrix}.$$

2. *The molecular orbitals of TRP*

The calculations were carried out in π-electron approximation, according to Fig. 3. The atomic distances were taken from [10]. The molecular orbitals ψ_i were taken as linear combinations of the χ_q $(q=1, 2, \ldots, 10)$ $2p_z$ atomic orbitals:

$$\psi_i=\sum_{q=1}^{10} c_{iq}\chi_q.$$

The coefficients (c_i) and the energies (E_i) relating to the molecular orbitals were determined with a computer by the Löwdin method, from the matrix equation

$$\hat{H}\hat{c}_i = E_i\hat{S}\hat{c}_i.$$

The overlap between non-neighbouring atoms was neglected in the overlap matrix S. The p-th element in the main diagonal of the energy matrix $\hat{H}$ is the ionization energy H_{pp} of the electron of the

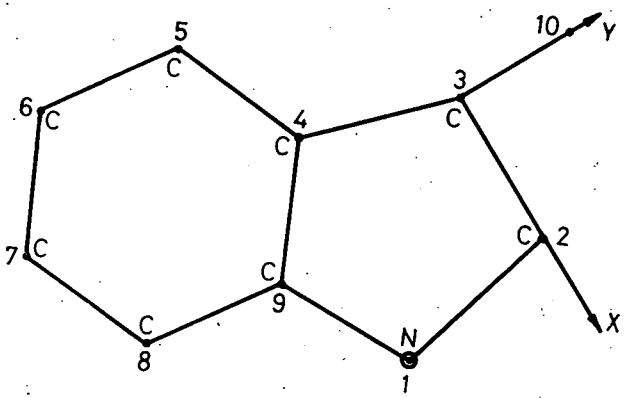

Fig. 3. Scheme of the indole-ring with the coordinate system fixed to the ring

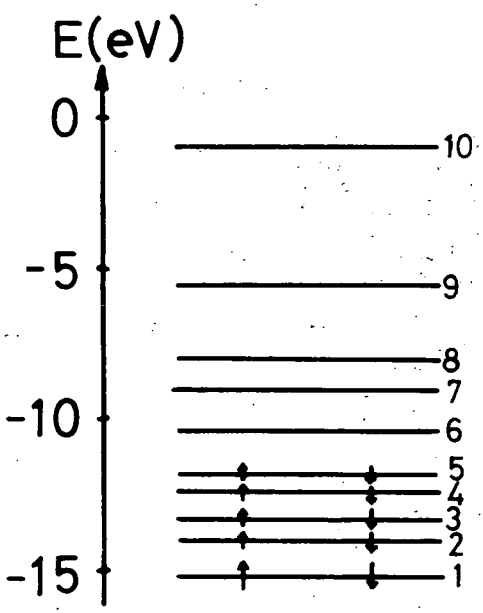

Fig. 4. Energy diagram of tryptophan

p-th atom participating in the molecular orbital. The element H_{pq} outside the main diagonal can be given for aromatic compounds according to *Hoffmann* as

$$H_{pq} = 1.75 \frac{H_{pp}+H_{qq}}{2} S_{pq}.$$

In our case $H_{11} = -14.16$, $H_{22} = \ldots = H_{10.10} = -11.31$, $H_{12} = H_{19} = -4.45$, $H_{13} = \ldots = H_{8,10} = -4.93$, $H_{3,10} = -3.78$, $H_{4,9} = -4.93$ eV.

The energy diagram is shown in Fig. 4. The excitations from the 5-th level to levels 7 and 8 give the two nearer electronic excited states of TRP (and indole derivatives), L_a and L_b, often studied experimentally.

Further the charge densities of the π-electrons localized on the atoms were calculated. The results for the ground state S_0 and excited state L_a are tabulated (Table III).

Table III

π-electron densities in the ground state S_0 and excited state ^1L_a of tryptophan around different atoms (for the notation see Fig. 3)

atomic notation	1	2	3	4	5	6	7	8	9	10
S_0	1.59	0.74	1.07	1.05	1.00	1.06	1.02	1.07	0.97	0.50
^1L_a	1.59	0.78	0.96	1.06	1.08	1.09	0.99	1.15	0.96	0.37

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МИГРАЦИЯ ЭЛЕКТРОННО-ВОЗБУЖДЕННОЙ ЭНЕРГИИ МЕЖДУ ТРИПТОФАНАМИ, НАХОДЯЩИМ ИСЯ НА АКТИВНЫХ МЕСТАХ ЛИЗОЦИМА

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Известно, что 35—40 % от полной люминесценции водного раствора лизоцима происходит от триптофана 62 и 63. Химическими методами разделение люминесценции не осуществимо, поэтому мы установили кинетическую модель, применимость которой мы проверяли поляризационными исследованиями. Установили, что одна треть люминесценции происходит от триптофана 63, и две третих от триптофана 62. Использованием кристаллографических данных определили относительное положение индольных колец, определили полную энергию взаимодействия (1,75 эв) и частоту миграции ($3{,}7 \cdot 10^{12}$ сек$^{-1}$).