

Quantum-chemical study of spectral-luminescent properties of indole

V.A. Pomogaev and V.Ya. Artyukhov

V.D. Kuznetsov Siberian Physical Technical Institute at the Tomsk State University

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The quantum-chemical study of spectral and photophysical properties of indole by the Intermediate Neglect of Differential Overlap (INDO) method is carried out. The rate constants of radiative degradation k_r , internal transition k_{int} , and intercombination conversion k_{ST} are calculated. Various models of the indole molecule in neutral and aqueous solutions at low temperature are considered. The obtained results agree well with experimental data. In aqueous solutions, the indole absorption and fluorescence bands are shifted toward longer waves relative to neutral solutions.

Analysis of spectral-luminescent characteristics of proteins is one of the efficient methods for studying their structure and conformations. Only three amino-acid residues have absorption and fluorescence spectra in the near-UV region (tryptophan, tyrosine, and phenylalanine). In this list, tryptophan is of particular interest, since it most actively participates in various photophysical and photochemical reactions and its contribution to the fluorescence spectra of proteins is predominant (under the condition that tryptophan is included in a protein chain), and the position and intensity of tryptophan fluorescence spectrum may vary significantly depending on characteristics of a medium.

The shape of tryptophan absorption and luminescence spectra and the position of maxima are largely determined by the chromophore center of the molecule and indole ring without marked contribution of side groups. Therefore, the study of indole is rather urgent. The detailed study of various characteristics of indole in the ideal case should yield the "topography" of the emitting protein center, its molecular environs, and the form of the polypeptide chain. The knowledge of the absorption properties of protein is necessary for revealing the molecular structure, drawing the energy level diagram, selecting the spectral region for excitation of protein luminescence, and estimating true quantum yields. Fluorescence spectra give an idea on changes in the protein structure and molecular environment.

Photophysical properties of indole were studied in numerous experimental papers.¹⁻⁴ In particular, it was found that indole in non-polar solvents has two fundamental absorption bands: shortwave one with $\nu \approx 46500 \text{ cm}^{-1}$ and the molecular extinction coefficient $\epsilon \approx 25200 \text{ M}^{-1}\cdot\text{cm}^{-1}$ and longwave one splitting into two overlapping bands 1L_a and 1L_b in the region of 37600 and 35700 cm^{-1} with $\epsilon \approx 6300$ and $4100 \text{ M}^{-1}\cdot\text{cm}^{-1}$, respectively. In polar solvents, all absorption bands experience a small red shift. At the room temperature, the peak of the indole fluorescence spectrum is located near $\nu = 32470\text{--}32260 \text{ cm}^{-1}$ in non-polar solvents and

significantly (by $3000\text{--}4000 \text{ cm}^{-1}$) shifted toward longer waves at addition of polar components (the shift in ν as large as 4600 cm^{-1} was observed in water). In the frozen state, the fluorescence spectra of indole in various media were close to the fluorescence spectrum of indole in a neutral solvent, and alcohol and acid solutions have a high quantum yield of fluorescence. Fluorescence of amino-acids can be observed at low temperature in the region of $20000\text{--}25000 \text{ cm}^{-1}$ depending on pH of the medium. Some questions are still unclear: from what state does indole fluorescence originate in different media; what intermolecular and internal mechanisms favor the change of energy levels, and what factors affect the change of the quantum yield of indole and tryptophan luminescence.

Contradictions arising at interpretation of various experimental data can be resolved in some cases based on thorough theoretical calculations. But, unfortunately, there are only few works devoted to the study of spectral and luminescent characteristics of the indole molecule, and this work tries to meet this lack at least partly. The study was conducted within the framework of the standard quantum-chemical Intermediate Neglect of Differential Overlap (INDO) method with spectroscopic parameterization.⁵ Parameters were fitted for the molecule being in the ground state in non-polar aprotic solvent like cyclohexane at low temperature.⁶ The following transitions correspond to the absorption spectra of indole in a neutral medium: two close longwave weakly resolved transitions of the bands S_1 ($\nu = 34630 \text{ cm}^{-1}$, $\epsilon = 1160 \text{ M}^{-1}\cdot\text{cm}^{-1}$) and S_2 ($\nu = 37203 \text{ cm}^{-1}$, $\epsilon = 8570 \text{ M}^{-1}\cdot\text{cm}^{-1}$); then in the region of $43300\text{--}48800 \text{ cm}^{-1}$ four allowed transitions S_4 ($\nu = 43309 \text{ cm}^{-1}$, $\epsilon = 25465 \text{ M}^{-1}\cdot\text{cm}^{-1}$), S_7 ($\nu = 45137 \text{ cm}^{-1}$, $\epsilon = 27440 \text{ M}^{-1}\cdot\text{cm}^{-1}$), S_9 ($\nu = 46887 \text{ cm}^{-1}$, $\epsilon = 13250 \text{ M}^{-1}\cdot\text{cm}^{-1}$), and S_{12} ($\nu = 48456 \text{ cm}^{-1}$, $\epsilon = 14450 \text{ M}^{-1}\cdot\text{cm}^{-1}$). The molar extinction coefficient was calculated by the approximate equation $\epsilon_{\max} = 4.63 \cdot 10^4 f$ (Ref. 7). The dipole moment is equal to 2.2 D ; this value coincides with the experimental data and is connected with the presence of unshared

electron pair located at the nitrogen atom. The calculated ionization potential is 7.9 eV; this value also is very close to the experimental one 7.8 eV.

The general scheme of photophysical processes in the indole molecule is depicted in Fig. 1. According to the accepted classification of electronic states in neutral solvents, the level S_1 (α -band) corresponds to the transition ${}^1L_b \leftarrow A$, and the level S_2 (p -band) corresponds to ${}^1L_a \leftarrow A$. The information about polarization of these transitions is contradictory.¹ It is assumed that the direction of polarization of the transition to the α -band makes up an angle from zero to several tens of degrees with the long molecular axis, and the transition to the p -band is mostly oriented along the short axis. Analysis of calculations has shown that the angle between the directions of the both transitions is only 11.8° (${}^1L_b \leftarrow A$ makes up the angle of 11.6° relative to the long molecular axis, while ${}^1L_a \leftarrow A$ makes up the angle of 23.4°).

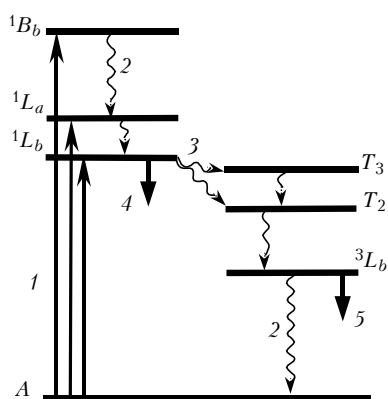


Fig. 1. General scheme of lower excited electronic states of the indole molecule and photophysical processes occurring in it: absorption (1), internal conversion (2), intercombination conversion (3), fluorescence (4), phosphorescence (5). Processes of vibrational relaxation are omitted in the scheme.

Shortwave transitions falling within the considered region are more intense and characterized by $\log \epsilon > 4.5$; they correspond to β -bands. Among four electronic states, only the transition to the S_{12} level is clearly polarized along the long molecular axis (1.6°) and is assigned as ${}^1B_b \leftarrow A$. Other levels can be classified only with respect to one another. The transition to S_4 occurs at the angle of 21.5° to the long molecular axis and it is assigned as ${}^1B_b \leftarrow A$, and the transition to S_9 (71.0°) is assigned as ${}^1B_a \leftarrow A$. The most intense transition to S_7 (40.1°) is assigned by us to the ${}^1B_b \leftarrow A$ type, because it makes up the angle smaller than 45° with the long molecular axis. The absorption band corresponding to this transition partly overlaps other shortwave absorption bands, whose effect is reduced to only elongation of the arm of spectrum. Just this transition (see Fig. 1) was assigned to the experimentally observed band 1B_b . Energy levels, transitions to which are forbidden, are omitted in the scheme.

The change in the indole absorption spectrum in aqueous solutions is connected with the effect of the hydrogen bond. To determine the place of formation of the hydrogen bond, the Molecular Electrostatic Potential (MESP) method was used. It was assumed that the water molecule is oriented with respect to the indole molecule in such a way that one O–H bond is directed through the point of minimum to an atom or bond, while another O–H bond makes up the angle of 106° with the first one. The separation between molecules is restricted by the Van der Waals spheres of C and O atoms (its value is taken to be 2.85 \AA [Ref. 8]). In this case, the closest hydrogen atom of the water molecule cannot be separated by less than 1.9 \AA from the carbon atom.

For the indole ground state, two deep minima (crosses in Fig. 2) are present at such a distance normally to the molecular plane near the C_3 atom (-58.8 kJ/mol) and the C_5 – C_6 bond (-46.8 kJ/mol).

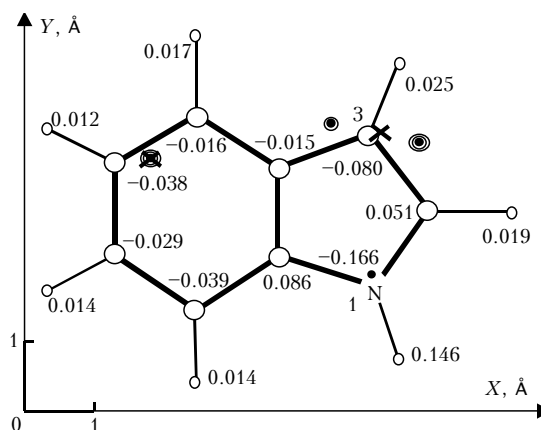


Fig. 2. Geometry, MESP minima ($Z = 1.9 \text{ \AA}$ – crosses, $Z = 1.5 \text{ \AA}$ – concentric rings, each ring corresponding to 20 kJ/mol) and effective charges of atoms for the indole molecule in the ground state.

Two minima of the C_3 atom (-47.8 and -55.4 kJ/mol) and one minimum of the C_5 – C_6 bond (-63.4 kJ/mol) are located closer to the indole plane (1.5 \AA). Thus, the O–H bond in the region of the C_5 – C_6 bond is directed normally to the indole plane through the points of minimum, and the O–H bond in the region of the C_3 atom is directed through the point of minimum (separated by 1.9 \AA from the molecule) to the atom. Two other H-bonds are symmetric about the molecular plane. An interesting fact is the absence of a deep minimum (-29.4 kJ/mol) near the nitrogen atom. This minimum should be caused by the presence of unshared electron pair. But the high negative charge density at nitrogen (-0.166 e) is compensated by the high positive charge of its hydrogen (0.146 e), while the MESP minimum near the C_3 atom is caused by the charge density difference between carbon (-0.080 e) and its hydrogen (0.025 e) (see Fig. 2). With allowance for four H-bonds, the following absorption transitions were obtained: ${}^1B_b \leftarrow A$ (44520 cm^{-1}), ${}^1L_a \leftarrow A$ (36305 cm^{-1}), ${}^1L_b \leftarrow A$ (34090 cm^{-1}). When comparing the results

obtained in a neutral solvent and in water, one can see that all bands experience the red shift, while the mutual arrangement of the levels keeps unchanged. This molecule has no centers with high localized charge density, and this causes low values of MESP minima and, consequently, very weak hydrogen bonds. The proposed model is a limiting case of interaction with water and therefore it gives the largest red shift in the spectrum. Under actual conditions, establishment of all four H-bond is likely impossible because of their high liability to destruction due to other intermolecular processes.

It is well-known that after the absorption process (Franck–Condon transition), the vibrational relaxation occurs with molecular transition to an excited equilibrium state. In this case the geometric structure of the molecule may change. Since the indole molecule is plane, the change in its geometry involves only a change of bond lengths, which can be calculated as $\Delta R_{AB} \approx -0.46 \Delta P_{AB}$ (Ref. 5), where ΔR is the change of the interatomic separation, and ΔP is the change of the bond population (order) at transition from one state to another. The calculated dipole moment in the 1L_b state is 3.1 D (in Ref. 3 the values of 2.9–3.3 D and even 5.0 D can be found, but the latter is likely overestimated). The calculations have shown that the change of the molecular geometry in the excited state leads to the change of radiation polarization. The direction of polarization of the radiative decay of the level 1L_b with $\nu = 32445 \text{ cm}^{-1}$ almost coincides with the long molecular axis, making up a small angle with it ($\alpha = 4.8^\circ$), and the rate constant of this process is $k_r = 2.5 \cdot 10^7 \text{ s}^{-1}$ (see Fig. 1). The radiative transition from 1L_a is directed at an angle ($\alpha = 28.9^\circ$) to the long axis, but it is improbable, because the rate constant of the internal conversion ($k_{\text{int}} = 3.5 \cdot 10^{11}$) to 1L_b more than three orders of magnitude exceeds $k_r = 1.6 \cdot 10^8 \text{ s}^{-1}$. Population of triplet levels is provided by the spin-orbit coupling (rate constants of intercombination conversion $k_{ST3} = 2.5 \cdot 10^8$ and $k_{ST2} = 1.8 \cdot 10^8$), what provides for the possibility of phosphorescence from the lower triplet band 3L_b with $\nu \approx 21000 \text{ cm}^{-1}$.

At excitation to the first absorption band, the electron density re-distributes from the five-member cycle to the benzene ring (Fig. 3). As this occurs, the charge density at C_3 ($\Delta Q = 0.031 \text{ e}$) and the nitrogen atom ($\Delta Q = 0.059 \text{ e}$) decreases. This re-distribution of the density leads to the strong growth of the charge at the C_5 atom ($\Delta Q = -0.080 \text{ e}$), as well as increase of the charge at the C_7 ($\Delta Q = -0.039 \text{ e}$) and C_8 ($\Delta Q = -0.043 \text{ e}$) atoms. Besides, the charge is transferred inside the five-member cycle from nitrogen to C_2 ($\Delta Q = -0.047 \text{ e}$) and inside the six-member cycle from C_6 ($\Delta Q = 0.030 \text{ e}$) to other atoms. As a result of such processes, the MESP minimum near this atom disappears completely, while the MESP minima in the region of the C_5 – C_6 bond become deeper (-78.2 kJ/mol) and a minimum (-79.3 kJ/mol) arises near the C_7 atom at the height of 1.6 \AA above the

molecular plane. It was assumed that the hydrogen bond establishes through these points of MESP minimum in the first excited state. The MESP minima located 1.2 \AA above the molecular plane were not considered as reaction centers: first, their values are lower than those considered earlier, and the interaction through them is improbable, and, second, they are too close to the molecular surface, what strongly hampers the interaction with water.

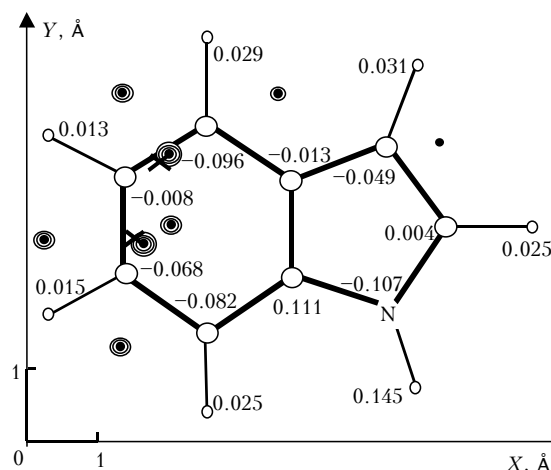


Fig. 3. Geometry, MESP minima ($Z = 1.9 \text{ \AA}$ – crosses, $Z = 1.5 \text{ \AA}$ – concentric rings, each ring corresponding to 20 kJ/mol) and effective charges of atoms for the indole molecule in the first excited state.

The separation between the MESP minima is roughly 1 \AA . Naturally, this does not allow two water molecules to be brought to one side of the plane because of steric resistance to approach of oxygen atoms. Three situations are possible for establishment of hydrogen bonds: the first and second – two water molecules come to one of the points of minimum above and below the plane, the third – two water molecules come to different points of minimum, one below and another above the plane. The calculations for all the three versions have shown that the radiation frequency shifts by up to 100 cm^{-1} . The fact of existence of two close MESP minima leads to the situation that different configurations of the indole molecule with a solvation sphere can arise. Under the effect of different solvents and temperature variations, the radiation frequency can shift by more than 100 cm^{-1} . The situation with double fluorescence is possible, but radiation originates from one state in different molecular environs. It should be noted that the reactivity of the indole molecule in the excited state increases with the increase of the values of MESP minima, and this defines the possibility for establishment of a stronger relation with the solvent.

The close agreement between the experimental and calculated results allows the following conclusions. Some red shift of the spectrum actually occurs in a polar solvent, the indole molecule in the excited state shows higher reactivity, the fluorescence spectrum in the aqueous medium experiences a slight red shift, what corresponds to the frozen state of the solution, and

double fluorescence can arise due to emission of different configurations of indole-solvent complexes.

Acknowledgments

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