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Electronic transitions in molecules in static external fields

I. Indole and Trp-59 in ribonuclease T_1

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Electronic transition properties of indole perturbed by its environment were calculated by use of quantum-mechanical semi-empirical numerical methods. The environment was represented by a discrete set of charges placed at different positions around the indole ring. Wavelength shifts and transition intensity changes in indole were evaluated for several, specifically modeled geometries of external charges. This methodology was employed to estimate the extent of spectroscopic changes induced by small nonprotein polar species on the Trp-59 residue in the anisotropic environment of the protein ribonuclease T_1. The geometry of the residue environment was obtained from dynamically equilibrated X-ray crystallographic data of the protein.

1. Introduction

Probing a molecule with light allows a wealth of information about the energy and dynamics of its electronic structure to be obtained. Techniques based on measuring the absorption, and particularly, the emission of absorbed light thus represent major research tools for biophysical studies of protein structure and function. The assumption that the perturbation caused by the absorption of light is small in comparison with the gross energetic and dynamic parameters of the protein is essential to such studies. Exchange of one, two or three photons of light [1] with a protein chromophore can be analyzed in terms of the electronic structure properties of (i) the optically responsive portion of the chromophore, (ii) the adjacent peptide chain, and (iii) groups in the protein adjacent to the chromophore. The latter category consists of submolecular protein fragments which, although well removed from the chromophore in terms of the protein's linear (primary) sequence, are brought closer by the folding of the main chain; solvent molecules and other solutes which have access to the chromophore would also be included. Within the energy domain of common excitation sources (250–300 nm) the indole moiety of the tryptophan residue is the principal intrinsic protein chromophore and as such has been the subject of extensive experimental and theoretical research. The electronic and nuclear structure of indole resembles somewhat the isoelectronic molecule naphthalene, yet it has a number of peculiar properties [2,3]. Two features distinguish indole from symmetric molecules of similar structure and size which, to some extent, have more predictable electronic structures and properties: the asymmetry of the nuclear framework and an apparent closeness (or even a pseudo-degeneracy) of the two lowest excited singlet states. Gross absorption

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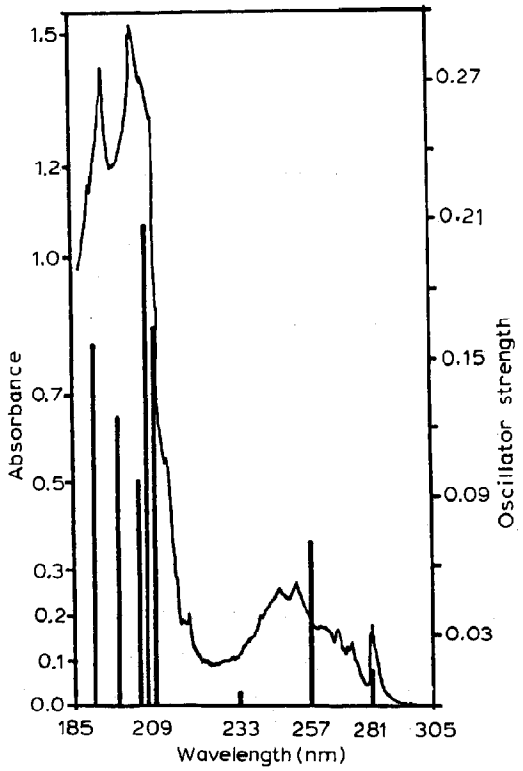

Fig. 1. Absorption spectra of isolated indole in the gas phase and positions and strengths of electronic singlet transitions in indole in a vacuum calculated by INDO/S with single and double excited configuration interaction (CISD) method.

spectroscopic features of isolated indole [4] are shown together with theoretical numerical results (P. Ilich and P.R. Callis, unpublished data) in fig. 1. The third property of the indole ring, which probably results from the previous two, is an apparent susceptibility of the highest, frontier electronic states to substituent and environmental perturbations. It is the latter property of indole that we consider in this paper.

In this, the first study of this type on indoles, we attempt to represent the environment-induced changes in the electronic structure properties related to spectroscopic characteristics of indole by a more quantitative method. The theoretical method used is simple and we consider specific, electrostatic-charge-induced perturbations of the absorption spectra of indole.

2. Technical part

Calculation of electronic transitions was performed by use of a spectroscopically parameterized version of the Intermediate Neglect of Differential Overlap (INDO/S) program [5,6] with relatively extensive use of configuration interaction. All calculations were conducted on a DEC Micro-Vax II computer. A reasonably good match between the calculated and the experimentally observed absorption spectrum of indole [4] was obtained by slightly varying overlap parameters in the INDO/S routine [7] (fig. 1). The geometry of indole used for the electronic transition calculations was derived from the best available crystal structure of indole [8] fully optimized by quantum mechanical semi-empirical routines. The environment of the indole ring in RNase T_1 was determined from the protein crystal structure [9,10] enhanced by adding all hydrogen atoms and subsequently optimizing the geometry with a molecular mechanics routine, CHARMM [11]. Geometric accessibility of this residue to nonprotein species such as water, quenching agents, was estimated by use of a modified version of the molecular contact surface routine [12–14]. Complex molecular structures (1454 atoms for RNase T_1) were displayed by use of the HYDRA molecular graphics routine (R.E. Hubbard, unpublished data: program HYDRA, University of York, U.K.) on an Evans and Sutherland PS330 graphics system.

3. Results and discussion

It is a difficult and challenging problem to model the environment of a fluorophore in quantum-mechanical calculations. This problem has been carefully considered (for example, see ref. 15 and reference cited therein) and several theoretical approaches have been proposed and numerically evaluated. In one approach a solute molecule is placed in a spherical cavity in a continuous isotropic dielectric representing the surrounding medium. The solute molecule is expressed as a dipole [16] or, in a more refined variant, as a multipole in an ellipsoidally shaped cavity. If short-range interactions at the solute/solvent in-

terface are considered more important, or if there is a high anisotropy of the surrounding medium, a set of discrete molecules, fragments or point charges can be used to simulate the environment [17]. Hybrid methods employ a discretized immediate environment enveloped in a continuum with bulk dielectric properties [18]. The strengths and weaknesses of most of these methods are well recognized though not yet fully explored, and the whole field is characterized by a need for intensive numerical experimentation and theoretical modeling.

The basic approach in the studies given here was: (a) to calculate electronic structure properties of a molecule of the size of indole; (b) to evaluate electronic transition parameters of the indole ring embedded in a highly anisotropic environment; and (c) to approximate closely the environment by considering structural characteristics of a specific protein. To achieve our objectives we have (i) limited our choice of theoretical tools to empirically parameterized computational quantum-mechanical methods; (ii) represented the environment by discrete electric charges; and (iii) evaluated the electronic transition energies and intensities with external perturbers arranged in accordance with chemical topology and the three-dimensional geometry of the protein RNase T_1, the latter having been dynamically equilibrated by use of molecular mechanics calculations.

The representation of an isotropic environment by use of discrete charges is a simple and intuitively appealing idea, probably first employed in quantum-mechanical calculations of electronic structure properties by Clementi [19]. A complete system in this model consists of the quantum part, indole in this case, and the classical part, electric charges, and is therefore quantum-mechanically inconsistent. 'Environmental' effects in this formalism can be easily visualized by employing the usual partition of the total solute energy to the following additive parts:
a nuclear-charge term,

$$\Delta E_n = \frac{Z_A Z_X}{R_{AX}} \tag{1}$$

a charge-electron term,

$$\Delta E_1 = \frac{\langle \phi_A | \phi_A \rangle}{R_X} \tag{2}$$

and, in the case where the external perturber is an ion,
the electron-electron term,

$$\Delta E_2 = \frac{\langle \phi_A \phi_X | 1 - P_{AX} | \phi_A \phi_X \rangle}{R_{AX}} \tag{3}$$

In eqs. 1–3, subscript A denotes molecular entities while X is the counter for charges; $< \phi |$ are electron wave functions, R the distance between centers and P_{AX} a transposition operator needed to generate the electron exchange term. For calculations of electronic transitions in a common approximation of fixed equilibrium nuclear frame, or Condon approximation [20], the term (eq. 1) makes no contribution. The contribution of two-electron integrals (eq. 3) is in principle smaller, and this becomes perceptible when the external perturbers are molecular fragments [21] for, within the INDO/S formalism (see, for example, ref. 22), two-electron repulsions are employed to approximate one-electron terms. In what follows, the field of external charges is effected through additive corrections to the diagonal elements of the one-electron integral prior to iterative, self-consistent generation of electron energy values and wave functions. Once the type of interaction has been identified, selected and discretized, there is the problem of determining the number, position and magnitude of external charges. The magnitude of charges, the only additional parameter in our calculations, is equal or proportional to the ground-state atomic charges in indole calculated by the INDO/S method. We thus introduce no additional parameters and make no assumptions beyond the INDO/S method and, consequently, we term our approach an a priori quantum-mechanical semi-empirical calculation. In determining the positions and the number of external charges we were guided by our perception of indole environments in particular systems. We considered several models of such environments, in accordance with the type and degree of exposure of the chromophore in different proteins, and used several methodologies to evaluate the effects of the environment on characteristic electronic transition parameters. A full account of this work will be presented in a separate communication [21]. The focus in this particular case has been on a

variable, less predictable component of external perturbation that is possibly experienced by the tryptophan residue in a shielded anisotropic enclosure within the interior of a protein. For this purpose we conducted a series of simple systematic calculations of indole electronic structure with single positive and negative charges, or clouds of small, mutually noninteracting charges, at different positions in the local, indole coordinate system. At the SCF level the induced effects are simple and easily predictable.

The effect of a single pointless charge on the INDO/S Hartree-Fock energy levels is given in fig. 2.

Positive and negative charges, when allowed to act simultaneously on all atoms, produce energy shifts which are equal in magnitude but opposite in sign. However, while the HOMO-LUMO gap remains unchanged, the relatively strong positive field induces an apparent oversize energy stabilization and dramatically increases the overall electrophilicity of indole. For our purpose, it is worth noting that for equal magnitude and distance, a charge perpendicular to the indole ring induces larger energy shifts than one placed laterally. The reason lies in the relative geometry of the perturbant rather than in the directionality of atomic orbitals. Another reason for this effect, even more pronounced beyond the SCF computational level, is that hydrogen nuclei and adjoining electrons which are largely localized to (1s) hydrogen atomic orbitals are most affected by a lateral approach to the perturbant. The energy of these states is relatively high and, within Roothaan's LCAO MO formalism [23], they contribute little to the low-energy states determining the optical transition gap of indole. We can say that, in this wavelength domain, short-range electrostatic interactions within the indole ring plane are less relevant.

Beyond the Hartree-Fock levels, at the configuration interaction level, the effects on the optical transition domain are more complex and essentially depend on the number and choice of configurations. We have routinely used over 200 canonical configurations which is a 5–20-times larger set than has been used in previous vacuum calculations of indole electronic structure [24–26]. Those configurations were preselected from about 2000 single and double excited configurations by use of perturbation expansion criteria [27].

Not surprisingly, a priori calculations of the electronic transition properties – energies, line strengths, intrinsic lifetimes, transition dipole moments and two-photon cross-sections in indole exhibit strong dependence on the position, magnitude and sign of the external perturbant. A substantial effort in model calculations was focused on selecting a set of distance-charge variables which would be computationally sensible and physically intuitive. We eventually limited the surrounding shell to 2–5 Å away from the indole, and the perturbing charge to the range from one tenth to one half of the unit charge. This distance domain is in accord with characteristic dimensions in (at least some) protein 'cavities' in which tryptophan side chain resides, while the second parameter is comparable with the calculated INDO/S net atomic charges. For example, the X-ray data for the RNase T_1 crystal structure [9,10] place the Pro-60 oxygen 2.06 Å away from the Trp-59 nitrogen atom. Combined with the range of INDO/S oxygen atomic charges (up to −0.6e)

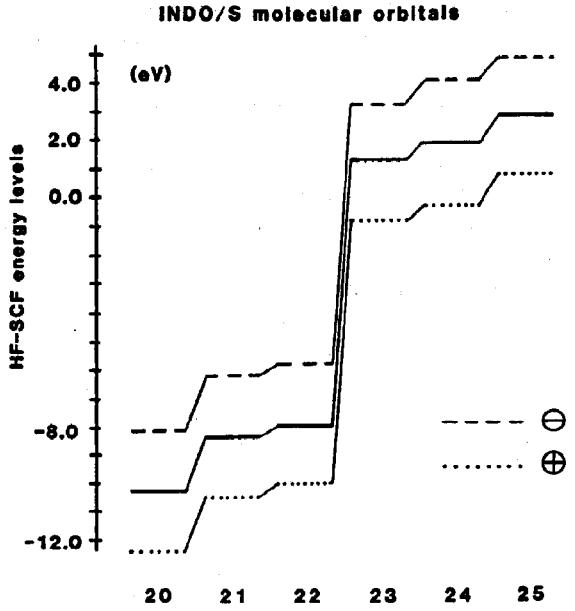

Fig. 2. Generalized effects of point charges on SCF MO energy levels in indole calculated by INDO/S method.

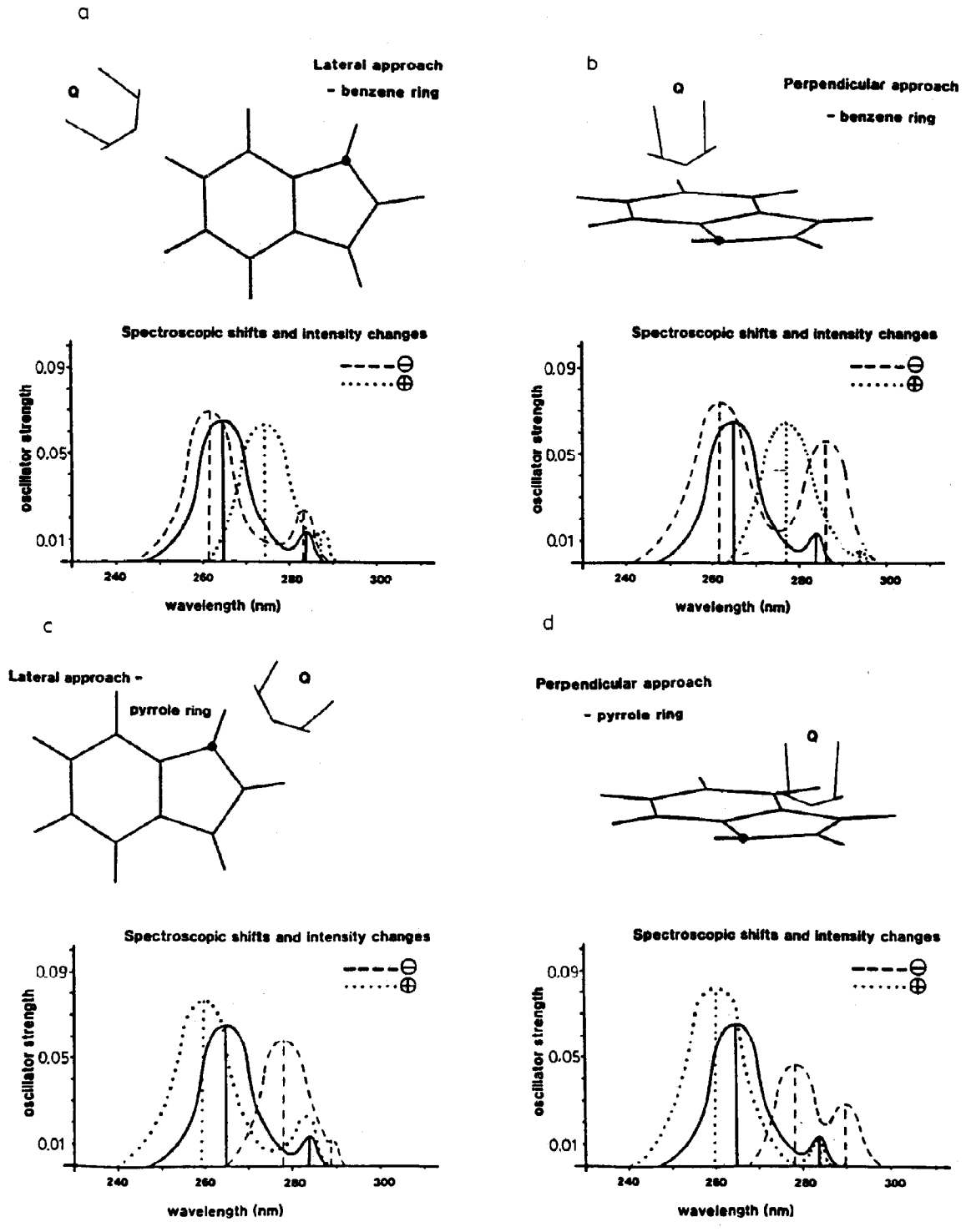

Fig. 3. Four different positions of external charges with nominal field $\phi = q/(4\pi\epsilon_0 R) = 3.5$ V/m and the corresponding energy shifts in indole calculated by INDO/S CISD method; simulated absorption curves are Gaussian envelopes with full-width at half-maximum (fwhm) = $f_{osc} \cdot 45$ kK.

this would result in a much stronger field than we routinely used in our calculations. We have limited our analysis of energy levels and oscillator strengths in indole to the two lowest singlet transitions, $S_{1\leftarrow 0}$ and $S_{2\leftarrow 0}$. (These are frequently, if somewhat inappropriately, labeled $^1L_b \leftarrow {}^1A_1$ and $^1L_a \leftarrow {}^1A_1$ transitions, respectively, after the Platt-Murrell [28,29] notation for electronic transitions in hexacenes.)

There are at least three reasons for selecting that spectroscopic domain: (i) $S_{1\leftarrow 0}$ and $S_{2\leftarrow 0}$ are experimentally the most accessible transitions; (ii) they are computationally more accessible than higher energy transitions which would require larger configuration interaction (CI) basis sets, beyond the power of most computers; and (iii) these transitions are quite susceptible to environmental perturbations and have been extensively exploited for that purpose [30–34].

The results of our preliminary calculations [35] as well as typical geometries of tryptophan residues in proteins suggest four preferential directions for the approaching perturbant: lateral and perpendicular to the benzene part (fig. 3a,b) and lateral and perpendicular to the pyrrole part of the ring (fig. 3c,d).

Several effects are prominent in the calculated transition energies and in the oscillator strengths for the first two singlet transitions in indole perturbed by external point charges:

(i) There is a distinct dichotomy between transitions induced by perturbing the pyrrole part and those induced by perturbing the benzene part of the indole ring;

(ii) The effects of positive and negative charges are generally, but not strictly, of opposite sense;

(iii) As already noticed for the HF-SCF levels, external charges perpendicular to either part of the indole ring are more effective than those placed laterally;

(iv) For all charges, distances and geometries, the calculated bathochromic (red) shifts in transition energies are several times larger than the comparable hypsochromic ones and that ratio is generally proportional to the nominal field strength of the external perturber.

In the next step we applied this methodology to the tryptophan residue in the fungal (*Aspergillus oryzae*) RNase T_1. The only tryptophan in RNase T_1 is the 59th amino acid in the total sequence of 104 [36]. According to the X-ray structure data [9,10] Trp-59 is positioned inside the protein in an oblate cavity of approximate dimensions 6×7.5 Å (fig. 4).

RNase T_1 has been regarded as a protein with a relatively inaccessible tryptophan, and this inference is corroborated by the X-ray crystal structure data [9,10] and by the characteristics of the fluorescence emission [37]. Two variants of the protein structure were analyzed for 'solvent' accessibility to tryptophan: the original X-ray data structure and an all-hydrogen representation geometrically adjusted by use of a molecular mechanics routine [11]; we termed the latter structure a canonical newtonian geometry of the protein. While the gross geometries are similar, there are significant differences between these two representations of RNase T_1 in terms of accessibility as well as in terms of the intrinsic environment of Trp-59. The knowledge of the latter was necessary for evaluation of the electronic transition parameters of indole placed in the Trp-59 environment of RNase T_1 in a vacuum. In the case of the X-ray

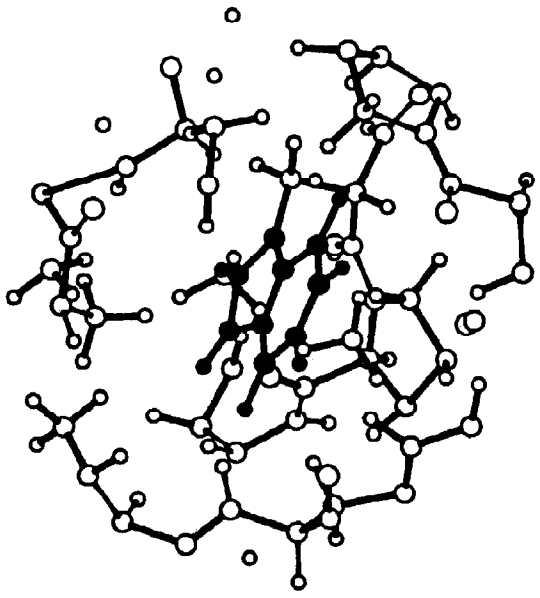

Fig. 4. X-ray structure of the Trp-59 immediate environment in the RNase T_1 with all hydrogen atoms explicitly represented.

structure the shift of the absorption bands would be largely dominated by the influence of the very close oxygen (barely over 2 Å!) of the Pro-60 residue. According to our calculations, the position of the $S_{2\leftarrow 0}$ band would then be shifted some 16 nm to the red. In the case of the X-ray structure with explicitly added all-hydrogen atoms and subsequently dynamically equilibrated, the proline fragment retreats back and resides most of the time some 3.5 Å away from the indole ring. The vacuum calculations, in the crude adiabatic approximations of this structure, indicate actually slight blue shifts in both $S_{1\leftarrow 0}$ and $S_{2\leftarrow 0}$ band positions while transition strengths remain essentially the same with respect to indole devoid of any environment. Here we present the results for the canonical structure where the tryptophan residue accessibility was evaluated by a van der Waals probe algorithm [12–14]. For this purpose, the original Trp-59 residue was replaced with the structure obtained by quantum-mechanical optimization of the X-ray structure of the indole moiety. There is a simple explanation for this seemingly impractical procedure: both the protein X-ray data and the CHARMM optimized bond distances and angles appear to be too coarse for the electronic structure calculations. One may question why the indole molecule was used in calculations instead of a tryptophan residue connected to a chain long enough to represent faithfully the electronic structure of the whole protein. While the resonant states, distribution of ring rotamers, polarization and polarizability of the adjacent peptide chain do influence the electronic structure in indole, even within a crude INDO/S representation [21], we simplified our model in order to expose more clearly the effects the variable nonprotein species exert on Trp-59 in RNase T_1. We feel that at this level of representation the tryptophan residue can be reasonably well approximated by indole.

The surface probe algorithm revealed two free-space regions in the vicinity of Trp-59: one, proximal to the benzene part, and the other more oriented toward the pyrrole part of the ring. As our INDO/S calculations show, the surface proximal to the pyrrole part plays little role in the response to externally applied charge. However, perturbation is to be expected from the pocket directed toward the H_6, C_6 and H_7 on the benzene part of the ring (here and throughout the text we follow IUPAC recommendations for labeling of the indole ring). A diffuse negative charge in that pocket would induce slight, blue transition shifts and an apparent increase in the transition strength of the lowest singlet. Positive charge, on the other hand, would cause red shifts and a decrease in transition strengths. Significant energy transition effects would be induced by strong fields (e.g., those caused by ions) in that region. Free ions, with high charge density per unit volume, are unlikely to be encountered inside the RNase T_1 without prior structural changes of the protein. Hydrated ions, on the other hand, are too bulky to be accommodated within the pocket directed toward the benzene part of the residue. A small polar molecule such as acrylamide might be a perturber. The results represented in fig. 5 are calculated for a charge equivalent to the highly polarized oxygen end in acrylamide and occupying the space between 1.9 and 3.1 Å in the pocket oriented toward the benzene part of Trp-59.

Interestingly, planar ground-state acrylamide is too branched a structure for the limited space of the pocket and a better fit is achieved by twisting the molecule around the middle bond at an energy expense of some 200 cm^{-1} *. In terms of sphere-packing requirements, a water molecule is an even better fit but the group proximal to the H_6, C_6 region in Trp-59 is most likely the hydrogen end of the water molecule. In either case the electrostatic effects are moderate at best.

In quantitative terms the results from fig. 5 are but a combination of the model geometries from fig. 3. In qualitative terms, these results represent relative changes in the electronic absorption spectrum of Trp-59 in RNase T_1 likely to be induced by a nonprotein probe. The calculated transition energy changes, although small, could be observable in the absorption spectrum of RNase T_1. Unfortunately, due to the absorption predomi-

* This value, calculated by NDO-structured semiempirical quantum mechanical routines, is probably somewhat low for the ground state acrylamide (but not for excited state!) (P. Ilich, unpublished calculations).

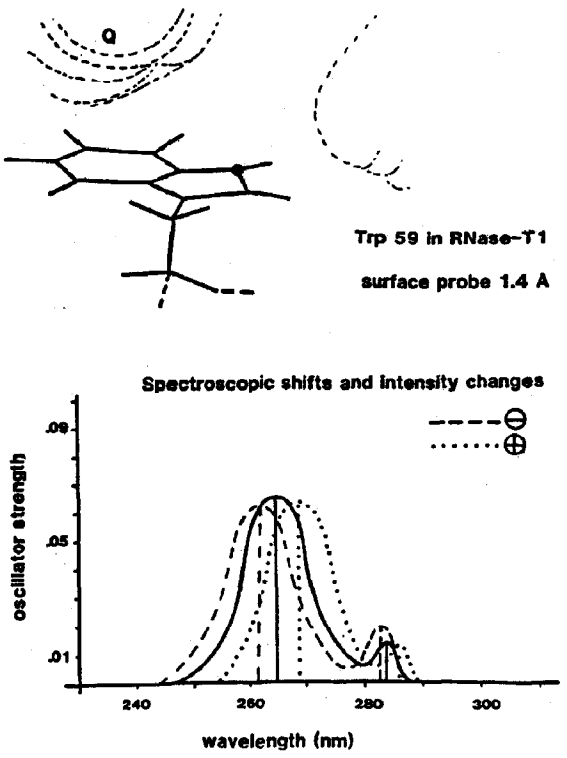

Fig. 5. Proximal surfaces to the Trp-59 and transition shifts and oscillator strength changes induced by a 0.7 unit charge diffused over the 'left' pocket within the 1.9–3.1 Å layer; the simulated absorption curve is a Gaussian envelope with fwhm $= f_{osc} \cdot 45$ kK.

nance of the nine tyrosyl residues in the RNase T_1, this prediction cannot be tested directly by absorption spectroscopy. One, though not trivial, test would be to look for a change of the intensity fo the Franck-Condon active C-C stretching band (mode 32 according to our calculations) observed in resonance Raman spectrum of tryptophan residue [38]. Our results do enable us, however, to make a zero-order prediction for the spectroscopic shifts in the protein emission spectrum which originates largely from the single tryptophan residue [39]. If the excitation is limited to the $S_{1 \leftarrow 0}$ domain, then, in the presence of a negatively charged perturbant in the vicinity of Trp-59, the emission should be relatively intense and unshifted to lower energies. On the other hand, if this negatively charged entity is also an effective fluorescence quencher then, in principle, the chromophore-quencher pair should exhibit a significant acoustic contribution in the excitation spectrum. If the specific perturber is acrylamide, the static geometrical parameters restrict the electrostatic influence; this, in conjunction with a large mismatch in energies of electronic transitions between the tryptophan residue and acrylamide (see footnote on p. 347), devalues the latter species as an effective perturbant of either the absorption or the emission of light in RNase T_1 (cf. refs. 37 and 40).

4. Summary

Absorption energies and intensities of indole perturbed by environment were evaluated by quantum-mechanical semi-empirical methods. The environment consisted of a discrete set of point charges or charged submolecular fragments and was designed, with respect to positions and magnitudes of charges, to simulate the anisotropic environment a tryptophan residue may experience in the protein interior. It was demonstrated that, within the formalism of a predominantly one-electron-type interaction, electronic transition energies and strengths are sensitive to the proximity, position, sign and magnitude of the external perturber. These properties were employed to evaluate spectroscopic energy shifts and changes in intensity induced by charged nonprotein species on the Trp-59 residue in RNase T_1. Molecular mechanics refinements of the X-ray structure of the protein confirmed that tryptophan in RNase T_1 is well screened by adjacent and proximal residues and that the benzene part of the indole ring is partially accessible to solvent or quencher species. INDO/S calculations with extensive configuration interaction showed that transition energies in the residue would be slightly blue-shifted by a negatively charged species in this region. We anticipate that this method will be very useful for the spectroscopic properties of intrinsic chromophores in native protein with respect to geometry, electrostatics and electrodynamics of the environment and in predicting specific spectroscopic changes induced on intrinsic or artificial chromophores in protein mutants.

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