A Biochemical Logic Gate Using an Enzyme and Its Inhibitor. 1. The Inhibitor as Switching Element†

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Molecular-scale logic systems will allow for further miniaturization of information processing assemblies and contribute to a better understanding of brain function. Of much interest are the pertinent biological systems, some of the basic components of which are biomolecular switching elements and enzyme-based logic gates.In this series of accounts, results of investigations are presented on the implementation of an enzyme/inhibitor logic gate operating under the rules of Boolean algebra. In this report (part 1 of the series), consideration is given to the experimental conditions—particularly the irradiation mode—that affect the performance of proflavine as inhibitor of α-chymotrypsin. Also, assessments are made on the reversibility of the process involved and the long-term stability of the system. Moreover, using a theoretical conformational analysis of proflavine and its reduction products, detailed features were established regarding their three-dimensional structure, partial charge distribution, and hydrophobicity. Accordingly, an understanding was reached as to the factors affecting the interaction between these compounds and the enzyme. In part 2 of this series, the actual implementation of an AND logic gate will be presented. This gate involves proflavine and a chemically derivatized α-chymotrypsin, and its operation relies on the conclusions reached in this report regarding the optimal mode for controlling the inhibitory activity of proflavine.

1. Introduction

Biomolecular electronics, as part of molecular electronics, is an emerging field to which chemistry, biophysics, molecular biology, electrical engineering, solid-state physics, and computer sciences contribute to a large extent. A growing interest is now developing in utilization of biological molecules in molecular electronics and, particularly, in artificial intelligence systems. This interest stems from the understanding that, by using currently available technologies, further miniaturization of electronic devices is severely limited by basic physical phenomena (*1*−*12*). Hence, to allow for progress to be made, it is intended to take advantage of the unique abilities of biological systems, such as pattern recognition, learning, self-assembly, and self-reproduction, as well as high-speed and parallel information processing.

The fundamental concepts for using biomolecules in logic gates were developed by the pioneering work of Rosen (*13*). He introduced a "two-factor model" based on the idea that the dynamic behavior of physiological and biochemical systems is regulated by the combined action of two factors, an excitatory one and an inhibitory one.

Enzyme-based logic gates (ENLOGs) contain—as biochemical functional elements—an enzyme and its substrate, two different enzymes, or an enzyme, its substrate, and an inhibitor and are capable of performing basic operations such as input, switching, processing, and output (see refs *13*−*15* and the references quoted therein). Depending on the particular mode of assembly and regulation of the composing elements, ENLOGs operate according to the rules of Boolean logic or of similar ones.

Results of preliminary studies on the effect of proflavine (i.e., 3,6-diaminoacridine) on the activity of α-chymotrypsin (αCT) were reported in the past (*16*). Thus, it was shown that proflavine is a powerful inhibitor of αCT, affecting the activity of this enzyme at concentrations as low as in the micromolar range. However, unlike proflavine, the products obtained upon its reduction with ascorbic acid are devoid of inhibitory activity. Based on this difference, it was suggested that the proflavine/reduced proflavine system can be used as switching elements of the enzyme. Also, it was indicated that the product of proflavine reduction is 3,6-diaminoacridan. Moreover, it was postulated that the difference in the inhibitory activity of 3,6-diaminoacridine and 3,6-diaminoacridan is due to a difference in their three-dimensional conformation, which is planar for the former and nonplanar for the latter (*16*).

In this series of accounts, we adduce results of studies on a biochemical logic gate in which an αCT derivative and proflavine are the key elements. For its function, this gate uses two input sources. The first is phenylazobenzoyl-α-chymotrypsin (PABαCT). This derivative was prepared by reacting αCT with phenylazobenzoyl chloride (*12*,*17*) and, in it, the PAB moiety is attached close to the entrance of the active site. Upon irradiation at appropriate wavelengths, reversible isomerization of the azo bond in the PAB moiety takes place and, as a result, the conformation of this moiety is reversibly changed between the cis and the trans forms. In the cis form, PAB

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obstructs the access of substrate to the active site, and this is the "OFF" state of the enzyme derivative. On the other hand, upon isomerization into the trans form, PAB swings away from the active site, access of substrate to the active site is now possible, and this is the "ON" state of the enzyme derivative. A detailed investigation of this system was reported earlier (*12*, *17*).

For the second input source of the logic gate considered, we use an inhibitor of the enzyme. To this aim, we rely on the reversible interconversion between 3,6-diaminoacridine (proflavine) and 3,6-diaminoacridan, mentioned above. Proflavine is an inhibitor of αCT and PABαCT, prevents enzymic activity, and, as such, is used as the "OFF" state of this input. On the other hand, 3,6-diaminoacridan is not an inhibitor, allows for enzymic activity and, as such, is used as the "ON" state of this input.

In this report (part 1 of this series), we present the results of a thorough investigation of the 3,6-diaminoacridine/3,6-diaminoacridan system and, particularly, of the chemical and physicochemical characteristics that allow for its use as a switching element of αCT. In a subsequent report (*15*), we describe the use of proflavine and an appropriately modified αCT derivative in the implementation of a logic gate performing the AND logic operation under the rules of Boolean algebra. The function of this gate is obtained by appropriate combination of the "ON" and "OFF" states of the two inputs, and this is achieved by appropriate manipulation of the input controlling signals.

2. Materials

2.1. Materials. α-Chymotrypsin (αCT), type II from bovine pancreas, and *p*-nitrophenylcaproate were purchased from Sigma Chemical Co. (USA), proflavine dihydrochloride dihydrate from Mann Research Laboratories (USA), and ascorbic acid from Aldrich Chemical Co. (USA). All reagents were of analytical grade and were used without further purification.

Two groups of optical filters were purchased from Ditric Optics (USA): (a) narrow band interference filters with transmission peaks at 334, 405, 420, 436, 450, 460, 470, or 490 nm, all having a bandwidth of 8 ± 2 nm at 50% peak height, and (b) a wide-band filter with a transmission peak at 453 nm and a bandwidth of 50 nm at 50% peak height. All these filters were further characterized in terms of their actual transmission characteristics (*12*).

2.2. Nomenclature. A distinction is here made with regard to the nomenclature of some of the materials employed. Thus, two "forms" of proflavine, which are actually two different compounds, were considered: the oxidized form, which is 3,6-diaminoacridine, acting as competitive inhibitor of αCT, and the reduced form, which is 3,6-diaminoacridan, having essentially no inhibitory characteristics toward this enzyme (*16*). The term "proflavine", without additional indication, is used for the oxidized form only.

3. Methods

3.1. Thin-Layer Chromatography (TLC). TLC separations were performed on F_{254} silica gel on aluminum plates (Merck, Germany), using various chloroform/methanol mixtures as the mobile phase. Spots were detected by irradiation at 254 nm using a Fluotest 5340 UV lamp (Hanau Co., Germany).

3.2. Spectrophotometry. Absorption spectra were measured using a Spectronic-2000 spectrophotometer (Bausch & Lomb, Rochester, NY). The same instrument was used also for characterization of the optical filters employed and for kinetic measurements. The latter were carried out using quartz cuvettes (1-cm light path length), before or after the irradiation process. No additive effects were noted to be due to the irradiation performed in the spectrophotometric procedure itself, most probably due to its much lower intensity compared to that of the optical control system.

3.3. Irradiation Procedure. ***3.3.1. The Optical Control System.*** The optical control system (*17*) includes a 100-W high-pressure Hg lamp, two liquid light guides transparent to UV, and the optical filters. This system was built so as to allow concomitant irradiation through one or two filters, as required. When irradiation was performed with "white light", no filters were used.

3.3.2. Irradiation Cycle. In this study, irradiation of test solutions was performed as a series of cycles. Each cycle involved a period of actual irradiation (control time), followed by a period of rest, when no irradiation was performed and during which optical and kinetic measurements were carried out.

3.3.3. Irradiation Mode. Using the optical control system mentioned above (section 3.3.1), the functional characteristics of proflavine were studied in relation to the irradiation mode and, particularly, to the following.

(A) Irradiation Time. Differentiation is herein made between the control time of irradiation in each cycle (up to 10 min) and the cumulative irradiation time, which is the sum of all control time periods (up to 60 min) in a given experiment.

(B) Irradiation Environment. The transformation of proflavine to its reduced form and vice versa was carried out at room temperature, in a capped quartz cell and under continuous stirring, in a controlled environment. The photoreduction was achieved by irradiation under a continuous flush of nitrogen (*16*), while back-oxidation was performed outside the optical control system, under air.

3.4. Optical Characteristics of Proflavine. An absorption spectrum of proflavine was measured, using a 0.02 mM solution in 0.2 M acetate buffer, pH 4.0. Two absorbance maxima were noted, at 260 and 444 nm, as also reported in the literature (*18*). The pertinent molar extinction coefficients are $\epsilon_{260} = 49\ 600\ M^{-1}\ cm^{-1}$ and $\epsilon_{444} = 34\ 800\ M^{-1}\ cm^{-1}$ (*18*).

3.5. Stability of Proflavine Solutions. Stock solutions of proflavine (4 mM in 0.1 M acetate buffer pH 4.0) were prepared weekly and stored in the dark. Under these conditions, no decomposition of the dye and no appearance of precipitate was noted, as also reported in the literature (*18*, *19*).

Solutions containing proflavine and ascorbic acid (0.02 and 2 mM, respectively, or 0.2 and 20 mM, respectively) in 0.1 M acetate buffer, pH 4.0, were prepared daily and stored in the dark. Under these conditions, no changes in the spectra of these solutions were observed.

3.6. Reduction of Proflavine and Back-Oxidation. The procedure employed is essentially the one described in the literature for irradiation with white light (*16*). Solutions containing 0.2 mM proflavine and 20 mM ascorbic acid (solution A) or 0.02 mM proflavine and 2 mM ascorbic acid (solution B), both in 0.1 M acetate buffer, pH 4.0, were employed. The processes were followed by measuring the absorbance at 444 nm, using the values $\epsilon_{444} = 34\ 800\ M^{-1}\ cm^{-1}$ for proflavine (3,6-diaminoacridine) (*18*) and $\epsilon_{444} = 0$ for the reduced form (3,6-diaminoacridan) (*19*). Reference solutions had the same composition with no proflavine included.

3.7 Activity of αCT. The activity of αCT (2×10^{-7}M) was measured using, as substrate, 4.3 mM *p*-nitrophenylcaproate in 0.02 M borate buffer, pH 9.1, also containing 0.1 M KCl and 5% (v/v) dimethyl sulfoxide (DMSO). All concentrations indicated are in the final assay mixture of a total volume of 1.25 mL. The actual pH of the assay solution was 8.6−8.7, and the reaction was followed by measuring the changes in absorbance at 400 nm. Under these conditions, the reaction rate was constant for up to 120 min. Throughout this study, the assay was run for 5 min, and calculations were made using a molar extinction coefficient of $\epsilon_{400} = 1.8 \times 10^4$ M^{-1} cm^{-1} for the *p*-nitrophenolate ion (*20*).

Kinetic parameters were determined from activity measurements at initial substrate concentrations in the range 4.3×10^{-7} -4.3×10^{-3} M and using the Lineweaver−Burk procedure.

3.8. Activity of αCT in the Presence of Proflavine. The activity of αCT in the presence of proflavine (3,6-diaminoacridine) was measured essentially in the same manner as in its absence (section 3.7), with the only difference that various concentrations of proflavine (2×10^{-7}−2×10^{-4} M; these are final concentrations in a 1.25-mL assay mixture) in 0.1 M acetate buffer, pH 4.0, were used. Reference solution contained no enzyme. Kinetic constants were determined using Lineweaver−Burk plots (*21*).

3.9. Conformational Analysis. Conformational analysis was carried out using the Macromodel Version 3.0 software (Columbia University, NY). This software allows one to establish the most probable conformation of a given molecule (i.e., the conformation of lowest energy) and to depict it in a stereoview. It also allows one to calculate the distribution of partial electric charges on each atom in the otherwise neutral molecule, as well as to assess the hydrophobicity of the molecule. The latter characteristic is deduced from the energy required for transferring the molecule considered from vacuum to aqueous environment, this being calculated as the difference between the conformational energies in the two environments. The more positive (or less negative) this difference is, the more hydrophobic is the molecule.

The numerical calculations required were performed on a system involving a 9120 VAX computer (Digital, USA), an IBM PS/2 system as terminal, and the Tgraf-07 emulation software (Grafpoint, USA).

4. Results

4.1. Chemical and Biochemical Characteristics of Proflavine. ***4.1.1. Purity of Proflavine.*** The purity of proflavine was assessed by TLC, using chloroform/methanol mixtures of various compositions (3:7, 7:3, or 5:5 v/v) as the mobile phase. In all cases, only one spot was detected. Proflavine from the same supplier was previously found to be homogeneous upon electrophoresis at pH 4.6 or chromatography in *n*-butanol/acetic acid/water (4:1:5 v/v) (*18*). Hence, proflavine was used as such, without any further purification.

4.1.2. Inhibition of αCT by Proflavine. Kinetic measurements were performed both in the absence and in the presence of proflavine, as described in sections 3.7 and 3.8 above. Representative results are presented in Figure 1, where we note the following.

(a) The line representing results obtained in the absence of inhibitor intercepts the 1/[S] axis at -4×10^4 M^{-1} and the 1/*v* axis at 13.2 (Δ absorbance units at 400 nm)$^{-1}$ × min. From these values, we have calculated $K_M = 2.5 \times 10^{-5}$ M and $V_M = 0.076$ (Δ absorbance units at 400 nm)/min.

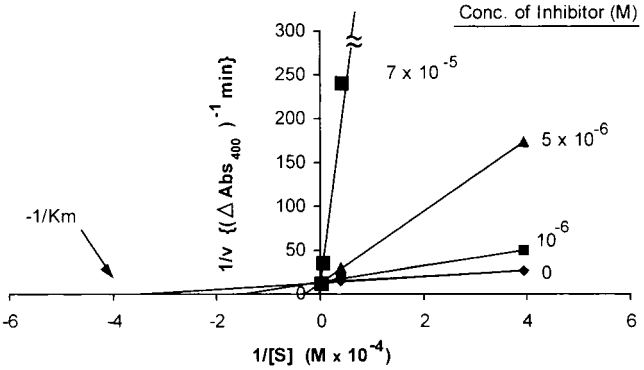

Figure 1. Activity of αCT in the absence and in the presence of proflavine as inhibitor. Representative data are presented in terms of Lineweaver−Burk plots, and the concentration of inhibitor is indicated for each plot.

(b) The line representing data obtained in the absence of inhibitor and the lines representing data obtained in the presence of several concentrations of inhibitor all meet in one point located on the 1/*v* axis. This feature is characteristic for the inhibition taking place by the competitive mechanism (*21*).

For processes taking place by the competitive mechanism, as documented under (b) above, the inhibition constant, K_i, can be calculated from the slopes of individual lines presented in Figure 1 (*21*). To this aim, we take cognition of the fact that the slope of the line representing results obtained in the presence of inhibitor is increased by a factor of $\{1 + [I]/K_i\}$, compared to the slope of the line representing results obtained in the absence of inhibitor (*21*). Here, [I] stands for the concentration of inhibitor in the particular set of experiments considered. Based on the above, the inhibition constant was calculated to be $K_i = 0.4 \times 10^{-6}$ M.

4.2. Photochemical Characteristics of Proflavine. ***4.2.1. Reduction of Proflavine and Back-Oxidation: Preliminary Studies.*** Photoreduction of proflavine was carried out using the optical control system described in section 3.3.1. As proton donor, ascorbic acid in 0.1 M acetate buffer, pH 4.0, was used, as recommended (*19*). In these preliminary studies, the proflavine-containing solution B was irradiated using white light, and the reduction−oxidation cycle was followed as indicated in section 3.6.

The results obtained are shown in Figure 2. We note that the absorbance decreases during the photoreduction process and increases back during reoxidation, as also indicated in the literature (*16, 19*). In addition, a slight shift of the spectrum from 444 to 426 nm was observed upon reduction, and it is reversed upon oxidation.

4.2.2. Irradiation Regimen. To assess the performance of proflavine as a switching element and to optimize its efficiency, a detailed investigation was carried out regarding the effects of experimental conditions prevailing during irradiation. In particular, we considered the effects of the concentration of the solution employed (section 4.2.2-A), as well as of the duration of irradiation (section 4.2.2-B) and its spectral characteristics (section 4.2.2-C). Based on the results obtained, experimental conditions were delineated for use in further studies, and these are described in section 4.2.2-D.

4.2.2-A. Effect of Concentration. Proflavine was photoreduced upon irradiation through the 453-nm wide-band filter and back-oxidized by air. The absorbance of the solution was measured at 444 nm, at 5−10 min intervals (i.e., control time). The reference solution contained no proflavine. At the end of the photoreduction

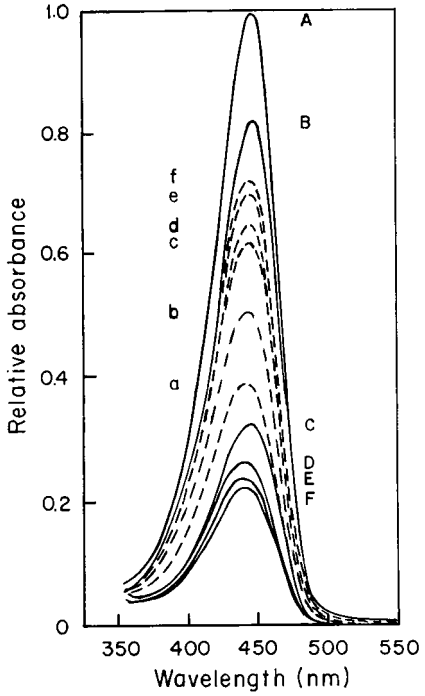

Figure 2. Changes in the visible absorption spectrum of proflavine during photoreduction upon irradiation with white light (—) and back-oxidation (- - -). The letters on the right- and left-hand sides of the spectra indicate the sequence time when the pertinent spectra were recorded during reduction and back-oxidation, respectively.

period (cumulative time of 60 min), back-oxidation was performed for 60 min, and this process was also monitored at 444 nm, at 5−10 min intervals.

Experiments were carried out with both solution A (in which the concentrations of proflavine and ascorbic acid are higher than those of solution B) and solution B. The results obtained are depicted in Figure 3A and expressed in terms of optical density values relative to the OD of the starting solution. We note that, upon reduction, solution A exhibits a fast change in relative OD after only 10 min cumulative time, reaching a limiting value $LA_{red} = 0.3$. With solution B the process is even more extensive, reaching the pertinent limiting value $LB_{red} = 0.1$. A similar difference between the two solutions is also noted for the back-oxidation process, the pertinent limiting values being $LA_{ox} = 0.7$ and $LB_{ox} = 0.8$ for solutions A and B, respectively. Thus, lower concentrations of reagents allow for more extensive changes in the proflavine and the reduced form molecules. Moreover, the results obtained with solution A are in line with the ones reported in the literature (*16*) for irradiation with visible light.

4.2.2-B. Effect of Irradiation Time. This section considers the effects of both control time and cumulative time of irradiation on the photoreduction of proflavine. Solution B was photoreduced using the 453-nm wide-band filter and at control times of 1−3 and 5−10 min. Figure 3B shows that a more rapid decrease of absorbance is obtained using long irradiation periods.

4.2.2-C. Effect of Spectral Characteristics of Irradiation. The results presented in sections 4.2.2-A and 4.2.2-B were obtained using a wide-band filter. Its optical characteristics (i.e., maximal transmission at 453 nm and bandwidth of 50 nm) match very well the wide optical absorption range in the visible region of the proflavine spectrum. However, the possibility exists that this absorption range of proflavine represents the overlap of a

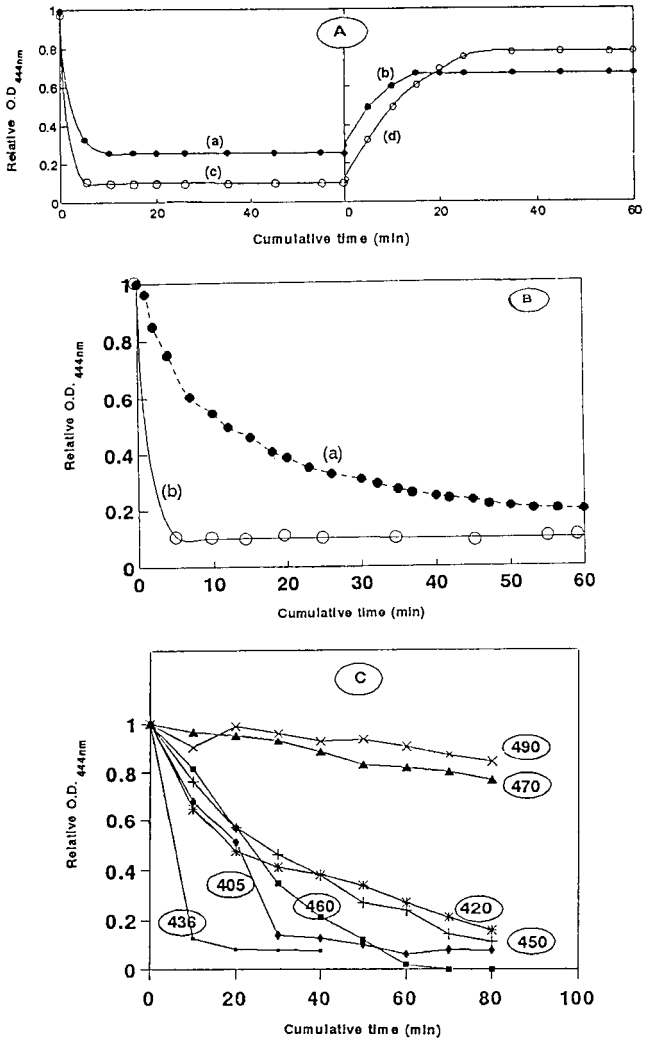

Figure 3. Changes in absorbance of the proflavine solution. (A) Dependence on solution concentration for control time periods of 5−10 min. (a and c) Reduction upon irradiation through the 453-nm wide-band filter; (b and d) oxidation using air. ●, Solution A; ○, solution B. (B) Dependence on time of irradiation during reduction upon irradiation through the 453-nm wide-band filter, using solution B. (a) Control time 1−3 min; (b) control time 5−10 min (taken from panel A). (C) Dependence on the wavelength of irradiation during reduction, using solution B and control time periods of 5−10 min. The numbers on the graphs indicate the wavelength characteristic of the narrow-band filters used.

series of narrow absorption bands. Hence, it was of interest to carry out the photoreduction process upon irradiation within narrow spectral ranges. It was intended that, in so doing, one can identify individual bands that are responsible for the photoprocess considered and, therefore, one can control the activity of the proflavine switch with minimal interference from overlapping effects.

Toward this aim, studies were conducted using the narrow-band filters listed in section 2.1. All other experimental conditions were kept constant and as determined above to be optimal; thus, for reagents' concentrations, we used the ones for solution B, and the control time was 5−10 min.

The results obtained were assessed on the basis of three criteria.

(a) Efficiency. Experiments were performed as described above, and the results obtained are presented in Figure 3C. We note that irradiation at 470 or 490 nm

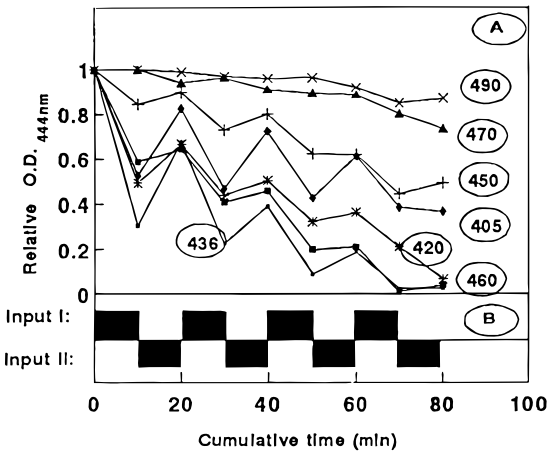

Figure 4. "Switching" of proflavine and its survival characteristics during alternating periods of 10 min of photoreduction (panel B, input I) and 10 min of back-oxidation (panel B, input II) using solution B. The numbers on the graphs indicate the wavelength characteristic of the narrow-band filters used.

has almost no effect, irradiations at 405, 420, 450, or 460 nm are more efficient, and the most pronounced effect is achieved at 436 nm.

Based on these measurements, the "efficiency" is defined as the relative amount of dye that undergoes reduction during the first 10 min of irradiation, and this is quantitatively expressed as the pertinent decrease in relative OD at 444 nm. For example, upon irradiation at 436 nm, the decrease is from 1.0 to 0.15; therefore, the efficiency is 0.85. The values thus obtained and their interpretation are presented in section 4.2.2-D.

(b) Reversibility. The reversibility of the process is measured for consecutive periods of irradiation of solution B in anaerobic conditions followed by oxidation with air. Experiments were carried out using the narrow-band filters, and the results obtained are depicted in Figure 4. From these data, the reversibility is calculated by summation of the absolute differences in the relative OD values between successive states of reduced and oxidized dye and vice versa, for a cumulative time of 60 min. We note that, by this criterion, too, the effects of irradiation at 470 or 490 nm are negligible, while irradiation at 436 or 405 nm is optimal. These results are also summarized and interpreted in section 4.2.2-D.

(c) Survival. This quantity expresses the tolerance of proflavine to a sequence of irradiation and aeration stages. The experiments are carried out as a series of irradiation (10 min) and aeration (10 min) periods, and the results obtained are the ones presented in Figure 4. The survival is defined as the relative OD at 444 nm reached after a total treatment time of 60 min. The results obtained and their interpretation are also presented in section 4.2.2-D.

4.2.2-D. Irradiation Regimen: Summary. The data on efficiency and reversibility of the process, as well as the survival of the system upon irradiation, are collected and compared in Figure 5, where they are also related to the visible absorption spectrum of proflavine. We note that the spectral distributions of the efficiency and reversibility match the absorption spectrum, suggesting that a band centered in the 436-nm region is, indeed, responsible for the phenomena observed. In this respect, however, the efficiency at 450 nm is somewhat lower than expected. For the time being, we have no explanation for this feature.

The spectral distribution of survival is essentially the mirror image of that of the efficiency: the higher the

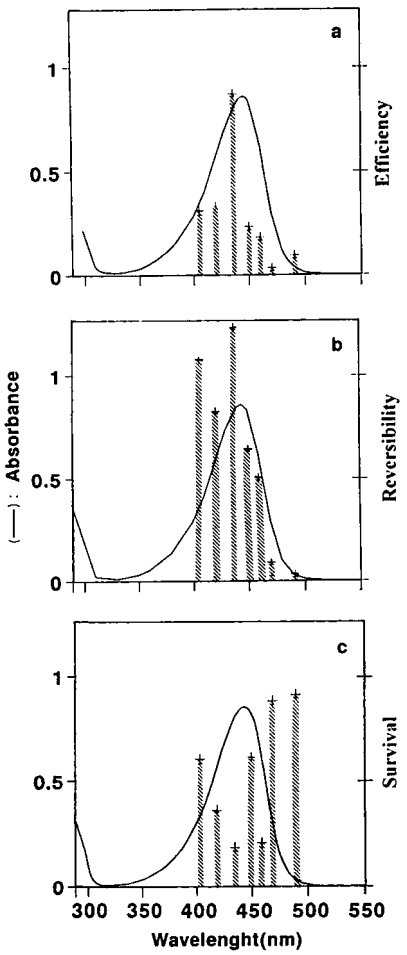

Figure 5. Functional characteristics of the proflavine switch, as related to its visible absorption spectrum. Efficiency, survival, and reversibility (hatched bars) are defined in section 4.2.2-C in text.

efficiency, the lower the survival of the system, and vice versa. These results fully support the conclusion mentioned above. Here, too, the results at 450 nm are rather unexpected.

As to the reversibility of the process considered, we note that this is very high almost throughout the entire region of the visible absorption band, with the exceptions at 470 and 490 nm.

As a result of these analyses, irradiation at 405 or 436 nm was considered optimal and was selected for all further studies (*15*).

4.2.3. Conformational Analysis. The characteristics of proflavine as a competitive inhibitor of αCT have been investigated in the past (*16*, *18*, *22*). As a result of these studies, it was proposed that the loss of this inhibitory ability upon irradiation is due to the photocatalyzed reduction from the acridine to the acridan form (*16*). Moreover, it was postulated (*16*) that this transformation is associated with a change in the molecule from a planar conformation to a nonplanar one. This particular conclusion is reconsidered here, using a comprehensive conformational analysis of the compounds involved.

Reduction of proflavine (3,6-diaminoacridine) may, in principle, yield three products, and their structures are shown in Figure 6 as products **I**, **II**, and **III**. These products differ from one another by the position—in the central ring—where the two newly introduced hydrogen atoms are located. To establish which of these products is most probably formed, we subjected them to a detailed

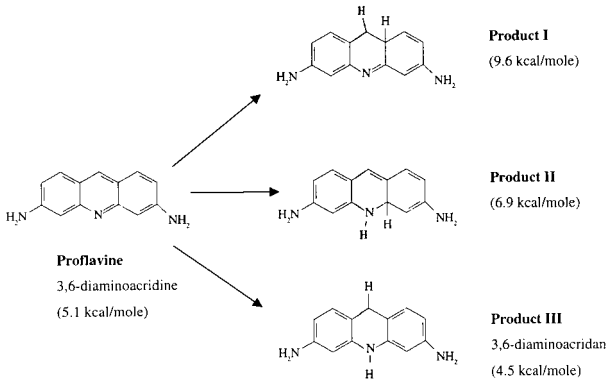

Figure 6. Chemical structures of proflavine (3,6-diaminoacridine) and of its possible reduction products. For each compound, the lowest potential energy is indicated.

conformational analysis using the powerful Macromodel software. For each of them, as well as for the 3,6-diaminoacridine starting material, the lowest potential energy is also indicated in Figure 6. Considering these values, we conclude that the most probable reduction product is product **III** (3,6-diaminoacridan), as also suggested in the literature (*23, 24*).

Furthermore, the conformational analysis carried out also allowed us to depict the three-dimensional structure of the compounds considered. Their most probable conformation is shown in Figure 7, where each compound is presented in stereoview, both from above the plan and as a side view. We note that proflavine is, indeed, planar, as also suggested in the literature (*16*). On the other hand, the products having a low probability of formation (i.e., products **I** and **II**) are the only ones that are nonplanar, and to a slight extent only. However, 3,6-diaminoacridan (product **III**), the most probable product of the reduction process, is fully planar, like proflavine. This particular conclusion is in contradiction to what was previously assumed (*16*).

In view of the fact that both proflavine and 3,6-diaminoacridan assume a planar conformation, this particular characteristic cannot be invoked to explain the difference in the inhibitory potency of these two components toward αCT. As such, the search was extended by considering two other characteristic features.

(a) Distribution of Partial Charges. The results of calculations are presented in Figure 8. We note that, upon reduction, rather large changes occur at the atoms forming the central ring of proflavine and, particularly, at the nitrogen heteroatom and at the atoms attached to it.

(b) Hydrophobicity. Calculations were performed as described in section 3.9. As indicated in Figure 6, the conformational energies of proflavine (P) and 3,6-diaminoacridan (AN) in vacuum are 5.1 and 4.5 kcal/mol, respectively. For the same two compounds, the conformational energies in an aqueous environment were calculated to be 11.6 and 12.8 kcal/mol, respectively. Thus the energies for transferring P and AN from vacuum (representing the nonpolar medium) to an aqueous environment are $\Delta E_P = 6.5$ kcal/mol and $\Delta E_{AN} = 8.3$ kcal/mol, respectively. Based on these values, we conclude that 3,6-diamonoacridan is more hydrophobic than proflavine.

The results presented above lead us to the understanding that the lack of inhibitory ability of 3,6-diaminoacridan is not due to a three-dimensional conformation formerly (*16*) assumed to be different from that of the 3,6-diaminoacridine inhibitor. Rather, it may be due to

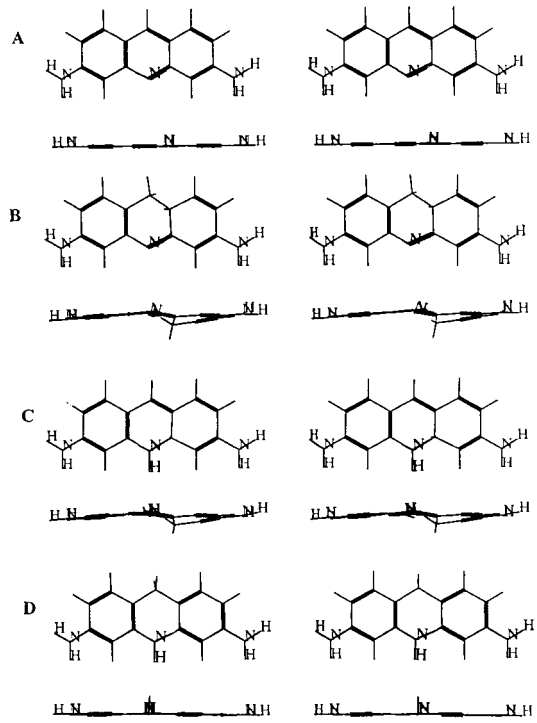

Figure 7. Three-dimensional stereoview of the most probable conformation of proflavine and of the three possible reduction products shown in Figure 6. In each panel, the top section presents the view from above the plan, while the bottom section presents the side view. (A) 3,6-Diaminoacridine; (B) product **I**; (C) product **II**; (D) product **III** (3,6-diaminoacridan). In all the structures presented, the heavy bar stands for a double bond.

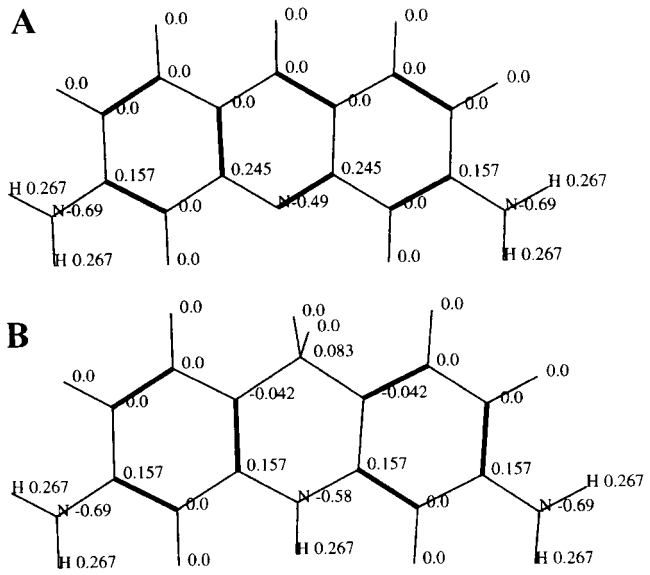

Figure 8. Partial charge distribution in 3,6-diaminoacridine (A) and 3,6-diaminoacridan (B). In both structures presented, the heavy bar stands for a double bond.

a different charge distribution pattern and a different hydrophobicity, and most probably these are the factors that lead to a much weaker interaction with the enzyme.

5. Conclusions

In this study, we used a combination of experimental and theoretical methodologies in order to assess and optimize the ability of proflavine to serve as a switching element in biochemical logic systems.

The experimental investigation allowed us to delineate optimal conditions for operating this switch and, par-

ticularly, the concentration of reagents as well as timing and optical characteristics of irradiation.

The theoretical conformational analysis led us to the understanding that both 3,6-diaminoacridine and 3,6-diaminoacridan assume planar three-dimensional conformations. Hence, the difference in the inhibitory characteristics of the two molecules stems from a difference in their charge distribution pattern, leading to different capabilities to interact with the enzyme.

These conclusions are implemented in further studies (*15*).

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