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A molecular switch for biochemical logic gates: conformational studies

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Abstract: This report presents the computer-assisted design of a molecular switching element, in which a molecular switch regulates the enzymatic activity of Ribonuclease A (RNase A). The molecular switch, an appropriately modified amino acid residue, is constructed with an electron donor group and an electron acceptor group, connected to one another with a conjugated double bond bridge. The switching mechanism is based on the azonium-hydrazo tautomerization, by which a charge separation induced in the excited state causes a rearrangement of the molecular electronic structure, resulting in the exchange of locations of single and double bonds. This rearrangement of bonds leads to different three-dimensional conformations of the switch. Using the electrostatically driven Monte Carlo (EDMC) method and the empirical conformational energy progam for peptides (ECEPP/3) potential energy function, we carried out an exhaustive search of the conformational space of the switching element. The results of these calculations reveal two sets of conformations: in one set the access to the active site of the enzyme is preferentially blocked, while in the other set the active site is preferentially accessible. Integration of the designed element into biochemical logic gates operating under the rules of threshold value, and experimental implementation of this system, are considered. © 1996 Elsevier Science Limited

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1 INTRODUCTION

By a currently accepted definition (Birge, 1994), molecular electronics is a multi-disciplinary area in which molecular systems are employed for coding, manipulation and retrieval of information. Accordingly, the logic gates involved, as well as their constituent switching elements, are molecular species and molecular assemblies. As part of this undertaking, bio-molecular electronics relies on the use of biological molecules and, in so doing, takes advantage of their most unique characteristics and specific modes of action.

Theoretical and experimental evidence is accumulating about the ability of biomolecular systems to carry out logic-type operations (Lotan *et al.*, 1993; Tuchman, 1993; Adleman, 1994; Birge, 1994, 1995; Tuchman *et al.*, 1994; Bray, 1995; Sivan, 1995). Thus, for example, we have

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previously described the design of enzyme-based logic gates (ENLOGs)* working under the rules of Boolean algebra (Lotan *et al.*, 1993; Tuchman, 1993; Tuchman *et al.*, 1994; Sivan, 1995). More recently (Adleman, 1994), it was shown that one can envisage a sequence of DNA manipulations for which critical performance characteristics, such as operation speed, energy efficiency and information storage density, far exceed those reached even by the currently available supercomputers. These manipulations can thus be used for solving highly complex mathematical problems.

In order to accommodate the particular features of biological computations properly, as well as to take full advantage of its high complexity and capability, appropriate mathematical frameworks are being developed. In this respect, representative examples are the bio-algebras, described recently (Reischer & Simovici, 1990). Obviously, even such computations are subject to fundamental physical constraints and/or limitations, particularly to the ones of thermodynamic origin (Margolus, 1992; Adleman, 1994).

For the experimental implementations of some of the concepts related to biological computing and biochemical logic gates, enzyme-based molecular switching elements (ENMOSEs)† are ideal as basic components. For them to carry out their predetermined function under defined environmental conditions, efficient techniques for their rational design must be developed. To reach this goal, molecular mechanics, conformational analysis and statistical thermodynamics procedures are appropriate.

We are currently involved in a research program aimed at designing ENMOSEs in which enzymic activity can be switched ON and OFF by outside controllers, such as optical signals. For this purpose, we focus on the use of enzymes in which a particular amino acid residue is replaced by an appropriately designed amino acid derivative. This derivative is chosen so that the outside optical signal will cause it to undergo a predictable conformational change which, in turn, will afffect the biological activity of the enzyme. Thus, when such an amino acid derivative is incorported in the enzyme, it performs as the molecular switch (MOS)* component of the ENMOSE.

Previously (Ashkenazi, 1992; Lotan *et al.*, 1993), we have used some basic molecular modeling approaches and designed ENMOSEs involving lysozyme and α-chymotrypsin. The MOS moieties employed there function on the principle of soliton propagation, and operate between two stable conformations, both of the ground state type. The switching act involves a 'stroke' of an appropriately designed arm (the molecular switch). Similar phenomena, such as muscle contraction and cell movement (Spudich, 1994), and the function of an allosteric enzyme (Joseph *et al.*, 1990) and of the trans-membrane Na^+ channel gate (Catterall, 1993), were suggested as being involved in these natural systems.

In the present study, we extend these design activities and use Ribonuclease A (RNase A) as the working enzyme. This choice was prompted by the fact that RNase A can be cleaved selectively by subtilisin (Richards & Wyckoff, 1973) to yield two fragments, both enzymically inactive: the S-Peptide (amino acid residues 1–20) and the S-Protein (amino acid residues 21–124). Under appropriate conditions, these two fragments can be reassembled to form an enzymically active complex (Richards & Wyckoff, 1973). Thus, when a suitably located, yet non-essential amino acid residue, is identified in the S-Peptide fragment, its substitution by an appropriately designed MOS is realistically feasible, even using only the currently available techniques of peptide chemistry. These unique features of RNase A make it a most appropriate system for the task considered.

The previous studies (Ashkenazi, 1992; Lotan *et al.*, 1993) are extended here for two principal reasons: to provide a basis for implementing the concept of soliton-based switching; and to use the most advanced procedures for molecular mechanics computations, thereby allowing us to consider systems of higher complexity. Thus, unlike in the previous study, the MOS is built here from an electron donor and an electron acceptor group, connected to one another by means of a conjugated double bond bridge. Fur-

* An ENLOG is a multi-component assembly containing ENMOSEs, capable of performing logic operations of Boolean or other types.

† An ENMOSE is an enzyme into which a MOS was built, capable of performing as a component of ENLOGs.

* A MOS is a modified amino acid residue, built into the enzyme, and specially designed for carrying out ON–OFF switching operation of the enzymatic activity, as directed by an outside signal.

thermore, the switching process involved relies here on the operating principles of the 'push–pull polyenes', displaying non-linear optical characteristics (Roth, 1986, 1987; Gohring *et al.*, 1987; Blanchard–Desce *et al.*, 1988; Roth *et al.*, 1989; Shen *et al.*, 1990; Lehn, 1991; Buckley, 1992). A charge-separated form (i.e. the excited state) is induced by an outside optical signal, leading to an intramolecular rearrangement of the electronic structure, and to interchange in the locations of single and double bonds. It is the latter that is the main cause for the desired conformational change occurring in the MOS.

The basic mechanism of the process described above can be expressed either in terms of an electron transfer from donor to acceptor (Effenberger *et al.*, 1988; Maier *et al.*, 1989; Slama-Schwok *et al.*, 1990; Effenberger & Wolf, 1991), or in terms of soliton propagation, i.e. creation of positively and negatively charged solitons out of the electron–hole pair photogenerated in the conjugated double bond bridge (Su & Schrieffer, 1980; Roth, 1986, 1987; Yu, 1988; Roth *et al.*, 1989; Bredas *et al.*, 1990; Bartolo, 1992). The electric dipole moment induced in the charge-separated excited state thus attained leads to a structure higher in energy relative to the gound state. Since this excited state is metastable, spontaneous decay to the ground state will occur. However, by appropriate molecular design of the system, one can stabilize the polarized excited state (Buckley, 1992), and thereby delay its spontaneous decay. Thus, for example, based on experimental results obtained with a variety of dye molecules (Griffiths, 1976; Fabian & Hartman, 1980; Zollinger, 1987; Peters & Freeman, 1991), it appears that stability of the polarized excited state can be enhanced by using a strong electron donor and a strong electron acceptor, where the donor strength is given approximately by the first ionization potential of the moiety involved, and the acceptor strength is indicated by the pertinent Hammet constant. Alternatively, stabilization can be achieved by incorporating aromatic residues adjacent to the donor and acceptor, or by extending the conjugated double bond bridge connecting these two moieties. After attaining the situation in which both the ground state and the excited state are stable entities, switching back and forth from one to another can be promoted by illumination at appropriate wavelengths (Roth *et al.*, 1989), thereby achieving the desired control of the switching timing.

When a reversible interchange of a single bond–double bond location is induced in a MOS moiety, interconversion of the latter between two tautomeric forms—a ground state and an excited state—takes place. This reversible process may be associated with an equally reversible conformational change, but only if the potential energies for the two tautomers differ from one another, and also if an energetically accessible conformational path exists between the two conformations.

The molecular switch designed in this study contains a hydrazo moiety, and the operation of this MOS relies on an azonium-hydrazo type tautomerization in the electronic structure of this functional group. This is associated with a planar-to-pyramidal change in the geometry of one of the nitrogen atoms involved, and this leads to a change in the three-dimensional conformation of the MOS. Azonium-hydrazo tautomerizations have been shown to take place in a number of dye compounds (Zollinger, 1961, 1987; Allen, 1971; Patai, 1975; Griffiths, 1976; Gordon & Gregory, 1983), for which the relative stabilities of the two extreme forms could be controlled by solvent polarity and temperature, as well as by the presence of appropriate chemical substituents in the molecule. Therefore, when an azonium (or hydrazo) moiety is present in an appropriate location in the MOS, promotion of the above-mentioned tautomerization will cause the tilting of one part of the MOS relative to the the other part. Such a process is now thought to occur also in natural bio-systems.

In this part of our studies, computer modeling was carried out to design a molecular switching element based on RNase A. This ENMOSE is intended to operate between two forms: one in which the active site of the enzyme is blocked and, therefore, enzymic activity cannot be expressed (the OFF form), and another form in which the active site of the enzyme is not blocked and, therefore, the protein functions as an active enzyme (the ON form). The detailed structural moieties built into the MOS component of this ENMOSE were chosen to allow for the desired conformational change, by taking into accunt the specific structure of the active site of the enzyme and the location of the MOS in the protein. As for the stabilization of the metastable excited state, a variety of approaches—such as incorporation of aromatic rings and a sufficiently long conjugated double bond bridge between donor

and acceptor—were used. Finally, we report here an exhaustive search of the conformational preferences of the ENMOSE considered.

For building the MOS in the RNase A molecule, the position of residue ALA 4 in the S-Peptide fragment was selected as the most appropriate one. The selection was based on both the location and the orientation of this particular residue relative to the active site of the enyzme.

2 METHODS

2.1 General approach

The design of the MOS (i.e. of the modified amino acid residue), the choice for its location with respect to the active site of the enzyme, and the final design of the ENMOSE rely on the extensive use of molecular modeling techniques.

The choice of the detailed structure of the MOS component of the ENMOSE was made according to the following general considerations:

(a) relatively strong electron donor and acceptor groups, an aromatic moiety such as a phenyl group, and an extended polyene bridge between donor and acceptor should be incorporated, in order to enhance the stability of the excited state;
(b) the length of the polyene bridge should be such as to provide sufficient blocking of the active site of the enzyme when the MOS is in the 'closed' position (i.e. in the ground state);
(c) a five-membered ring is to be included in the MOS, in order to provide it with the conformational rigidity required for this section of the molecule, as well as with the desired orientations relative to the active site, both in the 'open' and in the 'closed' states.

2.2 Basic data

Bond lengths and bond angles for each tautomer of the molecular switch were obtained from crystallographic data on model compounds (Trotter, 1960; Fan & Lin, 1965; Tsukuda *et al.*, 1969; Wong, 1978; Allen *et al.*, 1983, 1991; Ahmed, 1984; Enders *et al.*, 1986; Fischer *et al.*, 1988; Keefer, 1988; Valle *et al.*, 1988; Toniolo *et al.*, 1989; Hesse & Jansen, 1990a, b). The rotational barriers associated with some bonds were taken from previously reported values (Mannschreck & Koelle, 1967; Kalinowski & Kessler, 1973; Testa, 1977; Lister *et al.*, 1978; Armstrong & Walker, 1987; Lambert & Takeuchi, 1992). Partial charges were calculated by using the CNDO method (Pople & Segal, 1966), and are given in Table A1 of the Appendix. Initial models for both ground and excited states of the switch were built with these geometrical parameters by using the Biosym Insight-II software. Finally, a model of the RNase A molecule was provided by Dr Sehkar Talluri (personal communication). This model was built by adapting the X-ray structure to one satisfying ECEPP/3 geometry (Momany *et al.*, 1975; Némethy *et al.*, 1983, 1992; Sippl *et al.*, 1984), i.e. having standard bond length and bond angles.

Molecular modeling studies on the ENMOSE considered here were carried out in several stages. Initially, models for the two forms of the molecular switch were generated from crystallographic information and refined using the program Insight II (Biosym Inc.). Figure 1 shows the atomic connectivity for the hydrazo tautomer. The atomic coordinates of these models were used to construct the data files for the ECEPP program, with the computed partial charges being listed in Table A1 of the Appendix. The bonds for which explicit torsional terms were included and their corresponding torsional parameters are listed in Table A2 of the Appendix.

2.3 Conformational search and energy evaluation

To test the validity of the design of the MOS, we carried out a conformational search using the EDMC method and the ECEPP/3 potential energy function. In this search, it was assumed that the ENMOSE would remain enzymically active, i.e. the ENMOSE would still adopt the native-like structure of RNase A. Consequently, the search was directed to identify energetically favorable conformations of the ENMOSE that are also compatible with a native-like conformation.

The ECEPP/3 (Momany *et al.*, 1975; Némethy *et al.*, 1983, 1992; Sippl *et al.*, 1984) program was used to generate the structures of the molecules considered, and to evaluate their confor-

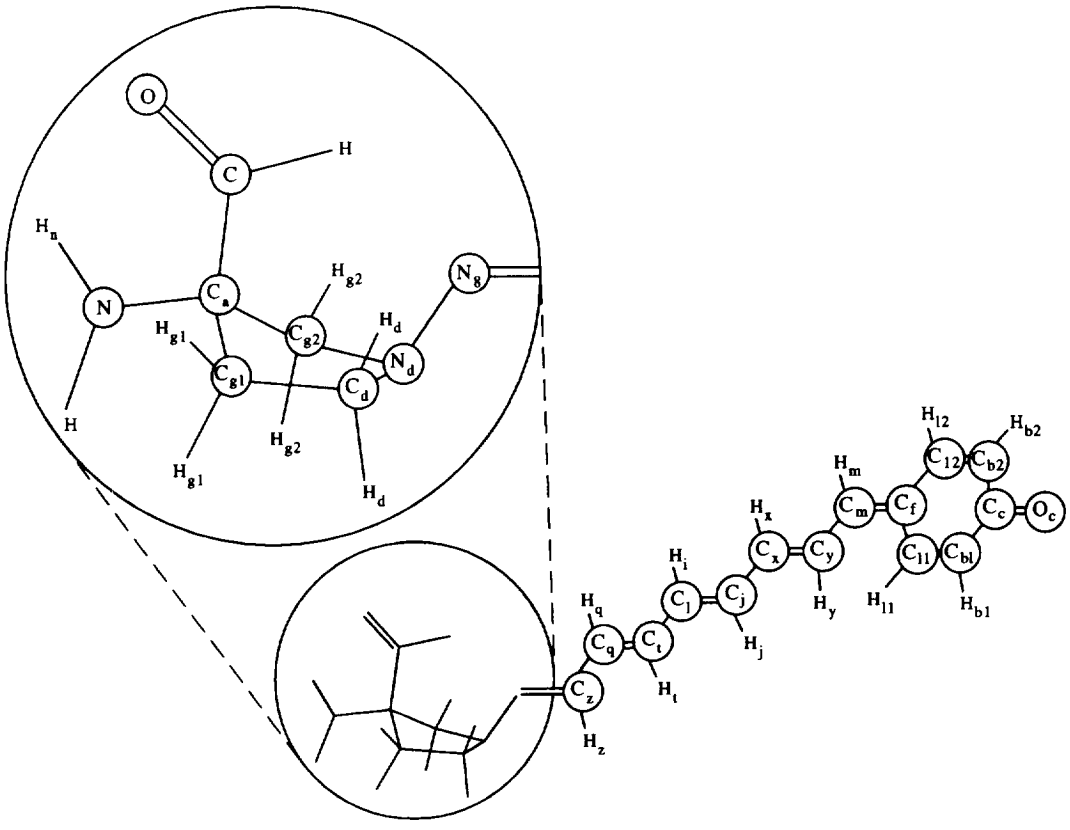

Fig. 1. Atomic connectivity and atom names in the molecular switch (hydrazo tautomer). Inset: an enlarged view of part of the structure.

mational energies. Energy minimizations were carried out with the gradient-based secant unconstrained minimization solver (SUMSL) algorithm of Gay (Gay, 1983). Conformational searches were carried out with the EDMC method (Ripoll & Scheraga, 1988, 1989; Ripoll *et al.*, 1995). This procedure is used for iteratively searching the conformational hyper-surface of polypeptide molecules. The multiple-minima problem is surmounted by following a conformational pathway representing successive improvements over an initial conformation. A parallel version of the algorithm has recently been implemented (Ripoll *et al.*, 1995) on the Kendall Square Research KSR1 computer at the Cornell Theory Center. Most of the calculations described in this report were carried out with this parallel code. During the EDMC runs, the standard-geometry backbone of the protein was kept fixed, and only the side-chain dihedral angles of the switch and of residues in the neighborhood of the active site, i.e. LYS 1, GLU 2, THR 3, ALA 5, ALA 6, LYS 7, ARG 10, GLU 11, LYS 41, ASN 67, GLN 69, ASN 71, GLU 111, VAL 118 and HIS 119, were allowed to vary. In accordance with our assumption that the ENMOSE should retain a native-like structure, the conformations of buried side chains, such as those of residues PHE 8, HIS 12, THR 45 and PHE 120, that would probably be involved in crucial interactions, were kept fixed.

3 RESULTS AND DISCUSSION

After the MOS positioning decision was made, its detailed structure was delineated, and is shown in Figure 2. The hydrazo tautomer is the ground state, while the azonium tautomer is the excited state. Upon illumination of the hydrazo form at an appropriate wavelength, an electron transfer takes place from the donor (the hydrazo group) to the acceptor (the carbonyl group in the benzoquinone moiety). As a result, a charge-separated structure (i.e. the excited state) is obtained in which the azonium and the phenolate moieties

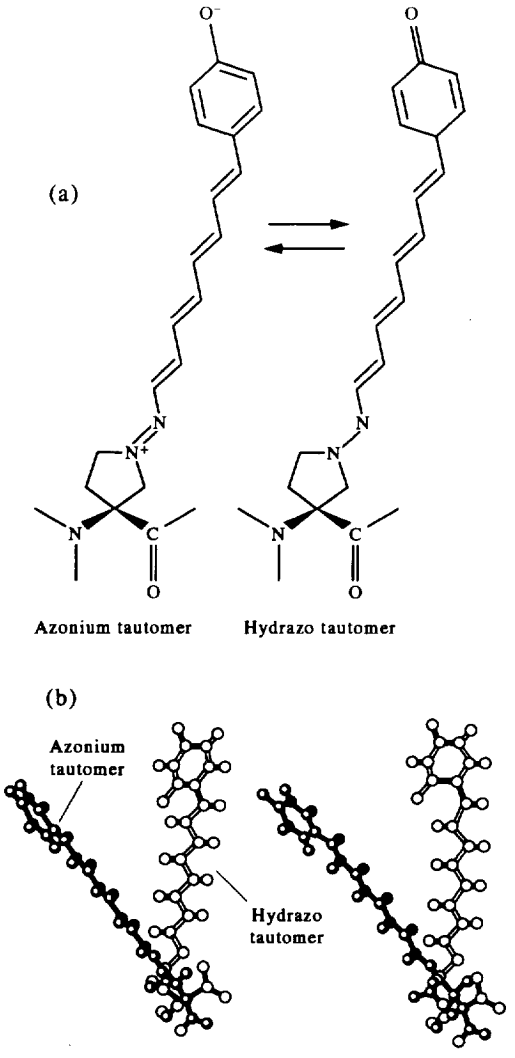

Fig. 2. Molecular switch based on azonium-hydrazo tautomerization. The two low-energy forms are shown separately in (a), and (in stereo) superimposed in (b). The side chains are shown in an extended conformation. For convenient comparison, the backbone atoms are displayed in the same conformation; hence the swing of the side chain by approximately 30° can be noted.

thus produced now carry the positive and negative charges, respectively. This process is associated with a conformational change at the ring nitrogen, the latter changing from a pyramidal sp^3 configuration in the hydrazo tautomer to a charged, planar sp^2 configuration in the azonium tautomer. The final outcome is a tilting movement of the polyene bridge relative to the five-membered ring (see Figure 2(b)).

Information concerning the torsional barriers was incorporated at this stage. The constructed models show that, due to the azonium-hydrazo tautomerization, a swing of the polyene arm of about 30° (assuming an extended conformation for both forms) is achieved (see Figure 2(b)). This displacement of the arm was consistent with our main objective.

The next stage was to generate a model with the MOS incorporated into the protein. Using the standard-geometry model of RNase A, residue ALA 4 was replaced by the 'closed' (hydrazo) structural state of the MOS with its side chain in an extended conformation. The initial values of the backbone dihedral angles of the new residue were selected to satisfy the following conditions: (a) to be compatible with the right-handed α-helical structure prevailing in this region of the protein; and (b) to avoid severe atomic overlaps. This initial conformation of the ENMOSE was then subjected to energy minimization, using the ECEPP/3 potential energy function. Since the objective of this calculation was to relieve the remaining atomic overlaps originating from the amino acid substitution of ALA 4 by the chosen MOS, only those dihedral angles corresponding to the backbone of residues 1–12 were allowed to vary.

During the subsequent conformational searches, the entire set of backbone dihedral angles that resulted from the calculation mentioned above was kept fixed. This decision was based on the assumption that the modified enzyme molecule should still fold into a native-like conformation. Figures 3(a) and (b) display the open (azonium) and closed (hydrazo) conformations of the ENMOSE, assuming extended conformations for the MOS side chain. Under these conditions, the hydrazo and the azonium tautomers can be viewed as functioning as the 'closed' (i.e. enzyme inactive) and 'open' (i.e. enzyme active) states, respectively.

Subsequently, a conformational search of the ENMOSE was carried out using the EDMC method. During these runs, 62 side-chain dihedral angles, corresponding to residues in the neighborhood of the active site of the enzyme (i.e. side-chain dihedral angles of residue nos 1, 2, 3, 4, 5, 6, 7, 10, 11, 41, 67, 69, 71, 111, 118 and 119), were considered as variables. All the conformational search runs, eight for the 'open' state and five for the 'closed' state, were started from randomly chosen values for the variable dihedral angles, with the exception that two runs were started from the extended side-chain confor-

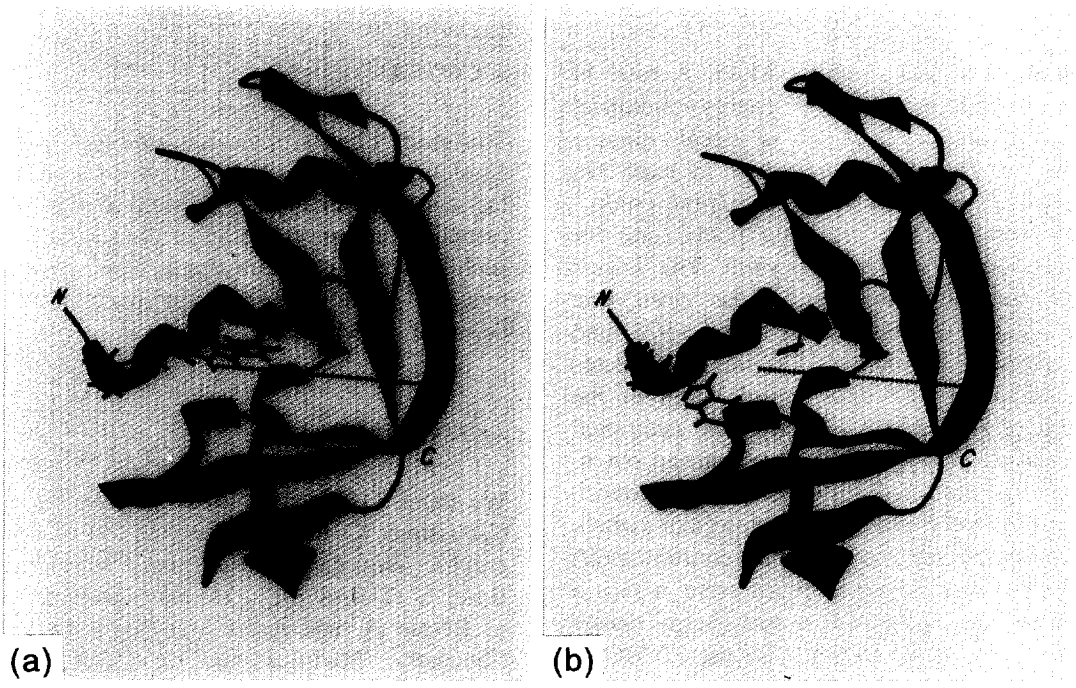

Fig. 3. Representative conformations for the 'closed' (hydrazo) state (panel a) and 'open' (azonium) state (panel b) of the ENMOSE designed in this study. The C^{α} trace of the X-ray crystallographic data (Nachman et al., 1990) for RNase A (EC 3.1.27.5) displayed here has been colored as follows: blue ribbon for the β-sheet regions, light blue 'worm' for turns, and green ribbon for the α-helical regions. The amino-terminus (N) and carboxyl-terminus (C) of the protein are also indicated. The catalytic residue HIS 12 is shown in orange. Residue ALA 4 has been replaced by the molecular switch, shown in red. For clarity, a dotted line (black) was drawn to indicate the active site region (groove) of the enzyme.

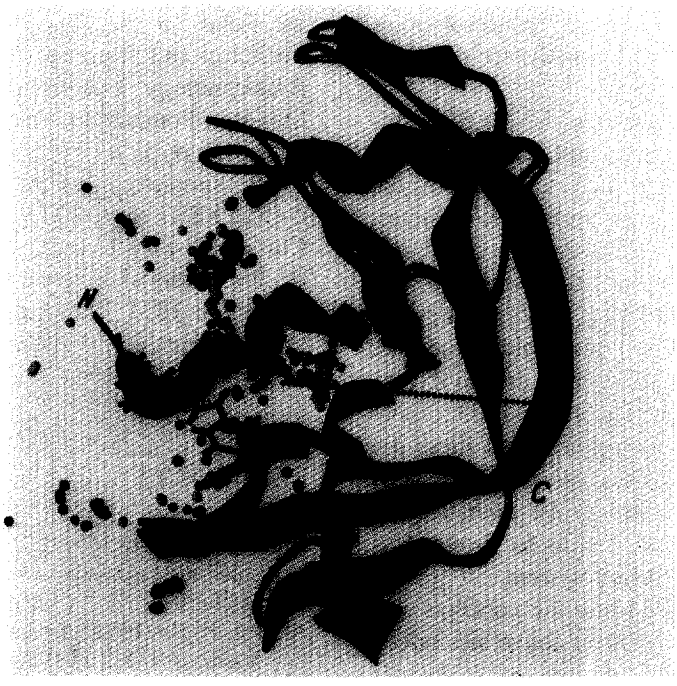

Fig. 4. Ensembles of conformations for the 'open' and 'closed' states of the ENMOSE. Two sets of points, representing the position of the oxygen atom (O_{ζ}) at the tip of the molecular switch, are superimposed on Fig. 3(b). These positions correspond to conformations accessible for the switch, as calculated with the EDMC program. The purple and yellow dots represent the 'open' and 'closed' conformations, respectively, of the molecular switch. The green-yellow worm corresponds to the ECEPP-generated structure for RNase A (fixed bond lengths and bond angles). This structure is shown for comparison, superimposed onto the one obtained by X-ray crystallography.

mations of both states of the MOS. A total of 29,000 conformations were energy-minimized during this study. The set of local minima accepted by the EDMC procedure in each run was collected for further analysis. These conformations were merged into two pools, one for each state of the molecular switch. The lowest ECEPP/3 energies identified for the 'open' and 'closed' states of the switch were −972 and −956 kcal/mole, respectively. Figure 3 illustrates representative 'closed' and 'open' states of the ENMOSE designed in this study. The additional analysis of the EDMC results was carried out to determine the statistical probability of occurrence for each of the two states of the molecular switch. For this purpose, we considered all conformations of each tautomer whose energies were within a 30 kcal/mol range from the lowest-energy ones.

Figure 4 shows the structure of the ENMOSE and, superimposed, the positions of the oxygen atom (O_ϵ) at the tip of the molecular switch for all conformations within a 30 kcal/mole range from the lowest energy ones located for each tautomer. It can be seen that, for most cases in the ensemble of 'closed' conformations, the oxygen atom is indeed located in close proximity to the HIS 12 residue in the active site. On the other hand, in the ensemble of 'open' conformations, the oxygen atom is found mostly in positions further removed from HIS 12.

Obviously, for the particular ENMOSE considered in this study, we do not have only two limiting states (i.e. 'open' and 'closed') of the MOS. Instead, we have two ensembles of states, one 'preferentially open' and the other one 'preferentially closed'. However, this situation can nevertheless be handled by any logic gate operating under the rules of threshold value and, from this point of view, ENLOGS are no exception (Lotan *et al.*, 1993; Tuchman, 1993; Tuchman *et al.*, 1994). For such gates (and these are their most general and most useful form), it is sufficient for the two states to be operationally different from one another, i.e. to be detected as such by the output measuring system.

It can thus be concluded that the concepts and calculation procedures described above are indeed basic techniques for the design of molecular switching elements and, accordingly, of enzyme-based logic gates. In the future, we will implement the results of such calculations with experimental tests.

4 CONCLUSIONS

This study illustrates the concepts and approaches employed in the design of enzyme-based molecular switching elements, as well as the pertinent computational methodologies. In addition, results such as the ones obtained here provide the required guidelines for experimental implementation of ENMOSEs.

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APPENDIX

Table A2. Torsional parameters* assigned to bonds in the side chains of both tautomers of the molecular switch

Bond	$U_0/2$ *(kcal/mole)*	
	Hydrazo tautomer	*Azonimum tautomer*
N_d–N_e	0·00	35·00
N_e–C_z	29·30	2·25
C_z–C_q	3·58	21·50
C_q–C_r	21·50	3·58
C_r–C_i	3·58	21·50
C_r–C_j	21·50	3·58
C_j–C_x	3·58	21·50
C_x–C_y	21·50	3·58
C_y–C_m	3·58	21·50
C_m–C_f	13·00	2·45

*The form of the ECEPP/3 torsional potential function, U_{tor}, used in this study is: $U_{tor} = (U_0/2)\cdot(1 - \cos n\theta)$, where U_0 (in kcal/mole) was taken from the literature (see Section 2·2) using experimental data on model compounds; θ is the value of the dihedral angle associated with the bond; n defines the symmetry of the barrier. In all cases, we used $n = 2$. This value takes into consideration the nature of the bonds involved, and the electronic structure of the atoms involved in these bonds.

TABLE A1. Computed CNDO charges (in e.s.u.) for the two tautomers of the molecular switch

*Atom name**	*Hydrazo tautomer*	*Azonium tautomer*
N	−0·356	−0·356
H_n	0·176	0·176
C_a	0·045	0·039
C_{g1}	−0·034	−0·036
C_{g2}	0·085	0·192
C	0·450	0·450
O	−0·384	−0·384
H_{g1}	0·035	0·037
H_{g1}	0·035	0·037
H_{g2}	0·029	0·043
H_{g2}	0·029	0·043
C_d	0·090	0·133
N_d	−0·247	−0·259
H_d	0·038	0·032
H_d	0·038	0·032
N_e	0·190	0·012
C_z	0·100	−0·146
H_z	0·045	0·057
C_q	−0·080	0·095
H_q	0·058	0·057
C_r	0·001	−0·154
H_r	0·061	0·052
C_i	−0·050	0·074
H_i	0·046	0·041
C_j	−0·044	−0·151
H_j	0·047	0·057
C_x	−0·029	0·068
H_x	0·054	0·042
C_y	−0·072	−0·161
H_y	0·058	0·069
C_m	0·019	0·095
H_m	0·056	0·048
C_f	−0·101	−0·183
C_{f1}	−0·002	0·038
C_{f2}	0·001	0·013
H_{f1}	0·059	0·041
H_{f2}	0·059	0·041
C_{b1}	−0·135	−0·148
C_{b2}	−0·140	−0·119
H_{b1}	0·078	0·069
H_{b2}	0·078	0·069
C_c	0·288	0·239
O_c	−0·295	−0·394

*See Figure 1 for designation of atom names.