directed measurement of energy thresholds to conformational isomerization in tryptamine

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Stimulated emission pumping (SEP)—hole filling spectroscopy and SEP-induced population transfer spectroscopy have been used to place narrow bounds on the energy thresholds for isomerization between individual reactant-product isomer pairs involving the seven conformational minima of tryptamine. The thresholds for isomerizing conformer A to all six other conformations divided into three groups at 750 wavenumbers (cm⁻¹) (A → B, F), 1000 cm⁻¹ (A → C(2)), and 1280 to 1320 cm⁻¹ (A → D, E, and C(1)). The appearance of the first band and the absence of the band below it were used to place upper and lower bounds to the barrier heights for each process. The thresholds for A → B and B → A isomerizations were also combined to determine the relative energies of these two lowest energy minima. The combined data from all X → Y isomerizations identify important isomerization pathways on the potential energy surface linking the minima.

The folding of polymers and proteins depends on the flexibility of sites shared by much smaller molecules, with energetic barriers dictated largely by hindered rotation about single bonds. When there are several flexible sites linked to one another, the number of low-energy conformational minima and barriers soars. In such circumstances, it quickly becomes difficult to predict the important pathways between any two minima. Furthermore, a treatment of the dynamics as a series of one-dimensional torsional motions ignores coupled motion along more than one flexible coordinate, which may provide a more efficient pathway to isomerization. The experimental determination of these barriers would allow the complex potential energy surfaces (PESs) for conformational changes to be mapped out (1, 2).

Although kinetic studies can provide phenomenological activation energies, there have been few direct experimental determinations of barrier heights in isomerization reactions (3), particularly in cases where there are several minima separated by many barriers. In such circumstances, not only is conformation-specific detection difficult, but activation energies extracted from temperature-dependent rate measurements cannot easily be connected with specific features on the potential energy surface governing the process.

We recently have described an approach to studying the dynamics of conformational isomerization in molecules with many flexible coordinates, which uses conformation-specific excitation and detection to selectively probe individual reactant-product X → Y pairs (4–6). In those studies, infrared (IR) excitation was used to selectively excite a single conformation of the molecule of interest in a well-defined NH stretch oscillator. The energy of one IR photon (~10 kcal/mol) is well above many of the barriers to conformational isomerization. By carrying out the IR excitation early in a supersonic expansion, it was possible to re-cool the isomerized products into their vibrational zero-point levels where they could be interrogated downstream in the expansion by a second laser. The observed population changes were used to determine quantum yields for isomerization for each of the NH stretch fundamentals of each conformation.

Because the IR excitation was to regions of the potential energy surface well above the lowest barriers to conformational isomerization (~3 to 5 kcal/mol), the IR studies did not directly probe the thresholds to isomerization. Here, we introduce an alternative vibrational excitation scheme, namely, stimulated emission pumping (SEP) as a means of probing these thresholds (7). The method shares much in common with the SEP-resonant two-
photon ionization studies of Leutwyler and co-workers (8). SEP is a double resonance pump-dump scheme (Fig. 1B) in which a fraction of the population of one conformer is excited by the pump laser ($\lambda_p$) to its electronically excited zero-point level and then stimulated back down into a specific vibrational level in the ground state with the dump laser ($\lambda_d$). SEP has two primary advantages over IR excitation as a means of producing vibrationally excited single conformers capable of isomerization. First, unlike IR excitation, SEP can produce vibrationally excited conformational isomers with a wide range of energies that extend from the zero-point level up through the regions where isomerization barriers typically are located. Second, SEP has better conformation selectivity than IR excitation because any unique vibronic band of a given conformer can be chosen for the pump step, thereby avoiding interference from transitions caused by other conformers.

We applied our method to tryptamine [3-indole ethylamine (TRA)] (Fig. 1B), a close analog of tryptophan and serotonin, that has seven conformational minima with measurable population (9–14). The experimental arrangement shares in common with earlier studies (4–6) the cool-excite-cool-probe configuration (Fig. 1A). TRA was heated to 400 K in a sample holder, entrained in helium gas at a total pressure of 2 or 3 atmospheres, and expanded through a 2-mm-diameter nozzle to create a supersonic expansion. A single conformation of TRA was selectively excited (using SEP) 4 mm downstream from the expansion orifice, where laser-induced fluorescence (LIF) scans had established that the majority of the TRA population was collisionally cooled into the zero-point levels of the seven lowest energy conformational minima (Fig. S1). After the selective excitation of a single conformation of TRA (e.g., TRA conformer A), the vibrationally excited molecules were collisionally re-cooled either back into the original well or into the zero-point levels of other minima after isomerization. The products were then detected downstream with the use of LIF via the ground to first excited singlet state ($S_1$ to $S_0$) vibronic transitions of TRA.

Two complementary laser tuning schemes have been used (Fig. 1B). In SEP-hole filling spectroscopy (SEP-HFS), $\lambda_p$ and $\lambda_d$ were fixed in wavelength to excite a single conformer and stimulate the population down into a specific vibronic level of the conformer for the excited molecules to traverse the distance between the laser beams. As the beams positioned 1.0 cm downstream from the SEP lasers and delayed in time by 2.2 ns pumped, frequency-doubled dye lasers (~0.1 mJ/pulse, 6 ns duration) that spatially and temporally overlap. The beams from $\lambda_p$ and $\lambda_d$ cross the supersonic expansion 4 mm downstream from the exit of the pulsed valve. $\lambda_p$ is tuned to the $S_1$ to $S_0$ origin transition of conformer A, whereas $\lambda_d$ stimulates a fraction of the excited population back down to a specific vibrational level in the ground electronic state. If the energy of this vibrational state is below all barriers to conformational isomerization, then the vibrationally excited conformer is collisionally cooled back into the zero point level as it proceeds downstream in the expansion. However, once the barrier to isomerization to form a given product (e.g., C) is exceeded, the isomerization followed by collisional cooling will result in an increase in the population of the zero-point level of conformer C downstream in the expansion. This change is detected via LIF using a third pulsed tunable UV laser (also a Nd–YAG pumped frequency-doubled dye laser) that is tuned to the $S_1$ to $S_0$ origin of conformer C ($\lambda_s$) that is positioned 1.0 cm downstream from the SEP lasers and delayed in time by 2.2 ns, the time required for the excited molecules to traverse the distance between the laser beams. As the $\lambda_s$ wavelength is tuned, the rate-limiting barriers to other isomerization pathways are overcome, producing gains in population in other conformational zero-point levels. All seven conformations can be approximately described by two flexible internal coordinates: the C–N internal rotation angle that specifies the position of the amino group (NH$_2$) relative to the indole ring and the C–N internal rotation angle that specifies the orientation of the amino group. The position of the amino group (NH$_2$) is defined as anti (pointing away from the indole ring), gauche on the pyrrole side of the indole ring (Gpy, pointing toward the five-membered ring), or gauche on the phenyl side of the indole ring (Gph, pointing toward the six-membered ring). The orientation of the amino group is specified by locating the direction of the nitrogen lone pair (e.g., up, out, in, ph, or py) relative to the indole ring (10).

Fig. 1. (A) Schematic diagram of the spatial and temporal arrangement and (B) energy-level diagram for the SEP-HFS experiment. The conformers of TRA are cooled in the first part of the expansion into their zero-point vibrational levels. A single conformer (e.g., conformer A) is selectively excited using SEP. This is achieved by a pair of high-powered UV lasers pulses from two Nd–YAG (Nd–yttrium-aluminum-garnet) pumped, frequency-doubled dye lasers (~0.1 mJ/pulse, 6 ns duration) that spatially and temporally overlap. The beams from $\lambda_p$ and $\lambda_d$ cross the supersonic expansion 4 mm downstream from the exit of the pulsed valve. $\lambda_p$ is tuned to the $S_1$ to $S_0$ origin transition of conformer A, whereas $\lambda_d$ stimulates a fraction of the excited population back down to a specific vibrational level in the ground electronic state. If the energy of this vibrational state is below all barriers to conformational isomerization, then the vibrationally excited conformer is collisionally cooled back into the zero point level as it proceeds downstream in the expansion. However, once the barrier to isomerization to form a given product (e.g., C) is exceeded, the isomerization followed by collisional cooling will result in an increase in the population of the zero-point level of conformer C downstream in the expansion. This change is detected via LIF using a third pulsed tunable UV laser (also a Nd–YAG pumped frequency-doubled dye laser) that is tuned to the $S_1$ to $S_0$ origin of conformer C ($\lambda_s$) that is positioned 1.0 cm downstream from the SEP lasers and delayed in time by 2.2 ns, the time required for the excited molecules to traverse the distance between the laser beams. As the $\lambda_s$ wavelength is tuned, the rate-limiting barriers to other isomerization pathways are overcome, producing gains in population in other conformational zero-point levels. All seven conformations can be approximately described by two flexible internal coordinates: the C–N internal rotation angle that specifies the position of the amino group (NH$_2$) relative to the indole ring and the C–N internal rotation angle that specifies the orientation of the amino group. The position of the amino group (NH$_2$) is defined as anti (pointing away from the indole ring), gauche on the pyrrole side of the indole ring (Gpy, pointing toward the five-membered ring), or gauche on the phenyl side of the indole ring (Gph, pointing toward the six-membered ring). The orientation of the amino group is specified by locating the direction of the nitrogen lone pair (e.g., up, out, in, ph, or py) relative to the indole ring (10).

Fig. 2. (A) LIF excitation spectrum of TRA in the region of the $S_1$ to $S_0$ origin. The LIF spectrum contains contributions from seven conformers of TRA. The origin transitions of each conformer are labeled with a letter and an abbreviation that describes the conformation of the ethylamine side chain relative to the indole (13, 14). (B to D) SEP-HFS after selective excitation of conformer A of TRA to vibrational levels with the indicated energies (748, 1219, and 1411 cm$^{-1}$). These scans reflect the changes in populations in the conformers of TRA induced by the SEP laser. The difference in fluorescence intensity induced by $\lambda_d$ (20 Hz) with or without $\lambda_s$ present was recorded. $\lambda_p$ (20 Hz) was fixed on the $S_0$ origin of A, and $\lambda_s$ (10 Hz) was fixed to a transition from the $S_1$ origin to a ground state vibrational level with the indicated energy. Under typical conditions, nearly 50% of the population of a given conformation is excited with $\lambda_s$, whereas up to 50% of this excited population is stimulated back down to the ground state level on a strong SEP transition. The detected signal is spread out over the open product channels (from one to seven conformations). The band marked with an asterisk at 34,899 cm$^{-1}$ is a hot band of A, which appears due to incomplete cooling of a small fraction of the SEP-excited A conformers.
of choice. The probe laser ($\lambda_3$) was then tuned to determine where the population went after the SEP and cooling steps. Alternatively, in SEP-induced population transfer spectroscopy (SEP-PTS), $\lambda_1$ was fixed to excite a particular conformation, and $\lambda_2$ was fixed to detect population changes in a second conformation induced while $\lambda_3$ was tuned. In either type of scan, $\lambda_1$ and $\lambda_2$ were operated at 20 Hz, whereas $\lambda_3$ operated at 10 Hz. The difference in the LIF signal from $\lambda_3$ was then monitored in real time using the active baseline subtraction mode of a gated integrator.

The $S_r \leftrightarrow S_0$ origin transitions of the conformers of TRA are shown in an LIF scan which detects total fluorescence (Fig. 2A). These transitions are double-labeled with a letter designation A to F (on the basis of the relative intensities of the transitions) and a descriptive abbreviation (explained in the caption to Fig. 2) for the conformational assignments given previously to these transitions (9–11, 13, 14). There are two origin transitions underneath C that are unresolved at the present resolution (10).

An SEP scan of conformer A (Fig. 3A) was recorded with the pump laser fixed on transition A (from Fig. 1) while $\lambda_3$ was tuned over the range from 440 to 1580 cm$^{-1}$ above the zero-point level in the ground state (1 kcal/mol = 350 cm$^{-1}$). Based on the density functional theory calculations carried out previously (11), SEP scans over this energy region should produce vibrationally excited A conformers with energies ranging from below to well above the thresholds to isomerization. The SEP scan shows dense vibronic structure throughout this energy range, necessary for obtaining narrow bounds on the isomerization thresholds. The SEP spectra of conformers B and C (15) are nearly identical to that for A, indicating that the Franck-Condon factors responsible for the transitions arise primarily from indole ring vibrations, as expected for the $\pi \leftrightarrow \pi^*$ transition involved in the SEP steps.

A series of SEP population transfer spectra (Fig. 3, B to F) show the excitation of conformer A while monitoring transitions B to F from Fig. 2A, respectively, with the probe laser. Positive signals show an increase in the population of the indicated conformer downstream in the expansion. A comparison of the population transfer spectra with the SEP spectrum (Fig. 3A) shows a sudden turning on of each spectrum at a particular threshold energy that is unique to each conformer. These thresholds for isomerization are located at 748 (A→B, F), 1000 (A→C), and 1280 to 1320 cm$^{-1}$ (A→D, E).

Two conformers [labeled C(1) and C(2)] contribute to band C in the LIF spectrum (10). Despite their overlapping origin transitions, these conformers have well-separated vibronic transitions 413 and 422 cm$^{-1}$ above the C origin, which can be used to separate out the thresholds to isomerization from the two conformers (13). The population transfer spectra taken while monitoring these non-overlapped transitions (fig. S2) determine the threshold for C(1) at 1316 cm$^{-1}$ and for C(2) at 1000 cm$^{-1}$.

An important consequence of these sharp thresholds (at 748, 1000, and 1280 cm$^{-1}$) is that they can be used to exert some control over which isomerization products are formed by the SEP step. When $\lambda_3$ is fixed at wavelength corresponding to 748 cm$^{-1}$ of energy above the zero-point level of A, only conformers B and F are formed as products (Fig. 2B). At 1219 cm$^{-1}$, conformer C(2) appears in the product spectrum (Fig. 2C); at 1411 cm$^{-1}$, C(1), D, and E are added as well (Fig. 2D). Because the scans in Fig. 2 are taken with a 20/10/20 Hz configuration, the SEP hole-filling signal in transition A can be either positive (750 cm$^{-1}$ scan) or negative (1215 and 1410 cm$^{-1}$ scans), depending on the competition between the fate of the fluorescing molecules ($\lambda_3$ off) relative to those undergoing the SEP process ($\lambda_2$ on). When the probe signal due to conformer A is positive, SEP causes more of the conformer A population to refill the A zero-point level than does fluorescence; when its signal is negative, SEP is less efficient than fluorescence in refilling A. Thus, as the energy of the laser-excited A population increases, the depletion in A grows.

The identified energy thresholds for isomerization are closely related to the energies of the barriers to isomerization on the potential energy surface. In most circumstances, the turn-on in the SEP-HFS constitutes an upper bound to the barrier to conformational isomerization, whereas the absence of the next strong SEP transition below this energy constitutes a lower bound. However, the measured threshold could differ from the energy barrier if there is a marked kinetic shift or if tunneling is substantial.

A kinetic shift would occur if the rate of isomerization just above threshold is so slow that collisional cooling back below the barrier competes with isomerization. This effect would lead to an overestimation of the barrier height based on the upper bound. However, the thresholds appear to be sharp and reach the full intensity of the SEP spectrum in the first observed transition, suggesting that collisional cooling is not effectively competing with isomerization, even at threshold. This situation may arise because the states accessed by the SEP process are inherently conformationally mixed due to anharmonic coupling between the level carrying the oscillator strength in the dump step and background states at that energy (16, 17).

The internal rotation of the NH$_2$ group is one of the flexible coordinates closely associated with isomerization. Because this coordinate involves hydrogen motion, tunneling.

![Fig. 3. (A) SEP spectrum of conformer A of TRA, $\lambda_1$ was fixed on the S$_r$→S$_0$ origin transition of A (34910 cm$^{-1}$) while $\lambda_2$ was tuned over the wavenumber range from 34 470 to 33 330 cm$^{-1}$, corresponding to vibrational energies from 440 to 1580 cm$^{-1}$ above the ground state zero-point level of conformer A, as marked along the ordinate. (B to F) SEP-PTS recorded by monitoring the corresponding transitions B to F with $\lambda_3$. All transitions except C correspond to the S$_r$→S$_0$ origin transition of a single conformation. These scans monitor the change in population of the zero-point level of the conformers B to F induced by the SEP excitation of conformer A, followed by collisional cooling. To selectively excite conformer A, we fixed $\lambda_1$ (20 Hz) on the S$_r$→S$_0$ origin of A while $\lambda_2$ was tuned. The difference in fluorescence intensity induced by $\lambda_3$ (20 Hz) with or without $\lambda_2$ (10 Hz) present was recorded as $\lambda_3$ was tuned in wavelength. The SEP-PTS show thresholds at 748 [(B) and (F)], 1000 (C), and 1280 to 1320 cm$^{-1}$ [(D) and (E)], signifying the energy at which the SEP step overcomes the barrier to isomerization to the indicated product conformation. The overlapped transition C has contributions from two conformers [C(1) and C(2)] with thresholds at 1316 and 1000 cm$^{-1}$, respectively.](www.sciencemag.org)
through the barrier could produce a threshold below the classical barrier height. The magnitude of this effect in TRA has been assessed by comparing the measured thresholds for the A→B isomerization in NH2 and ND2 isotopomers of TRA, which are related by an internal rotation of the amino group. The TRA-ND2 A→B threshold is identical to that for TRA-NH2 (748 cm⁻¹), indicating that tunneling does not have a measurable effect on the observed threshold.

The combined data on isomerization thresholds pumping from different minima also determines the relative energies of the conformational minima in favorable cases. For instance, the lower and upper bounds on the barrier to isomerization of conformer A into conformer B are located at 688 and 748 cm⁻¹, respectively, whereas the upper bound on the barrier for isomerizing B into A is at 562 cm⁻¹. Because these two measurements probe the same transition state, their energy difference constitutes a direct measure of the relative energies of the conformational minima, with B at least 126 cm⁻¹ above A.

We have summarized the observed upper and lower bounds for isomerization in tryptamine (Fig. 4), plotted on a schematic potential energy source for the two primary isomerization coordinates, internal rotation about the Cα–N and Cα–Cβ single bonds. The conformational assignments on the diagram are largely those from Carney et al. (13, 14), which drew on the combined data from rotational band contour analysis (10, 11), rotational coherence studies (12), and single-conformation IR and ultraviolet (UV) spectroscopy (13, 14). These assignments were made more firm by the recent rotationally resolved vibronic spectra of Pratt and co-workers (18).

The results from additional SEP-PT scans are included (Fig. 4) in which conformer B was excited in the SEP step while monitoring products A, C, and F. Similarly, the threshold for pumping conformer C [either C(1) or C(2)] back to B was also measured (19). In principle, one could carry out a complete characterization of all the relevant stationary points on the potential energy surface by exciting each of the seven “reactant” conformers while monitoring any one of the other six “product” conformers. However, in practice, it is not possible to observe the effects of moving a small fraction of a minor population conformer into a product well that already contains a large population (e.g., an F→A scan). Furthermore, any reactant conformer connected to the global minimum “A” by a low barrier will have much of its population routed toward A as soon as this threshold is overcome, making it difficult to see higher energy thresholds for pathways from this reactant.

The six product conformers formed by isomerization out of A have only three unique thresholds at 748 (B and F), 1000 [C(2)], and 1280 to 1320 cm⁻¹ [C(1), D, and E]. Two possible explanations are consistent with these results. (i) The rate-limiting barriers for each isomerization reaction occur at different locations on the multidimensional PES, but these barriers happen to have similar energies for other reasons. (ii) A common energetic threshold could signal that the same transition state acts as the rate-limiting step in each case. This latter explanation seems more likely to us, given the accuracy of the threshold measurements. If the same transition state is rate-limiting, it provides insight to the isomerization pathway(s) that dictate how population moves between the conformational minima. For instance, because the A→F and A→B thresholds are the same height (688 to 748 cm⁻¹) and are larger than that for B→F (561 to 688 cm⁻¹), it seems likely that the isomerization pathway from A to F passes through B. Similarly, the common threshold for A→C(1), A→D, and A→E isomerizations probably reflects a common rate-limiting barrier for all three pathways, involving motion along the Cα–Cβ coordinate associated with hindered rotation of the amino group from the Gpy to the anti position.

The association of a given threshold with a particular transition state on the PES must be made with some care, because even in TRA the routes between the minima might not proceed along the arrows indicated on Figure 4. The full potential energy surface for TRA is 63-dimensional (3N – 6 where N = 23 atoms), and the lowest energy pathways may involve flexing the molecule along several of these coordinates. In particular, there is a third flexible coordinate (internal rotation about the Cβ–Cα bond) that is not represented in the figure, which may play an important role in the isomerization pathways. Thus, the experimental measurements will provide fertile ground for testing state-of-the-art ab initio calculations, which in turn could shed further light on the pathways involved.

Our data allow us to set upper and lower bounds on the barriers to isomerization, determine the relative energies of key minima, and provide insight into the isomerization pathways in TRA. The results raise several important issues warranting further study. First, the application of these methods to molecules containing more flexible degrees of freedom should probe in some detail the connectivity of the potential energy surface and the way in which isomerization proceeds within subspaces of the
Formation of Secondary Organic Aerosols Through Photooxidation of Isoprene

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Detailed organic analysis of natural aerosols from the Amazonian rain forest showed considerable quantities of previously unobserved polar organic compounds, which were identified as a mixture of two diastereoisomeric 2-methyltetros: 2-methylthreitol and 2-methylerythritol. These polyols, which have the isoprene skeleton, can be explained by OH radical–initiated photooxidation of isoprene. They have low vapor pressure, allowing them to condense onto preexisting particles. It is estimated that photooxidation of isoprene results in an annual global production of about 2 teragrams of the polyols, a substantial fraction of the Intergovernmental Panel on Climate Change estimate of between 8 and 40 teragrams per year of secondary organic aerosol from biogenic sources.

Aerosols are of climatic interest because they act as cloud condensation nuclei (1) and scatter and absorb solar radiation (2). It has been well established that photooxidation products of monoterpenes (e.g., α- and β-pinene) (3, 4), which are biogenic volatile organic compounds (VOCs) emitted mainly by terrestrial vegetation, contribute to the aerosol budget (5, 6). However, it has been assumed that the much larger emissions of isoprene (7) do not result in secondary organic aerosol (SOA) formation in the atmosphere (8). Knowledge of the degradation mechanisms of isoprene, which represents almost 50% of all biogenic non-methane hydrocarbons on the global scale (7), is of considerable interest for air quality modeling (9). It has recently been proposed that the heterogeneous reaction of isoprene on acidic particles could be an important source of humic-like substances, which contribute 20 to 50% of the water-soluble organic aerosol at urban and rural sites in Europe (10). Here, we report evidence that photooxidation of isoprene is a substantial source of SOA, contrary to previous assumptions.

As part of the Cooperative Large-Scale Biosphere-Atmosphere Experiment in Amazonia Airborne Regional Experiment (CLAIRE) 1998 and 2001 experiments, atmospheric aerosols were collected at Balbina (1°55’S, 59°24’W), 125 km north of Manaus, Brazil, during the wet season (11). Backward air mass trajectories indicated that this site was not affected by anthropogenic sources, given that surface air masses originated from the northeast to east and had traveled 1000 km over the remote regions of the Amazonian rain forest for almost a week before being sampled. The organic compounds present in the aerosol samples are, therefore, believed to be characteristic of local and regional atmospheric chemical phenomena rather than of long-range transport. The Amazon basin contains the world’s largest humid forest ecosystem, which is known to emit large quantities of VOCs (7, 12). Because solar radiation and the production of OH radicals are at a maximum in the tropics, the formation of photooxidation products from natural VOCs is expected to be important.

Selected aerosol samples were subjected to analysis by gas chromatography–mass spectrometry (GC-MS) for detailed characterization of organic compounds (11). Figure 1 presents a GC-MS total ion current chromatogram of the trimethylsilylated (TMS) extract of the fine size fraction (PM2.5) of a typical aerosol sample collected during the CLAIRE 2001 campaign with a high-volume (Hi-Vol) air sampler. Compounds 1 and 2 correspond to the newly found compounds, which were identified as diastereoisomeric forms (threo and erythro) of a polyol, specifically 2-methylthreitol and 2-methylerythritol. The structures of these compounds were elucidated with a combination of electron ionization (EI) and methane chemical ionization GC-MS, and then confirmed by a comparison of the GC-MS data with data from synthesized reference compounds (11).

References and Notes