Manipulation of Origin of Life Molecules: Recognizing Single Molecule Conformations in β-Carotene and Chlorophyll-a - β-Carotene Clusters

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ABSTRACT

Carotenoids and chlorophyll are essential parts of plant leaves and are involved in photosynthesis; a vital biological process responsible for the origin of life on Earth. Here, we investigate how β-carotene and chlorophyll-a form mixed molecular phases on a Au(111) surface using low temperature scanning tunneling microscopy and molecular manipulation at the single molecule level supported by density functional theory calculations. By isolating individual molecules from nanoscale molecular clusters with a scanning tunneling microscope tip, we are able to identify five β-carotene conformations including a structure exhibiting a 3-dimensional conformation. Furthermore, molecular resolution images enable direct visualization of β-carotene-chlorophyll-a clsuters with intimate structural details highlighting how they pair: β-carotene preferentially positions next to chlorophyll-a and induces switching of chlorophyll-a from straight to several bent tail conformations in the molecular clusters.

Keywords: β-Carotene, Chlorophyll-a, scanning tunneling microscopy, molecule manipulation, Au(111) surface, density functional theory

Among many organic molecular systems, β-carotene and chlorophyll-a are the key ingredients for light harvesting complexes and biological systems in nature.[1-5] β-carotene takes multiple responsibilities in photosystems; as an antioxidant, and as a charge and energy transfer agent.[5-7] It also functions as a protector of excessive light by adsorbing a region of the visible light spectrum not accessible by chlorophyll.[8] Chlorophyll-a can be found in photosynthetic complexes in plant leaves like spinach, algae, and bacteria, and has an absorption spectrum for all the wavelengths of the visible light except that of the green light.[1-5] Both molecular systems are also considered to be acting as molecular wires for charge transport.[9] Although β-carotene and chlorophyll-a are vital for photosystems associated to the origin of life, a direct visualization of their detailed structures in the molecular complexes is still elusive. Scanning probe microscopy is useful to image complex molecular structures with sub-molecular resolutions[10-15], however acquiring detailed molecular structures inside β-carotene-chlorophyll-a complexes are hindered by a high degree of complexity in their conformations. Single molecule imaging of β-carotene-chlorophyll-a molecular complexes will have a tremendous impact on the fundamental understanding of molecular systems vital for the origin of life on Earth. Moreover, incorporation of plant molecular systems into solid state devices will also be greatly beneficial for the development of environmental friendly solar cells[16-18], which may require formation of plant molecular films on materials surfaces. In this work, we systematically investigate conformations of individual β-carotene and chlorophyll-a using molecular manipulation schemes with a low temperature scanning tunneling microscope, which enables identification of their intimate structures in the molecular clusters.

RESULTS AND DISCUSSIONS

β-carotene on Au(111)

β-carotene has a chemical structure of $C_{40}H_{56}$ and it consists of a hydrocarbon-chain with nine conjugated double bonds and two β-ionone rings at the opposite ends[19-21] (Fig. 1*a*). On Au(111) surface, β-carotene does not form ordered arrangement in the clusters at our deposition condition, and the clusters are mostly located at the corners of the herringbone surface reconstructions due to a high mobility of the molecules on this surface (Fig. 1*b*, 1*c*). Remarkably, a whole β-carotene cluster can be moved across the Au(111) surface just by manipulating at a single point of the cluster with the STM tip (Fig. 1*d*, 1*e*) indicating that the molecule-molecule interactions in the β-carotene clusters are stronger than the molecule-substrate interactions.

In STM images, it is difficult to recognize the conformations of individual molecules in the β-carotene clusters because of their disordered nature and complexity (Fig. 1*b*). To circumvent this hurdle, we decompose the clusters by removing individual molecules one at a time with the STM tip.[22,23] This procedure enables us to isolate the molecules in order to examine their conformations and mechanical stability. An example is presented in Fig. 2 where separation of a β-carotene molecule from a cluster reveals a curved shape structure (see an STM movie in Supporting Information). Such removal of individual β-carotene molecules are realized by using the STM lateral manipulation procedure[24] with 5.3 MΩ to 16 MΩ tunneling resistance range and from 40 mV to 80 mV bias. Manipulation of the molecular clusters can involve both *pulling* and *pushing* modes[24] while manipulation of individual molecules mostly proceeds *via* pulling mode. Pushing is triggered when the tip makes a direct contact with the molecule during manipulation and pulling is originated from the attractive tip-molecule interactions (Supporting Information).

Combining STM lateral manipulation scheme with STM imaging, we are able to distinguish five distinct β-carotene conformations including the known conformations, *trans*, *cis*, and *twist*, and previously unreported conformations, *v-shape* and *v-twist* (Fig. 2*c*). In the *trans* conformation the β-ionone rings appear as two protrusions positioning opposite ends of the straight central carbon-chain[21] while the *cis* conformation appears with its carbon-chain bent and the two end protrusions are located at the same side. The measured bent angle of the carbon-chain in the *cis* conformation is 120°, and it is consistent with the structure of 15-*cis*-β-carotene.[25] In the *twist* conformation, the carbon-chain has 120° double tight turns (Fig. 2*c*) involving turning of C12-C13, and C'12-C'13 bonds. In the fourth structure of β-carotene, its carbon-chain is tightly bent for almost 60° and appears as a "V" shape. Thus this conformation is named *v-shape*. Here the two β-ionone rings at the end of the carbon chain appear as two protruding lobes in STM images. The last stable structure of β-carotene also appears as a "V" shape with an added twist feature in the carbon-chain, and hence, it is named *v-twist*. Energetically, the most stable β-carotene structure is the *trans* conformation however, various *cis* and isomer structures have been found when they are irradiated, thermally heated, or in solution[25]. To our knowledge, the *twist*, *v-shape*, and *v-twist* structures have not been directly imaged before.

In order to check energetics of the observed β-carotene conformations, we have performed quantum chemical calculations using the Gaussian 09 software with two functionals, B3LYP and PPE, and two basis sets, 6-31G* and 6-311+G(2d,2p). In addition, calculations were done at the second order perturbation level (MP2), which accounts for dispersion effects that may be important in the twisted forms. The calculations confirm all five β-carotene conformations as stable structures (Fig. 2*c*). For the fourth structure, *v-shape*, the calculations reveal a tightly bent carbon chain with a 60° angle and it can be drawn in 2-D plane (Fig. 3*a*). On the other hand, the *v-twist* conformation also has a tightly bent carbon chain but it has a 3-D structure (Fig. 3*b*, 3*c*, 3*d*). Here the carbon chain is bent and it turns upward for 60° from the x-y plane (Fig. 3*c*) while it is also tilted ~25° in the 'z'

direction (Fig. 3*d*). The trans has the lowest total energy and thus, it is the most stable structure. Using the total energy of trans conformation as the basis, the amount of an increased total energy of other conformations (ΔE) with respect to the trans are plotted in Fig. 3e. All the calculations using different basis sets show 0.1 eV and 0.2 eV increase in the total energy respectively for the *cis* and *v-shape* conformations. The increased in total energies for the *twist* and *v-twist* conformations are varied depending on the basis set used. This is especially notable in *v-twist* structure where the total energy increases up to 0.8 eV. However, when second order perturbation level (MP2) is considered, the total energies of the *twist* forms are greatly reduced (Fig. 3e).

The calculated conformations for the β-carotene adsorbs on Au(111) surface (Fig. 4) reveal that all five conformations are stable in accordance with the STM results of Fig. 2c, and the carbon chain in all conformations except the *v-shape* (Fig. 4*d*) mostly align along a close-packed surface row directions, *i.e*. [110] surface directions on Au(111). However the minimum energies for other adsorption sites on Au(111) are not much different due to the weak molecule-surface binding. For the *v-twist* conformation, both bent parts of the carbon chain adsorb on Au(111) surface. Here, the two end rings and the closely folded carbon chain appear as protrusions giving an impression of a twist in STM images (indicated with red circles in Fig. 4*e*). Energetically, the trans is the most stable after adsorption on Au(111) and have the lowest total energy. Then the total energy increases for the *cis*, *twist*, *v-shape*, and *v-twist* (Fig. 4f). Unlike the gas phase calculations presented in Fig. 3, the second level perturbation method is not available for the DFT calculations if the metal surface is considered as the case of Fig. 4.

In order to qualitatively test the structural integrity and stability of the observed β-carotene structures, we use STM manipulation schemes. Figure 5 shows sequences of STM images acquired before and after manipulations of four β-carotene conformations; *cis*, *twist*, *v-shape*, and *v-twist*. An inelastic electron tunneling (IET) process[26,27] is used for the rotation of a *cis* conformation (Fig. 5*a*

and 5*b*). For this manipulation, the STM tip is positioned above the molecule with a fixed height and then electrons with energy up to 1.0 eV are tunneled to the molecule. The IET excitation results in the rotation and displacement of the whole molecule while the *cis* conformation remains intact. For the rotations of the *twist* (Fig. 5c), the STM lateral manipulation procedure is used.[24,28,29] During the manipulation process, the STM tip height is reduced to enhance the tip-molecule interactions, and then the tip is laterally moved to pull the molecule. The manipulation results in a clockwise rotation and displacement of the molecule (Fig. 5d). Similar STM lateral manipulation scheme is used to manipulate the *v-shape* (Fig. 5e, and 5f), and *v-twist* conformations (Fig. 5g, and 5h). Rotation of v-twist molecules can also be performed by IET (Supporting Information). However, we do not observe rotation of *trans* and *twist* molecules using IET process. For the *trans* molecule, tilting of the end pi ring can be induced by IET (Supporting Information). All the conformations are preserved after manipulations indicating that these structures are rather stable on Au(111) surface in accordance with the DFT results.

After investigating the molecular shapes in the STM images, we are now able to identify individual β-carotene in the nanoscale molecular clusters. An example is presented in Fig. 6*a* where individual β-carotene conformations can now be assigned in the molecular cluster presented in Fig. 1*c*. This cluster is composed of 13 β-carotene molecules; 8 *cis*, 2 *trans*, 2 *v-twist*, and 1 *twist*. Statistical distribution of the β-carotene conformations is determined from 1172 molecules in 115 clusters across different locations on the surface (Fig. 6*b*) and it is revealed that the *cis* conformations appear the most frequent (36.8 %) followed by the *trans* (30.0%) and *twist* (16.2%) while the *v-twist* and *v-shape* conformation appears the least frequent (10.2% and 6.8%, respectively). Although *trans* is the most stable conformation (Fig. 4f), the *cis* appears as the majority in the clusters, which might be induced by the interactions between the neighboring molecules. Moreover, the molecules in the clusters do not follow particular adsorption sites due to strong interactions with neighbors. We note here that the calculations are performed for isolated molecules without

considering neighboring molecules and the energy differences between the different adsorption sites are less than 0.1 eV.

Chlorophyll-a on Au(111)

Chlorophyll-a has a chemical structure consisting of $C_{55}H_{72}MgN_4O_5$, and it is composed of a porphyrin head-group and a hydrocarbon-chain tail[30] (Fig. 7*a*). In order to form β-carotene-chlorophyll-a molecular complexes, we first deposit chlorophyll-a on Au(111) surface and investigate their structures. Unlike β-carotene, chlorophyll-a deposited by using the same experimental conditions as β-carotene grows expitaxially on Au(111) and forms clusters with an ordered close-packed structure (Fig. 7*b*). Within the clusters, chlorophyll-a molecules are arranged in a head-to-head and tail-to-tail alignment (Fig. 7*c*) and the head porphyrin unit is adsorbed on Au(111) surface while the carbon chain tail is lifted up by CH_3 units in a straight tail conformation.[30] Here, their carbon-chain tails are aligned along [211] surface direction. For chlorophyll-a conformations on Au(111) surface, three bent-tail conformations (Fig. 7*d*) are known to exist on Au(111)[30], which can be induced by IET process with the STM tip. The chlorophyll-a conformations are labeled as (0) for the straight tail, (1) for the 60° bent tail, (2) for the 120° bent tail, and (3) for the 180° bent tail conformations, respectively.

β-carotene–chlorophyll-a mixed phases

Mixed β-carotene–chlorophyll-a molecular clusters are formed by depositing β-carotene onto the Au(111) sample having chlorophyll-a clusters as shown in Fig. 7. Before deposition of β-carotene, the chlorophyll-a clusters on Au(111) surface have a well ordered molecular arrangement. Remarkably, the deposition of β-carotene completely alters the appearance of the surface, and the STM images acquired at 77K reveal three distinct regions: Well-ordered chlorophyll-a cluster regions, mixed β-carotene-chlorophyll-a regions, and bright (high intensity) regions (Fig. 8*a*). The

comingled β-carotene-chlorophyll-a areas are located next to the highly ordered chlorophyll-a clusters while the high intensity or noise like regions are located next to the mixed molecular complexes. These bright regions are attributed to the mobile β-carotene molecules producing noise-like patterns during STM tip scanning, and no molecular resolution is resolved in these regions. In previous measurements (Fig. 1,2,5 and5), the movements of the of β-carotene is suppressed due to a low substrate temperature of 5K. β-carotene becomes mobile on Au(111) surface at 77K due to thermal excitations.

Our key interest is the mixed β-carotene-chlorophyll-a regions, which should provide direct information for how the two species coexist. The close-up STM images of the mixed molecular clusters show recognizable single molecules of chlorophyll-a and β-carotene (Fig. 8*b*). Interestingly, some of the chlorophyll-a molecules now appear with different conformations than the straight tail conformation. A careful analysis of the chlorophyll-a conformations in the mixed β-carotene-chlorophyll-a region reveals four chlorophyll-a structures; the straight tail and the three bent-tail conformations as shown in Fig. 7*d* while the β-carotene conformations fall into one of the five structures as previously discussed (Fig. 2*c*). Statistical analysis of the observed β-carotene and chlorophyll-a conformations from the STM images reveals that the straight tail conformation of chlorophyll-a, and the *trans* conformation of β-carotene are the majority while all other conformations of these two molecules are present but in less than 10% (Fig. 8*c*).

Isolated chlorophyll-a molecules are rarely observed on Au(111) before the β-carotene deposition. Interestingly, a large number of chlorophyll-a molecules are now involved in the mixed β-carotene-chlorophyll-a cluster formation. Thus the chlorophyll-a molecules in the mixed molecular clusters should be originated from the ordered chlorophyll-a clusters initially formed on Au(111). It appears that the sides of the ordered chlorophyll-a clusters are being destroyed by β-carotene while the center part of the clusters remains intact. Conceivably β-carotene strongly interacts with

the chlorophyll-a located at the vulnerable sites such as the edges of the clusters in order to form the mixed molecular phases. Moreover, the bent-tail conformations of chlorophyll-a are appeared mainly in the mixed molecular regions indicating that these structures are induced by β-carotene. On the other hand, unlike in β-carotene pure clusters, the *trans* conformation contributes the largest portion among β-carotene conformations in the mixed molecular regions indicating that β-carotene conformations are also altered after interaction with chlorophyll-a as well.

In the mixed molecular regions, β-carotene and chlorophyll-a molecules tend to position next to each other. Among many pairing structures of β-carotene and chlorophyll-a observed in molecular complexes, two pairing arrangements are commonly found. In the first pairing arrangement, both β-carotene and chlorophyll-a are in *trans* conformations (Fig. 8*b*). Here, the carbon chains of the two molecular species are located parallel to each other. In the second arrangement, either chlorophyll-a head or β-ionone ring is positioned next to the middle of the carbon chain of the other molecule. In this case, the carbon chain of the other molecule is bent to accommodate the chlorophyll-a head or β-ionone ring. Both arrangements provide a maximum area to interact between the two molecules. We also observe positioning of the molecules over the same or other type of molecules thereby allowing a direct contact between them.

CONCLUSION

In summary, molecular clusters composed of origin of life molecules, β-carotene and chlorophyll-a, are formed on an atomically clean Au(111) surface by vacuum deposition under UHV environment, a technique compatible for fabrication of thin-films for solid-state devices. By isolating individual molecules from molecular clusters using the STM tip, we are able to identify five β-carotene conformations. Previously unreported β-carotene conformations are found in STM experiments and their structural integrity are checked by single molecule manipulation with STM tip. Geometrically

relaxed DFT calculations confirm the stability of all five β-carotene conformations including the 3-D structure in the gas phase and on Au(111) surface. Furthermore, the sub-molecular resolutions STM images of mixed β-carotene-chlorophyll-a molecular clusters enable direct visualizations of molecular pairs and provide insight information such as induction of structural changes by one another. Moreover, the formation of mixed β-carotene and chlorophyll-a molecular clusters on a materials surface under UHV condition opens possibilities to incorporate plant based systems into solid state devices for future electronic and green energy applications.

METHODS

Experimental Details

For the experiments, Au(111) surface was atomically cleaned by repeated cycles of neon ion sputtering and annealing to 600 K in ultrahigh vacuum (UHV). Sub-monolayer coverages of β-carotene (*trans*, Sigma-Aldrich) and chlorophyll-a (Sigma-Aldrich) were separately deposited onto atomically clean Au(111) surface held at 120 K using a custom-built Knudson cell in UHV environment. All the STM data for β-carotene were measured at ~5K substrate temperature. For the formation of mixed β-carotene-chlorophyll-a clusters, β-carotene was deposited onto chlorophyll-a pre-deposited Au(111) surface. The substrate and source temperatures during the molecular deposition were ~120 K, and ~400 K, respectively. The samples were then transferred to STM chamber directly attached to the preparation chamber under UHV. The STM data for the mixed β-carotene – chlorophyll-a clusters were measured at ~77K substrate temperature using a custom-built low temperature UHV STM system.

Computational Details

For the gas phase carotene structures, quantum chemical calculations were performed using the Gaussian 09 software with two functionals, B3LYP and PPE, and two basis sets, 6-31G* and 6-311+G(2d,2p). In addition, calculations were done at the second order perturbation level (MP2). For all of the isolated molecule calculations, structures were optimized at the 6-31G* level and frequency calculations were performed to make sure there were no imaginary frequencies. The larger basis set calculations [6-311+G(2d,2p)] were done on the optimized 6-31G*. For the gas phase calculations, known structures of *trans*, *cis* and *twist* are used as the starting points while the bent structures closed to the experimental shapes are used as the starting points for the *v-shape* and *v-twist*.

Density Functional Theory calculations for the β-carotene adsorbed on Au(111) surface were carried out with the Vienna ab initio simulation package (VASP) code[31] with core electrons described by the projected augmented wave method (PAW).[32] Exchange-correlation was treated in the PBE[33] in Generalized Gradient Approximation (GGA). Because of the relative importance of bonding and non-bonding molecule-surface interactions, the van der Waals D3 functional was used.[34] The plane wave basis was expanded to a cutoff of 600 eV. Because of the large size of the system, the first Brillouin zone was sampled at the *Γ* point only. The Au(111) surface was modelled by three-layer slabs (432 atoms) with a vacuum space of 20 angstroms. The bottom two layers of Au were kept fixed in all geometry optimizations. This calculation scheme is useful for large molecular systems adsorbed on Au(111) surface.[35] For the relaxed structural calculations, the optimized structures obtained from the gas phase calculations were used as the initial structures. The relaxation allows the molecules to orient on the surface as well. Geometry optimizations were converged within $1x10^{-5}$ eV and all the geometries were optimized until the residual forces became less than $1x10^{-2}$ eV/Å.

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge on the ACS publication website. It contains additional information on lateral manipulation of β-carotene clusters, lateral manipulation of single β-carotene, IET induced manipulation of single β-carotene, STM tip induced conformation switching, molecular jigsaw puzzle, and an STM movie caption.

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FIGURE CAPTIONS

Figure 1. β-carotene clusters. (**a**) Chemical structures of β-carotene. (**b**) STM image of β-carotene clusters on Au(111) together with background herringbone surface reconstruction [44 nm x 44 nm, I_t = 0.17 nA, V_t = -1.5V]. (**c**) A zoomed STM image of a β-carotene cluster [8.6 nm x 7.0 nm, I_t = 0.1 nA, V_t = 0.2V]. (**d**) A β-carotene cluster is laterally rotated by STM tip manipulation along the direction indicated by the white arrow. (**e**) Consecutive STM image recorded after the manipulation reveals that the whole cluster is displaced. [23.8 nm x 19.5 nm, I_t = 0.15 nA, V_t =-1.5V].

Figure 2. β-carotene conformations. (**a**) A β-carotene molecule is removed from the cluster along a white arrow using STM tip resulting in separation of a molecule with the cis conformation (**b**). [12.6 nm x 7.7 nm, I_t = 0.17 nA, V_t = 1.5V]. (**c**) STM images five β-carotene conformations [I_t = 0.2 nA, V_t = 0.5V] together with corresponding DFT calculated structures; trans (1), cis (2), twist (3), v-shape (4), and v-twist (5).

Figure 3. β-carotene conformations. (**a**) The v-shape conformations has a 60° bent carbon chain and it can be drawn in a 2-D plane. (**b**) The v-twist conformation has a 3-D structure. (**c**) By rotating a part of the carbon chain, the bent angle of v-twist is determined as 60°. (**d**) Another rotated view of the molecule reveals 25° tilt from the ‚z' axis. (**e**) Total energy difference, ΔE as a function of the five β-carotene conformations shown in Fig. 2c: (1) trans, (2) cis, (3) twist, (4) v-shape, and (5) v-twist. [$\Delta E = E_N - E_1$ where N = 2,3,4 and 5, and E_1 is the energy of the conformation 1].

Figure 4. Calculated β-carotene conformations on Au(111). (a) trans, (b) cis, (c) twist, (d) v-shape, and (e) v-twist conformations. The red circles in (e) indicate the protrusions observed in STM

image of v-twist. (f) The total energies of β-carotene conformations on Au(111). [$\Delta E = E_N - E_1$ where N = 2,3,4 and 5, and E_1 is the energy of the conformation 1].

Figure 5. Structural integrity test. Individual β-carotene molecules are manipulated with the STM tip to check their mechanical stability. The arrows indicate manipulated directions. STM images show before and after molecular manipulation of cis (**a**, **b**), twist (**c**, **d**), v-shape (**e**, **f**), and v-twist (**g**, **h**). [I_t = 0.2 nA, V_t = 0.5V].

Figure 6. β-carotene conformations in clusters. (**a**) Using known β-carotene conformations, the molecules can be assigned in the cluster shown in Fig. 1c. (**b**) A plot of average composition of β-carotene in the clusters. Here, the five conformations are labeled as 1,2,3,4 and 5 as in Fig. 2c. The error is induced by statistical uncertainty in assignment of the molecules in molecular complexes.

Figure 7. (**a**) Chemical structure of Chlorophyll-a. (**b**) A large area STM image showing Chlorophyll-a clusters on Au(111) together with the background herringbone surface reconstruction of the surface [48 nm x 48 nm, I_t = 0.17 nA, V_t = 2V]. (**c**) Head-to-head and tail-to-tail ordered arrangement of chlorophyll-a inside a self-assembled molecular cluster [11 nm x 9.2 nm, I_t = 0.21 nA, V_t = 0.4V]. (**d**) STM images show various chlorophyll-a conformations; a straight tail conformation (0), and three bent-tail conformations (1, 2, and 3). [I_t = 0.2 nA, V_t = 0.5V].

Figure 8. β-carotene-chlorophyll-a mixed structures. (**a**) A large area STM image show 3 distinct regions after deposition of β-carotene on Chlorophyll-a/Au(111) surface [92 nm x 92 nm, I_t = 0.2 nA, V_t = 1.2V]. Ordered cluster region (1), disordered regions (2), and noise regions (3). (**b**) A molecular resolution STM image shows mixed β-carotene (yellow) and chlorophyll-a (green) with various conformations [20 nm x 20 nm, I_t = 0.1 nA, V_t = 1.2V]. The red and white ovals indicate the straight

and bent pairing schemes of the molecules. (**c**) The plot of conformation statistics of chlorophyll-a and β-carotene in the mixed regions. Here, 0, 1, 2, and 3 represent the straight tail, and the three bent-tail chlorophyll-a conformations, respectively. The β-carotene conformations are 1 (trans), 2 (cis), 3 (twist), 4 (v-shape) and 5 (v-twist), respectively. The % error is induced by statistical uncertainty in assignment of the molecules in mixed phases.

Author Contributions

S.W.H. conceived and designed the experiments: V.I. and T.S. performed the STM experiments; T.S., V.I. and S.W.H analysed the data, A.N., P.C.R. and L.A.C performed the quantum chemical and DFT calculations. All the authors discussed the results and commented on the manuscript.

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