Organophosphorus Hydrolase at the Air−Water Interface: Secondary Structure and Interaction with Paraoxon

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The secondary structure of organophosphorus hydrolase (OPH) at the air−water interface was studied using polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). The shape and position of the amide I and amide II bands were used to estimate the surface conformation and orientation of OPH. The PM-IRRAS results indicated that the enzyme did not unfold for the range of surface pressure used (0−30 mN/m). At low surface pressures, the signal of amide I was very weak and the intensity was almost the same as amide II. Upon further compression, the PM-IRRAS signal and the ratio of the intensity of amide I and amide II both increase, implying an increased interfacial concentration of the enzyme. From the amide I/amide II ratio and the band position, it was deduced that the enzyme adopts a conformation which gives a higher occupied surface at low surface pressure and rotates to a more vertical orientation at high surface pressures. The compression and decompression of the OPH monolayer indicated that the fingerprint of the secondary structure at the air−water interface was reversible. PM-IRRAS was also used to investigate the pH effect of the subphase on the secondary structure of OPH. The secondary structure of OPH at the air−water interface was well defined when the pH of the subphase was near its isoelectric point (IP, pH 7.6). However, it adopted a different orientation when the subphase pH values were higher or lower than the IP with formation of random coil structure. The hydrolysis of organophosphorus compound paraoxon by OPH was also studied at the air−water interface by PM-IRRAS. The pH effect and the interaction with paraoxon both seem to orientate the enzyme more in the plane of the interface and to produce random coil structure.

Introduction

The acquisition of structural information from Langmuir monolayers began in the mid-1980s with the use of epifluorescence microscopy to study domain formation and the X-ray reflection and diffraction to investigate molecular orientation.[1,2] Later, Brewster angle microscopy was employed for the analysis of domain structure.[3,4] These methods were limited because not much information could be obtained on the biophysical parameters such as headgroup structure and enzyme secondary structure. However, infrared reflection absorption spectroscopy (IRRAS) has provided several well-known advantages for the molecular characterization of enzymes. The IRRAS could monitor molecular vibrations that produce dipole moment oscillations. The observed frequencies were dependent on molecular conformations and configurations.[5,6] However, the IRRAS method presented some difficulties for the study of Langmuir monolayers because the strong absorption of the water vapor hid the spectral region where the most useful molecular information was located. To overcome this problem, a differential IR reflectivity technique using polarization modulation of the incidence light (PM-IRRAS) was developed.[7−10] This technique has been proven to be almost insensitive to the strong IR absorption of water vapor, and only important bands arising from the Langmuir monolayer were observed.[7]

Organophosphorus hydrolase (OPH) is the most well characterized enzyme that can catalyze the hydrolysis of a wide range of organophosphorus pesticides as well as nerve agents by cleaving various phosphoryl bonds (P−O, P−F, P−CN, and P−S).[11,12]Such a wide catalytic effect of OPH on the organophosphorus neurotoxins is unmatched by any other enzymes.[13] The X-ray crystalline structure of OPH showed a globular biomacromolecule (a dimer with two identical monomers) with overall dimensions of approximately 51 Å × 55 Å × 51 Å. Its architecture consists of a distorted α/β barrel structure which is comprised of 2 parallel β-strands, 8 antiparallel β-strands and 14 α-helix.[14] In our research group, a detailed surface chemistry study of OPH at the air−water interface and secondary structure of OPH in aqueous solution and Langmuir−Blodgett (LB) film using circular dichroism (CD) spectroscopy have already been reported.[15,16] The studies on the organization of OPH after immobilization using layer-by-layer (LBL) and LB film deposition techniques showed that well-ordered, stable OPH films with high strength were formed and could be used to interact with paraoxon.[17−20] Interaction between OPH and paraoxon at the air−water interface was studied by epifluorescence microscopy and in situ UV−vis and fluorescence spectroscopies. The results indicated that continuous growth of the OPH aggregates was a distinct phenomenon associated with paraoxon hydrolysis.[21,22] We recently used (CdSe)ZnS quantum dots and OPH bioconjugates to detect paraoxon, and it was found that the quenching of the photoluminescence intensity was caused by the conformational change in OPH.[23] Although much work has been done using various surface chemistry techniques, the information on

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conformation and orientation of OPH at the air−water interface still remains unknown. How should the distinctive features of OPH be correlated under a different surface environment with its own secondary structure? It would be very interesting yet important if we can reveal the secondary structure change directly at the air−water interface with the change of subphase pH and in the presence of paraoxon.

In this paper, we report a study of the OPH secondary structure at the air−water interface using PM-IRRAS. The stability of OPH Langmuir monolayers was observed by a compression−decompression cycle. On the basis of spectral features of amide I and II bands, the orientation of OPH at the air−water interface was characterized, and the secondary structure at different pH values was investigated. Finally, the interaction between the OPH Langmuir monolayer and paraoxon dissolved in the subphase was also studied.

Experimental Section

Materials. Purified OPH (E. C.3.1.8.1) was obtained from the U. S. Army Laboratory (Edgewood Chemical and Biological Center, APG, MD) with a purity of 85−90%. OPH stock solution (1.24 mg/mL) was prepared in 100 mM bis-tris-propane (BTP), pH 7.5, containing 10 μM Co^{2+}. The stock solution was kept in the refrigerator at 4 °C.

The enzyme solutions for PM-IRRAS measurements were freshly prepared on the day of the experiment at a concentration of 0.21 mg/mL. The water used as subphase was purified by a Modulab 2020 water purification system (Continental Water Systems Corp., San Antonio, TX). The pure water has a specific resistance of 18 MΩ · cm and a surface tension of 72.6 mN m^{-1} at 20 ± 1 °C. Buffer solutions with different pH values were prepared: pH 5.0 and pH 7.6 (0.1 M KH_2PO_4 and 0.1 M NaOH), pH 8.0 and 9.0 (0.1 M Tris and 0.1 M HCl) and were used as subphase. KCl was added into the buffer solution as an electrolyte. All of the chemicals including paraoxon were purchased from Sigma Chemical Co. (St. Louis, MO).

Methods. All of the experiments were performed in a clean room class 1000, with a constant temperature of 20.0 ± 0.5 °C and a relative humidity of 50 ± 1%. PM-IRRAS spectra were recorded on a Bruker EQUINOX 55 FT-IR spectrometer (Bruker Optics Inc., Billerica, MA) using the external accessory (XA-511) for the air−water interface that is equipped with a liquid-nitrogen-cooled mercury cadmium telluride (MCT) detector. Ninety scans were collected for each spectrum with a resolution of 8 cm^{-1} and a two level of interpolation. The incident IR beam was polarized by a BaF_2 polarizer and modulated by a photoelastic modulator (PEM, Hinds Instruments, Inc., Hillsboro, OR) between parallel (p) and perpendicular (s) polarization to the plane of incidence. Then the IR beam was focused onto the water surface of the μS trough (Kibron Inc., Helsinki, Finland) with a surface area of 115 cm^2 (5.9 cm × 19.5 cm) between the two barriers. The two-channel processing of the detected IR signal gives the differential reflectivity spectrum $\Delta R/R = (R_p - R_s)/(R_p + R_s)$ where R_p and R_s were the polarized reflectivities.[8] To remove the contribution of the water absorption, each enzyme IR spectrum was divided by the corresponding spectrum of the subphase. The optimal value of the angle of incidence for the detection was 75° relative to the optical axis normal to the interface. At this angle of incidence, a negative band indicated a transition moment oriented preferentially perpendicular to the surface, whereas a positive reflection absorption band was related to a transition moment oriented preferentially in the plane of the surface.[9,10]

A volume of 40 μL of the OPH solution was spread dropwise at the air−water interface resulting in an initial surface pressure of 0 mN/m and 10 min was allowed for the equilibration of the Langmuir monolayer. PM-IRRAS spectra were collected during compression and decompression of the OPH Langmuir monolayer. It would take 2 min for each spectrum collection at a specific surface pressure. The interaction between OPH and paraoxon was studied by dissolving paraoxon in the buffer of pH 7.6 and using this solution directly as a subphase.

All of the spectra recorded were followed by baseline correction and 9-point smoothing.

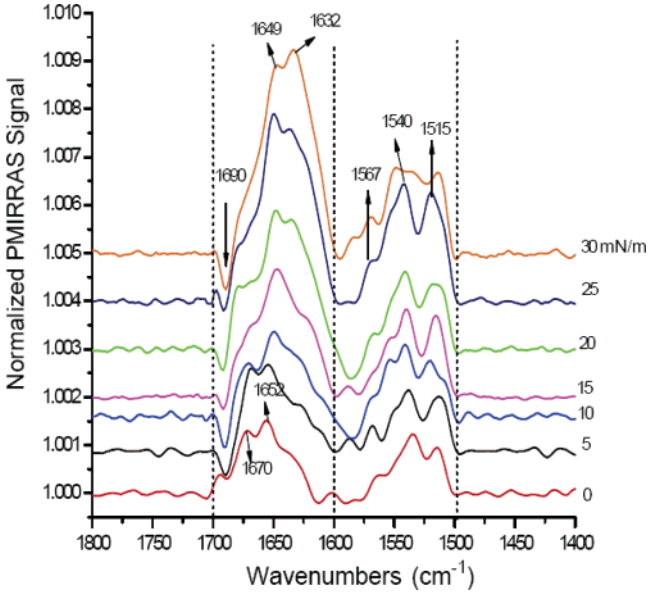

Figure 1. Normalized PM-IRRAS spectra of OPH Langmuir monolayer collected at the air−water interface at different surface pressures.

Results and Discussion

PM-IRRAS spectra collected during compression of the OPH monolayer at the air−water interface at pH 7.6 are shown in Figure 1. In all spectra, vibration bands were observed in amide I (1700−1600 cm^{-1}) and amide II (1600−1500 cm^{-1}) regions. The amide I contains the most useful information for the analysis of enzyme secondary structure, thus it is the region of interest. Careful examination of the amide I band revealed that the OPH contains β-turn (1670 cm^{-1}), α-helix (1652 and 1649 cm^{-1}), parallel (1632 cm^{-1}), and antiparallel β-sheet (1690 cm^{-1} and shoulder at 1625 cm^{-1}) structures.[24−28] The spectra also indicated that the axis of the α-helix should be mainly in the plane because of the positive direction of α-helix bands and the direction of antiparallel β-sheet chains should be perpendicular to the plane because of the negative direction of the band at 1690 cm^{-1}.

At low surface pressures (from 0 to 10 mN/m), the signal of amide I was very weak and the intensity was almost the same as amide II. Upon further compression, the PM-IRRAS signal increased, and so did the ratio of the intensity of amide I and amide II bands indicative of an orientational change of the enzyme. In fact, the PM-IRRAS signal is dependent on the interfacial concentration of the enzyme.[29] As the Langmuir monolayer was compressed, the surface pressure increased and the enzyme surface concentration also increased, resulting in an increase of the signal intensity. During the whole compression procedure, no changes were observed concerning the shape of the amide I and amide II bands and the proportions of the secondary structure appeared identical at low and high surface pressures which indicate that the enzyme kept its conformation.

It has to be noted that the band at lower frequency (1632 cm^{-1}) was a shoulder at low surface pressures, but it became dominant upon further compression. Concomitant, the intensity of the band at higher frequency (1652 cm^{-1}) decreased from

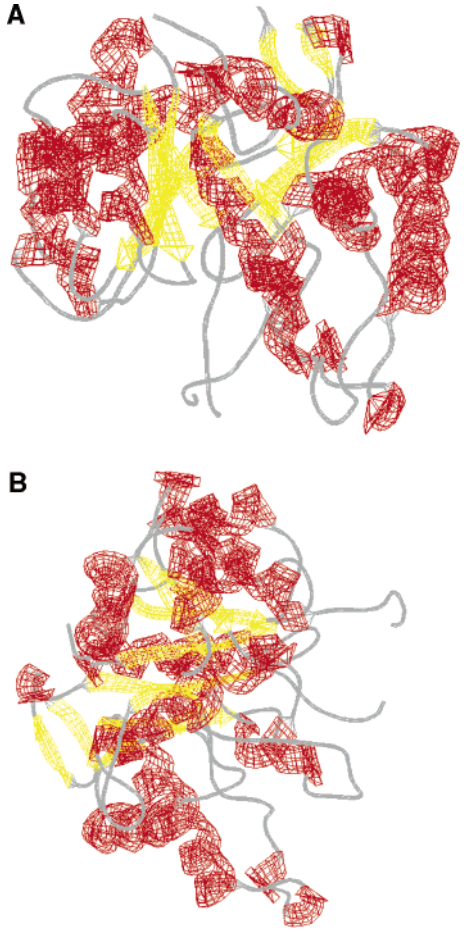

Figure 2. Illustration of the orientation of OPH at low (0−10 mN/m) and high surface pressure (15−30 mN/m) (from Swiss Protein Data Bank, accession number: 1PTA).

low to high surface pressures. Moreover, the 1652 cm^{-1} band shifted to 1649 cm^{-1} with the compression of the monolayer, implying that the axis of the α-helix was closer to a vertical orientation. It was shown earlier that the orientation of a helical structure has an effect on the position of the amide I band.[10,30,31] If the helix tilt angle is varied from 0° (α-helix perpendicular to the interface) to 90° (α-helix parallel to the interface), the amide I band position should shift from 1649 to 1656 cm^{-1}. Therefore, changes in the orientation of α-helix structure may cause the shift of the amide I band. As in our compression experiments, the α-helix band was located at 1652 cm^{-1} when the surface pressure was 0 and 5 mN/m. The spectra showed that the change of the α-helix orientation was done simultaneously with the change of orientation of the β-sheets and produced an intensification of the low-frequency component at 1632 cm^{-1}, consequently contributing to the apparent shift of the amide I band. At higher surface pressure, the full enzyme seems to be rotated to adopt a lower surface occupation. Figure 2 showed as model the orientations for OPH at low and high surface pressures. At zero surface pressure, the enzyme had its side chains lying flat at the air−water interface, whereas an increase in the surface pressure favored the orientation of the polar groups toward the aqueous phase and the nonpolar away from the interface which implies a change of the α-helix orientation at the interface.

Three major bands were observed in amide II region, 1540, 1567, and 1515 cm^{-1}, assigned to α-helix (1540 and 1567 cm^{-1}) and random coil structure (1515 cm^{-1}), respectively.[32] No

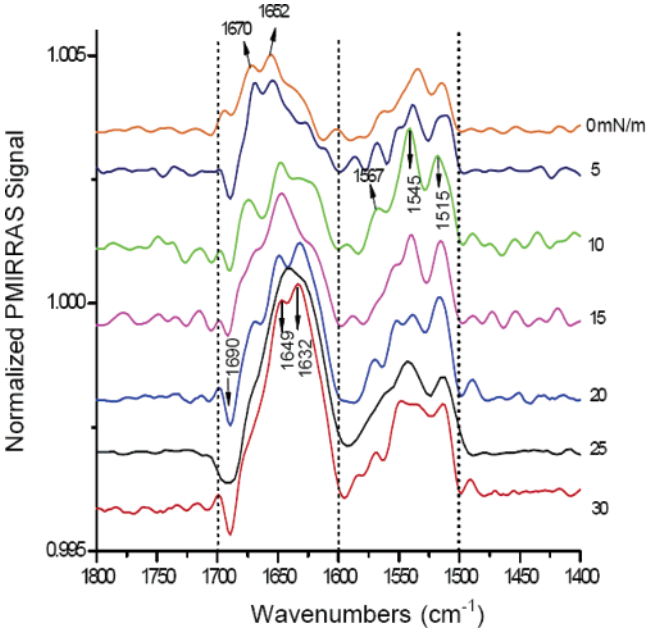

Figure 3. Normalized PM-IRRAS spectra of OPH Langmuir monolayer collected during the decompression at the air−water interface at different surface pressures.

significant changes were observed in the band positions during the monolayer compression.

The same OPH Langmuir monolayer was decompressed from surface pressure 30 to 0 mN/m, and the PM-IRRAS spectra were collected during the decompression to investigate if there was any conformational change of the enzyme. Figure 3 showed that the band shape changed at some surface pressures, but the enzyme adopted the same overall conformation during the decompression. The band at 1632 cm^{-1} reappeared with a well-defined shape when the Langmuir film was decompressed to 20 mN/m and the intensity was higher than the one at 1649 cm^{-1}. Upon further decompression, the α-helix peak became dominant and shifted to 1652 cm^{-1}. This result showed a reversibility of the secondary structure of the enzyme upon decompression.

The OPH secondary structure dependence on pH at the air−water interface was also investigated below and above the isoelectric point of OPH (pH 7.6), and the results were shown in Figure 4, panels A (pH 8.0), B (pH 9.0), and C (pH 5.0). The PM-IRRAS spectra were collected from surface pressures 0 to 25 mN/m because the OPH had a lower collapse surface pressure when the subphase pH was changed from the isoelectric point. When the pH of the subphase changed from 7.6 to 8.0, the intensity of the amide I band was stronger even at low surface pressures, indicating a different conformation of the enzyme Langmuir monolayer at the interface under this pH. The positions of the amide I and II bands were similar at different surface pressures. A new band appeared at 1645 cm^{-1} which was associated with the formation of a random coil. This may be due to the disorder in the hydrogen bonds when the subphase pH was different than the isoelectric point. The strong intensity of the amide I band and the very weak intensity of amide II band suggested a favorable orientation of the α-helix in the plane of the film but also specific orientation of the amide groups in the secondary structure. When the pH of the subphase increased to 9, the amide I band centered at 1634 cm^{-1} was assigned to the formation of a β-sheet structure. The amide II band was still weak at all surface pressures, as the β structure was dominant and taking into account the selection rule of PM-

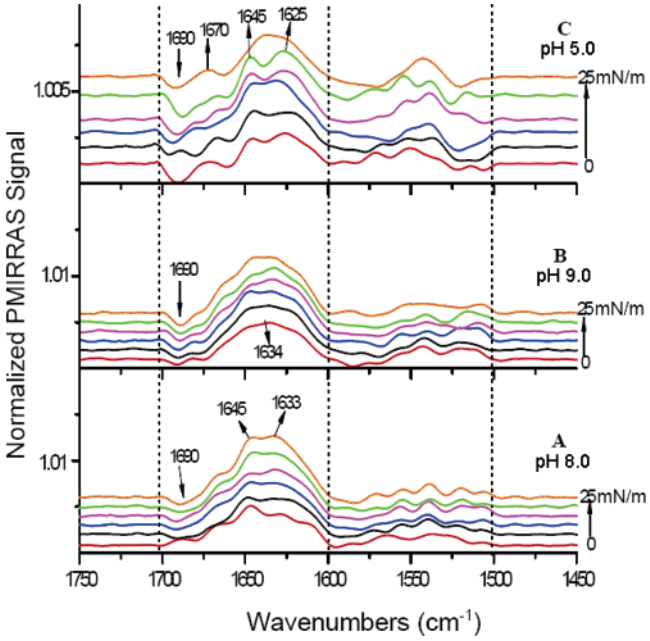

Figure 4. Normalized PM-IRRAS spectra of OPH Langmuir monolayer collected at the air−water interface at different surface pressures under different subphase pH values: pH 8.0 [A], pH 9.0 [B], and pH 5.0 [C].

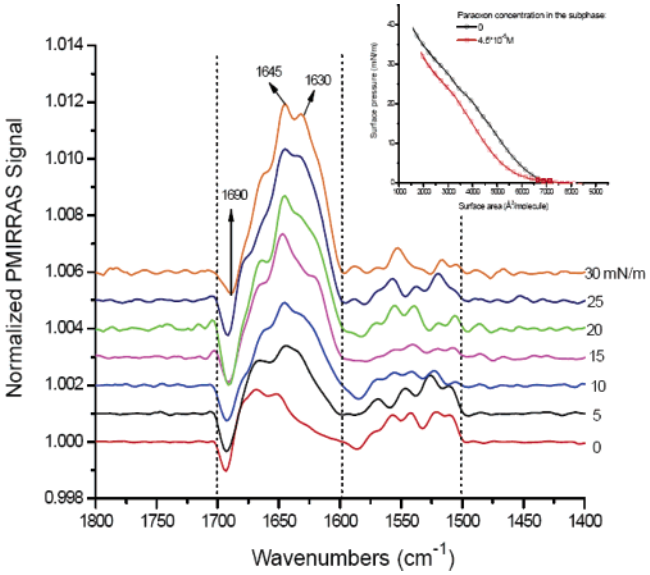

Figure 5. Normalized PM-IRRAS spectra of OPH Langmuir monolayer collected at the air−water interface at different surface pressures in the presence of paraoxon (4.5×10^{-5} M). The inset presents the surface pressure−area isotherms of OPH in the absence and presence of paraoxon (4.5×10^{-5} M).

IRRAS, the axis of the chains in the β-sheet should adopt an angle close to 40° with the optical axis normal to the interface.[33] This indicates that the orientation of the enzyme at the interface was very sensitive to the variation of pH.

At pH 5, the IR signal was noisier and the intensity of the amide I and amide II bands decreased. At this pH, the visual observation during the compression of the OPH monolayer showed some turbidity at the interface, which indicated an aggregation of the enzyme leading to an insoluble enzyme at the interface. The evidence for aggregation was showed by the formation of the antiparallel β-sheet at 1625 cm^{-1}.

The variation of the subphase pH from the isoelectric point of OPH led to changes in the intensity and shape of PM-IRRAS spectra, dramatically distorting the enzyme secondary structure. In fact, when the pH of the subphase changes, the electrostatic interactions between charged amino acids were also changed, so that the enzyme molecules encounter a very different environment at the interface.

The molecular interaction between OPH and paraoxon was also studied at the air−water interface by dissolving the paraoxon in the subphase (pH 7.6) at a concentration of 4.5×10^{-5} M, then spreading the enzyme solution as a Langmuir monolayer. The PM-IRRAS spectra in the presence of paraoxon are shown in Figure 5. The intensity of the amide I band was very strong and the band at 1645 cm^{-1} was dominant at all surface pressures because of the inability of OPH to form a random coil structure. It should be emphasized that the amide II band has almost disappeared, indicating that the intensity ratio of the amide I to amide II bands was much higher than that in the absence of paraoxon. The PM-IRRAS selection rule at the dielectric interface showed that of the α-helix axis is parallel to the interface it will give a strong positive amide I band and a weak positive amide II band.[7] It was clear from the recorded PM-IRRAS spectra in the presence of paraoxon not only favored an orientation of the α-helix parallel to the air−water interface but also a random coil conformation, which might be the cause of the decrease in the intensity of the amide II band. This organization at the interface is comprehensible because the random coil structure was adopted by enzymes so that the molecules of solvent (water from the subphase in this case) interact more favorably with the amide groups. The alterations in enzyme secondary structure had an effect on the substrate accessibility to the active site, making the limiting molecular area smaller than in the absence of paraoxon as shown in the inset of Figure 5. The mechanism of this change is not clear yet, we believe that the active site of the enzyme should be located in the vertical part of the molecule, the presence of the substrate paraoxon rendered the α-helix more parallel to the surface, making it harder for the substrate to access the active site.

Conclusion

The characterization of OPH secondary structure at the air−water interface was studied by PM-IRRAS. The position of the amide I and amide II bands showed that OPH monolayer contained α-helix, parallel and antiparallel β-sheets, and β-turn. The orientation of the β-sheets and α-helix at the air−water interface changed upon compression and decompression of the OPH Langmuir monolayer at pH 7.6. The pH effect on the secondary structure of OPH Langmuir monolayer showed that the most stable secondary structure can be obtained when the subphase pH was 7.6, which is the isoelectric point of OPH. Molecular interaction between OPH and paraoxon caused the enzyme to adopt different orientations, favoring the α-helix in the plane of the air−water interface and the formation of random coil structure.

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