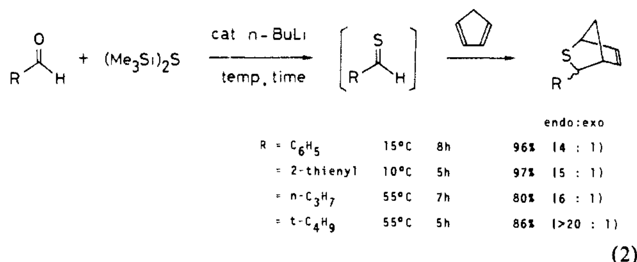
are converted to thioaldehydes in good to excellent yields (eq 2).



(2)

The described method opens a new route to the generation of seleno- and thioaldehydes and will permit further studies of the reactivity of these exotic molecules. We are currently exploring the various possibilities offered by these preliminary results.

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Supplementary Material Available: Full characterization including spectral and elemental analysis for all compounds described (8 pages). Ordering information is given on any current masthead page.

Intrinsic Fluorescence and HPLC Measurement of the Surface Dynamics of Lysozyme Adsorbed on Hydrophobic Silica

Xiao-Ming Lu, Alvaro Figueroa, and Barry L. Karger*

Barnett Institute and Department of Chemistry
Northeastern University, Boston, Massachusetts 02115
Received July 20, 1987

This paper presents results on the direct intrinsic fluorescence and liquid chromatographic measurement of the rate of change of state (weaker to stronger binding) of lysozyme adsorbed at the interface of a hydrophobic surface (*n*-butyl silica gel, C4-RP). To our knowledge, this is the first reported study on the direct kinetic measurement of changes of state while a protein is in contact with an adsorbent surface. There is a great deal of interest within various fields in the study of protein adsorption.[1,2] It is well known that protein conformation and reorientation can take place when the molecule is in contact with a surface.[3] Such changes have been observed by various techniques including intrinsic fluorescence spectroscopy[2,4-7] and chromatography.[8-12] The understanding of these protein surface changes is important in elucidating protein mechanisms of adsorption. In the present case, a first-order rate constant of change of state for lysozyme of 2.7 $\times 10^{-2}\ s^{-1}$ was measured in a 1% methanol buffer at 4 °C. The fluorescence measurements reveal a fast step accompanied by a red shift of the emission wavelength maximum, followed by a slower step associated with a small blue shift and an increase in emission intensity for which the rate constant is determined. In order to demonstrate that this slow step leads to a different state, a C4-RP packed fluorescence cell was incorporated into an HPLC system, and the rate of conversion was separately determined by HPLC.[8-10] The rate constants from the spectroscopic and HPLC methods were found to be in close agreement.

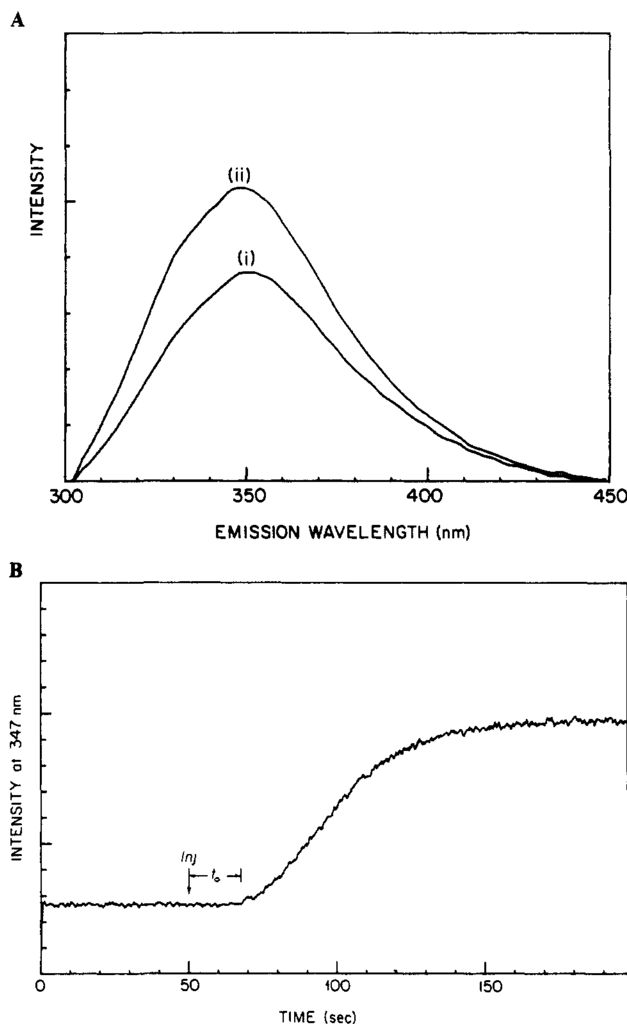

Figure 1. (A) Emission spectra of adsorbed lysozyme collected 15 s after contact (i) and after 90 s incubation (ii): excitation wavelength, 295 nm; slit width, 4 nm; scan rate, 2.5 nm/s; flow rate, 0.3 mL/min. (B) Intensity change as a function of time at an emission wavelength of 347 nm. The arrow indicates the injection time, and t_0 is the time needed for the sample to reach the column. Conditions: 1% methanol in 10 mM phosphoric acid, pH 2.3, 0.3 mL/min, 4 °C, 5 μg of lysozyme injected.

The instrument consisted of a DuPont 8800 liquid chromatograph (DuPont Co., Wilmington, DE) connected to a Suprasil quartz spectroscopic flow cell (35 μL, 11 $\times$ 2 mm i.d.) of an SPF-500 spectrofluorometer (SLM-Aminco, Urbana, IL). The flow cell (maintained at 4 °C) was utilized both for fluorescence measurements and as a chromatographic column by slurry packing roughly 25 mg of Vydac silica gel (Separations Group, Hesperia, CA) bonded with *n*-butyltriethoxysilane[13] (particle size 5 μm, pore diameter 300 Å, specific surface area 78 m^2/g). The outlet of the flow cell was connected to an HP 1046A fluorescence HPLC detector (Hewlett Packard, Palo Alto, CA). The sample consisted of 10 μL of 0.5 mg/mL of chicken egg white lysozyme (Sigma Chem. Co., St. Louis, MO) purified by size exclusion chromatography. For the surface fluorescence studies (measured at 90° to the excitation beam), wavelength maxima shifts were determined from a series of spectra taken in the region of 345–360 nm

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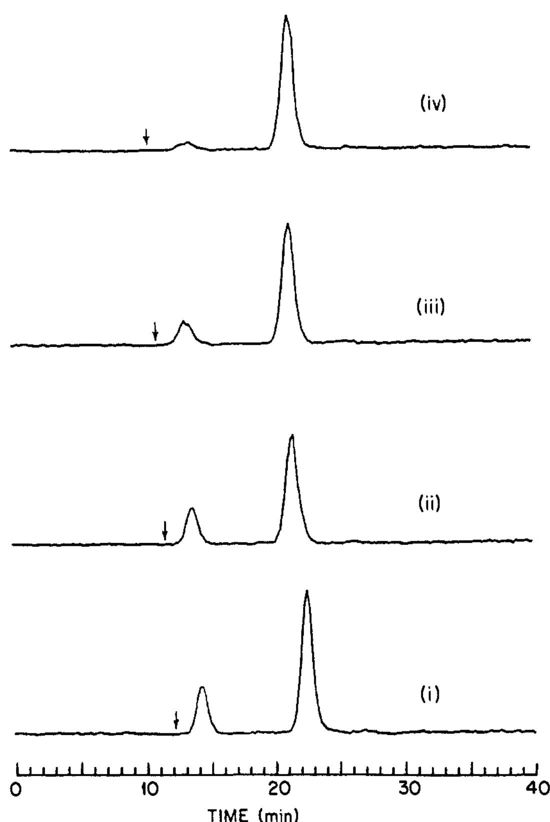

Figure 2. Chromatographic profile of lysozyme as a function of incubation time on the 35μL C4-RP column. Incubation time: (i) 0 min, (ii) 0.5 min, (iii) 1.0 min, (iv) 1.5 min, 5 μg injected, 0.3 mL/min, 4 °C, EX wavelength, 295 nm; EM wavelength, 350 nm; solvent A: 10 mM phosphoric acid, pH 2.3; solvent B: 90% (v/v) methanol in 10 mM phosphoric acid, pH 2.3; gradient: 1–100% solvent B in 10 min. The arrow indicates the time of injection.

over a 6-s period. Kinetic changes were followed from the emission intensity changes as a function of time at a fixed wavelength of 347 nm and a flow rate of 0.3 mL/min. For chromatography, a 10-min linear solvent gradient was employed from solvent A (1% methanol in 10 mM H_3PO_4, pH 2.3) to 100% solvent B (90% methanol in solvent A), both solvents being sparged by helium.

Figure 1A shows the emission spectra (corrected for the blank) of lysozyme adsorbed on the C4-RP surface under solvent A conditions. Spectrum i was collected 15 s after the protein contacted the top of the column and spectrum ii after 90 s. Care was exercised to eliminate photooxidation of Trp. Environmental changes of one or more of the six Trp's (tryptophans) of lysozyme must have occurred upon adsorption, since a fast red shift (solution 346–354 nm) followed by a slower blue shift of λ_{max} (354–349 nm) with an emission intensity increase is observed. These changes clearly arise from the influence of the stationary phase. Figure 1B shows a typical intensity versus time plot obtained at fixed wavelength (347 nm). A least-squares analysis of the plot of log($I - I_f$) versus time (I, intensisty at 347 nm at time t, and I_f, intensity at 347 nm after the blue shift has been completed) yielded a rate constant of 2.7×10^{-2} s^{-1} for the slower step. Figure 2 shows the gradient chromatographic profile as a function of the incubation time of lysozyme on the small C4-RP cell column. Two peaks are observed, the second peak growing at the expense of the first one. The rate constant was determined by the chromatographic method previously described[8] with a value of 1.9×10^{-2} s^{-1}, in close agreement with the fluorescence result.

The emission λ_{max} of the lysozyme solution in solvent A (1% methanol) at 4 °C was 346 nm, in agreement with the data reported in the literature for a pure aqueous phase.[14,15] As soon as the protein adsorbed onto the surface, an 8-nm red shift occurred, indicating that some Trp's of the lysozyme became rapidly exposed to solvent. This result suggests that one or more Trp's in the native protein had become exposed to a more hydrophilic microenvironment. After the first contact of the protein with the surface, the exposed Trp's moved toward a more hydrophobic environment, causing a blue shift in the peak maximum and an increase in the intensity of the fluorescence spectrum.[14–17]

The solvent exposure of Trp residues after the initial contact of lysozyme with the hydrophobic surface is undoubtedly related to a conformational change of the protein. The subsequent conversion, during a period of 2 min, of exposed residues to a more hydrophobic region (most likely contact with the hydrophobic surface) may be due to a further conformational change and reorientation (rearrangement) of the molecule. The agreement between the first-order rate of conversion of lysozyme as measured chromatographically with that measured by surface fluorescence suggests that the two processes have a similar origin. The weaker binding state (i.e., earlier eluting species) can be associated with λ_{max} of 354 nm and the stronger binding state with that of 349 nm. This assignment is reasonable if it is accepted that exposed Trp's contact the n-alkyl surface for the later eluting species.

In conclusion, the combination of surface intrinsic fluorescence and chromatography has been shown to provide an effective means of probing the surface dynamics of a protein in contact with an adsorbent. Work is continuing in this area, and full details will be reported subsequently.

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Evidence for a Cytochrome P-450 Catalyzed Allylic Rearrangement with Double Bond Topomerization

Robert H. McClanahan, Alain C. Huitric, Paul G. Pearson, John C. Desper, and Sidney D. Nelson*

Department of Medicinal Chemistry
University of Washington, BG-20
Seattle, Washington 98195

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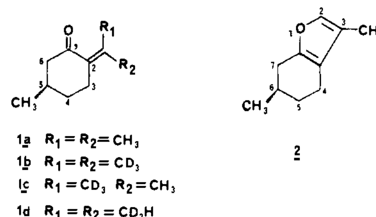
Pennyroyal oil is a volatile plant oil which has been used as an abortifacient.[1,2] However, the high doses required can cause hepatic necrosis and death.[3] Toxicity studies in mice have revealed that (R)-(+)-pulegone (5-methyl-2-(1-methylethylidene)cyclohexanone), **1a**, the major constituent terpene of the oil, is re-



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