Unambiguous Demonstration of Compression Promoted Dipeptide Synthesis in a Monolayer Film

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Introduction

Chemical reactions in monolayer and Langmuir−Blodgett films have potential applications in the development of surfaces with novel properties.[1] Although reactions in monolayers are generally analyzed by means of surface techniques, these methods are not suited to elucidating the molecular structures of components of a film. In order to probe compound structure and distribution in complex monolayer systems, we needed definitive characterization of individual reaction products. In this preliminary report, we describe the first use of flash chromatography, ^{1}H NMR, and mass spectroscopy for the isolation and identification of the product of a reaction in a monolayer film.

Background

For a bimolecular or higher order reaction, the effective molarities of the components of a monolayer film are significantly increased with respect to solution reactions, and, if the relative geometry of reactive species is suitable, reaction rates are accelerated.[2] On the other hand, because the architecture of a film controls translational and rotational degrees of freedom of molecules at the surface, reaction pathways for the surface reaction can be different from those for related reactants in solution.[3]

Mono- or multilayer films provide an environment in which the condensation of amino acids, derivatized with long alkyl chains and functionalized as active esters, is spontaneous.[4−6] Infrared data suggest, but do not prove, that amide bonds are formed. However, rigorous characterization of these products has not been pursued.

While others have focused on the use of monolayers for the preparation of stable thin films, our own interest is in the design of a nonenzymatic model of ribosomal peptide synthesis. Experiments in our laboratory have shown that amino acids derivatized with either long alkyl chains or with hydrophobic derivatives of nucleotides form monolayers which can be compressed to high packing densities. When held in proximity in the monolayer, the derivatized amino acids react to form a large number of new products.

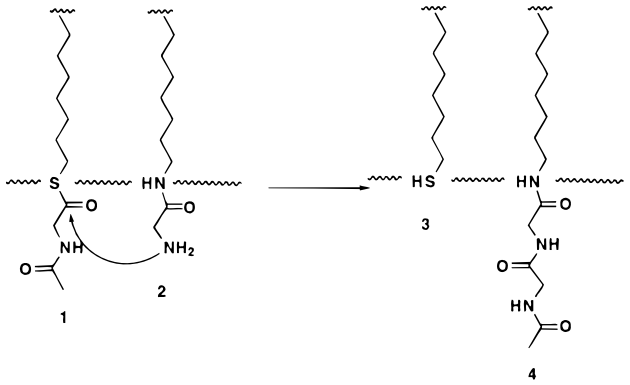

Figure 1. Reaction scheme of derivatized amino acids designed to give a single product at the air/water interface.

For our purposes, surface measurements and IR spectroscopy, analytical techniques employed in previous work, are inadequate for structure determination. Therefore we examined the possible advantages of solution characterization. In order to establish the utility of our analytical procedure in a relevant system, we first focused on characterizing a reaction that should yield a discrete product.

The components and products of the test system are shown in Figure 1. The free amino group of **2** is the only nucleophile present in the monolayer, and the thioester of **1** is the only reactive electrophile. Nucleophilic attack by the free amine should give the dipeptide derivative **4**. This dipeptide has a stable amide linkage to the hydrophobic portion of the molecule so that the condensation product **4** will remain at the interface during the experiment. In addition, the N-terminus is protected, precluding further reaction.

Behavior of the Two-Component Monolayer

In order for reaction to proceed to any significant extent within the monolayer, the two components (**1** and **2**, Figure 1) which make up the monolayer must be miscible. The isotherm for the two-component system indicates that the monolayer is a homogeneous mixture. Isotherms for each of the components and for a 1:1 mixture are shown in Figure 2. Each of the pure components has a high collapse pressure with departure from maximum slope at surface presssures of 60−70 mN/m and molecular areas at collapse of 23−25 Å^2 per molecule. The absence of a decrease from maximum slope at 65 mN/m for the mixture indicates that component **2** is not selectively extruded from the surface. A more complete study of the equilibrium spreading pressure as a function of monolayer composition[7] for the mixture was not undertaken as the extent of reaction of the mixture at the monolayer surface also provides evidence that the monolayer is a single phase.

Reaction in the Monolayer

In the experiment, an equimolar mixture of **1** and **2** in $CHCl_3$ was deposited on an aqueous subphase. After evaporation of the solvent, the monolayer was com-

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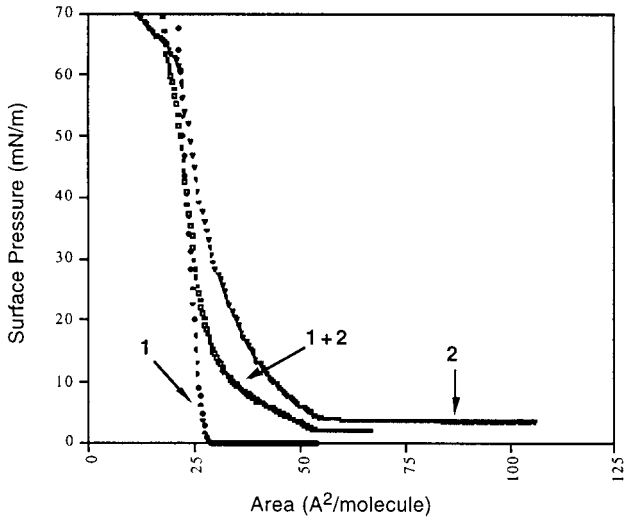

Figure 2. Isotherms of **1**, **2**, and an equimolar mixture of **1** and **2**.

pressed to a surface pressure of 25 mN/m by movement of Teflon barriers at the aqueous surface.[8] The surface pressure was maintained at 25 mN/m during reaction by computer-controlled movement of the barriers. At constant pressure during reaction, the surface area decreased by approximately 25%.[9] Reaction in the monolayer resulted in the almost complete loss of starting material within 2 h.[10] Concurrent with the disappearance of starting material, several new products were produced as observed by TLC. A product with a R_f on TLC the same as that of independently synthesized **4** was produced. In addition hexadecanethiol (**3**) and traces of glycine, presumably a product of hydrolysis of **1** by water, were observed. The monolayer film was isolated, and the component with a R_f identical to that of authentic **4** was isolated by flash chromatography. The purified reaction product was characterized by ^{1}H NMR (Figure 3a). The ^{1}H NMR spectrum of compound **4**, shown in Figure 3b, is essentially identical to that of material independently synthesized from hexadecanethiol (**3**). When D_2O was added to the sample from the monolayer, the signal at 3.26 ppm collapsed to a triplet and the signal at 3.92 ppm collapsed to two singlets, behavior consistent with coupling to exchangeable amide protons.[11] Likewise, the high resolution FAB mass spectrum of the product of reaction in the monolayer is identical to authentic **4**.

Our results show that compound **4** is the only product formed on the monolayer. No resonances for starting materials are observed in ^{1}H NMR spectra of crude reaction mixtures from a single, 2 h-compression. However, TLC of the crude reaction mixture indicates that traces of starting materials (**1** and **2**) are still present. The signal to noise ratio in these spectra allow us to conclude that the extent of reaction is greater than 85%.[12] We are currently using this NMR technique to investigate the kinetics of the reaction.

Conclusion

Long chain thioesters of amino acids spontaneously undergo acyl transfer reactions with amphiphilic nucleophiles when the reactants are constrained in a monolayer film. The chemical structure of the peptide product has been proven unambiguously. The use of solution characterization techniques is of great utility, though underutilized, for analyzing interactions and reactions in Langmuir monolayers. Recent results indicate that the kinetics of formation of the dipeptide can be determined by obtaining ^{1}H NMR spectra of a crude reaction mixture from a single experiment. Future studies will determine if the size, sequence and rate of peptide formation in more complex condensation reactions can be controlled in a predictable fashion by manipulating the monolayer.

Experimental

Reagents and solvents used were of reagent grade or better and were obtained from Aldrich Chemical Co. or Fluka Chemie AG. Deuterated solvents were obtained from Cambridge Isotope Laboratories. Analtech silica gel plates (60 F_{254}) were used for analytical TLC, and the spots were examined with UV light. Column chromatography was carried out on 200−400 mesh silica gel. Flash column chromatography was performed as described by Still.[13]

Reaction in Monolayer. Water for the subphase was purified with a Millipore Multi-Q system to a resistivity of at least 18 MΩ-cm. The subphase pH was adjusted to 7.8−8.0. Amphiphiles were spread from ~1 mg/mL solutions in 5% MeOH/$CHCl_3$ with a syringe by applying the solution at a minimum of 10 different locations on the subphase in a film balance. Solvent was allowed to evaporate for 20 min before commencing compressions. Film trough barrier speed was 20 mm/min. The pressure was maintained at 25 mN/m over the course of the reaction. Reaction times varied from 2−10 h after which the monolayer was compressed beyond collapse. Monolayer material on the surface of water was removed with a glass slide. The resulting aqueous mixture was extracted with CH_2Cl_2 and CH_3OH. The organic layers were combined and evaporated. The crude reaction mixture was subjected to flash column chromatography (2% acetone/8% CH_3OH/CH_2Cl_2) to isolate the dipeptide product. This compound was characterized by ^{1}H NMR and FAB MS. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.49 (s, 1H), 6.10 (s, 1H), 5.90 (s, 1H), 3.92 (dd, 4H, J = 5.40 Hz, 5.68 Hz), 3.26 (q, 2H, J = 6.80 Hz), 2.06 (s, 3H), 1.25 (s, 26H), 0.87 (t, 3H, J = 6.56 Hz). FAB-HRMS (NBA/NaI-matrix) calculated for $[C_{22}H_{43}N_3O_3 + Na]^+$: 420.3202; found: 420.3187.

***N*-Acetylglycine Hexadecyl Thioester (1).** To a stirred solution of *N*-acetylglycine (400 mg, 3.4 mmol) in CH_2Cl_2 (15 mL) and pyridine (190.7 μL, 3.4 mmol) was added hexadecane thiol (1.07 mL, 3.5 mmol). The mixture was cooled to 0 °C for 15 min. Dicyclohexylcarbodiimide (701.5 mg, 3.4 mmol) was added, and the reaction was stirred at 0 °C for 1 h and then at room temperature for 3 h. The flask was stored overnight in a refrigerator to precipitate urea. The reaction was quenched by the addition of CH_3COOH (2 mL). Urea was removed by vacuum filtration and the residue washed repeatedly with CH_2Cl_2. The resulting solution was washed with 5% HCl (2 × 20 mL), 5% $NaHCO_3$ (2 × 20 mL), and brine (2 × 20 mL). The organic layer was dried over Na_2SO_4 and filtered, and solvent was removed under reduced pressure. The crude reaction mixture was subjected to flash column chromatography (5% CH_3OH/CH_2Cl_2) to give 898 mg (74%) of a white solid. ^{1}H NMR (400 MHz,

(8) The surface pressure for reaction is lower than the equilibrium spreading pressure of any of the components. Surface pressures higher than 30 mN/m will result in extrusion into the subphase or crystallization at the surface of one or more components from the surface over long periods of time.

(9) The decrease in the surface area during reaction is due to at least two effects. The first is a decrease in the mean molecular area in the monolayer due to reorganization of the monolayer. The second reason may be a decrease in the mean molecular area as the reaction proceeds. It should be noted that the decrease in surface area is not due to loss of material from the surface as at these pressures the monolayer is stable and is not extruding material into the subphase.

(10) If the mixture of **1** and **2** was not compressed, compound **4** was not formed.

(11) See Supporting Information for spectrum following addition of D_2O.

(12) We have determined that there is no selective loss of the starting materials or the dipeptide product from the monolayer during reaction or during isolation of material from the surface.

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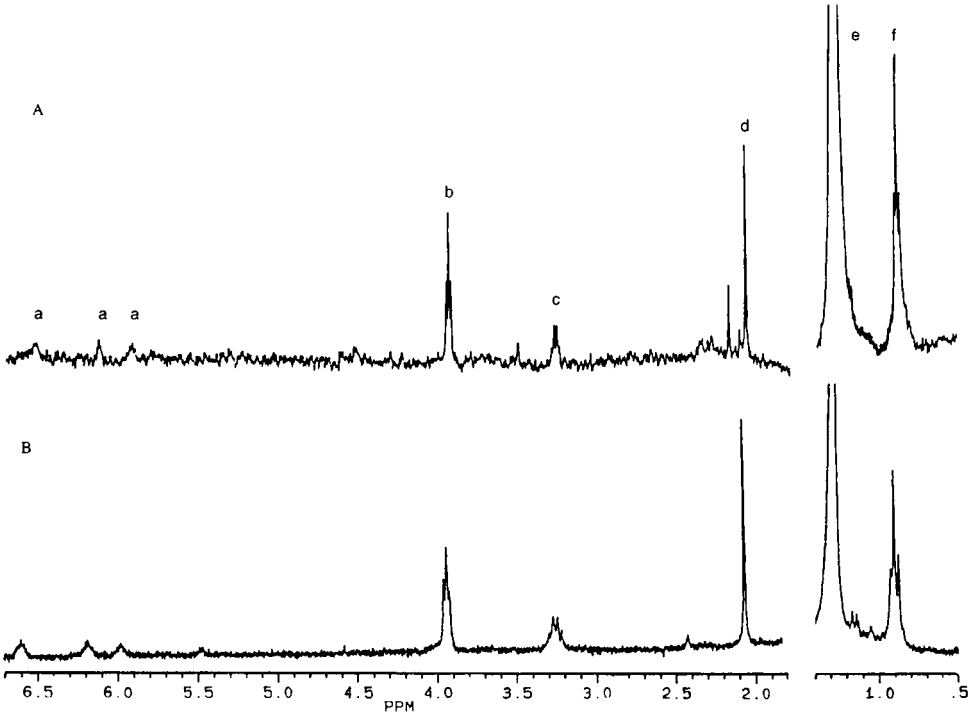

Figure 3. ^{1}H NMR spectra of compound **4** from (a) reaction of **1** and **2** in a monolayer surface and (b) solution synthesis. A large water peak appearing at a chemical shift of 1.5 ppm was present in both samples. Solvent peaks used in purification of the monolayer derived sample are observed at 2.18 ppm (acetone), 3.5 (methanol).

$CDCl_3$) δ 6.20 (s, 1H), 4.20 (d, 2H, J = 8.92 Hz), 2.91 (t, 2H, J = 11.56 Hz), 2.06 (s, 3H), 1.57 (quin, 2H, J = 11.64 Hz), 1.25 (s, 26H), 0.87 (t, 3H, J = 10.92 Hz). ^{13}C NMR (100.614 MHz, $CDCl_3$) δ 196.91, 170.07, 49.26, 49.10, 33.92, 31.88, 29.64, 29.62, 29.59, 29.53, 29.41, 29.31, 29.04, 28.78, 28.70, 25.61, 24.91, 22.93, 22.64, 14.06. FT-IR (CH_2Cl_2, ν, cm^{-1}) 3285, 2917, 2843, 1679, 1649, 1626, 1543, 1465, 1368. FAB-HRMS (NBA/NaI-matrix) calculated for $[C_{20}H_{39}NO_2S + Na]^+$: 380.2599; found: 380.2609.

***N*-Carbobenzyloxyglycine Hexadecyl Amide.** To a stirred solution of carbobenzyloxyglycine (300 mg, 1.45 mmol) in freshly distilled CH_2Cl_2 (15 mL) and pyridine (80.2 μL, 1.43 mmol) was added hexadecylamine (362 mg, 1.5 mmol). The mixture was cooled to 0 °C for 15 min followed by the addition of dicyclohexylcarbodiimide (295.05 mg, 1.43 mmol). The reaction was stirred at 0 °C for 1 h and then at room temperature for 2 h. The mixture was stored overnight in a refrigerator to precipitate urea. The reaction was quenched by the addition of CH_3COOH (1 mL). Urea was removed by vacuum filtration and the residue washed repeatedly with CH_2Cl_2. The resulting solution was washed with 5% HCl (2 × 15 mL), 5% $NaHCO_3$ (2 × 15 mL), and brine (2 × 10 mL). The organic layer was dried over Na_2SO_4 and filtered, and solvent was removed under reduced pressure. The crude sample was subjected to flash column chromatography (3% CH_3OH/CH_2Cl_2) to give 470 mg (76%) of a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 5H), 5.87 (s, 1H), 5.34 (s, 1H), 5.13 (s, 2H), 3.83 (d, 2H, J = 5.72 Hz), 3.24 (q, 2H, J = 6.69 Hz), 1.48 (qu, 2H, J = 6.64 Hz), 1.25 (s, 26H), 0.88 (t, 3H, J = 7.04 Hz). ^{13}C NMR (100.614 MHz, $CDCl_3$) δ 168.59, 136.09, 128.58, 128.30, 128.13, 110.45, 67.26, 44.75, 39.62, 31.91, 29.68, 29.66, 29.64, 29.58, 29.52, 29.50, 29.35, 29.25, 26.84, 22.68, 14.10. FT-IR (CH_2Cl_2, ν, cm^{-1}) 3333, 2911, 2844, 1688, 1638, 1527, 1455, 1344, 1261. FAB-HRMS (NBA/NaI-matrix) calculated for $[C_{26}H_{44}N_2O_3 + Na]^+$: 455.3249; found: 455.3251.

Glycine Hexadecyl Amide (2). A 50 mL, two-necked, round bottom flask was charged with a solution of *N*-carbobenzyloxyglycine hexadecyl amide (620 mg, 1.43 mmol) in CH_2Cl_2 (10 mL) and CH_3OH (5 mL) and $Pd(OH)_2/C$ (10 mg). The reaction was stirred under H_2 (1 atm) for 12 h. Catalyst was removed by filtration through Celite. The solvents were evaporated to give a white crystalline material. The desired product was obtained in quantitative yield (427 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1H), 3.34 (s, 2H), 3.27 (q, 2H, J = 6.94 Hz), 1.53 (quin, 2H, J = 7.14 Hz), 1.25 (s, 26H), 0.88 (t, 3H, J = 6.68 Hz). ^{13}C NMR (100.614 MHz, $CDCl_3$) δ 172.45, 44.80, 39.01, 31.91, 29.68, 29.66, 29.64, 29.58, 29.54, 29.34, 29.31, 26.97, 22.67, 14.08. FT-IR (CH_2Cl_2, ν, cm^{-1}) 3319, 2918, 2850, 1687, 1641, 1557, 1459, 1407, 1368, 1316. FAB-HRMS (NBA/NaI-matrix) calculated for $[C_{18}H_{38}N_2O + Na]^+$: 321.2882; found: 321.2888.

***N*-Acetylglycyl-Glycine Hexadecyl Amide (4).** To a stirred solution of *N*-acetylglycine (76 mg, 0.65 mmol) in freshly distilled CH_2Cl_2 (10 mL) and dry pyridine (36 μL, 0.65 mmol) was added glycine hexadecyl amide (200 mg, 0.67 mmol). The mixture was cooled to 0 °C followed by the addition of dicyclohexylcarbodiimide (134 mg, 0.65 mmol). The reaction was stirred at 0 °C for 1 h and then at room temperature for 2 h. The flask was kept overnight in a refrigerator to precipitate urea. The reaction was quenched by the addition of CH_3COOH (1 mL). Urea was removed by vacuum filtration and the residue washed repeatedly with CH_2Cl_2. Solvents were removed under reduced pressure. Flash column chromatography of the residue eluting with 5% CH_3OH/2% acetone/CH_2Cl_2) afforded 181 mg (70%) of a white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.58 (s, 1H), 6.16 (s, 1H), 5.96 (s, 1H), 3.93 (dd, 4H, J = 4.75 Hz, 4.08 Hz), 3.25 (q, 2H, J = 6.4 Hz), 2.06 (s, 3H), 1.59 (quin, 2H, J = 4.77), 1.25 (s, 26H), 0.87 (t, 3H, J = 6.62 Hz). ^{13}C NMR (100.614 MHz, CD_3OD) δ 174.26, 172.32, 171.4, 44.00, 43.36, 40.49, 33.04, 30.75, 30.67, 30.42, 30.36, 27.93, 23.70, 22.44, 14.40, 10.08. FT-IR (CH_2Cl_2, ν, cm^{-1}) 3280, 2918, 2845, 1730, 1634, 1544, 1459, 1419, 1374. FAB-HRMS (NBA/NaI-matrix) calculated for $[C_{22}H_{43}N_3O_3 + Na]^+$: 420.3202; found: 420.3200.

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Supporting Information Available: Proton and carbon NMR spectra, IR spectra, and mass spectra are available for all compounds described in the Experimental Section (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering.

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