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Tetrahedron 64 (2008) 186–190
Tetrahedron
www.elsevier.com/locate/tet

Two component hydrogel with γ-amino butyric acid as potential receptor and neurotransmitter delivery system

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Received 7 August 2007; received in revised form 3 October 2007; accepted 18 October 2007
Available online 23 October 2007

Abstract

The molecular recognition and self-aggregation between two types of therapeutic agents having discrete complementary functions have been detected. γ-Amino butyric acid and Fmoc-lysine self-assemble into nanofibers in water to formulate a new supramolecular hydrogel scaffold.

Keywords: Self-assembly; Nanofibers; Hydrogel; γ-Amino butyric acid; Fmoc-lysine

1. Introduction

Low molecular weight hydrogels have attracted intensive research attention in recent years because of their biocompatibility, biodegradability, and resemblance to the extracellular matrix for tissue engineering and drug delivery.[1] Most of the low molecular weight hydrogelators that have been discussed so far are composed from one component small molecules[2] but recently interest in multi component hydrogels has increased.[3] Molecular recognition and intermolecular interactions occur between the components to produce a complex, which subsequently self assembles into a fibrous supramolecular network that entraps solvent to produce gel. Zhang et al.[4] and Stupp et al.,[5] have reported the successful biomedical applications of hydrogels. We are involved in developing smart biomaterials by incorporating molecular recognition and self-association of low molecular weight peptide based systems.[6] To this end, I have designed a two component supramolecular hydrogel as a potential biomaterial directly from the self-assembly of pharmaceutical small molecules like γ-amino butyric acid (GABA) and Fmoc protected lysine or arginine. GABA is of interest because it is the well-known inhibitory neurotransmitter in the mammalian central nervous system where it exerts its effects through ionotropic receptors and metabotropic receptors and altered GABAergic neurotransmission has been implicated in major neurological and psychiatric disorders like epilepsy, brain ischemia, mood disorders, schizophrenia, and Alzheimer's disease.[7] As a result, the development of GABA receptors is of great therapeutic interest. The GABA receptor used in this study belongs to a novel class of anti-inflammatory agents and displays effective activity in tissue engineering.[8] As stated by Hibert and co-workers in a molecular modeling study, the receptor has both amine and carboxylate binding sites and the molecular dipole moments play a key role in GABA binding and receptor activation.[9]

2. Results and discussion

The structures of the three small molecules used in this study are shown in Figure 1. The receptors *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine[10] **1** and *N*-(fluorenyl-9-methoxycarbonyl)-L-arginine[11] **2** belong to a novel class of anti-inflammatory agents[8] and γ-amino butyric acid **3** displays effective neurotransmitter activity.[7] Each of the compounds is highly soluble in water and produces a transparent colorless clear solution. Neither **1** nor **2** acts as a hydrogelator in a neutral aqueous solution up to a concentration of 150 mg/ml (15 wt %). γ-Amino butyric acid **3** was chosen as the second component to provide hydrogen bond networks with receptor **1** or **2**. The addition of

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doi:10.1016/j.tet.2007.10.074

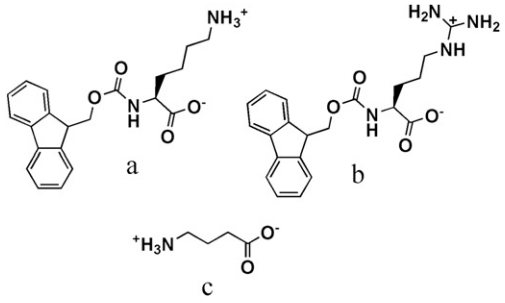

Figure 1. The schematic presentation of compounds (a) *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine **1**, (b) *N*-(fluorenyl-9-methoxycarbonyl)-L-arginine **2**, and (c) γ-amino butyric acid **3**.

γ-amino butyric acid **3** to the solution of *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine **1** leads to the formation of a hydrogel at pH 6.9 without any heat–cool cycle. Soft gels could only be obtained in deionized water with 1 equiv of receptor **1** and 1 equiv of compound **3** at 2 wt % and could be observed with the naked eye. The hard, clear, homogeneous gels appeared at high gelator concentrations, indicating that most of **1** and **3** are utilized to form the matrices of the hydrogel. The gelation has been confirmed by the inverted test-tube method. The gels displayed very good stability over time, as no changes were observed in these systems for more than two months. However, 1 equiv of compound **2** and 1 equiv of compound **3** failed to form any gel. They do not even form gels at high concentrations and other molar ratios.

The macroscopic properties of the gel were derived primarily from the tertiary structure using rheology—the study of flow that provides information about the type of network (tertiary structure) responsible for the observed gelation.[12] By measuring how the material responds to an applied oscillatory stress, several variables can be determined. Both the storage modulus (G', a measure of the elastic response of the material) and the loss modulus (G'', a measure of the viscous response) were measured at 20 °C as a function of time. The storage modulus (G') of the hydrogel (2 wt %) was found to be approximately an order of magnitude larger than the loss modulus (G''), indicative of an elastic rather than viscous material (Fig. 2).[13] Both G' and G'' were essentially independent of

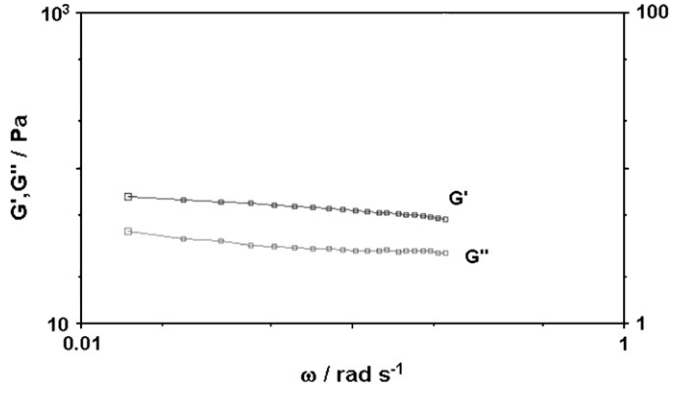

Figure 2. Mechanical response of 1 equiv of receptor **1** and 1 equiv of compound **3** gel at 20 °C with small oscillatory shear in the linear viscoelastic regime.

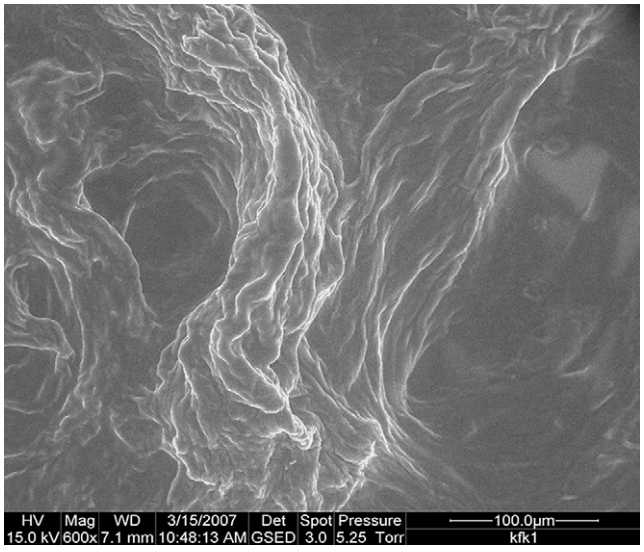

Figure 3. Environmental scanning electron micrograph of receptor–substrate hydrogel showing entangled three-dimensional fiber network.

frequency over the range 10^{-2}–10^{2} rad s^{-1}, which indicates that the dominant viscoelastic relaxations of the network are at lower frequencies; that is, the relaxation time, τ, of the network is long. Such rheological behavior is a characteristic, exhibited by networks that are physically cross-linked through weak cooperative interactions (hydrogen bonding, electrostatic, and/or hydrophobic interactions).[13]

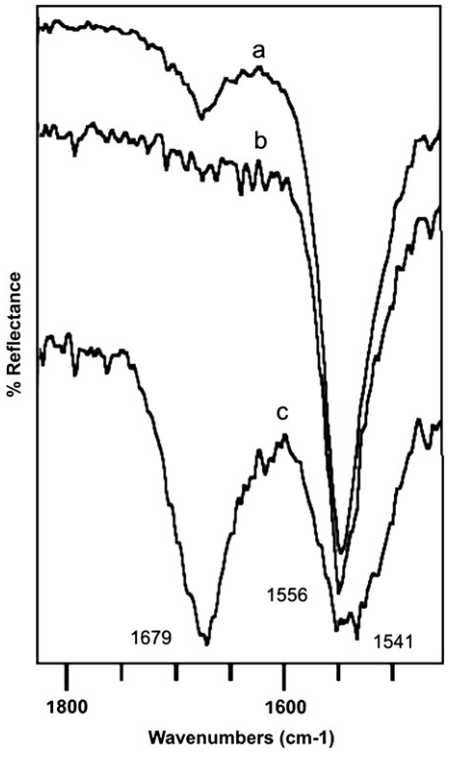

Figure 4. ATR-FTIR spectra of (a) 1 equiv of receptor **1** and 1 equiv of compound **3** hydrogels, (b) γ-amino butyric acid solution in water, and (c) *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine **1** solution in water.

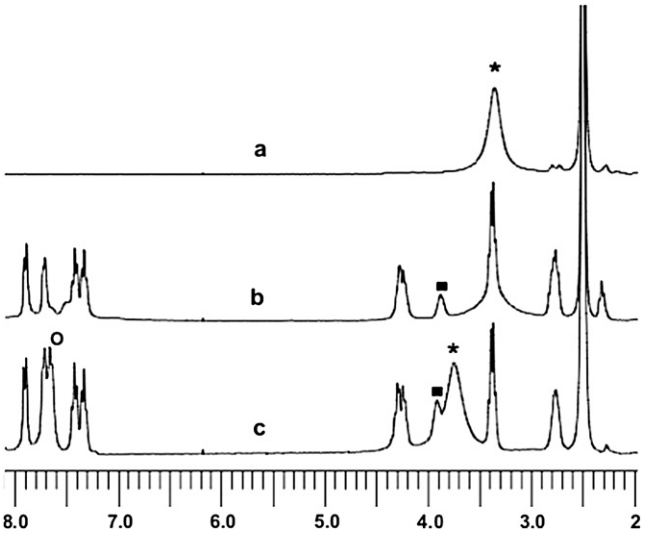

Figure 5. Sections of the ^{1}H NMR spectra (400 MHz, 298 K, 1 mM in DMSO-d_6) for (a) γ-amino butyric acid, (b) *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine **1**, and (c) 1 equiv of receptor **1** and 1 equiv of compound **3** showing the aromatic and amide resonances.

The morphology of the hydrogel was examined by environmental scanning electron microscopy (ESEM). The ESEM differs from conventional SEM or TEM in that it has a series of differential pumping zones, and is able to sustain a finite pressure around the sample. Hydrated samples can be imaged in their native state without fixing or drying, which substantially reduces the risk of artifacts being introduced or any change on morphology with evaporation of solvents.[14] A typical ESEM micrograph of the hydrogel is shown in Figure 3. It is evident that long fibers have formed and become entangled (physical crosslinks) to form a three-dimensional network, which entrap solvent molecules to form hydrogel.

The structure of the complex was studied by ATR-FTIR spectroscopy of the gels in their native state without drying (Fig. 4). The region 1800−1500 cm^{-1} is important for the stretching band of amide I and bending peak of amide II.[15] For both *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine **1** solution and the 1:1 *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine **1** and γ-amino butyric acid **3** complex (molar ratio) in the gel, the amide >C=O peak did not shift from 1679 cm^{-1}, indicating that there is no hydrogen bond between the amide >C=O groups of **1** and the amino or amide hydrogen atoms of **1** or **3**. The carboxylic acid >C=O peak shifted from 1679 cm^{-1} (solution) to 1550 cm^{-1} (gel) due to formation of complete carboxylates. Some peaks of interest (such as the NH stretch) were obscured by the OH stretch of water. No band was observed at around 3400 cm^{-1}, indicating that all amino H atoms are involved in intermolecular hydrogen bonding.[16]

Furthermore, a closer look at the NMR spectrum of the 1:1 complex of **1** and **3** revealed that the compounds self-aggregate: the NMR shifts are aggregation dependent (Fig. 5). The ^{1}H NMR spectrum of **1** in DMSO-d_6 (1 mM at 298 K) is consistent with a non-interacting species. The signals at δ=7.28, 7.39, 7.66, and 7.87 ppm are for the aromatic fluorenyl protons, a signal for the amide NH at δ=3.8 ppm, and for the amine NH at δ=3.35 ppm. However, in a 1:1 complex of **1** and **3** (1 mM at 298 K) solution in DMSO-d_6 the signal for the GABA amine NH protons has shifted to δ=3.7 from 3.35 ppm. A new signal at δ=7.6 ppm in the aromatic fluorenyl protons region is indicative of an intermolecular π−π interactions between two fluorenyl groups that provides a molecular recognition pathway for forming supramolecular structures.[17] Based on the above information and the molecular modeling studies, it is proposed that the γ-amino butyric acid molecules serve as a linkage to connect Fmoc-lysine. The electrostatic interactions, extensive hydrogen bonding networks, and π−π interactions have important role to construct the hydrogel scaffold (Fig. 6).

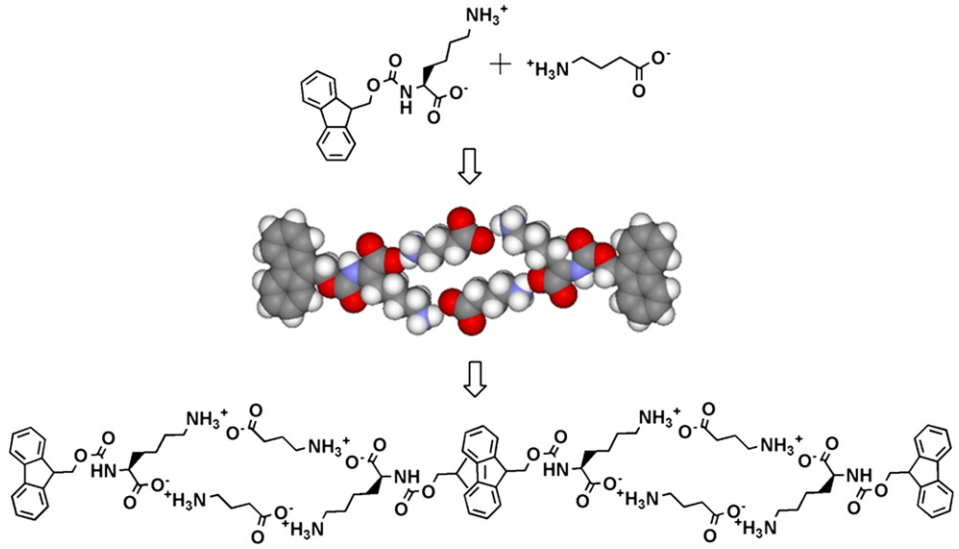

Figure 6. Schematic model of the *N*-(fluorenyl-9-methoxycarbonyl)-L-lysine-γ-amino butyric acid supramolecular complex.

3. Conclusion

In conclusion, the molecular recognition and self-aggregation with γ-amino butyric acid have been reported. The neurotransmitter GABA forms a two component hydrogel with the anti-inflammatory agent Fmoc-lysine in a 1:1 complex exploiting electrostatic interactions, hydrogen bonding networks, and π−π interactions. However, a gel is not formed with Fmoc-arginine. The understanding of such receptor−substrate supramolecular processes will allow the rational design of self-aggregating systems generated from important pharmaceutical small molecules and should offer an alternative to create useful biomaterials.

4. Experimental

4.1. General

All Fmoc protected amino acids, γ-amino butyric acid, and other reagents were used as received from Novabiochem, Sigma, and Aldrich.

4.2. Synthesis of compounds

Side-chain protecting groups of the amino acids (Boc, Pbf) other than Fmoc were removed by treating the protected amino acids (from Novabiochem) in trifluoroacetic acid (TFA)/anisole 19:1 (v/v). The TFA was removed by chilling the mixture with diethyl ether followed by centrifugation and successive washing with cold ether. The final compounds were obtained by multiple lyophilizing in a speed vacuum. Purity of the compounds was determined by analytical HPLC. The compounds were characterized by ^{1}H NMR, IR, and mass spectrometry.

4.2.1. Compound **1**

Yield=96%. Mp 26−28 °C. FTIR (aqueous solution): 1149, 1202, 1407, 1449, 1475, 1541, 1679, 2332, 2359, 2901, 2960, 3327 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.72 (br, 1H), 7.91 (d, J=7.2 Hz, 2H), 7.71 (d, J=6.4 Hz, 2H), 7.42 (t, J=7.3 Hz, 2H), 7.33 (t, J=7.5 Hz, 2H), 4.27 (m, J=10.5 Hz, 1H), 4.24 (m, J=10.5 Hz, 1H), 3.88 (t, J=7.0 Hz, 1H), 3.39 (q, J=9.5 Hz, 3H), 2.76 (q, J=5.5 Hz, 2H), 1.73−1.53 (m, 2H), 1.33 (m, 2H), 1.08 (m, 2H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 172.71, 156.25, 143.63, 143.77, 140.62, 127.63, 127.09, 125.11, 125.15, 120.17, 53.60, 51.85, 46.66, 38.57, 30.01, 26.32, 22.16 ppm. Mass spectral data $(M+H)^+$=369.6, M_{calcd}=368.4.

4.2.2. Compound **2**

See Ref. 11.

4.3. NMR experiments

All NMR studies were carried out on Brüker DPX 400 MHz spectrometer at 300 K in DMSO-d_6.

4.4. Mass spectrometry

Mass spectra of the compounds were recorded on a high resolution TOF mass spectrometer (Micromass Q-TOF) by positive mode electrospray ionization using a 1% solution of formic acid in acetonitrile/water (1:1) as liquid carrier.

4.5. Fourier transform infrared spectroscopy

Fourier transform infrared spectra were collected in transmission mode on a Nicolet Thermofisher 5700 FTIR spectrometer with smart orbit diamond ATR and OMNIC software. Incubated samples prepared in deionized water were placed on diamond ATR windows and covered with a Teflon spacer. Temperature was maintained at 298 K. For each sample 128 scans were collected and averaged in order to obtain a good signal-to-noise ratio. Spectra of deionized water were also collected as background and subtracted from the sample spectra.

4.6. Mechanical properties

Dynamic oscillatory experiments were performed on a stress-controlled rheometer (Bohlin C-VOR 200). Cone-and-plate (2° and 20 mm diameter) with a 0.7 mm gap was employed for all samples. Sample (1 mL) was loaded onto the stage and the upper cone was slowly lowered until the desired gap between plates was reached. The excess solution was then soaked away. To minimize solvent evaporation a thin layer of paraffin oil was placed around the periphery of the exposed sample and a solvent trap was used with wet tissue placed inside. To ensure the rheological measurements were done in the linear regime, a strain sweep at 2π rad s^{-1} was performed. It showed no variation in G' and G'' up to a strain of 10%. Frequency sweeps were carried out on gels between 10^{-2} and 10^2 rad s^{-1} at 20 °C. All measurements were repeated at least three times to ensure reproducibility.

4.7. Environmental scanning electron microscopy

Samples were examined by placing a drop of gel on a copper stub inside the microscope chamber of an FEI Quanta 200 ESEM. The samples were left to equilibrate at 5 °C (temperature controlled by a Peltier device under the copper stub). A few drops of distilled and deionized water were placed around the sample, before the chamber was sealed and evacuated to an initial pressure of 8 Torr. The chamber was flooded several times with water vapor before the chamber pressure was reduced to 5.25 Torr. Images were subsequently taken at an accelerating voltage of 10 kV.

Acknowledgements

I gratefully acknowledge financial support from the AVH foundation, Germany. I also thank Dr. P. Hill, School of Chemical Engineering and Analytical Science, The University of Manchester, U.K. for assistance with ESEM.

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