Enzymatic synthesis of a chiral gelator with remarkably low molecular weight

Vassil P. Vassilev, Eric E. Simanek, Malcolm R. Wood and Chi-Huey Wong*†

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

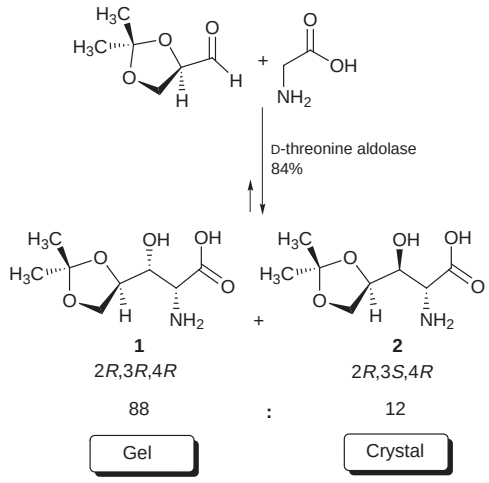
In one enzymatic step, two products of low molecular weight are obtained: one is a gelator of organic solvents, the other crystallizes to form an extended hydrogen-bondednetwork.

One product synthesized during our investigations of the enzymatic synthesis of β-hydroxy-α-amino acids using threonine aldolases was an optically-active, low molecular weight gelator of organic solvents.[1] Compound **1** is remarkably effective: only 0.2–0.4% of **1** is required for gel formation. Herein we report our investigations of **1** and **2** utilizing transmission electron microscopy, single crystal X-ray diffraction, and computation.

The products of the enzymatic aldol reaction between glycine and 2,3-*O*-isopropylideneglyceraldehyde (Scheme 1) are two diasteriomers which differ only in their absolute stereochemistry at C3. While **1** forms a gel in most organic solvents (Table 1), **2** crystallizes. This difference led us to investigate this system further.

While gel formation in aqueous and non-aqueous media by high molecular weight molecules is a well documented phenomenon, the study of low molecular weight gelators is in its infancy.[2] Their potential as nucleating agents for synthetic polymers and in health care products has been recognized.[3–8] All of these molecules contain chiral centers, hydrophobic regions, and groups capable of forming intermolecular interactions such as hydrogen bonds. Beyond such a description, our ability to predict which molecules will form gels in not possible: their discovery occurs serendipitously. In addition to the above cited small molecules, other recently published examples include derivatives of cholesterol,[9] 2,3-bis(*n*-decyloxy)anthracenes,[10] calixarenes,[11] and fluorinated hydrocarbons.[12] A majority of the gelators identified—especially those which give quartenary structure visible in electron micrographs—incorporate large aliphatic (hexadecyl, octadecyl) chains.[13–16]

Due to the low solubility of **1** in organic solvents, a MeOH quench strategy is used to form gels. Gels are obtained by first dissolving **1** in a small volume of MeOH, and then adding the solvent of choice warmed to ~ 40 °C: MeOH is typically present in < 10% v/v. Subsequent cooling of the solution to room or low temperature affords the gel. Excess solvent is removed by allowing the gel to stand at room temperature and decanting. After solvent release is complete, the molar ratio of **1**:solvent can be calculated (Table 1).



Scheme 1

Table 1 Compound **1** forms gels from a variety of solvents

Solvent	Phase formation	**1**/solvent (mol/mol)	**1**/wt%	*T*/°C[a]
H_2O	solution	—	20.5	—
H_2O–EtOH (1:5)	gel	125	4.5	3
MeOH	solution	—	2.4	—
MeOH	gel	117	4.9	3
THF	gel	702	0.4	room temp.
EtOH	gel	509	0.9	3
Bu^nOH	gel	1456	0.2	3
Pr^iOH	gel	1245	0.3	room temp.
MeCN	gel	1251	0.4	room temp.
Et_2O	gel	443	9	3
$PhCH_2CH_2OH$	gel	723	0.24	3
Bu^sOH	gel	1369	0.2	3
$C_5H_{11}OH$	gel	330	0.7	3
Isoamyl alcohol	solution	—	0.16	0
$C_8H_{17}OH$	gel	630	0.25	3
Solketal	solution	—	0.48	0

[a] Refers to temperature at which the solution of **1** in the indicated solvent was left to stand before gel formation was observed: 3 °C indicates a cold room at *ca.* 3 °C; 0 °C indicates an ice bath.

Compound **1** can be visualized by transmission electron microscopy following staining with OsO_4 vapor. At *ca.* 40 °C the gel becomes a viscous solution which can be transferred *via* pipet to a carbon-coated EM grid. After air drying for 5 min, the grid is placed in a sealed chamber next to a vial containing aq. OsO_4. After sitting overnight, the sample is examined in the EM. We observe fibers that are similar in appearance to those observed in related systems. Fig. 1(A) shows a low magnification image of the fibril network obtained. Fig. 1(B) shows a higher magnification image in which the dimensions of individual fibers can be measured. To our eyes, while the fibers all appear to be greater than 1 μm in length, they appear to terminate by forming a larger diameter fiber. Their widths also

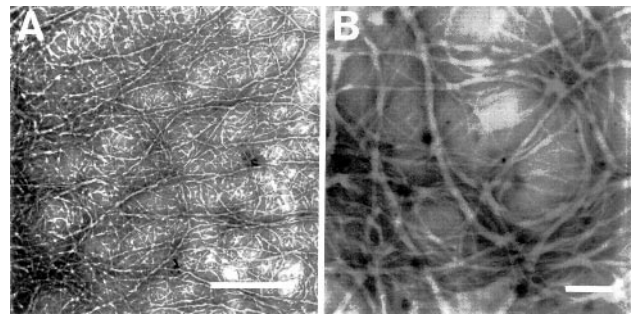


Fig. 1 Transmission electron micrographs of **1**. The scale bars shown represent (A) 2 μm and (B) 200 nm.

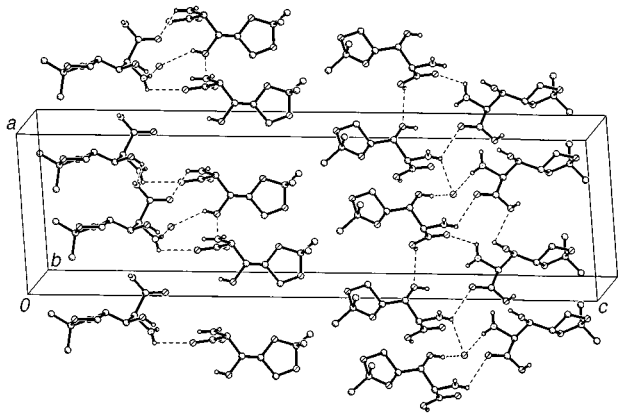


Fig. 2 Packing of **2** occurs with the inclusion of a water molecule (hydrogens omitted). Hydrogen bonds (calculated) are shown as dashed lines.

appear to be well defined. That is, we can identify three distinct sizes: narrow fibers (< 5 nm); medium fibers (20 nm); and wide fibers (> 20 nm). In Fig. 1(A) the wide fibers run from right to left, while medium fibers run between wide fibers. Narrow fibers are difficult to see at this magnification, but are seen in Fig. 1(B). Many of the gel fibers formed by higher molecular weight gelators containing one or more long chain alkanes show quarternary structure within a fibril: they appear woven like a rope. While some of the fibers suggest such a helicity, the images are not as satisfying as those reported by Thierry[3] and Demharter.[16d]

To infer reasonable patterns of intermolecular interactions in **1**, we investigated the crystal structure of **2**, which gave crystals suitable for single-crystal X-ray diffraction from an EtOH–water solution (similar to that in which **1** gives gels).‡ Fig. 2 shows the packing of these molecules in the solid state which contains a hydrophobic domain comprising the isopropylidene ring, and the hydrophilic domain comprising carboxylate, amine, and hydroxy groups. In general, hydrophobic groups of one molecule pack against the hydrophobic groups of neighboring molecules in either an edge-to-edge or face-to-face arrangement.[17] The hydrophilic regions pack to form a 3D network of interactions that are depicted as hydrogen bonds in Fig. 2.[18] Incorporated into the lattice is a highly-coordinated water molecule. All potential hydrogen bonding interactions are satisfied with the exception of the oxygen atoms of the isopropylidene group.

We envisioned that the difference between the gel and crystalline states might arise from the dimensionality of directional intermolecular interactions. That is, in the crystalline state, highly directional hydrogen bonds extend in three dimensions: one dimension is communicated along the hydroxy group at C3. Changing the geometry of this hydroxy group—changing from **2** to **1**—might change directional ordering in the third dimension leading to a gel state. To investigate this possibility we performed Monte Carlo calculations in Macromodel. Unfortunately, we find no conformational preference for the C3 hydroxy group in any of the four diasteriomers.[19]

To better evaluate the structure of the gel, we attempted to prepare gels from acidic or basic solutions of aq. MeOH and *n*-butanol—the solvent which most readily forms gels with **1**.[20]

We observed no gel formation even when *ca.* 10× the amount of **1** required for gel formation from 'pH-neutral' organic solvents was added. It seems very likely that the proton balance must be maintained for hydrogen bonding or ion-pairing to facilitate gel formation.

The molecular basis for the formation of a gel state instead of a crystal state remains unclear. We are pleased, however, that molecules like **1** and **2**—which contain three chiral centers and are available in one enzymatic step—display such interesting properties.

This work was supported by the Division of Materials Sciences of the U.S. Department of Energy under Contract No. DE-AC03-76SF-00098. The authors thank Dr Raj K. Chadha (Scripps) for crystallographic studies.

Notes and References

† E-mail: wong@scripps.edu

‡ *Crystal data* for **2**: $C_8H_{14}NO_5 \cdot 0.5H_2O$, M = 214.22, monoclinic, $C2$ (no. 5), a = 10.272(6), b = 5.795(4), c = 36.215(7) Å, β = 91.20(3)°, V = 2155(2) Å^3, T = 296(2) K, Z = 8, μ = 0.956 mm^{-1}, 3548 reflections collected, 2351 independent reflections (R_{int} = 0.1617), final R [$I > 2\sigma(I)$]: $R1$ = 0.0798, $wR2$ = 0.1875; (all data) $R1$ = 0.1390, $wR2$ = 0.2292. CCDC 182/954.

1 T. Kimura, V. P. Vassilev, G.-J. Shen and C.-H. Wong, *J. Am. Chem. Soc.*, 1997, **119**, 11 734. Compounds **1** and **2** were purified as indicated. Successive recrystallizations from EtOH–water gave **2** in > 97% purity (no impurities were observed by ^{1}H NMR analysis) and **1** in > 95% purity (< 5% of **2** was observed in the ^{1}H NMR spectrum of **1**).
2 J. M. Guenet, *Thermoreversible Gelation of Polymers and Biopolymers*, Academic Press, New York, 1992.
3 A. Thierry, C. Straupe, B. Lotz and J. C. Wittmann, *Polym. Commun.*, 1990, **31**, 299 and references cited therein.
4 F. M. Menger and K. S. Venkatasubban, *J. Am. Chem. Soc.*, 1978, **43**, 3413; F. M. Menger, Y.Yamazaki, K.K. Catlin and T. Nishimi, *Angew. Chem.*, 1995, **107**, 616; *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 585.
5 M. Jokic, J. Makarevic and M. Zinic, *J .Chem. Soc., Chem. Commun.*, 1995, 1723.
6 K. Hanabusa, Y. Matsumoto, T. Miki, T. Koyama and H. Shirai, *J. Chem. Soc., Chem. Commun.*, 1994, 1401.
7 E. J. De Vries and R. M. Kellog, *J. Chem. Soc., Chem. Commun.*, 1993, 238.
8 H. T. Stock, N. J. Turner and R. McCague, *J. Chem. Soc., Chem. Commun.*, 1995, 2063.
9 Y.-C. Lin, B. Kachar and R. G.Weiss, *J. Am. Chem. Soc.*, 1989, **111**, 5542.
10 T. Brotin, R. Utermohlen, F. Fages, H. Bouas-Laurent and J.-P. Desvergne, *J. Chem. Soc., Chem. Commun.*, 1991, 416.
11 M. Aoki, K. Murata and S. Shinkai, *Chem. Lett.*, 1991, 1751.
12 R. J. Twieg, T. P. Russel, R. Siemens and J. F. Rabolt, *Macromolecules*, 1985, **18**, 1361.
13 For amphiphilic agents, see: H. Hoffmann and G. Ebert, *Angew. Chem.*, 1988, **100**, 933; *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 902; J.-H. Fuhrhop and W. Helfrich, *Chem. Rev.*, 1993, 1565.
14 For arborols, see: G. R. Newkome, G. R. Baker, S. Arai, M. J. Saunders, P. S. Russo, K. J. Theriot, C. N. Moorefield, L. E. Rogers, J. E. Miller, T. R. Lieux, M. E. Murray, B. Philips and L. Pascal, *J. Am. Chem. Soc.*, 1990, **112**, 8458.
15 For more work from the lab of Hanabusa and colleagues, see: K. Hanabusa, K. Okui, K. Karaki, T. Koyama and H. Shirai, *J. Chem. Soc., Chem. Commun.*, 1992, 1371; K. Hanabusa, J. Tange, Y. Taguchi, T. Koyama and H. Shirai, *J. Chem. Soc., Chem. Commun.*, 1993, 390; K. Hanabusa, Y. Naka, T. Koyama and H. Shirai, *J. Chem. Soc., Chem. Commun.*, 1994, 2683.
16 For other systems, see: (*a*) C. S. Snijder, J. C. de Jong, A. Meetsma, F. van Bolhuis and B. L. Feringa, *Chem. Eur. J.*, 1995, **1**, 594; (*b*) J.-H. Fuhrhop, S. Svenson, C. Boetcher, E. Rossler and H.-M. Vieth, *J. Am. Chem. Soc.*, 1990, **112**, 4307; (*c*) S. Bhattacharya, S. N. Ghanashyam Acharya and A. R. Raju, *J. Am. Chem. Soc.*, 1996, **118**, 2101; (*d*) S. Demharter, H. Frey, M. Dreschler and R. Mulhaupt, *Colloid Polym. Sci.*, 1995, **273**, 661.
17 A similar pattern of packing has been observed by Menger in which the aromatic groups of ditoluoyl-L-cystine stack face-to-face (see ref. 4).
18 The hydrogen atoms are assigned and hydrogen bonds calculated using the SHELX package of programs. We cannot say with certainty that the carboxylate and amino groups exist as CO_2H and NH_2 groups as depicted to form the indicated hydrogen bond, or as an intimate ion pair.
19 The details of the Monte Carlo simulations are available from the authors.
20 When compound **1** (6.0 mg) was dissolved in 1 M NaOH (40 μl) and MeOH (80 μl), addition of 1 ml of *n*-butanol did not produce a gel. Dissolution of **1** (5.6 mg) in 1 M HCl (40 μl) and MeOH (80 μl), and addition of 1 ml of *n*-butanol also failed to produce a gel.

Received in Corvallis, OR, USA, 28th April 1998; 8/03230K