Gelatin Protein-Mediated Direct Aldol Reaction

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Gelatin protein was found to catalyze the aldol reaction between cyclohexanone and different aromatic aldehydes under mild reaction conditions. The aldol additions carried out in DMSO at 37° yielded the addition products with moderate diastereoselectivities favoring the *syn* isomers. Appropriate control experiments demonstrated the activity of the protein in the aldol reaction. The kinetic study of the model reaction between 4-nitrobenzaldehyde and cyclohexanone established a first-order rate constant $k = (7.4 \pm 0.5) \times 10^{-3}\ h^{-1}$. Moreover, the scale-up of the process was successfully achieved at 1-g scale in a yield comparable to that in small scale.

Introduction. – Since the first report in 1872 [1], the aldol reaction between two carbonyl compounds has become one of the most powerful C–C bond-forming methods in organic synthesis. During the last decades, a number of different systems, including small molecules, polymers, enzymes, and antibodies, have been reported to serve as efficient catalysts for this important reaction [2–5].

In this context, and motivated by the production of value-added products from sustainable resources, we decided to explore the potential of gelatin as a natural organocatalyst for C–C bond formation. Gelatin is a biodegradable protein (50–100 kD) obtained either by acid (type A) or alkaline (type B) processing of collagen, which is the main component of connective tissues and the most abundant protein in mammals. The average amino-acid composition often follows the pattern Gly-Pro-X and Gly-X-Hyp, where X is any other amino acid. In general, commercial samples contain Gly (*ca.* 23%), Pro (*ca.* 12%), and Hyp (*ca.* 11% of the total amino acid content). It should be noted that these values might vary depending on the source of the material and processing method [6]. The fact that some amino acids and peptides have been employed in different catalytic studies [2][7] prompted us to investigate the potential ability of gelatin to promote some organic reactions. This protein has been traditionally used as a general ingredient in food, cosmetic, pharmaceutical, and photographic industries [8], and only recently as a reducing ligand in the preparation of metal nanoparticles [9].

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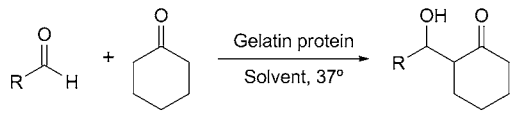
As a result of our studies, we have recently reported the addition of nitro alkanes to aldehydes (*Henry* or nitroaldol reaction) catalyzed by gelatin and collagen proteins in both aqueous and organic media [10]. We have expanded here the potential of the gelatin protein, reporting the results obtained from the direct aldol reaction between cyclohexanone and different aromatic aldehydes in the presence of commercial gelatin under mild reaction conditions.

Results and Discussion. – Based on our previous experience and recent reports [10][11], the reaction between 4-nitrobenzaldehyde (**1a**, 0.1 mmol) and cyclohexanone (**2**) at physiological temperature was chosen as the model aldol reaction to study the activity of the gelatin protein (*i.e.*, gelatin from porcine skin type-A (PSTA)). Preliminary screening of the reaction conditions (see *Exper. Part*) revealed that 10 mg of gelatin and tenfold molar excess of **2** with respect to **1a** were optimal to obtain the corresponding addition product **3a** in reasonable yields. Higher loadings did not significantly improve the results. For instance, the product yield obtained in the model reaction using 10 equiv. of **2** was *ca.* 62%, whereas the use of 20 equiv. afforded the product in *ca.* 64% yield. In contrast, the use of only 5 equiv. of **2** decreased the yield to 38%. Thus, further studies on the substrate scope were carried out using the mentioned pre-optimized conditions (*Table 1*).

Regarding the solvent, DMSO was found suitable for carrying out a comparative study. For instance, the product yield obtained in DMSO was *ca.* fourfold higher than in

Table 1. *Gelatin Protein-Catalyzed Aldol Reaction*[a])

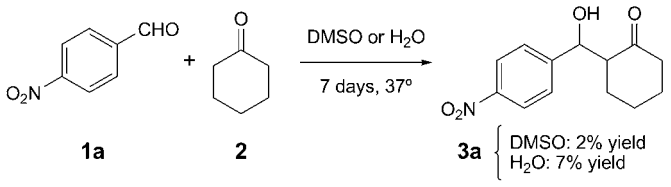


1 **2** **3**

Entry	R	**1**	Solvent	Yield[b]) of **3** [%]	dr[c]) [*syn*/*anti*]
1	4-NO_2–C_6H_4	**1a**	DMSO	62	3.9/1.0
2		**1a**	H_2O	14	1.3/1.0
3		**1a**	H_2O[d])	13	1.5/1.0
4	3-NO_2–C_6H_4	**1b**	DMSO	54	4.1/1.0
5	2-NO_2–C_6H_4	**1c**	DMSO	15	1.5/1.0
6	4-NC–C_6H_4	**1d**	DMSO	52	4.2/1.0
7	Pyridin-2-yl	**1e**	DMSO	24	3.6/1.0
8	Ph	**1f**	DMSO	20	2.3/1.0
9	4-Br–C_6H_4	**1g**	DMSO	34	2.8/1.0
10	4-Me–C_6H_4	**1h**	DMSO	10	2.2/1.0
11	4-MeO–C_6H_4	**1i**	DMSO	5	2.5/1.0

[a]) Reaction conditions: **1** (0.1 mmol), **2** (1.0 mmol), gelatin PSTA (10 mg), solvent (0.5 ml), 37°, and 7 d at 250 rpm. [b]) Yields (^{1}H-NMR) that correspond to the average values of two independent experiments (standard deviation, ±2%). [c]) Diastereomer ratio *syn*/*anti* determined by ^{1}H-NMR analyses of two independent experiments. Relative configurations were assigned by comparison with the data reported in the literature. [d]) Reaction carried out in the presence of Bu_4NBr (13 mg, 0.04 mmol).

Scheme. *Control Experiment in the Absence of Gelatin Protein*



H_2O under the same conditions (*Entries 1* and *2*). In contrast to our previous observations with the *Henry* (nitroaldol) reaction [10], the use of a phase transfer co-catalyst such as Bu_4NBr [12] did not improve the yield of the gelatin-mediated aldol reaction in H_2O (*Entries 2* and *3*).

It is important to highlight that appropriate control experiments confirmed the catalytic activity of the protein. For instance, only a tiny amount of the aldol product **3a** was detected when the model reaction between **1a** and **2** was carried out either in DMSO or in H_2O (pH *ca.* 4–5) for 7 d in the absence of gelatin (*Scheme*). In agreement with our previous studies on the *Henry* reaction [10], we could also exclude any catalytic effect on the aldol reaction possibly caused by other minor components (*ca.* 10%) such as metal and ash impurities in the gelatin samples [9a][13]. The aldol product was obtained in only 4% yield when the model reaction of **1a** and **2** was performed only in the presence of metal ions as *Lewis* acids at the concentration reported to be present in gelatin samples (*i.e.*, [metal] $\times 10^{-6}$ mol l^{-1}: 3.92 (Co^{2+}); 5.0 (Cu^{2+}); 9.42 (Fe^{3+}); 2.62 (Pd^{2+}); 9.92 (Ni^{2+})) [13]. Most importantly, although the specific mechanism of gelatin catalysis remains unclear, a simple acid or base catalysis was ruled out by *a*) controlling the pH of the reaction medium (pH *ca.* 5–5.5), *b*) the poor yields of the blank experiments (*vide supra*), and *c*) almost identical results obtained using gelatin samples with different isoelectric points (*i.e.*, PI gelatin type-A ≥ 6; PI gelatin type-B ≤ 5), in agreement with our previous observations [10].

We further studied the gelatin-mediated reaction between different aldehydes **1a**–**1i** and **2** (*Table 1*). Aromatic aldehydes with strongly or moderately deactivating groups (*i.e.*, electron-withdrawing substituents) were smoothly converted to the corresponding β-hydroxy ketones in modest yields (*Entries 1*, *4*, *5*, and *6*). The results also revealed the influence of the substitution position on the product yield, indicating a significant decrease with the *ortho*-substituted aldehyde **1c** (*Entries 1*, *4*, and *5*). On the other hand, low yields were obtained with pyridine-2-carbaldehyde **1e** (*Entry 7*), benzaldehyde (**1f**, *Entry 8*), and in the cases of aromatic aldehydes bearing weakly or moderately activating groups (*i.e.*, electron-donating substituents, *i.e.*, **1g**–**1i**; *Entries 9–11*). As observed with other aldol-like reactions, these yields could be improved by increasing the reaction time and/or the temperature [10]. Unfortunately, aliphatic aldehydes such as isovaleraldehyde were not converted after 7 d under the reported conditions.

Interestingly, a modest *syn* diastereoselectivity was found in all cases (*i.e.*, *ca.* two- to fourfold of *syn vs. anti*), which is an uncommon feature in this reaction [2][3]. The diastereoisomeric ratio (dr) was reduced either in H_2O, with *orto*-substituted aromatic aldehydes or with activated aromatic aldehydes. As expected due to the mild reaction

conditions, no potential by-products such as dehydrated or self-condensation products were observed.

In accordance with our previous studies [10], the direct use of collagen also afforded the desired aldol product (*e.g.*, **1a** (0.1 mmol), **2** (1.0 mmol), collagen (10 mg), DMSO (0.5 ml), 7 d, 37°: yield, 46%, dr (*syn*/*anti*), 3.4 : 1.0).

It is also worth mentioning that the reaction can be scaled-up with yields comparable to those of the small scale (*i.e.*, **1a** (1.13 g, 7.5 mmol), **2** (7.75 ml, 75 mmol), PSTA gelatin (0.75 g), DMSO (38 ml), 7 d, 37°; *ca.* 58% yield). In terms of recycling, gelatin was quantitatively recovered and reused in at least two consecutive runs with maintenance of stereoselectivity. Nevertheless, a major catalyst deactivation was observed in the third run (*Fig. 1*). This is presumably due to inefficient molecular desorption from the catalyst, instead of decomposition, as indicated by a visible gradual color change (from light-yellow to intense yellow-orange) and similar FT-IR spectra of the washed-dried catalyst after each run. Either acetone or EtOH could be used to precipitate the protein from DMSO, which was further separated by centrifugation. Alternatively, direct extraction of the aqueous solutions with AcOEt allows simple isolation and subsequent reuse of the protein-containing aqueous solution in the next cycle.

Despite the chiral secondary structure of gelatin and collagen proteins, chiral high-performance liquid chromatography of the reaction mixtures revealed almost negligible enantioselectivity (*i.e.* $<5\%$ ee for the major diastereoisomer, *syn*) during the aldol reaction. This lack of enantioselectivity has been also reported with other proteins or biopolymers when used as catalysts in aldol-like reactions [10][11][14]. In contrast to the results reported with other biocatalysts [15], reducing the temperature to 20° did not cause any increase of the enantiomeric selectivity.

The first-order kinetics analysis of the model reaction between **1a** and **2** established a slow rate constant $k = (7.4 \pm 0.5) \times 10^{-3}\ h^{-1}$ (*Fig. 2*). It is worthwhile to mention that the morphology and/or physical state of a protein catalyst may also play a significant

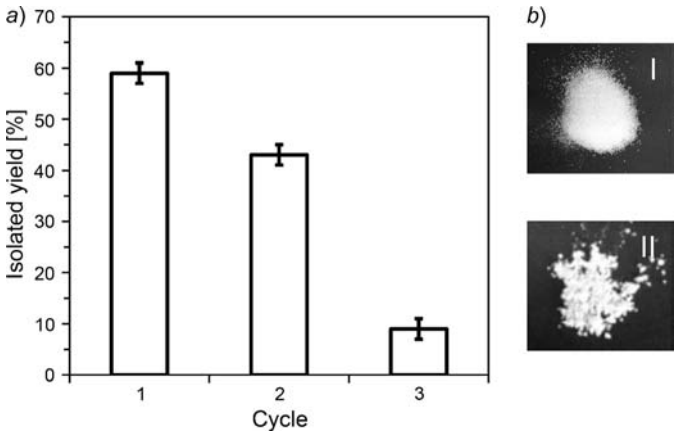


Fig. 1. *a*) *Recycling experiment for the model reaction between* **1a** (7.5 mmol) *and* **2** (75 mmol) *catalyzed by PSTA gelatin* (0.75 g) *in DMSO* (38 ml) *at 37° for 7 d. b*) *Gradual coloring and apparent clogging of gelatin.* I: Gelatin before 1st run; II: washed-dried gelatin after 3rd run.

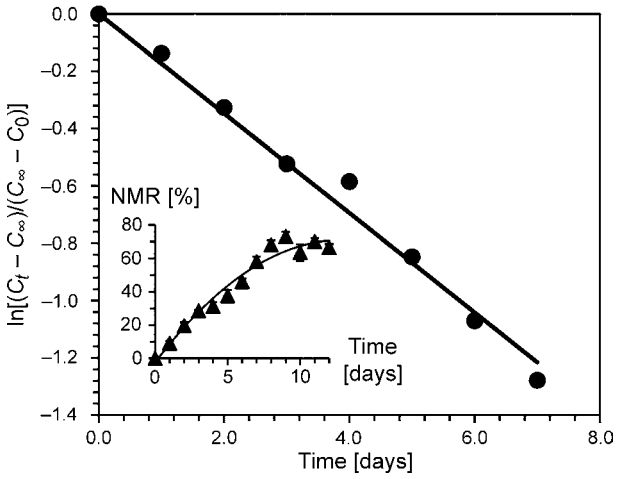


Fig. 2. *First-order kinetics plot of the model aldol reaction between* **1a** *and* **2** *catalyzed by gelatin.* C_∞, Final concentration, at infinite time; C_t, concentration at given time t; C_0, initial concentration, at $t = 0$. $R^2 = 0.98$. *Inset:* Evolution of the reaction over time

role in the kinetics of aldol-like reactions under heterogeneous or semi-heterogeneous conditions [10].

Conclusions. – In summary, we have demonstrated that C–C bond-formation through direct aldol reaction between cyclohexanone and different aromatic aldehydes can be promoted by commercial gelatin in organic medium under mild conditions. The corresponding addition products were obtained in modest yields governed by first-order kinetics. In general, modest *syn*-diastereoselectivity and complete chemoselectivity were also observed, demonstrating the applicability of the catalytic system in a g-scale experiment. Moreover, the protein could be recovered at the end of the reaction and reused in at least two consecutive cycles without major loss of the catalytic activity. Finally, we would like to emphasize that these and our previous investigations indicate that, although edible gelatin is not a competitive catalyst for this type of reactions from a synthetic perspective, the finding that it can mediate to some extent (even under aqueous conditions) the transformation of carbonyl compounds occuring in numerous foods or cosmetic formulations could be significant from a metabolic point of view.

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Experimental Part

Materials. Anal.-grade solvents and commercially available reagents were purchased from *Sigma–Aldrich* or *TCI Europe*, and used as received. *Milli-Q* H_2O was used for the experiments in aq. solns. Gelatin porcine skin type-A (PSTA) was purchased from *Sigma–Aldrich* (*CAS* 9000-70-8; Cat.

No. G2500-100G; Batch No. 128K0066; Type A, derived from acid-cured tissue; *ca.* 300 Bloom; 79% protein content by *Buiret*) and used without further purification. Collagen was purchased from *Sigma–Aldrich* (*CAS* 9007-34-5; Cat. No. C9879-1G; Batch No. 061M7015V; Type A, derived from bovine achilles tendon). A detailed description of the most important features of both gelatin and collagen can be found in the *Supporting Information* of our previous work focused on the nitroaldol (*Henry*) reaction catalyzed by these proteins [10].

Methods. TLC: Fluorescent-indicating plates (Al sheets precoated with silica gel *60 F254*; *Merck*); visualization by the use of the phosphomolybdic acid as stain soln. and UV light (254 nm). HPLC: *Varian 920-LC* chromatograph (column, *Phenomenex Lux Cellulose-2*, 4.6 × 250 mm, 5 μm; eluent, heptane/^{i}PrOH 70:30; flow rate, 1.0 ml/min; λ 254 nm). FT-IR Spectra: *Diamond ATR* (attenuated total reflection) accessory (*Golden Gate*).^{1}H-NMR Spectra: *Bruker Avance 300* spectrometer, at 25°.

Yields were determined by ^{1}H-NMR analyses of the crude product in $CDCl_3$ with Ph_2CH_2 (1 ml of a 0.1M stock soln.) as internal standard. The results were confirmed by a second experiment using directly *N,N*-dimethylacetamide (9.2 μl, added with a *Hamilton* syringe) as internal standard. Thus, possible concentration variations of the stock soln. of Ph_2CH_2 in $CDCl_3$ could be detected and the values cross-checked. Relative configurations were assigned by comparison with ^{1}H-NMR data reported in [16].

For kinetics calculations, the ^{1}H-NMR analyses of the mixtures were performed in the presence of Ph_2CH_2 (0.1 mmol) as internal standard. Each exper. point was the average of at least two independent experiments. Among various kinetics models, the straight line shown in the kinetics plot show best-fit of the first-order model (*i.e.*, [cyclohexanone] ≥ [aldehyde]).

NMR and IR spectra can be obtained directly upon request from the authors.

Preliminary Optimization Experiments. a) *Screening of Cyclohexanone* (**2**) *equivalents.* The model reaction between **1a** (15.1 mg, 0.1 mmol) and **2** (5, 10, and 20 equiv.) was carried out in DMSO (0.5 ml) at 37° for 7 d in the presence of PSTA gelatin (10 mg). That the aldol product **3a** was obtained in 38, 62, and 64% yield, resp.

b) *Screening of Catalyst Loading: Table 2* compiles the results of selected experiments for the reaction between **1a** (0.1 mmol) and **2** (2 mmol) in DMSO (0.5 ml) under different conditions of solvent temp., reaction time, and catalyst loading.

General Procedure for Gelatin-Catalyzed Aldol Reaction. Cyclohexanone (**2**; 104 μl, 1.0 mmol) was added in one portion to a 4-ml screw cap vial containing 4-nitrobenzaldehyde (**1a**; 15 mg, 0.1 mmol), gelatin PSTA (10 mg), and DMSO (0.5 ml). The mixture was stirred (250 rpm) for 7 d at 37°. The reaction was quenched by the addition of AcOEt (1 ml) and EtOH (1 ml), and subsequently the precipitated catalyst was filtered. The filtrate was rinsed three times with AcOEt (3 × 1 ml), and the combined org. phases were washed with H_2O (2 × 5 ml) and brine (5 ml), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to afford the crude product. Yield and diastereoisomer ratio (*syn*/*anti*) were determined by ^{1}H-NMR analyses of the crude product in $CDCl_3$ (300 MHz) using Ph_2CH_2 (1 ml of a 0.1M stock soln.) as internal standard after complete workup. Relative configurations were assigned by comparison with the spectroscopic data reported in the literature. For instance, in the model

Table 2. *Initial Screening of Catalyst Loading*[a])

Entry	Solvent	Temp. [°]	Time [d]	Catalyst loading [mg]	Yield of **3a** [%]
1	DMSO	25	14	0	2
2	DMSO	25	18	5	27
3	DMSO	25	14	10	62
4	H_2O	25	21	0	2
5	H_2O	37	7	0	7
6	H_2O/Bu_4NBr	37	7	0	5
7	H_2O	25	21	5	2
8	H_2O	37	7	10	14

[a]) The use of more than 10 mg of PSTA renders the workup more laborious.

reaction between **1a** and **2**, the *anti* diastereoisomer was clearly identified by a *doublet* at 4.85 ppm (J = 8.3, 1 H), whereas the *syn* diastereomer displayed the *doublet* at 5.41 ppm (J = 2.4, 1 H). Typically, for recycling experiments, acetone or EtOH (1 ml per 2 mg of catalyst) was added in order to precipitate the gelatin from DMSO. The protein was subsequently separated by centrifugation (10 min, 3800 rpm)–washing with AcOEt (2 ml)-centrifugation cycles. The obtained residue was finally dried under vacuum before the next catalytic cycle.

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