Technical Bulletin
AL-207

Aldolase Antibody 38C2

murine catalytic monoclonal antibody

Aldrich Catalog Nos. 47,995-0 and 48,157-2

5/99
4 pages

Introduction

Reactive immunization is a powerful method for the generation of efficient biocatalysts.[1] An antibody may be tailor-made and optimized by the immune system to covalently bind to the hapten. The binding pocket is not necessarily refined for noncovalent interactions with the immunogen, allowing for the production of catalysts that are broad in scope.[2] Antibody 38C2 (Ab 38C2) was generated by using the strategy of reactive immunization, which involves a hapten with a moderately reactive functional group that induces a chemical reaction with a functionalized amino acid residue in the active site of the antibody.[1a]

Professors Lerner, Barbas and their coworkers have demonstrated that Ab 38C2 catalyzes a wide variety of crossed and self aldol reactions, including intramolecular aldol reactions. Their groups have found that Ab 38C2 catalyzes reactions involving over 100 different substrate combinations with remarkably high rate enhancements. The biocatalyst can accept a variety of substrates with different physico-chemical properties (**Fig. 1**).

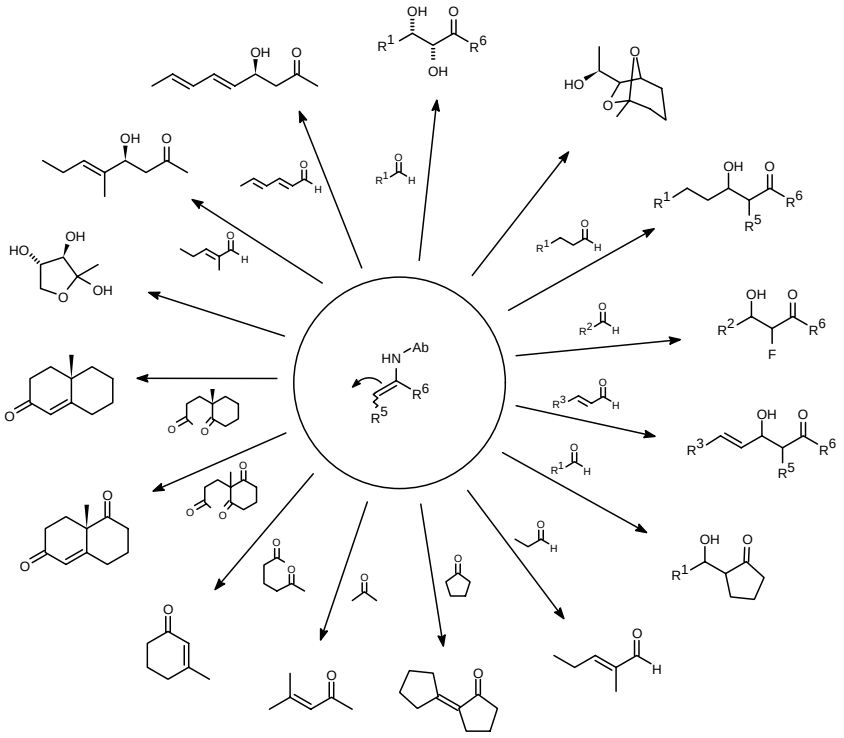


Fig. 1 Reactions catalyzed by Ab 38C2.

Mechanism

Ab 38C2 uses an enamine mechanism known from natural class I aldolase enzymes. Natural aldolases only tolerate minor changes in the structure of the donor substrate. Ab 38C2 accepts a wide variety of ketones as substrates for aldol reactions with aldehydes. The ε-amino group of a lysine residue in the binding pocket of the antibody reacts with the ketone substrate to form an enamine (see **Fig. 2**). This is followed by nucleophilic attack of the enamine on the aldehyde substrate and final hydrolytic release of the aldol product. Typical values for the Michaelis-Menten constants (K_M) of the donors range from 1 mM to 1 M, reflecting the ability of the antibody to accept a wide variety of different ketones.

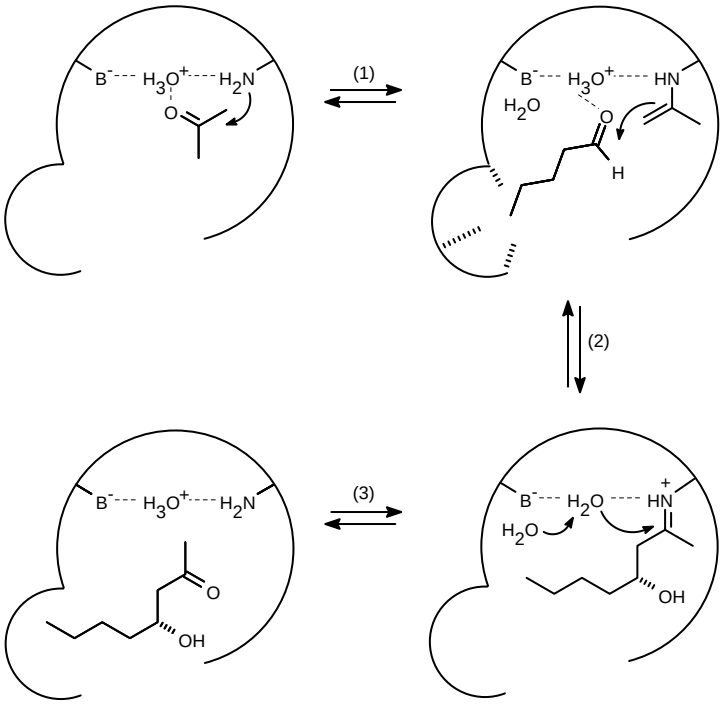


Fig. 2. Suggested mechanism for the aldol reaction as catalyzed by Ab 38C2.

Listings

47,995-0 **Aldolase antibody 38C2**,
murine catalytic monoclonal antibody
lyophilized (with PBS added) **10mg**

48,157-2 **Aldolase antibody 38C2**,
murine catalytic monoclonal antibody
lyophilized (without PBS) **10mg**

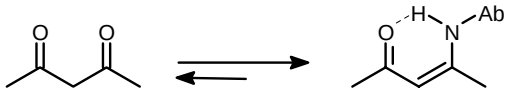
Antibody Stability

Stable at room temperature for up to 3 weeks when dissolved in different buffer solutions (pH = 5.5 to 8.5) and even pure water. No detectable activity loss was observed when the antibody was stored in stock solutions of 10 to 20 mg/mL in PBS (phosphate buffer saline) at -78 °C (stable for at least 1 year).

We recommend freezer storage of the lyophylized antibody to ensure the integrity of the product over a long period of time. The antibody will not lose activity during shipping at room temperature.

Antibody QC Assay

The aldolase Ab 38C2 can be checked for concentration and activity by incubating with acetylacetone[1] and monitoring the formation of the stable enaminone[2] using UV spectroscopy: λ_{max} = 318 nm, ε ~ 15,000 (molar absorptivity). Upon resuspension of the antibody lyophilized with buffer salts, some undissolved particulate solids may be observed.



1 **2**

λ = 318 nm

Experimental: 75 µL of a 10 mg/mL solution of antibody is dissolved in 425 µL PBS (phosphate buffer saline, pH = 7.4). 100 µL of this antibody solution is placed in a cuvette and the UV absorbance at 230 nm (A_{230}) recorded for calculation of antibody concentration. Next, 10 µL of a 1 mM solution of the acetylacetone in PBS is added to the solution in the cuvette. UV spectra are recorded at 1, 5, and 10 minutes. The A_{315} of the 10 minute spectrum is recorded and used for calculation of activity.

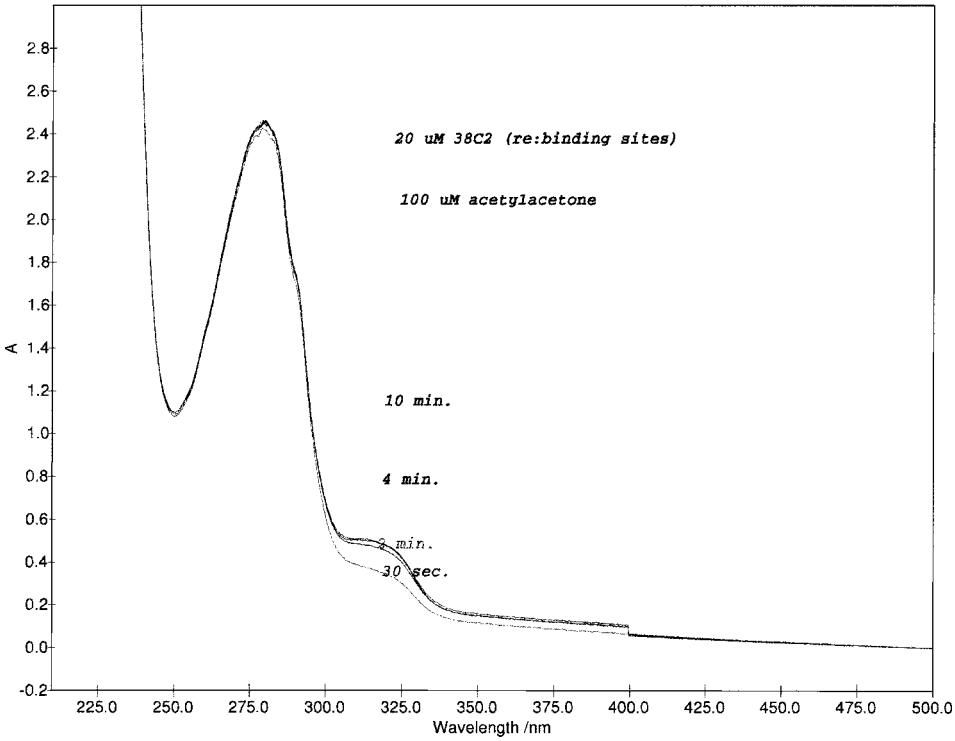


Fig.3 UV spectra of Ab 38C2 after incubation with acetylacetone at various time intervals.

Calculations: a) Determination of antibody concentration: The OD (optical density) value at 280 nm is divided by the ε of antibody (ε = 1.35) to give the concentration (mg/mL) of antibody in the diluted sample. Dividing this number by the molecular weight of the protein (M.W. ~150,000) gives µM of antibody in sample. Back calculation will then give concentration of stock solution. b) Determination of antibody activity: The OD value at 315 nm is divided by ε of enaminone (ε = 15,000) to give µM of antibody binding sites. This value should be 2x the determined antibody concentration.

Packaging

Lyophilized Ab 38C2 is packaged in sterilized (autoclaved), clear, low extractable borosilicate glass vials with gray butyl, 2-leg lyophilization stoppers and open-top, aluminum crimped seals.

Handling Precautions

Please refer to the Material Safety Data Sheet. This product is not for use in humans. This antibody is believed to be non-hazardous and non-toxic.

Instructions

Each vial contains 10 mg of lyophilized antibody (F.W. *ca*. 150,000) plus 5–6.5 mg of phosphate buffer salts (for 47,995-0). Prepare a stock solution by adding 1 mL of purified* water. Do not shake the stock solution— this can denature the protein. Other possible co-solvents (up to 5%) are acetonitrile and DMF, though the ketone donor may be used up to 10% v/v and will serve as co-solvent in most cases. No detectable activity loss was found when the antibody was stored in stock solutions of 10 to 20 mg/mL in PBS at -78 °C for up to one year. Syringes and reaction vessels do not require sterilization.

***Ultrafiltered and autoclaved (Fluka catalog # 95289) — the antibody does not contain any preservatives. Fluka products can be ordered through Aldrich (tel. 800-558-9160).**

Scope and Applications

Ab 38C2 catalyzes the following reactions:

- Aldol addition and, in some cases, the condensation reaction[1a,3]
- Crossed aldol[1a,3]
- Retro aldol[1a,3]
- Self aldol[1a,3]
- Decarboxylation of β-keto acids[4]
- Robinson annulation[5]
- Kinetic resolutions[6]

An excellent teaching tool in the laboratory for:

- HPLC characterization of the antibody catalyzed reaction
- Titration of antibody or enzyme active sites
- Analysis of the kinetics of the antibody catalyzed reaction
- Woodward UV rules
- pK_a
- Enzyme mechanisms

Typical Experimental[7]

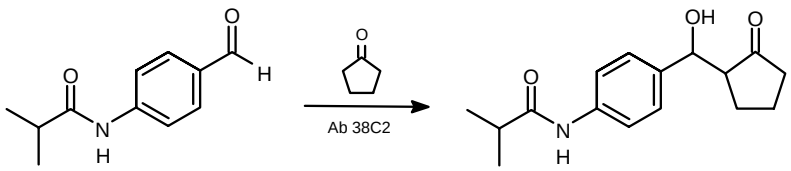
Reactions should be run with deoxygenated solvents and under argon to prevent oxidation of reagents and products to diketone inhibitors.

Analytical Scale

To a 6.25 mM solution of the aldehyde in 160 µL of PBS were added 10 µL of acetone and 30 µL of a 270 µM solution of antibody (regarding binding sites) in PBS. The final concentrations were 5 mM of aldehyde and 40 µM of antibody in a total volume of 200 µL containing 5% (v/v) of acetone. After 18 h, 12 mL of CH_2Cl_2 were added, the organic extract was dried ($MgSO_4$) and evaporated. The residue was redissolved in *ca*. 1 mL of *i*-PrOH and the *ee* was determined by normal phase HPLC using an appropriate chiral column for enantiomer separation.

Preparative Scale

Example 1



Cyclopentanone (1 mol, 88 mL) was dissolved in 912 mL of MOPS buffer (100 mM, pH = 7.4). Next, Ab 38C2 (0.67 mmol, 0.1 g[a]) was added and the first addition of aldehyde (1 mmol, 0.2 g) was made. The reaction was allowed to stir for 48 h followed by the next addition of aldehyde (1 mmol, 0.2 g). After another 48 h, the third and fourth additions of aldehyde (2.35 mmol, 0.45 g) were made for a total of 0.85 g in the reaction. The reaction progress was monitored by HPLC [Hitachi HPLC system (pump L-7100, UV detector L-7400 and integrator D-7500)] using a Rainin column (Microsorb-MV, C18, 300 Å, 5mm; 250 x 4.6 mm) and acetonitrile/water mixture[b] (containing 0.1 % trifluoroacetic acid) with eluents at a flow rate of 1.0 mL/min. When equilibrium was reached (*ca.* 21 days), the reaction mixture was saturated with NaCl and divided into two 500 mL portions for workup. Each portion was extracted with 3 x 250 mL ethyl acetate, dried ($MgSO_4$), and rotovapped to yield 1.46 g of crude product. Purification by FC (60:40, ethyl acetate/hexane) gave 0.9 g (yield = 72%) of pure product, *de* = 95%.

[a] 5 mL of a 20 mg/mL solution

[b] 20% CH_3CN / 80% water

Example 2

To a solution of 4-nitrocinnamaldehyde (110 mg, 0.61 mmol) in 15 mL of DMF and 31 mL of acetone, PBS buffer (571 mL, degassed and kept under argon) was added slowly. Ab 38C2 (8.0 mL of a 240 µM solution regarding binding sites) was added. The final concentrations of 4-nitrocinnamaldehyde and Ab38C2 were 1.0 mM and 3.0 µM, respectively, in a total volume of 625 mL containing 5% (v/v) of acetone. The reaction mixture was kept in a dark place at room temperature for seven (7) days under argon, then it was saturated with sodium chloride and extracted with 3 x 150 mL of ethyl acetate. The extracts were dried over $MgSO_4$ and rotavapped to yield 140 mg of crude product. Purification by FC (1:2, ethyl acetate/hexane) gave 96 mg (yield = 67%) of pure aldol product, *ee* = 91 %.

Kinetic Resolution

In a typical procedure, 5 µL of 100 mM stock solution of aldol in acetonitrile was added to 95 µL of a 40 µM solution of antibody in 50 µM PBS. After 50% conversion (time varied depended on the catalytic rate of the antibody corresponding to the specific aldol), the remaining aldol was purified on an analytical reverse phase HPLC column, and the solvent was removed by freeze-drying affording enantiomerically pure aldol. The product was redissolved in *ca*. 100 µL of *i*-PrOH and the *ee* was determined by normal phase HPLC using an appropriate chiral column for enantiomer separation.

Specific Rates

Please refer to data reported by Barbas and Lerner for specific rates [mol product • d^{-1}• mol^{-1}ab] for a variety of substrates.[5]

References

(1) (a) Wagner, J. et al. *Science* **1995**, *270*, 1797. (b) Wirshing, P. et al. *Science* **1995**, *270*, 1775.
(2) Barbas, C.F. III et al. *Science* **1997**, *278,* 2085.
(3) Hoffmann, T. et al. *J. Am. Chem. Soc.* **1998**, *120*, 2768.
(4) Björnestedt, R. et al. *J. Am. Chem. Soc.* **1996**, *118*, 11720.
(5) Zhong, G. et al. *J. Am. Chem. Soc.* **1997**, *119*, 8131.
(6) Zhong, G. et al. *Angew. Chem., Int. Ed. Engl.* **1998,** *37,* 2481.
(7) Shulman, A. et al., submitted for publication in *J. Chem. Ed.*

1001 West Saint Paul Ave., Milwaukee, WI 53233
Telephone 414-273-3850 • 800-231-8327
Fax 414-273-4979 • 800-962-9591
Internet http://www.sigma-aldrich.com
E-mail aldrich@sial.com