1st International Electronic Conference on Medicinal Chemistry

2-27 November 2015

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Inhibition of the cancer target human hyaluronidase Hyal-1 by natural substances

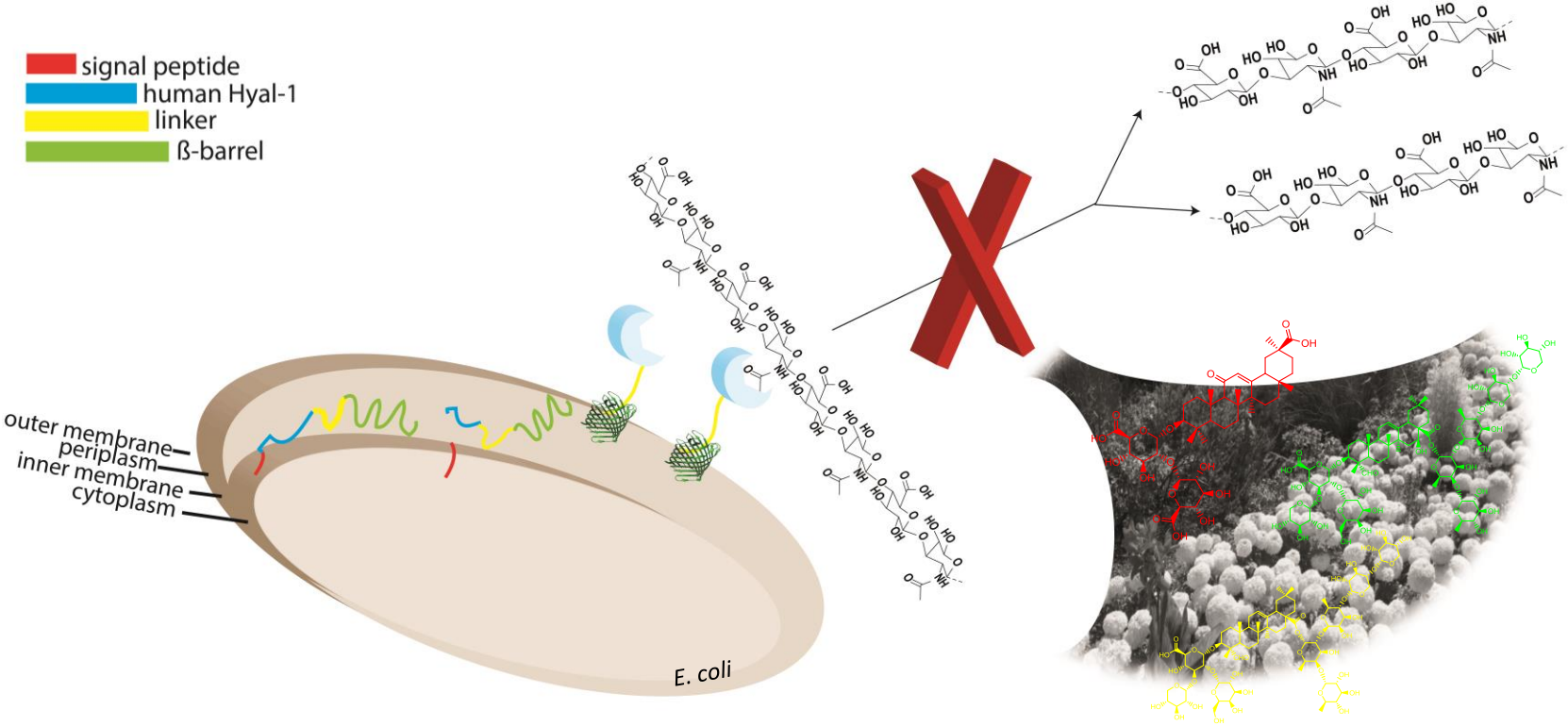
Isabelle Lengers[1,*], Zoya Orlando[1], Matthias F. Melzig[2], Armin Buschauer[3], Andreas Hensel[4] and Joachim Jose[1]

[1] Institute of Pharmaceutical and Medicinal Chemistry, and [4] Institute of Pharmaceutical Biology and Phytochemistry, PharmaCampus, Westfälische Wilhelms-Universität, Corrensstraße 48, 48149 Münster, Germany.
[2] Institute of Pharmacy, Freie Universität Berlin, Königin Luise Str. 2+4, 14195 Berlin, Germany.
[3] Institute of Pharmacy, Department of Pharmaceutical/Medicinal Chemistry II, University of Regensburg, Universitätsstr. 31, 93040 Regensburg, Germany.

* Corresponding author: isabelle.lengers@uni-muenster.de

PharmaCampus
Institut für Pharmazeutische und Medizinische Chemie

Inhibition of the cancer target human hyaluronidase Hyal-1 by natural substances



Abstract: The negatively charged polysaccharide Hyaluronic acid (HA) has diverse physiological and pathophysiological functions depending on its chain size. Space filling, anti inflammatory and antiangiogenic effects are triggered by high molecular weight HA (HMW HA) (>20 kDa). Hydrolyzation of HMW HA by Hyal-1 results in low molecular weight HA (LMW HA) (<20 kDa) which leads to inflammatory and angiogenic effects.[1] For this reason Hyal-1 is an interesting target for drug discovery. The surface display of active Hyal-1 on *Escherichia coli*, via Autodisplay, enables the screening for potential inhibitors in a whole cell system. Based on this technique we determined the inhibitory effect of different natural substances on human Hyal-1. The IC_{50} values of the plant extracts *Malvae sylvestris flos, Equiseti herba* and *Ononidis radix* were determined to be between 1.4 and 1.7 mg/mL. Furthermore, the IC_{50} values of four triterpenoid saponines were determined. The obtained IC_{50} value for glycyrrhizic acid, a known Hyal-1 inhibitor, was 177 µM. The IC_{50} values for the newly identified inhibitors gypsophila saponin 2, SA1641, and SA1657 were 108 µM, 296 µM and 371 µM, respectively.[2] For the synthesis of new small molecule inhibitors targeting human Hyal-1 these extracts and natural compounds could be used as a starting point.

Keywords: hyaluronic acid; hyaluronidase; cancer

[1] Stern R, Semin Cancer Biol, 2008, 18, 275-280.
[2] Orlando Z, *et al*. Molecules, 2015, 20, 15449-15498.

Why targeting human Hyaluronidase Hyal-1?

0022-5347/00/1631-0348/0
THE JOURNAL OF UROLOGY®
Copyright © 2000 by AMERICAN UROLOGICAL ASSOCIATION, INC.®

Vol. 163, 348–356, January 2000
Printed in U.S.A.

URINARY HYALURONIC ACID AND HYALURONIDASE: MARKERS FOR BLADDER CANCER DETECTION AND EVALUATION OF GRADE

VINATA B. LOKESHWAR,* CAN ÖBEK, HENRI T. PHAM, DAVID WEI, MARVIN J. YOUNG, ROBERT C. DUNCAN, MARK S. SOLOWAY AND NORMAN L. BLOCK

From the Departments of Urology, Cell Biology and Anatomy, and Epidemiology, University of Miami School of Medicine, Miami, Florida

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

Vol. 276, No. 18, Issue of April 13, pp. 11922–11932, 2001
Printed in U.S.A.

Stromal and Epithelial Expression of Tumor Markers Hyaluroni Acid and HYAL1 Hyaluronidase in Prostate Cancer*

Received for publication, September 14, 2000, and in revised form, January
Published, JBC Papers in Press, January 19, 2001, DOI 10.1074/jbc.M00

Vinata B. Lokeshwar‡§¶, Diego Rubinowicz‡, Grethchen L. Schroeder‡, Eva Forgacs‖, John D. Minna‖, Norman L. Block‡, and Mehrdad Nadji, and Bal L. Lokeshwar‡**

*From the Department of ‡Urology, §Cell Biology and Anatomy, and **Pathology, University of Miami School of M Miami, Florida 33101 and the ‖Hamon Center for Therapeutic Oncology Research, University of Texas Southweste Medical Center, Dallas, Texas 75390-8593*

The FASEB Journal • Research Communication

A novel role of low molecular weight hyaluronan in breast cancer metastasis

Man Wu,*,[1] Manlin Cao,†,[1] Yiqing He,* Yiwen Liu,* Cuixia Yang,* Yan Du,* Wenjuan Wang,* and Feng Gao‡,[2]

*Department of Molecular Biology, †Department of Rehabilitation Medicine, and ‡Department of Molecular Biology and Clinical Laboratory, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, People's Republic of China

Review

Seminars in Cancer Biology 18 (2008) 275–280

Hyaluronidases in cancer biology

Robert Stern*

Department of Pathology, Faculty of Medicine, Al Quds University, P.O. Box 20002, Abu-

frontiers in IMMUNOLOGY

MINI REVIEW ARTICLE
published: 14 April 2015
doi: 10.3389/fimmu.2015.00169

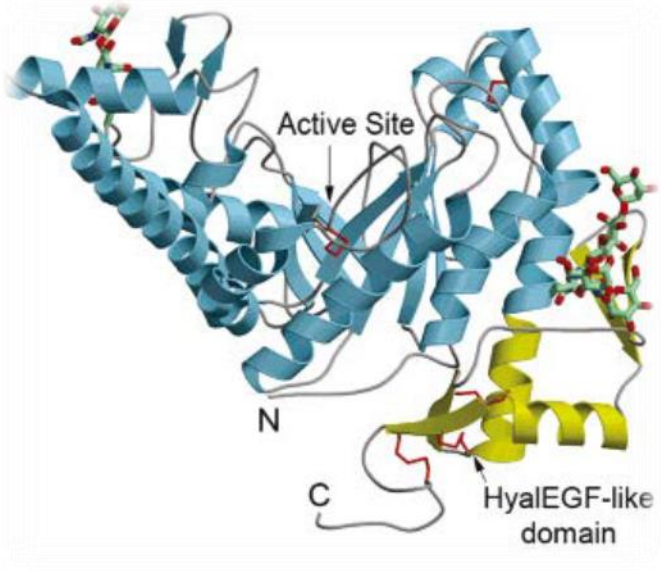
Cancer microenvironment and inflammation: role of hyaluronan

Dragana Nikitovic, Maria Tzardi, Aikaterini Berdiaki, Aristidis Tsatsakis and George N. Tzanakakis*

School of Medicine, University of Crete, Heraklion, Greece

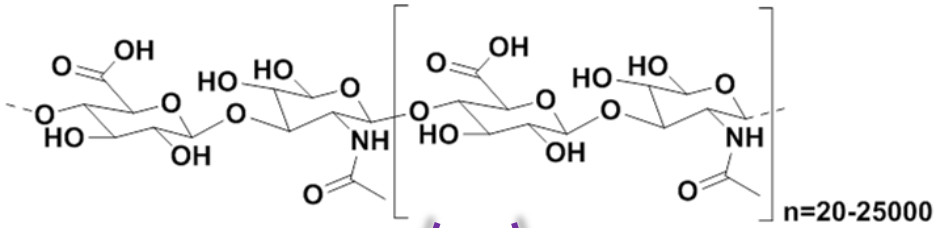
Human Hyaluronidase Hyal-1

- 57 kDa
- 4-Glycanohydrolase
- pH optimum 3.5
- Temperature optimum 37 °C
- Substrates: Hyaluronic acid
  Chondroitin
  Chondroitin sulfate



Chao *et al*. Biochemistry, 2007, 46, 6911-6920

Hyaluronic acid (HA)



Hyal-1

no degradation

Hyal-1

degradation

<u>High molecular weight HA (>20 kDa)</u>

immunosuppressive,
antiproliferative,
antiangiogenic,
space-filling

<u>Low molecular weight HA (<20 kDa)</u>

inflammatoric effects,
angiogenic

“Bottleneck”: Enzyme source

Problem:

- Eucaryotic production from *Drosophila Schneider-2* Cells (*DS*2-Cells):
  → **low yield; time and cost intensive**

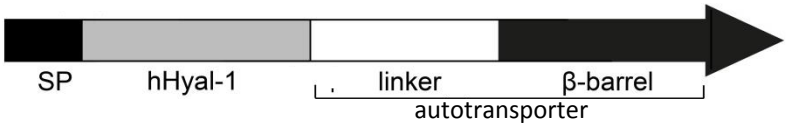
- Procaryotic production from *E. coli* cells:
  → **missing enzyme activity (misfolding/“Inclusion bodies”)**

Solution:

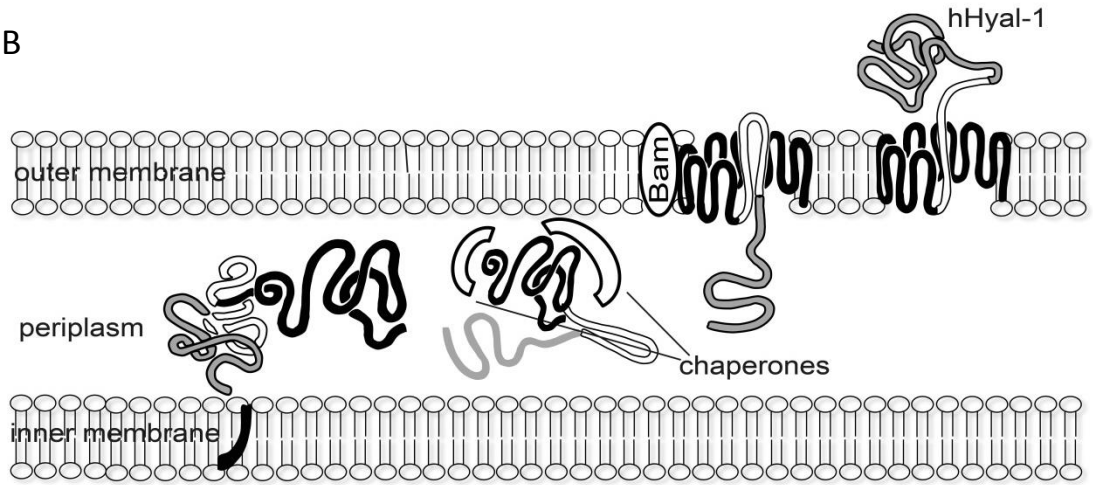
- Autodisplay:
  → **Surface expression of Hyal-1 on *E. coli***

Autodisplay of Hyal-1

A



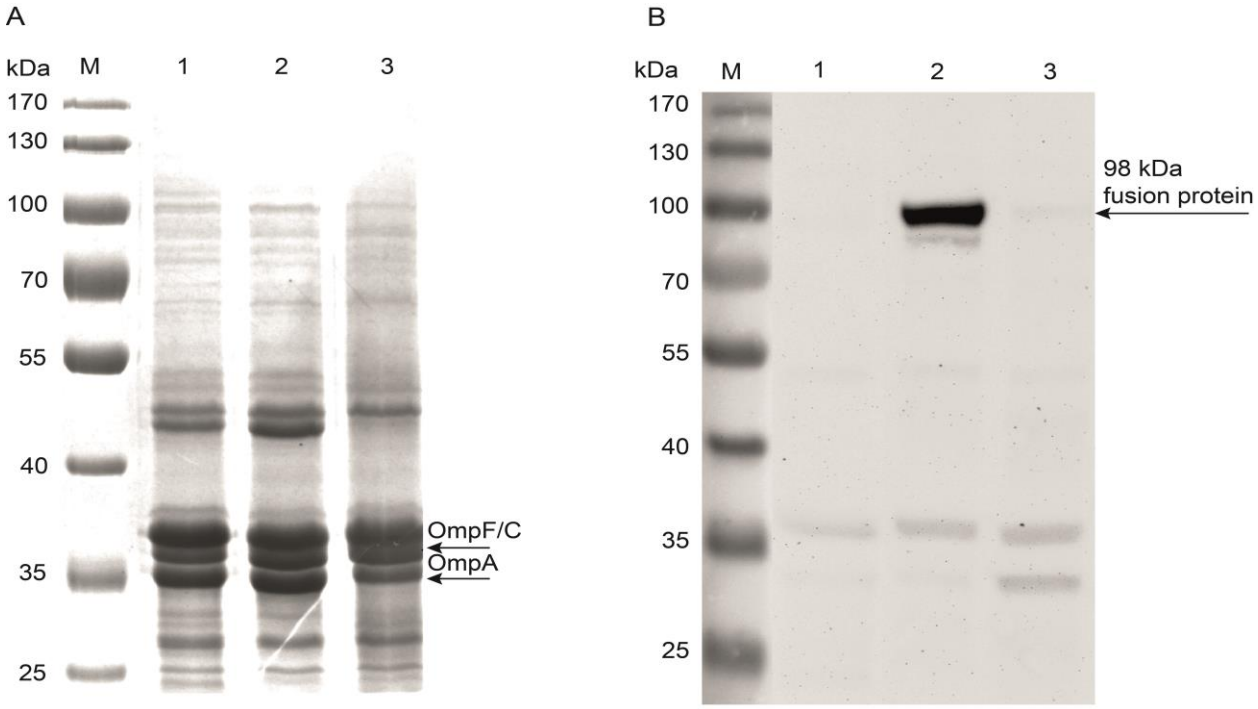
B



A Gene sequence encoding for the signal peptide (SP) the passenger domain Hyal-1, linker domain and the β-barrel.

B Surface expression of Hyal-1 via translocation of the unfolded enzyme through inner membrane and periplasm.

Surface expression of Hyal-1



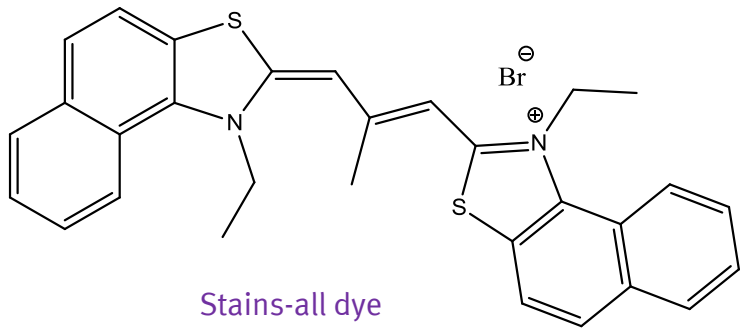
A: Polyacrylamid-gel (10%) and **B**: Western-blot analysis of outer membrane protein preparations from *E. coli* F470.

1. *E. coli* F470 without plasmid (control)
2. *E. coli* F470 pAK009 encoding Hyal-1
3. *E. coli* F470 pAK009 encoding Hyal-1 + proteinase K

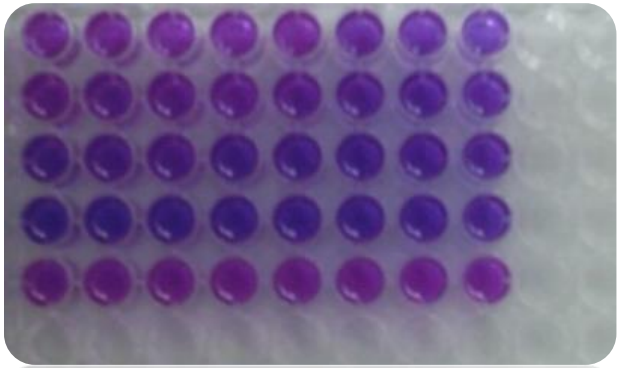
Photometric enzyme activity assay

- Positive charged dye
- Attachment to negatively charged HA
- Detection wavelength: 650 nm

→ High absorbance shows high hyaluronic acid concentrations and thus low Hyal-1 activity



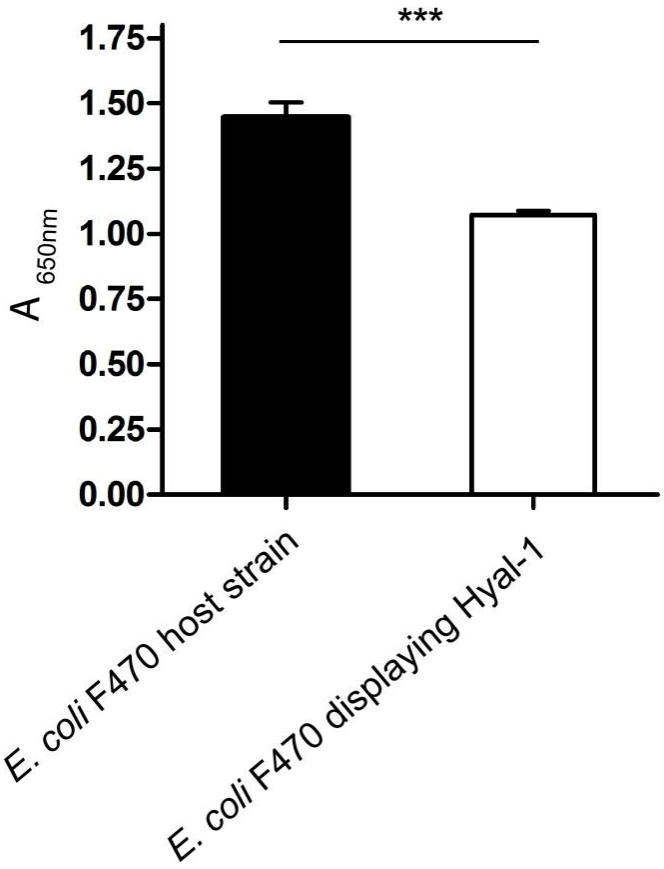
Stains-all dye



Reaction conditions:

- Temperature: 37 °C
- Sodium formate buffer [100 mM], pH 3.5
- HA: 0.11 mg/mL

Activity measurement of surface displayed Hyal-1

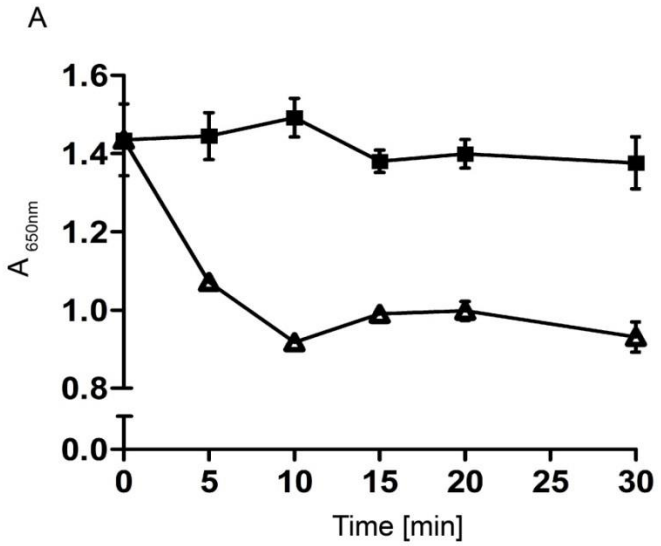


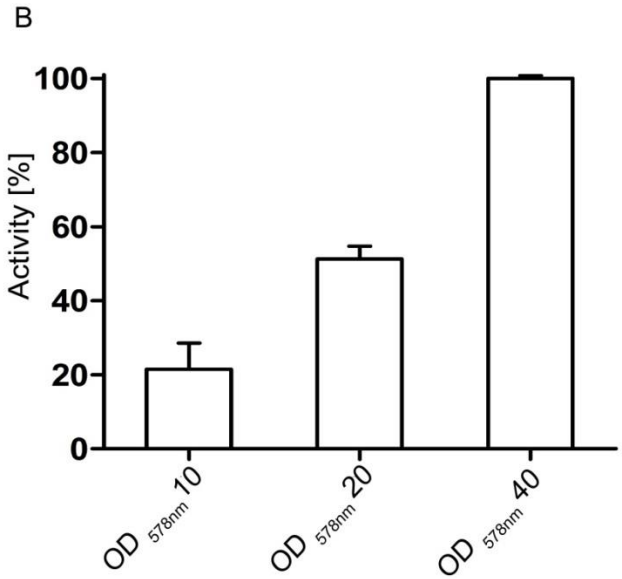
- HA: 0.11 mg/mL
- Measuring time point: 5 min
- OD_{578nm}: 10

Decrease in absorbance at a wavelength of 650 nm indicates degradation of hyaluronic acid and thus active surface displayed Hyal-1!

*** ≙ $\alpha < 0.001$

Activity measurement of surface displayed Hyal-1

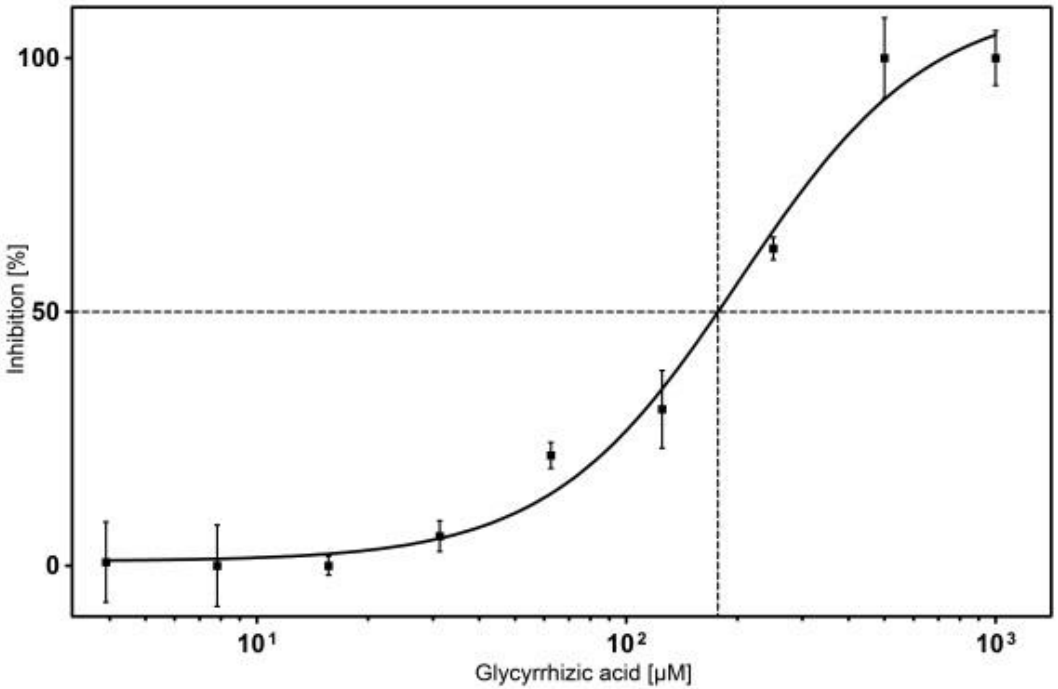




- HA: 0.11 mg/mL
- Measuring time point: 5 min

A Activity measurement of *E. coli* cells presenting human Hyal-1 (△) and control cells without plasmid encoding for Hyal-1 (■). The reaction of ‘‘stains-all’’ with undegraded hyaluronic acid results in a blue complex, which was measured at 650 nm. After digestion by Hyal-1 a decrease in absorbance is detectable. **B** Higher hyaluronidase activity was detectable by means of increasing concentrations of cells presenting Hyal-1.

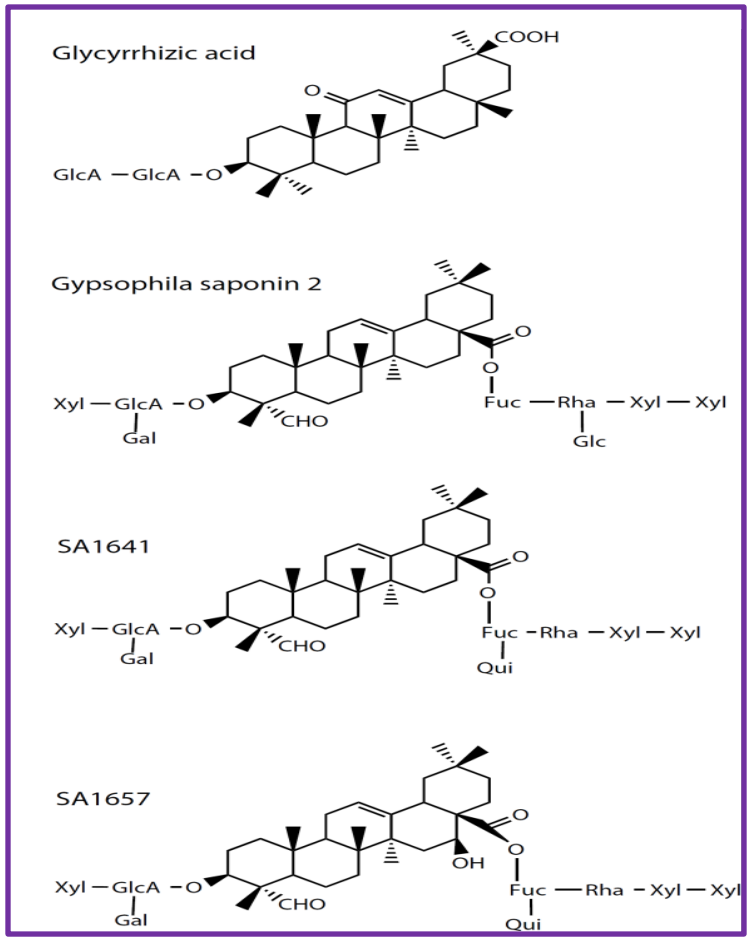
Triterpen saponins as Hyal-1 inhibitors



- HA: 0.11 mg/mL
- Measuring time point: 5 min
- OD_{578nm}: 10
- Glycyrrhizic acid: 0 – 1 mM

IC_{50}-value of Glycyrrhizic acid was determined. Glycyrrhizic acid showed an IC_{50} value of 177 µM towards surface displayed human Hyal-1.

Triterpen saponins as Hyal-1 inhibitors

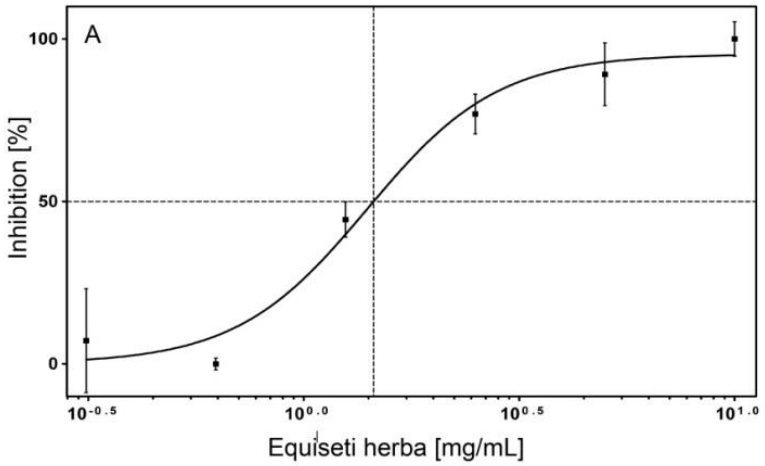


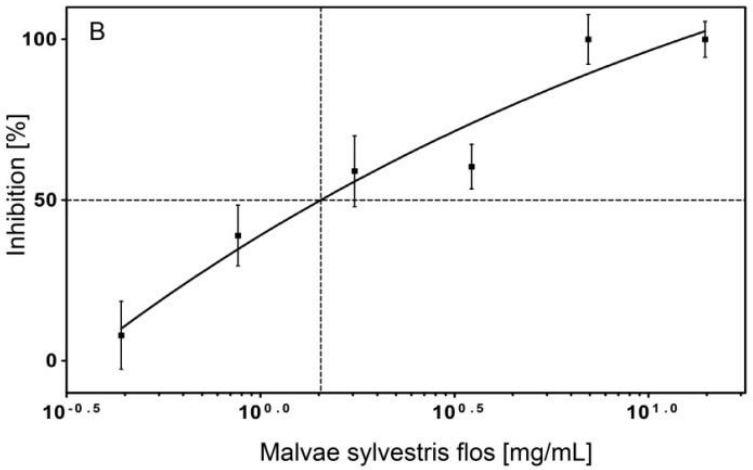
Fuc (fucose), Gal (galactose), GlcA (glucuronic acid), Glc (glucose), Qui (chinovose), Rha (rhamnose), Xyl (xylose)

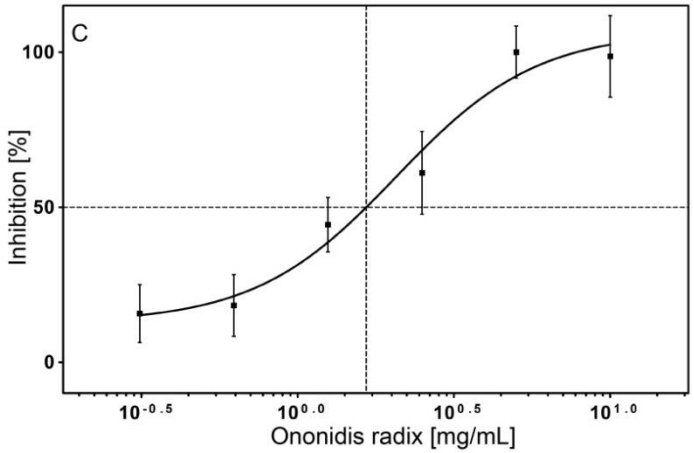
Compound	IC_{50} Value [µM]
Glycyrizic acid	177
Gypsophila saponin 2	108
SA1657	371
SA1641	296

- HA: 0.11 mg/mL
- Measuring time point: 5 min
- OD_{578nm}: 10
- Triterpen saponin: 0 – 1 mM

Inhibitory effects of plant extracts







- HA: 0.11 mg/mL
- Measuring time point: 5 min
- OD_{578nm}: 10
- Extract: 0 – 10 mg/mL

IC_{50}-value of *Equiseti herba, Malvae sylvestris flos* and *Ononidis radix* were determined to be between 1.4 and 1.7 mg/mL.

Inhibitory effects of plant extracts

plant extract	Inhibition % [10 mg/mL]	IC_{50} value [mg/mL]
Hennae folium	0	n. d.
Equiseti herba	100	1.5
Betulae folium	61	n. d.
Ononidis radix	81	1.7
Bucco folium	21	n. d.
Maydis stigma	47	n. d.
Malvae sylvestris flos	100	1.4
Solidaginis herba	100	4.9
Chebulae fructus	0	n. d.
Coptis rhizome	0	n. d.
Cranberry	10	n. d.
Althaeae radix	60	n. d.
Hydrastis rhizoma	7	n. d.
Mahoniae radix	26	n. d.

n.d: not determined

Conclusion

Surface display of active human Hyal-1 *via* Autodisplay makes the enzyme readily available for inhibitor screening. It offers the opportunity to screen a library of substances within a short time. Only few Hyal-1 inhibitors are known at this time. As a next step more compounds should be tested in order to determine a structure-activity relationship.

Acknowledgment

Thanks to:

- Prof. Jose and all members of our working group, especially Zoya Orlando

- Prof. Hensel
- Prof. Melzig
- Prof. Buschauer

PharmaCampus
Institut für
Pharmazeutische und Medizinische Chemie