Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2987–2990

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Preparation of biotinylated allosamidins with strong chitinase inhibitory activities

Shohei Sakuda,* and Masaru Sakurada

Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

Received 25 August 1998; accepted 25 September 1998

Abstract

$NaIO_4$ oxidation of allosamidin (**1**), a strong inhibitor of family 18 chitinases, followed by a coupling with Biotin Hydrazide^R afforded its mono- and dibiotinylated derivatives, **4** and **6**. Reduction of **4** by $NaBH_4$ afforded its reduced form **5**. Each of these three biotinylated derivatives maintained strong chitinase inhibitory activity. Especially, **6** inhibited a *Trichoderma* chitinase as strongly as **1**.

Keywords: Carbohydrates; Enzyme inhibitors; Fungi; Labelling

Allosamidin (**1**) is the first chitinase inhibitor isolated from mycelia of *Streptomyces* sp [1,2]. It has a unique pseudotrisaccharide structure and interesting biological activities toward chitin-containing organisms [3,4,5,6]. Since its aglycone moiety with an oxazoline skeleton was shown to interact with the active center of a family 18 chitinase [7], it is thought to be a mimic of the transition state intermediate in the enzyme reaction. Chitinases are classified into the two families,18 and 19 [8]. Allosamidin inhibited all family 18 chitinases, but did not inhibit family 19 chitinases. Family 18 chitinases are present in nature much more widely than family 19 chitinases, and they are thought to play an important role for growth of each organism containing them. A physiological role of the chitinase is, however, still unclear in many organisms such as in fungi or mammals. Study to clarify a histological localization of the chitinase is one of the important approach to investigate it. Allosamidin derivatives with a biotin residue retained chitinase inhibitory activity may be useful probes for the histological study. In this paper, we describe the preparation of three biotinylated

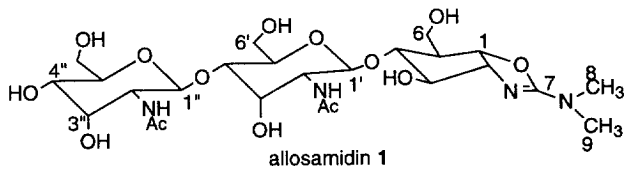
* To whom correspondence should be addressed. FAX: +81-3-5802-2932; E-mail: asakuda@hongo.ecc.u-tokyo.ac.jp

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00542-3

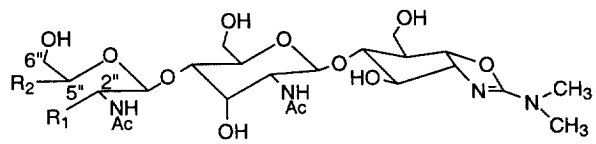
allosamidins, **4**, **5** and **6**, and their chitinase inhibitory activities as well as an application of **6** to an optical biosensor system.

Our previous study on structure-activity relationship of **1** indicated that the *N*-acetylallosamine moiety located at its non-reducing end is a possible target to prepare its derivatives retained relatively high activity [9]. Therefore, derivatization using a diol function at C-3" and C-4" was planned. First, **1** was oxidized with $NaIO_4$ to obtain a dialdehyde **2**. Because of the instability of the dialdehyde, its production was confirmed by its reduction to **3** with $NaBH_4$.[1] Next, **1** was oxidized with $NaIO_4$, followed by a coupling with 2 equiv of Biotin Hydrazide^R (Vector Laboratories Inc.). Purification of the crude products by HPLC under basic conditions afforded two main products, **4** and **6**.[2]

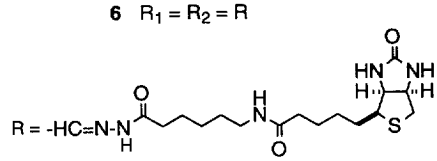
The FAB-MS spectrum of **6** indicated that it was a dibiotinylated derivative, and its structure was confirmed as **6** by analyzing its NMR spectra. On the other hand, **4** was shown to be a monobiotinylated derivative of **2** from its FAB-MS spectrum. Since the ^1H NMR spectrum of **4** was complicated probably due to tautomerization through an aldehyde group present in **4**, **4** was reduced by $NaBH_4$ to obtain **5**.[3] The $(M+H)^+$ ion observed in the FAB-MS spectrum of **5** was larger than that of **4** by two mass units, and the coupling between an imine proton and a proton at C-2" observed in the ^1H-^1H COSY spectrum of **5** clarified that a biotinyl chain is attached to C-3" position of **2**. By further analysis of NMR spectra, its structure was confirmed as **5**. Thus the structure of the monobiotinylated derivative before $NaBH_4$ reduction was assignable to **4**.



allosamidin **1**



2 R_1 = 3"CHO, R_2 = 4"CHO
3 $R_1 = R_2 = CH_2OH$
4 R_1 = R, R_2 = CHO
5 R_1 = R, $R_2 = CH_2OH$
6 $R_1 = R_2$ = R



The inhibitory activity of each allosamidin derivatives was measured against a fungal chitinase from *Trichoderma* sp. (Chitinase T-1, Takara Shuzo Co., Ltd.) [10]. The resulting IC_{50} values are listed in Table. Compoud **3** showed about five times weaker activity than **1** did, but this is the first case of an active allosamidin derivative having a non-sugar skeleton at its non-reducing end. Two monobiotinylated derivatives, **4** and **5**, showed a similar activity each other, which is a little weaker than **1** did. Very interestingly, the dibiotinylated derivative **6** inhibited the chitinase as strongly as **1**.

Table Inhibitory Activity of each Allosamidin Derivative toward *Trichoderma* Chitinase

Compound	IC_{50} (μg/ml)[a]
1	0.01
3	0.05
4	0.04
5	0.04
6	0.01

[a] Chitinase assay was performed with 4-methylumbelliferyl-β-D-*N,N',N''*-triacetylchitotrioside [11] in 50 mM citric acid-Na_2HPO_4 (pH 5.0).

[1] $NaIO_4$ (9 mg) was added to a solution of **1** (18 mg) in 100 mM AcONa-AcOH (pH 5.5, 1.8 ml) and stirred for 2 h at room temperature. After adjusting the solution pH to 9.0, $NaBH_4$ (5 mg) was added to the reaction solution and further stirred for 2 h at room temperature. The reaction solution was purified by HPLC (Capcell-Pak C_{18}, Shiseido, gradient elution of CH_3CN in 10 mM $AcONH_4$-NH_4OH at pH 8.9) to afford **3** (11.8 mg). **3**: FABMS (glycerol matrix) *m/z* 625 $(M+H)^+$; ^{13}C NMR δ_C (D_2O) 175.5(Ac), 174.5(Ac), 161.8(C-7), 103.2 and 100.5(C-1', C-1"), 86.6(C-1), 85.7(C-4), 81.5(C-3), 80.9(C-4'), 74.0 and 73.5(C-5', C-5"), 69.6(C-3'), 66.1(C-2), 62.1, 62.0, 61.6, 60.7 and 60.0(C-6, C-6', C-3", C-4", C-6"), 55.0(C-2"), 53.6(C-2'), 52.1(C-5), 38.1(C-8), 38.1(C-9), 22.8(Ac), 22.7(Ac); ^1H NMR δ_H (D_20) 5.27(dd, *J*=9, 5 Hz, H-1), 4.94(d, *J*=2.5 Hz, H-1"), 4.77(d, *J*=9 Hz, H-1'), 4.31(dd, *J*=4, 9 Hz, H-2), 4.29(t, *J*=3 Hz, H-3'), 4.22(dd, *J*=4, 5 Hz, H-3), 4.18(H-2"), 3.56-3.95(15H), 3.03(H-8, H-9), 2.47(H-5), 2.04(Ac), 2.04(Ac).

[2] After oxidation of **1** (22.5 mg) with $NaIO_4$ (10 mg) in 100 mM AcONa (pH 5.5, 2.25 ml) for 1 h at room temperature, Biotin (Long Arm) Hydrazide^R (25 mg) was added to the reaction solution. After being stirred for 4 h at room temperature, the reaction solution was purified by HPLC under the same condition as used in the isolation of **3** to afford **4** (7.3 mg) and **6** (24.0 mg). **4**: FABMS (glycerol matrix) *m/z* 974 $(M+H)^+$. **6**: FABMS (glycerol matrix) *m/z* 1327 $(M+H)^+$; ^{13}C NMR δ_C (D_2O+0.1%CD_3COOD) 176.0(Ac), 175.8(Ac), 162.9(C-7), 151.1 and 149.5(C-3", C-4"), 103.8 and 102.2(C-1', C-1"), 89.4(C-1), 87.6(C-4), 82.8(C-3), 80.5(C-4'), 76.0 and 75.0(C-5', C-5"), 70.5(C-3'), 66.8(C-2), 64.8, 63.2 and 61.6(C-6, C-6', C-6"), 56.1 and 55.5(C-2', C-2"), 54.1(C-5), 40.0 and 39.8(C-8, C-9), 24.4(Ac), 24.4(Ac), biotin moiety, 179.4, 178.9, 167.7, 64.6, 62.7, 57.9, 42.2, 41.5, 38.0, 36.3, 30.5, 30.4, 30.2, 28.0, 27.7, 27.1; ^1H NMR δ_H (D_2O+0.1%CD_3COOD) 7.48(d, *J*=4 Hz, H-3"), 7.32(d, *J*=7 Hz, H-4"), 5.31(dd, *J*=5, 9 Hz, H-1), 4.95(d, *J*=3 Hz, H-1"), 4.72(H-1'), 4.72(H-2"), 4.36(H-5"), 4.31(dd, *J*=4, 9 Hz, H-2), 4.24(H-3), 4.23(H-3'), 3.55-3.88(10H), 3.00 and 2.99(H-8, H-9), 2.47(H-5), 1.98(Ac), 1.97(Ac), biotin moiety, 4.52(2H), 4.33(2H), 3.25(4H), 3.07(2H), 2.91(2H), 2.69(2H), 2.25(4H), 2.16(4H), 1.21-1.69(24H).

[3] Compound **4** (6.0 mg) was reinjected to the HPLC and the eluate containing **4** was collected. Excess amount of $NaBH_4$ was added to the solution from the HPLC and stirred for 2 h at room temperature. The reaction solution was purified by HPLC under the same condition as used in the isolation of **3** to afford **5** (4.2 mg). **5**: FABMS (glycerol matrix) *m/z* 976 $(M+H)^+$; ^{13}C NMR δ_C (D_2O+0.1%CD_3COOD) 174.5(Ac), 174.2(Ac), 161.3(C-7), 148.7(C-3"), 103.2 and 100.6(C-1', C-1"), 87.5(C-1), 85.8(C-4), 81.1(C-3), 81.1(C-4'), 74.4 and 73.4(C-5', C-5"), 69.2(C-3'), 65.0(C-2), 62.1, 61.9, 61.5 and 59.9(C-6, C-6', C-6", C-4"), 53.6 and 53.5(C-2', C-2"), 52.3(C-5), 38.3 and 38.1(C-8, C-9), 22.7(Ac), 22.7(Ac), biotin moiety, 178.6, 177.9, 166.4, 62.9, 61.0, 56.2, 40.5, 39.8, 36.3, 34.6, 28.8, 28.6, 28.5, 26.3, 26.0, 25.4; ^1H NMR δ_H (D_2O+0.1%CD_3COOD) 7.52(d, *J*=4 Hz, H-3"), 5.37(dd, *J*=5, 9 Hz, H-1), 5.11(d, *J*=3 Hz, H-1"), 4.88(dd, *J*=3, 4 Hz, H-2"), 4.77(d, *J*=9 Hz, H-1'), 4.37(dd, *J*=4, 9 Hz, H-2), 4.33(t, *J*=3 Hz, H-3'), 4.29(dd, *J*=4, 5 Hz, H-3), 3.57-3.92(13H), 3.06(H-8, H-9), 2.52(H-5), 2.07(Ac), 2.04(Ac), biotin moiety, 4.60(1H), 4.41(1H), 3.32(1H), 3.17(2H), 2.98(1H), 2.77(1H), 2.31(2H), 2.23(2H), 1.28-1.75(12H).

Finally, we applied the biotinylated allosamidin to an optical biosensor system (IAsys, Affinity Sensors, UK). The dibiotinylated derivative **6** was immobilized through streptavidin on a sensor cuvette, and the binding interaction between the immobilized **6** and *Trichoderma* chitinase in solution (10 mM $AcONH_4$-AcOH containing 0.05% Tween 20, pH 4.5) was measured by the sensor. From the binding curves obtained at several concentrations of the chitinase, association equilibrium constant (K_D) for the interaction was calculated to be 5.3 x 10^{-8} M. This interaction was inhibited by addition of allosamidin into the chitinase solution dose-dependently. When allosamidin was added to the chitinase solution at a dose of 500, 125, 31, or 7.8 μg/ml under the constant concentration of the chitinase (2.5 μg/ml), the interaction between the immobilized **6** and the chitinase was inhibited by 65.6, 42.2, 21.9 or 7.8 %, respectively. These results suggested that the biotinylated allosamidin may be useful for a histological study using a biotin-avidin system. Its application on work to investigate a chitinase localization in a fungal mycelium is now in progress.

Acknowledgement

We thank Dr. A. D. Recklie of Shriners Hospital for Crippled Children for his very helpful initial discussion. This work was supported by grant-aid from JSPS Program for Research for the Future.

References

[1] Sakuda S, Isogai A, Matsumoto S, Suzuki A. J. Antibiot. 1987;40:296-300.

[2] Sakuda S, Isogai A, Matsumoto S, Suzuki A, Koseki K. Tetrahedron Lett. 1986;27:2475-2478.

[3] Sakuda S, Isogai A, Makita T, Matsumoto S, Koseki K, Kodama H, Suzuki A. Agric. Biol. Chem. 1987;51:3251-3259.

[4] Sakuda S, Nishimoto Y, Ohi M, Watanabe M, Takayama S, Isogai A, Yamada Y. Agric. Biol. Chem. 1990;54:1333-1335.

[5] Sakuda S, Isogai A, Suzuki A, Yamada Y. Actinomycetol. 1993;7:50-57.

[6] Sakuda S. Nippon Nogeikagaku Kaishi (in Japanese) 1995;69:147-153.

[7] Terwisscha van Scheltinga AC, Armand S, Kalk KH, Isogai A, Henrissat B, Dijkstra BW. Biochemistry;34:15619-15623.

[8] Henrissat B. Biochem. J. 1991;280:309-316.

[9] Kinoshita M, Sakuda S, Yamada Y. Biosci. Biotech. Biochem. 1993;57:1699-1703.

[10] Nishimoto Y, Sakuda S, Takayama S, Yamada Y. J. Antibiot. 1991;44:716-722.

[11] Kuranda MJ, Robbins PW. J. Biol. Chem. 1991;266:19758-19767.