# TA-3037A, A NEW INHIBITOR OF GLUTATHIONE *S*-TRANSFERASE, PRODUCED BY ACTINOMYCETES

## I. PRODUCTION, ISOLATION, PHYSICO-CHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITIES

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TA-3037A, a new inhibitor of glutathione *S*-transferase was discovered in the fermentation broth of *Streptomyces* sp. TA-3037. It was purified by chromatography followed by solvent extraction and then isolated as yellow needles. TA-3037A has the molecular formula of $C_{16}H_{11}NO_4$. It was competitive with the substrate, and the inhibition constant (*Ki*) was 4.9 $\mu$M.

The emergence of cancer cells acquiring resistance to anticancer drugs is serious problem in cancer chemotherapy. There are many resistant mechanisms in acquired resistant cells[1,2]. A lot of reports have suggested that increased glutathione *S*-transferase activity may be an important factor in resistant cell against a few anticancer drugs, for example, alkylating agents[3~6], cisplatin[7~9] and doxorubicin (adriamycin)[10]. Therefore, we have been screening inhibitor of glutathione *S*-transferase.

Recently, we found a new inhibitor named TA-3037A which was isolated from the culture broth of *Streptomyces* sp. TA-3037. In this communication we report the production, isolation, physico-chemical properties and biological activities.

### Materials and Methods

Chemicals

Glutathione was purchased from Sigma Chemical Co., and 2,4-dinitrochlorobenzene was from Tokyo Kasei Kogyo Co., Ltd. All other chemicals were analytical grade.

Enzyme

Glutathione *S*-transferase was prepared from doxorubicin-resistant P388 cells as follows. Namely, exponentially growing P388/ADR cells were homogenized using a Teflon-glass homogenizer in double distilled water. The homogenized cell was centrifuged at 105,000 × *g* for 1 hour at 4°C and the supernatant was used for the enzyme assay.

Glutathione *S*-Transferase Assay

The enzyme activity was measured according to the method of HABIG *et al.*[11]. The reaction mixture (total 2 ml) consisting glutathione 1 mM, 2,4-dinitrochlorobenzene 1 mM, potassium phosphate buffer 100 mM (pH 6.5) and 200 $\mu$l of glutathione *S*-transferase with or without inhibitors was incubated for 10 minutes at 30°C. The absorbance at 340 nm was recorded in a Beckmann spectrophotometer. The $IC_{50}$ value is the concentration of inhibitor at 50% inhibition of enzyme activity.

Microorganism

Strain TA-3037 was isolated from a soil sample collected in Nikko, Tochigi Prefecture, Japan and

has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Tsukuba-shi, Japan under the accession number FERM-P-11991.

Fermentation

The strain TA-3037 was inoculated into 100 ml of a seed culture medium consisted of glycerol 2.5%, beef extract (Kyokutō) 0.5%, Polypepton (Daigo) 0.5%, Bacto-yeast extract (Difco) 1.0%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CaCO_3$ 0.32% with a quarter strength artificial seawater (Jamarin S) in a 500-ml Erlenmeyer flask, and cultured at 27°C for 3 days on a rotary shaker (180 rpm). Two ml of the above seed culture was transferred to 100 ml of the same fresh medium in a 500-ml Erlenmeyer flask and cultured for 3 days under the same condition above until the production of inhibitor reached maximum.

Isolation

Culture filtrate (9 liters) was extracted at pH 2 with butyl acetate (9 liters). The solvent layer was dried over $Na_2SO_4$ and evaporated to dryness. The residue (6.6 g) was submitted to Sephadex LH-20 column chromatography and eluted with MeOH. The active eluate was concentrated under reduced pressure. The crude powder (206 mg), thus obtained, was then chromatographed on a silica gel column chromatography. The active fraction was eluted with chloroform - MeOH (50 : 1) and concentrated. The residue was crystallized from chloroform - MeOH to give yellow crystals of TA-3037A (125 mg).

Spectroscopic Methods

UV absorption spectra were measured with a Hitachi U-3210 spectrophotometer. IR absorption spectra were obtained with a Hitachi I-5020 FT-IR spectrometer. FAB-MS and EI-MS were obtained on a Jeol JMS-SX102 and a Hitachi M-80H mass spectrometer, respectively. NMR spectra were recorded on a Jeol JNM-GX 400 NMR spectrometer. The mp was measured by a micro mp apparatus, MP-3 (Yanagimoto). Optical rotations were taken by a Perkin-Elmer 241 polarimeter using a micro-cell (light path 10 cm).

## Results and Discussion

### Cultural and Taxonomical Characterization of the Producing Strain

Cultural characteristics of strain TA-3037 with various agar media were summarized in Table 1. The strain produces grayish white to light gray color aerial mycelia. No soluble pigment was produced on all media tested. The form of mature sporophores was *Rectiflexibiles*. The spores were cylindrical in shape with smooth surface. The hydrolyzed cell wall of the strain contained L,L-diaminopimelic acid. Table 2 summarizes the taxonomical characteristics of strain TA-3037. Based on the characteristics, strain TA-3037 is assessed to belong to the genus *Streptomyces*.

Table 1. Cultural characteristics of strain TA-3037.

| Agar medium | Growth | Aerial mycelium | Substrate mycelium | Soluble pigment |
|---|---|---|---|---|
| Waksman-1 | Poor | Grayish white | Grayish white | None |
| Waksman-2 | Poor | Grayish white | Grayish white | None |
| ISP-2 | Abundant | Light gray | Pale yellowish brown | None |
| ISP-3 | Abundant | Light brownish gray | Pale yellowish brown | None |
| ISP-4 | Abundant | Light gray | Light brownish gray | None |
| ISP-5 | Good | Light gray | Pale yellowish brown | None |
| ISP-6 | Moderate | None | Pale yellow | None |
| ISP-7 | Good | White | Dark yellow | None |
| ISP-8 | Moderate | None | Yellowish gray | None |
| Nutrient | Moderate | None | Yellowish gray | None |

Table 2. Taxonomical characteristics of strain TA-3037.

| | | | |
|---|---|---|---|
| Spore-chain morphology: | | Hydrolysis of: | |
| Section | Straight | Urea | Positive (weak) |
| Spore surface | Smooth | Starch | Positive (strong) |
| Cell wall type | I | Casein | Positive |
| Aerial mass color | Grayish white to light gray | Tween 60 | Positive |
| Reverse side color | Grayish white to dark yellow | Chitin | Negative |
| Soluble pigment | None | Tributyrin | Negative |
| Melanin formation | None | Alginate | Negative |
| NaCl conc range for growth | 0~3% | *p*-Nitrophenyl phosphate | Positive |
| Temperature range for growth | 7.0~32.5°C (18.0~23.5°C) | Carbon utilization: | |
| Coagulation of skim milk | Positive | D-Glucose | Positive |
| Peptonization of skim milk | Positive | L-Arabinose | Positive (doubtful) |
| Liquefaction of gelatin | Positive (weak) | D-Xylose | Positive (doubtful) |
| Nitrate reduction | Positive (weak) | D-Fructose | Positive |
| | | D-Mannitol | Negative |
| | | Inositol | Negative |
| | | L-Rhamnose | Positive |
| | | Raffinose | Negative |
| | | Sucrose | Positive |

Fig. 1. Time course of TA-3037A production.

● TA-3037A, ○ packed cell volume, △ pH.

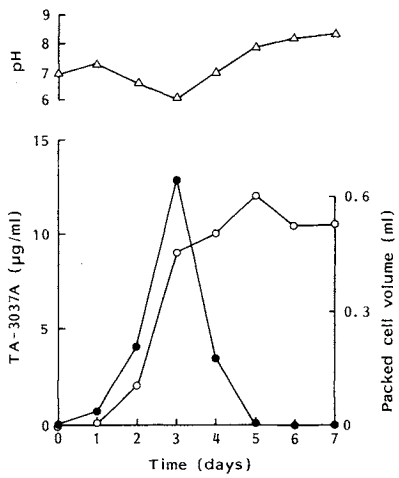


Fig. 2. Isolation of TA-3037A.

Culture filtrate (9 liters)
↓ extracted with BuOAc (9 liters) at pH 2.0
evaporated *in vacuo*

Crude paste (6.6 g)
↓ Sephadex LH-20 column (7.0 × 50 cm)
eluted with MeOH

Crude powder (206 mg)
↓ silica gel column (3.0 × 7.5 cm)
eluted with $CHCl_3$ - MeOH (50 : 1)

Yellow powder
↓ crystallized from $CHCl_3$ - MeOH (1 : 1)

TA-3037A yellow needles (125 mg)

## Production and Isolation of TA-3037A

The strain of TA-3037 was cultured in Erlenmeyer flasks at 27°C for 3 days on a rotary shaker. The time course of the production is shown in Fig. 1. The maximum peak of TA-3037A production in the flasks was obtained at 3 days, thereafter the production slowly decreased with a pH change to alkaline. The flow diagram for the isolation is shown in Fig. 2. The yield of pure TA-3037A was 125 mg from 9 liters of culture filtrate.

## Physico-chemical Properties of TA-3037A

The physico-chemical properties of TA-3037A are summarized in Table 3. The molecular weight and formula of TA-3037A was determined by EI-MS and HRFAB-MS. TA-3037A is soluble in THF, dioxane and DMF, but insoluble in $H_2O$, EtOAc and *n*-hexane. The detection of TA-3037A on silica gel TLC plates is visualized by molybdatophosphoric acid.

Determination of the structure of TA-3037A will be described in the following paper[12].

Fig. 3. Lineweaver-Burk plot of inhibition of glutathione *S*-transferase by TA-3037A.

□ I=4 μg/ml, △ I=2 μg/ml, ● I=1 μg/ml, ○ I=0 μg/ml.

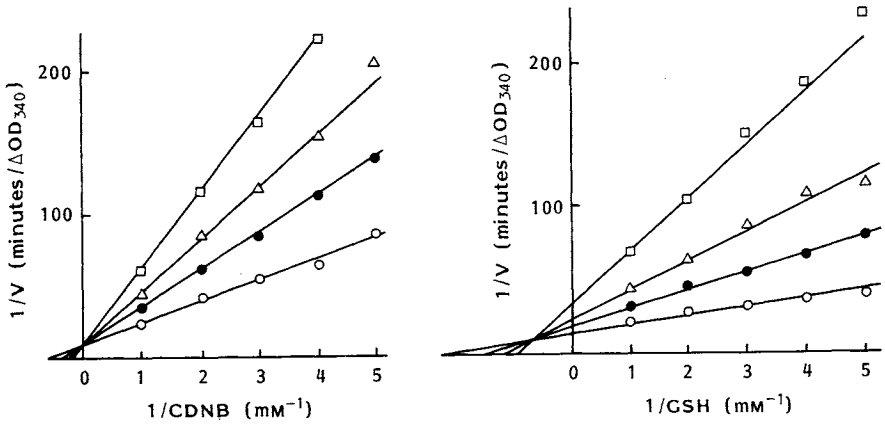


Table 3. Physico-chemical properties of TA-3037A.

| | |
|---|---|
| Appearance | Yellow crystals |
| Molecular formula | $C_{16}H_{11}NO_4$ |
| EI-MS (*m/z*) | 281 ($M^+$) |
| UV $\lambda_{max}^{EtOH}$ nm (log ε) | 204 (4.43), 242 (4.20), 282 (3.97), 383 (4.16) |
| $\lambda_{max}^{EtOH-HCl}$ nm (log ε) | 205 (4.34), 241 (4.26), 282 (3.96), 350 (sh, 3.94), 382 (4.16) |
| $\lambda_{max}^{EtOH-NaOH}$ nm (log ε) | 324 (3.96) |
| IR $\nu_{max}^{KBr}$ $cm^{-1}$ | 3440 (br), 2930, 1752, 1663, 1275, 1258, 758 |
| $[\alpha]_D^{23}$ | 0° (*c* 0.5, DMF) |
| MP (dec) | 248~250°C |
| Solubility | Soluble in THF, dioxane, DMF<br>Insoluble in $H_2O$, EtOAc, *n*-hexane, $CHCl_3$, MeOH |

Table 4. Inhibitory effects of TA-3037A and ethacrynic acid on glutathione *S*-transferase.

| Inhibitor | $IC_{50}$ (μM) |
|---|---|
| TA-3037A | 8.9 |
| Ethacrynic acid | 16 |

The assay method is described in the section of Materials and Methods.

## Biological Activities of TA-3037A

The inhibitory activities of TA-3037A and ethacrynic acid[13] are shown in Table 4. They showed $IC_{50}$ value of 8.9 μM, 16 μM against glutathione *S*-transferase, respectively. As shown in Fig. 3, inhibition of TA-3037A against glutathione *S*-transferase is competitive with the substrate, and the *Ki* and *Km* values were $4.9 \times 10^{-6}$ M and $1.7 \times 10^{-3}$ M, respectively. TA-3037A at 50 μg/ml had no antimicrobial activity. It has low toxicity; no deaths after intraperitoneal injection of 100 mg/kg to mice.

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