B309

# Direct synthesis of Neu5Ac from GlcNAc using NALase and GlcNAc 2-epimerase

이정규, 이정오, 이선구, 김병기
서울대학교 응용화학부 유전공학연구소
전화(02) 880-7528, FAX(02) 874-1206

**Abstract**

GlcNAc 2-epimerase gene from human was cloned. However GlcNAc 2-epimerase was expressed in *E. coli* as inclusion body formation. Several approaches were tried such as expression in low temperature and low concentration of IPTG. With these treatments production of active form of human GlcNAc 2-epimerase was enhanced. For the direct synthesis of NeuAc from GlcNAc and pyruvate, NALase and GlcNAc 2-epimerase were characterized in terms of temperature effect on activity, equilibrium and stability, inhibition by pyruvate etc. For cheap and ease preparation of both the NALase and GlcNAc 2-epimerase, pEN24ma vector was made, which express both the NALase and GlcNAc 2-epimerase simultaneously. In addition, E. coli BL21(DE3) harboring two plasmids was also made. Of the two systems, the latter was better for the expression of both enzymes.

**Introduction**

N-acetyl-D-neuraminic acid(Neu5Ac) is the precursor of sialic acids that are located at the terminal positions of glycoproteins and glycolipids. It acts as receptors for microorganisms, virus, toxin, hormone on cell surface. The biological importance of sialic acid demands large-scale production of Neu5Ac

Neu5Ac can be obtained from natural sources such as human serum, human milk, edible bird's nest substance, cow's milk, egg's yolk. Chemical synthesis of carbohydrate needs difficult and laboring processes such as repetitive sequential protection, reaction, deprodection, purification steps resulting in low yield.

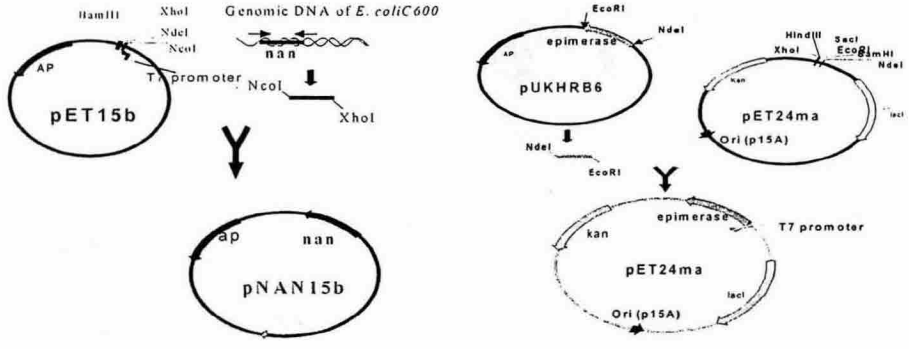
Enzymatic synthesis of Neu5Ac needs Neu5Ac lyase(NALase)[3] which using N-acetylmannosamine(ManNAc) and pyruvate as substrate.[1] Because ManNAc is expensive it is generally prepared from GlcNAc by epimerization. Direct synthesis of Neu5Ac from GlcNAc and pyruvate is possible by epimerization with GlcNAc 2-epimerase[4] coupled with aldol reaction using NALase.[2]

## Materials and Methods

### Materials

GlcNAc, ManNAc, Neu5Ac were obtained from Sigma Chemical Co. Ltd. Sodium pyruvate was obtained from Boehringer Mannheim. All other chemicals were analytical grade.

### Cloning of NALase and GlcNAc 2-epimerase




### Optimization of GlcNAc2-epimerase gene expression

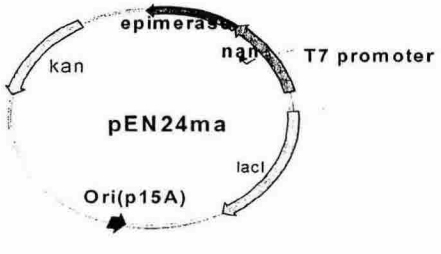
- Effect of temperatute on GlcNAc 2-epimerase gene expression
- IPTG concentration optimization

### Preparation of NALase and GlcNAc2-epimerase

| | induction O.D | IPTG(mM) | harvest O.D |
|---|---|---|---|
| NALase | 0.4~0.8 | 1mM | 3.6~4.0 |
| GlcNAc2-epimerase | 2.8~3.2 | 0.1mM | 4.5~5.0 |

### Neu5Ac synthesis

Construction of pEN24ma



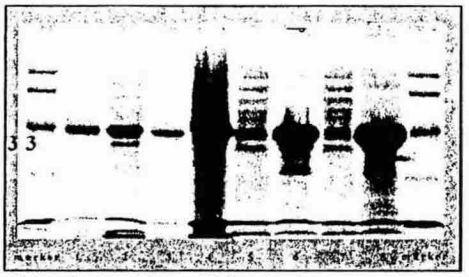

### Reaction conditions and analysis

The reaction solution was 100mM GlcNAc, 200mM pyruvate, 10mM $MgCl_2$ and 5mM ATP in 100mM, pH7.5 Tris-HCl buffer at 37℃. HPLC analysis was performed by means of Waters HPLC system with double AmineX HPX-87H

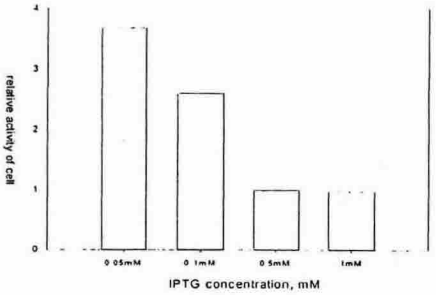
column in series to separate two epimers.

## Results and Discusstions

### GlcNAc 2-epimerase gene expression



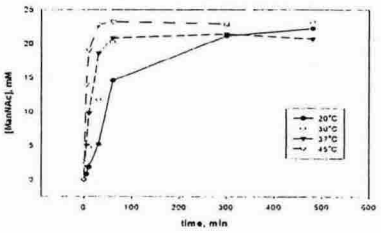
1: cell extract by permeabilization / 30°C
2: cell debris by permeabilization / 30°C
3: cell extract by permeabilization / 37°C
4: cell debris by permeabilization / 37°C
5: cell extract by sonication / 30°C
6: cell debris by sonication / 30°C
7: cell extract by sonication / 37°C
8: cell debris by sonication / 37°C




**Effect of IPTG concentration on the production of active GlcNAc2-epimerase**

Low IPTG concentration minimized the inclusion body formation, resulting in enhancement of active GlcNAc 2-epimerase production.

### GlcNAc 2-epimerase activity

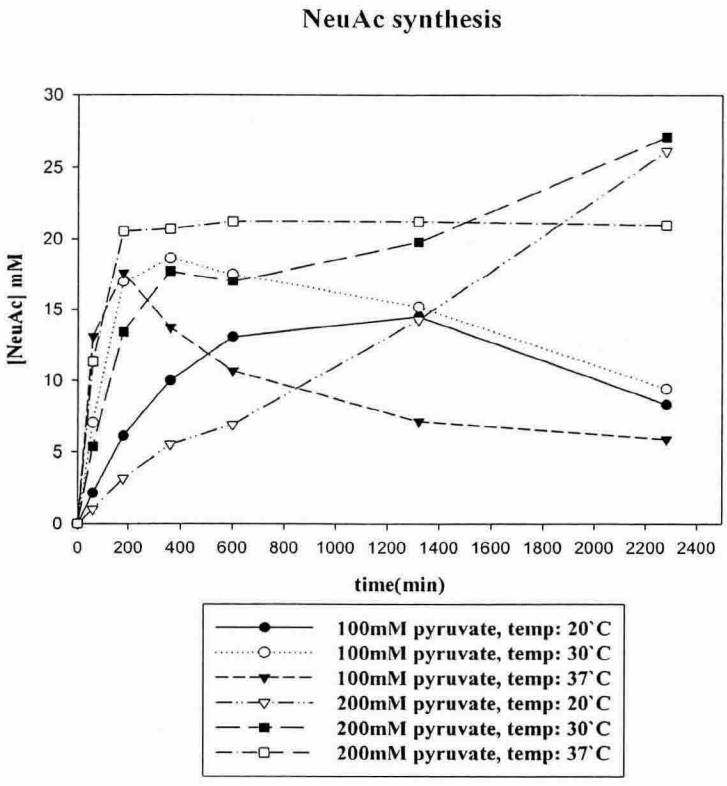



**Temperature effect on conversion of GlcNAc to ManNAc**

### NeuAc synthesis

Comparision of two methods for preparation of two enzymes

| | *E.coli* BL21 | *E.coli* BL21 -pEN24ma | *E.coli* BL21 -pNAN15b, pEPI24ma |
|---|---|---|---|
| NALase | 0 | 4.1U/ml | 8.8U/ml |
| GlcNAc 2-epimerase | 0 | 1.5U/ml | 5.2U/ml |




Two methods for preparing two enzymes with single culture were compared. *E.coli* BL21[pNAN15b, pEPI24ma] was superior to *E.coli* BL21[pEN24ma] for single preparation of both enzymes.

## References

1. Mahmoudian, M., "An efficient process for production of N-acetylneuraminic aicd using N-acetylneuraminic acid aldolase" Enzyme and microbial technology. 1997, 20, 393-400

2. Maru, I., "Simple and large scale production of N-acetylneuraminic acid from N-acetyl-D-glucosamine and pyruvate using N-acyl-D-glucosamine 2-epimerase and N-acetylneuraminate lylase", Carbohydrate research. 1998, 306, 575-578

3. Uchida, Y., "Purification and properties of N-acetylneuraminate lylase from *E. coli*", J.Biochem. 1986, 96, 507-522

4. Takahashi, S.,"Purification and characterization of renin binding protein (RnBP) from porcine kidney", J.Biol.Chem. 1990, 265, 6556-6561