

糖鎖分析メソッド集
ニワトリ卵白からオボアルブミンの精製

Ver. 20171005

Written by S. Natsuka

目的と方法の概要

ニワトリ・オボアルブミンはハイマンノース型とハイブリッド型を主に含む糖タンパク質であり、それら糖鎖を調製するために用いられる。卵白からグロブリン画分を塩析で除いた後、さらに塩濃度を上げて結晶化させ精製品を得る。

この方法を使った時に引用すべき論文

The hydrogen ion dissociation curve of the crystalline albumin of the hen's egg.
Ralph A. Kekwick, Robert K. Cannan. *Biochem. J.*, **30**, 227-234 (1936).

ニワトリのオボアルブミンがもつ糖鎖構造についてはこれらの論文を引用

- 1) Structural studies of two ovalbumin glycopeptides in relation to the endo-beta-N-acetylglucosaminidase specificity. Tai T, Yamashita K, Ogata-Arakawa M, Koide N, Muramatsu T, Iwashita S, Inoue Y, Kobata A. *J Biol Chem.* **250** (21) 8569-8575 (1975).
- 2) The substrate specificities of endo-beta-N-acetylglucosaminidases CII and H. Tai T, Yamashita K, Kobata A. *Biochem Biophys Res Commun.* **78** (1) 434-441 (1977).
- 3) Structures of the carbohydrate moiety of ovalbumin glycopeptide III and the difference in specificity of endo-beta-N-acetylglucosaminidases CII and H. Tai T, Yamashita K, Ito S, Kobata A. *J Biol Chem.* **252** (19) 6687-94 (1977).
- 4) The structures of the galactose-containing sugar chains of ovalbumin. Yamashita K, Tachibana Y, Kobata A. *J Biol Chem.* **253** (11) 3862-3869 (1978).
- 5) Sulfated asparagine-linked sugar chains of hen egg albumin. Yamashita K, Ueda I, Kobata A. *J Biol Chem.* **258** (23) 14144-14147 (1983).
- 6) Sialic acid-containing sugar chains of hen ovalbumin and ovomucoid. Yamashita K, Tachibana Y, Hitoi A, Kobata A. *Carbohydr Res.* **130**, 271-288 (1984).

プロトコル

~700 mL egg white from 20 hen eggs

- ↓ add equal volume of Na₂SO₄ solution (400 g Na₂SO₄ + 1 L DDW)
- ↓ mix by stirring
- ↓ stand still for 1 h
- ↓ centrifuge 10,000 rpm, 10 min, room temp.

sup.

- ↓ adjust pH to 4.7 by 0.1 M H₂SO₄
- ↓ add anhydrous Na₂SO₄ at 35°C until saturation
- ↓ decant to remove unsolved Na₂SO₄
- ↓ stand still for o/n
- ↓ centrifuge 10,000 rpm, 10 min, room temp.

ppt.

- ↓ dissolve in the same volume of DDW with the initial egg white
- ↓ re-salting out by anhydrous Na₂SO₄, x twice

ppt.

- ↓ dissolved in as little DDW as possible
- ↓ dialyze against DDW, x 7 times
- ↓ lyophilize

ovalbumin (~30 g)