Usefulness of new method for the preparation of yeast cell wall mannan using Benanomicin A

Takuya Kuraoka

It is noteworthy that the two pathogenic *Candida* species, *C. krusei* and *C. glabrata*, are producing resistant strains against azole antifungal agents. Accurate diagnosis is necessary to treat these infections with appropriate antibiotics, but a rapid and easy diagnostic methods for identifying the species among of genus *Candida* have not yet been clinically developed. What is considered as a rapid diagnostic method for mycoses including endogenous infections is immunochemical techniques using antigen-antibody reactions. Cell wall mannan is most important in the interaction between yeast and its host since this molecule is present in the outermost layer of the cell wall. For this reason, structural studies on mannan of pathogenic *Candida* species have been an active area of research, and the overall structure and antigenic determinants of this molecule have been reported in several clinically important *Candida* species.

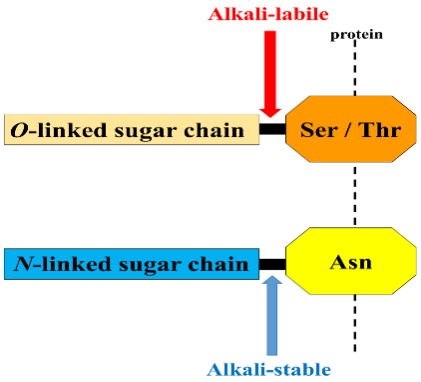


Fig. 1 Mannan-protein complex of yeast cell wall

One important requirement for the antigen analysis of *Candida* yeasts is the isolation of intact mannan molecules responsible for its antigenic activity. The most common procedure for purifying yeast-derived mannan is by precipitating this molecule after it forms a complex with copper in Fehling reagent. We previously isolated various mannans from several pathogenic *Candida* yeasts by this procedure and analyzed these molecules corresponding to various antigenic determinant. However, the use of the conventional mannan purification method using a strongly alkaline Fehling reagent results in a loss of *O*-linked sugar chains from mannan. In fact, as a result, most structural and immunochemical studies on yeast mannans so far have only mentioned the *N*-linked sugar moiety of the molecule. (**Fig. 1**).

The antibiotic Benanomicin A (molecular weight, 864) initiates antifungal action by selectively binding to the mannose residue of yeast cell wall mannan in the presence of Ca^{2+}. Therefore, Benanomicin A is regarded as an equivalent to lectin in its function even though it is not a protein (**Fig. 2**).

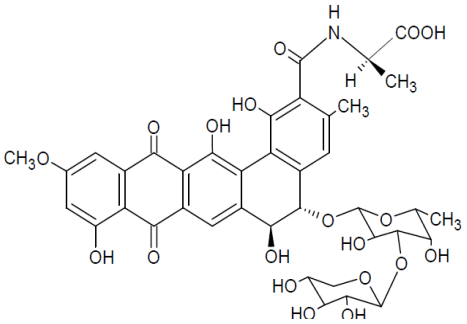


Fig. 2 Chemical structure of Benanomicin A

As mentioned above, *C. krusei* is a clinically important pathogenic yeast, but its immunochemical properties and chemical structure of its cell wall mannan remain unknown. The purpose of this study was to prepare cell wall mannan of *C. krusei* under rapid and mild conditions

using Benanomicin A.

1. Presence of *O*-glycosidically-linked oligosaccharides in the cell wall mannan-protein complex of *Candida krusei* prepared with Benanomicin A

Cell wall mannan of the pathogenic yeast *C. krusei* was prepared using antibiotic Benanomicin A, which has a lectin-like function. The chemical structure of this molecule was similar to that of mannan prepared from the same yeast by the conventional method using Fehling reagent. When the mannan prepared using Fehling reagent was subjected to alkali treatment (β-elimination), only a very small amount of degradation products was detected, but upon the same treatment of the mannan prepared with Benanomicin A, multiple α-1,2-linked oligosaccharides were detected (**Fig. 3**). These results showed that most of the *O*-linked sugar chains in mannan were lost under conventional conditions when exposed to the strongly alkaline Fehling reagent. In contrast, in the mild novel preparation method using Benanomicin A, the *O*-glycosidic bond in mannan was not cleaved and the *O*-linked sugar chains were maintained and almost intact. Therefore, it is considered that the new mannan-preparation method using Benanomicin A is superior to conventional methods. In addition, this study suggests that some yeast mannans, whose overall structure has already been reported, may contain more *O*-linked sugar chains than that previously recognized.

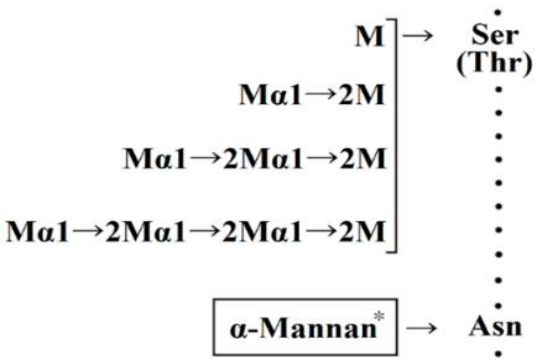
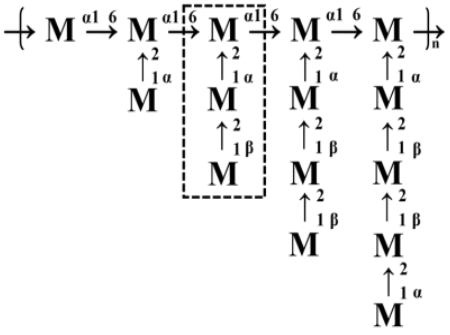


Fig. 3 Structure of *O*-linked sugar chains in the cell wall mannan of *C. krusei*. M indicate mannose residue. *The chemical structure of *N*-linked sugar chains in this mannan is not clear yet.

2. Distribution of oligomannosyl side chains in the cell wall mannan of *Pichia pastoris* purified by Benanomicin A

In this chapter, as the findings of two dimensional nuclear magnetic resonance (2D-HOHAHA) analysis of the resultant *P. pastoris* mannan to examine the distribution state of the side chains in this molecule, it was found that despite the existence of oligomannosyl side chains corresponding to pentaose, tetraose, and biose, in this moleculethe side chain corresponding to triose was not detected (**Fig. 4**). In previous study, a relatively large number of biosyl and triosyl side chains were detected in analyzes applying acetolysis to mannans prepared by the Fehling method from the same yeast cells. Such a difference can be explained as that the of some pentaosyl and tetraosyl side chains containing β-1,2-linkages were cleaved by acetolysis, and triose and biose occurred secondarily in large quantities.



Side chain distribution

(A)	9.60	:	26.36	:	-	:	36.85	: 27.19
(B)*	14.64	:	34.47	:	4.06	:	32.32	: 14.51

Fig. 4 Structure of *P. pastoris* mannan. (A) Side chain distribution was calculated based on the dimensions of characteristic H-1 signals of each side chain in the ^{1}H-NMR spectroscopy map. (B) Side chain distribution was calculated based on the peak-dimensions in the gel-filtration profile of the mild acetolysis products. M indicate mannose residue. *These values were referred from previous report (Kobayashi *et al*., 1988).