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Evidence for Oligomannosyl Residues Containing Both β-1,2 and α-1,2 Linkages as a Serotype A-Specific Epitope(s) in Mannans of *Candida albicans*

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In order to identify the branches containing both β-1,2 and α-1,2 linkages as the serotype A-specific epitope(s) in the mannans of *Candida albicans*, serotype A strains with oligosaccharides constituting the β-1,2 linkage, the α-1,2 linkage, and both the β-1,2 and the α-1,2 linkages were prepared from the mannans of *C. albicans* serotype A strains (NIH A-207 and J-1012) and tested for their inhibitory effects in the precipitin and slide agglutination assays. The results indicated that two oligosaccharides containing both β-1,2 and α-1,2 linkages, Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man and Manpβ1-2Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man, served as epitopes participating in the serotype A specificity of *C. albicans* strains.

Candida albicans is one of the important species of pathogenic fungi for humans, and therefore, identification of the yeast-form cells in clinical specimens is indispensable for the diagnosis of candidiasis. Hasenclever and Mitchell (2) showed that this species could be divided into two serotypes, A and B, and that the serological properties of whole cells of these serotypes resembled those of the closely related species *C. tropicalis* and *C. stellatoidea*, respectively.

We presumed (7, 9, 14) that the serotype A-specific oligomannosyl residue(s) might correspond to the relatively longer branches in the acid-stable domains of the parent mannans of two strains, NIH A-207 and J-1012 (called the A and J strains, respectively, in this report), because part of the longer branches contained both β-1,2 and α-1,2 linkages in their mannans. These assumptions can be supported by other structural findings; e.g., the mannan of *C. albicans* NIH B-792 (serotype B) (hereafter called the B strain) (8, 16, 17) and those of three *C. stellatoidea* type I strains (5, 19) did not possess any branches of this type in their acid-stable domains. Additionally, the findings obtained in a preceding study (4), that the mannans of two *C. albicans* serotype A strains grown at pH 2.0 lost the β-1,2 linkage and the phosphate group, provide additional evidence for the participation of β-1,2-linked oligomannosyl residues in serotype A specificity, because the cells lost agglutinability with a commercially available polyclonal factor serum 6, corresponding to the serotype A-specific epitope. The factor serum 6 was prepared in accordance with the description by Fukazawa and coworkers (1, 3, 18), who proposed the chemical structure of this epitope as a hexaosyl moiety, Manpα1-3Manpα1-2Manpα1-2Manpα1-2Manpα1-2Man, in a highly branched treelike structure of the parent J strain mannan. However, the results of our previous studies gave different findings for both the chemical structure of antigen 6 and the treelike structure of *C. albicans* mannan from those provided by Fukazawa and coworkers (3, 18). Thus, the serotype A-specific epitopes might contain both β-1,2 and α-1,2 linkages, and the chemical structures of the mannans were found to possess comblike structures (7, 9, 14).

In order to provide evidence for the identification of the serotype A-specific epitope(s), we conducted two series of serological assays, precipitin inhibition and slide agglutination inhibition reactions, with three series of oligosaccharides (Table 1): (i) homologous β-1,2-linked biose to hexaose, (ii) α-1,2- and α-1,3-linked tetraose, pentaose, and hexaose (tetraose is the homologous α-1,2-linked one), and (iii) pentaose, hexaose, and heptaose containing one to three β-1,2 linkages with α-1,2-linked tetraose, respectively. Mannans of the A, J, and B strains (mannans A, J, and B, respectively) and rabbit polyclonal antisera to yeast-form cells of the same *C. albicans* strains, designated antisera A, J, and B, respectively, were used. In our previous studies (7–9), antiserum B mainly contained antibodies against homologous β-1,2-linked oligomannosyl residues. Antisera A and J include antibodies that recognize both β- and α-linked oligomannosyl moieties.

Figure 1 shows the precipitin inhibition curves of 10 oligosaccharides obtained from A strain mannan against the antigen-antibody reaction, mannan A-antiserum A system (A system). AM_2-a, AM_3-a, and AM_4-a showed precipitin-inhibitory effects in a manner proportional to their molecular weight. However, the inhibitory effects of the homologs AM_4-a, AM_5-a, and AM_6-a were almost identical, suggesting that the sizes of the combining sites of the major molecular species of anti-β-1,2-oligomannosyl antibodies in antiserum A correspond to the β-1,2-linked tetraose (Fig. 1A). The precipitin-inhibitory effects of oligosaccharides containing both β-1,2- and α-1,2 linkages, AM_6-IIe and AM_7-IIe, and α-linked ones, AM_4-I, AM_5-I, and AM_6-I, against the same A system were compared (Fig. 1B). It is evident that AM_6-IIe and AM_7-IIe were potent inhibitors, exhibiting almost the same inhibition ratios as AM_4-a, AM_5-a, and AM_6-a. On the other hand, all α-linked oligosaccharides showed lower precipitin-inhibitory abilities. These findings imply the importance of β-1,2-linked oligomannosyl residues in mannan A as the epitopes dominating the stronger humoral antibody response. Because Kobayashi et al. (8) revealed the strong participation of β-1,2-linked oligomannosyl residues in the antibody-precipitating effects of the B

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TABLE 1. Chemical structures of three series of oligosaccharides used as the inhibitory hapten for the inhibition assays[a]

Linkage	Oligosaccharide from parent mannan: A	J
β-1,2-Linked series		
Mβ1-2M	AM_2-a	JM_2-a
Mβ1-2Mβ1-2M	AM_3-a	JM_3-a
Mβ1-2Mβ1-2Mβ1-2M	AM_4-a	JM_4-a
Mβ1-2Mβ1-2Mβ1-2Mβ1-2M	AM_5-a	
Mβ1-2Mβ1-2Mβ1-2Mβ1-2Mβ1-2M	AM_6-a	
α-Linked series		
Mα1-2Mα1-2Mα1-2M	AM_4-I	JM_4-I
Mα1-2Mα1-2Mα1-2Mα1-2M	AM_5-I	JM^5-I (1:1)[b]
Mα1-3Mα1-2Mα1-2Mα1-2M	AM^6-I (5:7)	JM^6-I (3:4)
Mα1-2Mα1-3Mα1-2Mα1-2Mα1-2M		
β-1,2- and α-1,2-linked series		
Mβ1-2Mα1-2Mα1-2Mα1-2M		JM_5-IIe
Mβ1-2Mβ1-2Mα1-2Mα1-2Mα1-2M	AM_6-IIe	JM_6-IIe
Mβ1-2Mβ1-2Mβ1-2Mα1-2Mα1-2Mα1-2M	AM_7-IIe	JM_7-IIe

[a] M denotes a D-mannopyranose unit. The β-1,2-linked oligosaccharides were obtained from the parent mannans by partial acid degradation (7, 9). The α-linked oligosaccharides were obtained from the partially acid-degraded mannans by acetolysis under conventional conditions (7, 9). The α-1,2- and β-1,2-linked oligosaccharides were obtained from partially acid-degraded mannans by acetolysis under mild conditions followed by enzymolysis with *Arthrobacter* GJM-1 exo-α-mannosidase (7, 9).

[b] Molar ratio of isomers.

mannan, this finding seems to provide additional evidence that these residues correspond to important epitopes. This result was supported by the findings that these oligosaccharides are distorted in conformation compared with the α-linked isomers (15).

The results of the present inhibition assay with the β-1,2-linked oligosaccharides JM_2-a, JM_3-a, and JM_4-a, the α-linked oligosaccharides JM_4-I, JM_5-I, and JM_6-I, and those containing both β-1,2 and α-1,2 linkages (JM_5-IIe, JM_6-IIe, and JM_7-IIe) against the antigen-antibody reaction, mannan J-antiserum J (J system), are shown in Fig. 2. It is noteworthy that JM_5-IIe, JM_6-IIe, and JM_7-IIe displayed practically identical inhibitory effects, and these effects were stronger than those of the other oligosaccharides corresponding to branches residing in mannan J. In other words, this fact implies the presence of large amounts of antimannan antibodies corresponding to oligomannosyl residues containing both β-1,2 and α-1,2 linkages in antiserum J. It is therefore noteworthy that a common structure in a mannotriosyl residue, Manpβ1-2Manpα1-2Man, at the nonreducing terminal or an intermediate position, is the key structure of serotype A-specific epitopes. In contrast to the above findings, oligosaccharides containing only an α-linkage exhibited low inhibitory powers. A similar tendency was observed in the results shown in Fig. 1B with the A system.

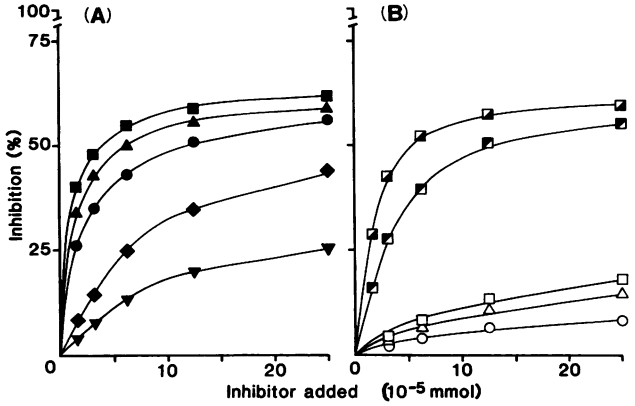

FIG. 1. Precipitin inhibition assay of the oligosaccharides obtained from mannan A in the A system. This assay was performed as described previously (8) as follows. Antiserum (0.1 ml) was preincubated with a known quantity of inhibitor (0.1 ml) for 1 h at 37°C. To the solution was added 0.5 ml of saline containing 25 μg of mannan. After reaction at 37°C for 1 h and at 4°C for 16 h, the amounts of precipitated protein were determined by the method of Lowry et al. (11). Symbols: (A) ▼, AM_2-a; ◆, AM_3-a; ●, AM_4-a; ▲, AM_5-a; ■, AM_6-a; (B) ○, AM_4-I; △, AM_5-I; □, AM_6-I; ◩, AM_6-IIe; ◪, AM_7-IIe.

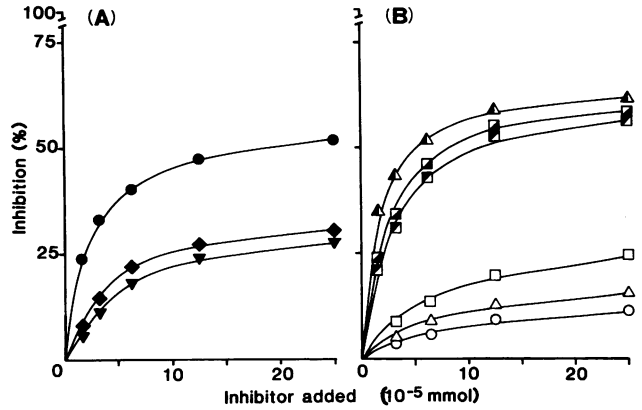

FIG. 2. Precipitin inhibition assay of the oligosaccharides obtained from mannan J in the J system. Symbols: (A) ▼, JM_2-a; ◆, JM_3-a; ●, JM_4-a; (B) ○, JM_4-I; △, JM_5-I; □, JM_6-I; ▲, JM_5-IIe; ◩, JM_6-IIe; ◪, JM_7-IIe.

Figure 3 shows the result of the precipitin inhibition assay with the oligosaccharides obtained from mannan J against the antigen-antibody reaction, mannan B-antiserum B (B system). It is evident that JM_5-IIe had a very weak inhibitory effect against the B system compared with its effect against the J system (Fig. 2B). Additionally, JM_6-IIe also showed a similar tendency, having a weaker inhibitory effect than

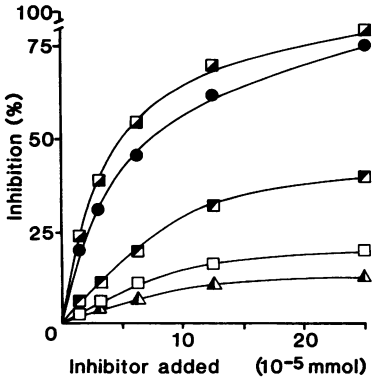

FIG. 3. Precipitin inhibition assay of the oligosaccharides obtained from mannan J in the B system. Symbols are the same as in Fig. 2.

TABLE 2. Inhibition of agglutination between factor serum 6 and cells of the J strain by three series of mannooligosaccharides obtained from the mannans of *C. albicans* serotype A strains[a]

Oligosaccharide	Agglutination[b] with inhibitor amt (μmol):							
	2^1	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	None
β-1,2-Linked series								
AM_4-a	+3	+3	+3	+3	+3	+3	+3	+3
AM_5-a	+3	+3	+3	+3	+3	+3	+3	+3
AM_6-a	+3	+3	+3	+3	+3	+3	+3	+3
α-Linked series								
AM_4-I	+3	+3	+3	+3	+3	+3	+3	+3
AM_5-I	+3	+3	+3	+3	+3	+3	+3	+3
AM_6-I	+3	+3	+3	+3	+3	+3	+3	+3
β-1,2- and α-1,2-linked series								
JM_5-IIe	±	±	+1	+1	+2	+2	+3	+3
JM_6-IIe	±	±	+1	+1	+2	+2	+3	+3
JM_7-IIe	+1	+1	+2	+2	+3	+3	+3	+3

[a] The assay was conducted as described by Miyakawa et al. (12) and Nishikawa et al. (13) as follows. Factor serum 6 (0.1 ml) was preincubated for 2 h at 37°C in the presence of inhibitor (0.1 ml). This solution (0.2 ml) and heat-killed cells (10^8/0.1 ml) were mixed, incubated at 37°C for 2 h, and kept overnight at 4°C.
[b] Agglutination was scored from high (+3) to low (±).

JM_7-IIe. On the other hand, JM_7-IIe showed a strong inhibitory power nearly identical to that of JM_4-a. This finding indicates that the oligosaccharides Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man and Man*p*β1-2Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man display different reactivities from those that the homologous β-1,2-linked oligosaccharides did against antisera A, J, and B, all of which contain anti-β-1,2-oligomannosyl antibodies.

We conducted a slide agglutination inhibition assay with the three series of oligosaccharides in an agglutination reaction system between J strain cells and a commercially available factor serum 6 (Iatron, Tokyo, Japan) (Table 2), because the agglutination of J strain cells with this serum was stronger than that of A strain cells. JM_5-IIe, JM_6-IIe, and JM_7-IIe each displayed a remarkable inhibitory effect. However, the other oligosaccharides containing β-1,2 linkages were less effective. These results indicate the presence of large amounts of an antibody corresponding to the branch(es) composed of α-1,2 linkages in the intermediate parts of JM_6-IIe and JM_7-IIe in factor serum 6.

Summing up all the findings obtained in the previous preceding studies, oligosaccharides containing both β-1,2 and α-1,2 linkages, Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man, Man*p*β1-2Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man, and Man*p*β1-2Man*p*β1-2Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man, and possessing a common structure, Man*p*β1-2Man*p*α1-2Man, correspond to an integral part of the specific epitopes composing antigenic factor 6, and the two former oligomannosyl residues containing one and two β-1,2 linkages are a major determinant for the specificity of the mannans of *C. albicans* serotype A strains.

Recently, we determined the presence of oligomannosyl residues containing the above common structure in the mannans of two other *Candida* species, *C. stellatoidea* type II (10) and *C. glabrata* (6), the cells of which are known to be agglutinated with factor serum 6.

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