Variation in Composition of Yeast Phosphohexosans

M. E. SLODKI, M. J. SAFRANSKI, D. E. HENSLEY, AND G. E. BABCOCK

Northern Regional Research Laboratory, Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

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Omitting of KH$_2$PO$_4$ from culture media leads to the production of altered phosphohexosans or neutral extracellular mannans by yeasts that otherwise elaborate phosphogalactans and phosphomannans.

Limitation of KH$_2$PO$_4$ or its omission from complex culture media leads to production of altered phosphohexosans or neutral extracellular mannans by yeasts that were previously found to elaborate phosphogalactans and phosphomannans. The extracellular phosphogalactan formed on orthophosphate-free medium by Sporobolomyces sp. NRRL Y-6493 contains less organic phosphate, but increased amounts of O-acetyl and D-glucose. Both Hansenula capsulata NRRL Y-1842 and H. holstii NRRL Y-2448 form neutral α-mannans when orthophosphate is omitted from the culture medium. A lightly phosphorylated product containing two polymeric components is formed when H. capsulata is grown in the presence of a limiting amount of orthophosphate.

Earlier work from the Northern Laboratory (1) showed that molar ratios of mannose to phosphorus in extracellular phosphomannans of H. holstii were not changed significantly by growth on different nitrogen sources. The media employed in those experiments routinely contained 0.5% KH$_2$PO$_4$. We now find that either limitation of KH$_2$PO$_4$ or its omission from the culture medium leads to production of altered phosphohexosans or neutral extracellular mannans by yeasts previously found to elaborate phosphomannans (7) and phosphogalactans (6). Table 1 compares the characteristics and yields of neutral mannans with those of corresponding phosphomannans.

The contrast is most striking for H. capsulata NRRL Y-1842. An α-mannan is produced when orthophosphate is omitted from the medium. The polymer formed in the presence of 0.5% KH$_2$PO$_4$ is primarily a polyphosphodiester of a β-linked mannose disaccharide (5). Evidently, a mixture of polymers is obtained when 0.05% KH$_2$PO$_4$ is present in the medium; however, as yet we have been unable to separate the components. Formation of neutral mannans is shown by (i) failure to detect organically bound phosphorus and (ii) precipitability from aqueous solution by a single volume of methanol in the absence of a salt. Precipitation of anionic polysaccharides in low concentrations of methanol normally requires the presence of a salt.

Omission of orthophosphate from the medium employed for phosphogalactan production did not lead to formation of a neutral polymer by Sporobolomyces species. Alcoholic precipitation of the product required the presence of potassium acetate. Even so, there was a fourfold reduction in degree of phosphorylation. Data in Table 2 show that other changes in polymer composition accompanied the fourfold decrease in phosphorylation. The amount of O-acetylation increased threefold and D-glucose made up half of the total hexose units. In view of this high proportion of D-glucose, its presence was investigated in phosphogalactan produced in medium containing 0.25% KH$_2$PO$_4$. D-Glucose constituted approximately 20% of the hexose residues from both Sporobolomyces species Y-6493 and Y-6502 polysaccharides. A separate glucan component was ruled out by ultracentrifugal analysis. Only single components were found in "phosphogalactans" produced on media with and without orthophosphate. Periodate oxidation destroyed over 95% of the glucose residues in both polymers. Acid hydrolysis after sodium borohydride reduction of both oxidized polymers gave rise to D-galactose. This result indicates the presence of periodate-resistant 1,3-linked D-galactose residues in both polymers. Immunochemical analysis of acetylated and deacetylated forms of intact and periodate-oxidized borohydride-reduced phosphogalactan has suggested that multiple sequences of acetylated 1,3-linked D-galactose are present in the highly phosphorylated phosphogalactan (3). Based on quantitative determinations of periodate consumption and formic acid production (6), it can be calculated that D-glucose residues, together with D-galactosyl-α-1-phospho-6-galactosyl end groups, account for most of the 1,6-like linkages in the more highly phosphorylated polymer.
**Table 1. **

| Organism       | Per cent of KH<sub>2</sub>PO<sub>4</sub> in medium | Molar ratio of mannose to P | [α]<sub>D</sub> (degrees) | Yield (g/100 ml) | Component polymers |
|----------------|-----------------------------------------------|-----------------------------|--------------------------|-----------------|-------------------|
| *H. capsulata* Y-1842 | 0.5                                           | 2.5                         | −2                       | 1.7             | 1                 |
| *H. capsulata* Y-1842 | 0.05                                          | 27.5                        | +61                      | 1.5             | 2                 |
| *H. capsulata* Y-1842 | 0                                             | P absent                     | +71                      | 2.1             | 1                 |
| *H. holstii* Y-2448   | 0.5                                           | 5.7                         | +106                     | 2.4             | 1<sup>b</sup>     |
| *H. holstii* Y-2448   | 0                                             | P absent                     | +96                      | 0.3             |                   |

* Methods for production, isolation, purification, and analysis of extracellular polymers are given in reference 7. The values cited for phosphomannans formed in the presence of 0.5% KH<sub>2</sub>PO<sub>4</sub> are also taken from this reference and are included for purposes of comparison. According to paper chromatography of hydrolyzates, D-mannose was the only sugar present. Yields are based on recovery of purified, lyophilized product. None of the products listed contain O-acetyl. Number of components was determined by ultracentrifugal analysis on 0.2% polymer in 4 M urea in 0.03 m tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.2 (F. R. Dintzis, G. E. Babcock, and R. Tobin, Carbohydr. Res., in press).

* See reference 4.

**Table 2. **

| Characteristic | Polymer formed in media |
|----------------|-------------------------|
|                | With KH<sub>2</sub>PO<sub>4</sub> | Without KH<sub>2</sub>PO<sub>4</sub> |
| Molar ratios  |                          |                                    |
| Hexose to P   | 8.7                      | 36.7                                |
| Hexose to O-acetyl | 20.2                  | 6.9                                |
| [α]<sub>D</sub> (degrees) | +118                  | +157                                |
| Yield (g/100 ml) | 0.8                    | 0.6                                |
| Hexose composition | 79% D-Galactose  | 46% D-Galactose                     |
|                  | 21% D-Glucose           | 54% D-Glucose                      |

* Data cited for phosphogalactan formed in presence of KH<sub>2</sub>PO<sub>4</sub>, methods for production, isolation, purification, and analysis are given in reference 6. Total carbohydrate content of polymer formed in the absence of KH<sub>2</sub>PO<sub>4</sub> was determined colorimetrically by the phenol-sulfuric acid procedure (2) with color yield factors derived from yields of standard mixtures of glucose and galactose, the composition of which was based on paper chromatograms of hydrolyzates and direct determination of D-glucose therein. D-Glucose content of hydrolyzates (4 n H<sub>2</sub>SO<sub>4</sub>, 100 C, 60 min) was estimated with a commerically available glucose oxidase reagent after neutralization. Ultracentrifugal analyses (see Table 1) of both polysaccharides showed single peaks.

These results demonstrate the potential for production of extracellular neutral hexosans by cultural manipulation of yeasts previously shown to elaborate phosphorylated polysaccharides.

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