ACYLGLUCOSES IN *ESCHERICHIA COLI, SACCHAROMYCES CEREVISIAE* AND *AGARICUS BISPORUS*

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1. Introduction

Acylglucoses – the simplest of the glycolipids – have now been identified in *Mycoplasma* sp., strain J, which contains a 3,4,6-triacyl-β-D-glucopyranose [1] and in several gram-positive bacteria: *Corynebacterium diphtheriae, Mycobacterium smegmatis, Mycobacterium tuberculosis* BCG and *Brevibacterium thiogenitalis,* all of which contain a 6-*O*-mycolylglucose [2, 3]. In *Streptococcus faecalis* the acylglucose has the probable structure, 3,4,6-tri-*O*-acetyl-2-*O*-laurylglucopyranose [4].

In this note we report the occurrence of acylglucoses in a gram-negative organism, *Escherichia coli,* and in two fungi, *Saccharomyces cerevisiae* and *Agaricus bisporus.* From this, and the work referred to above, it seems that acylglucoses are ubiquitous in micro-organisms when glucose is a major carbon source in the growth media.

2. Methods and materials

Escherichia coli B and *Saccharomyces cerevisiae* were grown in the medium described previously [2], containing 2% glucose. *Agaricus bisporus,* vegetative stage, was grown for about three months in a medium composed of 2% malt extract and 0.1% $CaCO_3$. Harvested cells were washed thoroughly and extracted several times by shaking at 20° with chloroform-methanol (2:1 and 1:2), except for *A. bisporus* which required grinding with sand in chloroform-methanol completely to extract the lipid. Lipid extracts were washed [5] to remove traces of medium glucose.

Lipids were applied to columns of silicic acid and eluted with chloroform, acetone in chloroform and finally methanol in chloroform [2]. Preparative thin-layer chromatography was achieved on wide (40 cm) plates of silica gel H in chloroform-methanol-water (65:25:4), chloroform-methanol (9:1) or chloroform-methanol (10:1).

Lipids were deacylated by mild alkali treatment [6] and the products chromatographed on Whatman No. 1 paper. Glucose, trehalose and mannitol were identified by comparison with authentic samples in several solvents [2], by the fact that trehalose on hydrolysis produced only glucose and by the non-reducing reaction of mannitol and trehalose to *p*-anisidine hydrochloride. Other experimental procedures have been described previously [2, 7].

3. Results

Portions of the washed free lipid from *E. coli*, *S. cerevisiae* and *A. bisporus* were subjected to mild deacylation and the water-soluble products chromatographed on paper (fig. 1). A glucolipid was obviously a major component of the lipids of all three. The conditions for deacylation do not result in glycosidic cleavage, and it is therefore inferred that the glucose originated in an acylglucose.

Free lipid from the three sources was applied to silicic acid columns. The acylglucoses of *E. coli* were completely eluted with 60% acetone in chloroform, and were the only apparent glycolipids in this fraction. Two acylglucoses (R_f 0.63 and R_f 0.95 in chloroform-methanol, 9:1) were purified by preparative thin-layer chromatography. The faster component showed a ratio of one glucose to 3.82 acyl groups and from this and its R_f value appears to be a tetraacylglucose. The number

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Fig. 1. Chromatograms of deacylated total soluble lipid from *E. coli* (A), *S. cerevisiae* (B), and *A. bisporus* (C). 1) Glucose; 2) deacylated lipid from *E. coli*; 3) glucose; 4) deacylated lipid from *S. cerevisiae*; 5) deacylated lipid from *A. bisporus*; 6) glucose. The obvious slow moving components among the products from *E. coli* and *S. cerevisiae* are largely glyceryl-phosphoryl derivatives, with some trehalose in the case of *S. cerevisiae*. Chromatography in ethyl acetate-acetic acid-formic acid-water (18:3:1:4) on Whatman No. 1. Spots were detected with a $AgNO_3$-NaOH dip [2].

of acylated positions in the other lipid has not definitely been established, but appears to be less than four.

A small amount of the acylglucoses of *S. cerevisiae* was eluted from a silicic acid column by 5% acetone in chloroform. These were not examined but presumably are polyacylated glucoses. The bulk of the acylglucoses were eluted with 60% acetone in chloroform. Preparative thin-layer chromatography in chloroform-methanol (10:1) revealed four lipids. The major one (R_f 0.43) was readily detected due to a bright blue fluorescence with an anthrone spray [8]. Analysis of this showed a ratio of one glucose to one acyl group. The infrared spectrum was very similar to those of the monomycolylglucoses of corynebacteria and mycobacteria [2], with strong hydroxyl group absorption at 3100 cm^{-1} and strong ester carbonyl group absorbtion at 1720 cm^{-1}, both of about equal intensities.

The bulk of the glycolipids of *A. bisporus* was eluted with 60% acetone in chloroform. Besides acylglucoses, two minor glycolipids were also present, which yielded trehalose and mannitol on deacylation. Free glucose, trehalose, and mannitol are found in aqueous extracts of *A. bisporus*, vegetative stage [9], and it now appears that the acylated forms of these carbohydrates are also present in the cells. Three acylglucoses each yielding glucose as the sole carbohydrate on acid and alkaline hydrolysis were separated from the other glycolipids by preparative thin-layer chromatography in chloroform-methanol (10:1) and in chloroform-methanol-water (65:25:4). One of them (R_f 0.92 in chloroform-methanol-water (65:25:4)) appears to be a tetraacylglucose. The other (R_f 0.70) reacts readily with the periodate-Schiff reagent [10], indicating unsubstituted vicinal hydroxyl groups.

4. Discussion

Two acylglucoses were isolated from *E. coli*, one of which appears to be a tetra-*O*-acylglucose. Welsh et al. [4] previously found free glucose among the products of alkali-treated lipid from several gram-negative bacteria, including *E. coli* B. However, it was suggested [1] that this glucose did not arise from acylglucose but from degradation of lipopolysaccharide, due to the vigorous extraction conditions used. In view of our results it appears that at least some of the glucose observed by Welsh et al. [4] originated in acylglucose.

Monoacylglucoses have been found in mycobacteria and corynebacteria [2]. In view of the fungus-like features of mycobacteria [11] it is not surprising that acylglucoses are also found in some fungi, and particularly a monoacylglucose in the case of *S. cerevisiae*.

A. bisporus contains free mannitol, glucose and trehalose [9]. The latter two carbohydrates are also present in *S. cerevisiae*, mycobacteria and corynebacteria [12]. The evidence above, and other work

[2, 13, 14], shows that these carbohydrates are also present in the acylated form. Therefore a criterion for the presence of acylglucoses in an organism may be an adequate provision of free intracellular glucose.

It now appears that acylglucoses are widespread in nature and unlike the glycosyl diglycerides [15], for instance, are not confined to any one group of organisms. Neither can any phylogenetic pattern be found in the number of acylated positions. Both gram-positive and gram-negative bacteria, including one corynebacterium [16], contain tetraacylglucoses while other corynebacteria, mycobacteria and *S. cerevisiae* have monoacylglucoses.

There is no evidence on the function of these compounds. We are investigating the possibilities that they are osmotically inactive storage forms of hexose or that they are involved in glucose transport into the cells.

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