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STARCH IN FUNGI IV. THE LIPOPROTEIN AMYLOSE-PRECIPITATING FACTOR

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SUMMARY

The amylose precipitating factor from *Neurospora* is a lipoprotein of 53,800 mw. The lipid component, which is responsible for the amylose precipitating activity, is mainly polar lipid and constitutes 14.5% of the lipoprotein molecule The binding of amylose to the amylose precipitating factor possibly results in the conversion of the amylose molecule from a random coil to an interrupted helix configuration.

INTRODUCTION

Certain fungi produce a unique starch which is composed of short chain amylose molecules that are deposited in the walls of the hyphae (1,2,3). Amylose production occurs in the cytoplasm (4) which contains starch/glycogen phosphorylase (EC 2.4.1.1) and ADPglucose glucosyltransferase (EC 2.4.1.21) (unpublished results). Either or both of these enzymes has the ability to form relatively long amylose molecules (5,6). Therefore the presence of only short chain amylose molecules in these fungi, even after incubation in glucose-1-phosphate (4), was unexpected. In a preliminary report an amylose precipitating factor was shown to be present in *Lentinellus* hyphae (7). However, this factor could not be characterized because of difficulties in obtaining pure preparations. In this report we describe a simple method for obtaining pure samples of this factor which could possibly be used with other soluble lipoproteins.

METHODS

Fresh or frozen *Neurospora crassa* (wild type OR23 1A) mycelia were floated in 1.5N NaOH overnight. After filtration through cheesecloth and centrifugation at 20,000x g for 10 min the pH of the resulting supernatant

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liquid was adjusted to 7. During this pH change a precipitate formed which was composed of amylose precipitating factor and the amylose endogenous to *Neurospora* (McCracken, Varkey, & Rutherford, unpublished results). This preparation was centrifuged again and the supernatant liquid containing soluble amylose precipitating factor was adjusted to pH 4.0. This step separated the amylose precipitating factor (insoluble) from glycogen (soluble). After centrifugation (10,000x g, 5 min) the pellet was redissolved in 0.1 M Tris-HCl buffer (pH 7.0) and used as the source of amylose precipitating factor for characterization. Amylose precipitating activity was measured as described previously (7). Protein was determined by the Lowry method (8).

Aliquots containing 50 µg protein were run on disc polyacrylamide gel electrophoresis according to Maurer (9) using gels with 7% acrylamide, 0.2% bisacrylamide and Tris-borate-EDTA (pH 8.4). Electrophoresis was performed at 20°C and 3mA per tube. Gels were treated to visualize protein, amylose-binding ability, and lipoprotein. Protein was stained with coomassie blue and lipoprotein with Oil red O (10). Amylose binding regions in the gels were demonstrated by incubation of the gel in 0.2% amylose for 1-2 h at room temperature. Afterwards the gels were rinsed in H_2O and stained with 0.2% I_2 - 2.0% KI. The electrophoresis was repeated with samples containing Triton X-100 (1 µl/100 µl

To determine the molecular weight of the amylose precipitating factor SDS-polyacrylamide gel electrophoresis was run using gels containing 7% acrylamide and 0.2% bisacrylamide (11).

As the results of observed interactions between amylose precipitating factor and bovine serum albumin standard on SDS electrophoresis, amylose precipitating activity was determined in the presence of 0.3% bovine serum albumin.

Amylose precipitating factor apoprotein was prepared by delipidation of factor preparations in ethanol-ether (12) and then used for both regular and SDS electrophoresis.

The ethanol-ether extracts were reduced in volume by evaporation under nitrogen. In order to determine to which class(es) of lipids the lipid moiety of amylose precipitating factor belongs, aliquots of the concentrated extract were spotted on Silica gel TLC plates (Supelco).

The molecular weights of the amylose precipitating factor and its apoprotein were determined by sedimentation equilibrium runs in an ultracentrifuge[2].

RESULTS AND DISCUSSION

Previously an attempt was made to isolate an amylose precipitating factor from *Lentinellus ursinus* fruit bodies (7). Partial success was obtained by precipitation with ammonium sulfate or low pH. However, the factor could not be isolated in pure enough form to allow its characterization. During an attempt to isolate amylose from *Neurospora crassa* (McCracken, Varkey, & Rutherford, unpublished results) we observed amylose precipitating factor activity in alkaline extracts of the mycelia. Adjustment of the pH to neutral-

2. Molecular weights were determined by Thomas Holzman, Biochemistry Department, University of Illinois.

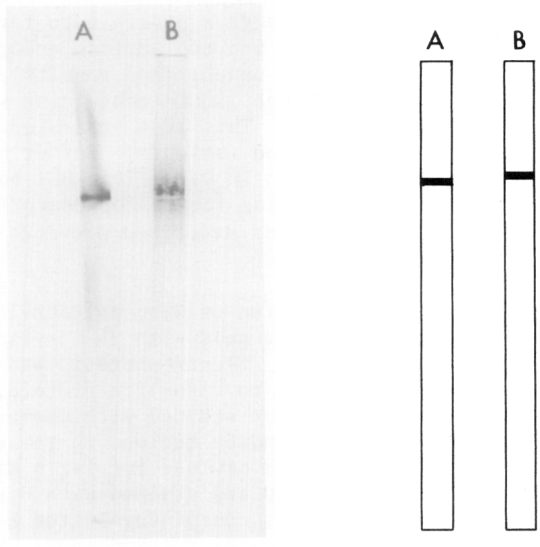

Fig. 1. Polyacrylamide gels treated to visualize protein (A) and amylose-binding ability (B).

ity allowed the removal of endogenous amylose complexed to amylose precipitating factor as a precipitate. Subsequent pH adjustment to 4.0 separated the factor (pellet) from endogenous glycogen (supernatant). The pH 4 pellet was soluble in 0.1 M Tris - HCl (pH 7.0) and on electrophoresis appeared as a single protein band which co-migrated with a band with amylose precipitating activity (fig. 1).

SDS electrophoresis was unsuccessful as no protein bands were observed other than the standards. The presence of amylose precipitating factor did, however, affect the mobility of the bovine serum albumin standard indicating an interaction between these two proteins. When bovine serum albumin (0.5%) was present in the assay system there was a complete inhibition of amylose precipitating activity. One possible explanation for these observations is that the amylose precipitating factor is a lipoprotein which is inactivated upon removal of the lipid moiety by bovine serum albumin. To test this hypothesis extracts were electrophoresed and stained with Oil red O. Also extracts were electrophoresed in the presence of Triton X-100. An Oil red O staining band was observed and corresponded to the protein and amylose-binding bands. After Triton X-100 treatment two protein bands were observed, one of which (the

major band) ran just behind the dye front and the other much more slowly. The slow-migrating band bound amylose but the fast-migrating band did not. Treatment of amylose precipitating factor with ethanol-ether abolished the amylose precipitating activity while causing the appearance of a fast-migrating protein band on electrophoresis. Consequently amylose precipitating factor is a lipoprotein whose lipid moiety is responsible for its activity. The involvement of lipids is not unexpected as fatty acids are known to be amylose precipitants (13,14).

The lipid moiety of amylose precipitating factor appears to be composed solely of polar lipids as the lipid only ran in solvents for polar lipids. Staining of the thin layer plates for phosphate residues did not indicate the presence of phospholipids.

The molecular weight of the amylose precipitating factor lipoprotein is 53,800 and the apoprotein is 46,000. Thus the lipid component constitutes 14.5% of the lipoprotein. Since the lipid moiety may be removed by ethanol-ether, Triton X-100, prolonged dialysis against water, and bovine serum albumin, the protein-lipid complex must be held together by noncovalent bonds. By analogy with the molecular model for plasma high density lipoprotein (17) we propose that the polar head of the lipid moiety forms ionic interactions with charged amino acid side chains in the protein and that the nonpolar chains of the lipid extend out from the protein.

The specificity of amylose precipitating factor for amylose seems to be absolute as there was no precipitation of amylopectin, cellulose, dextran, dextran sulfate, glycogen, or inulin. Maltose and glucose are not inhibitors of amylose precipitating activity (7), however, which indicates the absence of a sugar binding site such as is found in conconavalin A (15) which precipitates amylopectin and glycogen by binding to terminal glucan residues. Amylose precipitating factor apparently requires an α-1,4 glucan with a chain length sufficient for the formation of a helical configuration. This is demonstrated by its ability to precipitate very short chain amylose (average chain length

12-20) produced by limited acid hydrolysis of potato amylose (16) but not maltose (7). Since the lipid moiety of amylose precipitating factor is responsible for amylose precipitation and the mode of lipid (fatty acid) complexing with amylose is known (14), we have developed the following model for the action of amylose precipitating factor. We postulate that the lipid moiety has one or more free chains which extend from the lipoprotein as has been proposed by Segrest et al. for the high density lipoprotein of plasma (17). The free lipid chain would act as the stimulus for a portion of the amylose random coil (14) to assume the helical configuration. However, since the lipid chain is much smaller than the amylose molecule much of the molecule remains in a random coil. Association with other lipid chains would convert further portions of the amylose molecule to the helical configuration. The end result would be an amylose molecule in an interrupted helix (14) with each helical portion complexed with an amylose precipitating factor molecule.

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