When chlorophyll *a* was left on silica gel G under an atmosphere of nitrogen, conversion into the 'changed' chlorophylls was greatly diminished. This suggests that these compounds are oxidized chlorophylls. The chlorophyll C-10 hydrogen may be replaced in 'changed' chlorophylls *a*-1 and *b*-1 by a hydroxyl group (not a methoxyl as in certain chlorophyll 'allomers'), and 'changed' chlorophylls *a*-2 and *b*-2 may be the optical isomers at C-10 of 'changed' chlorophylls *a*-1 and *b*-1 respectively (Bacon & Holden, 1966). 'Changed' chlorophyll *a*-3, which is perhaps a mixture of isomers, may in addition have oxygen between C-9 and C-10 in view of its similarity to pigments described by Holt (1958). The structure of 'changed' chlorophyll *b*-3 is probably analogous to that of 'changed' chlorophyll *a*-3.

There have been many recent reports of the use of silica gel or kieselguhr for the chromatography of chloroplast pigments. The identities and purity of pigments separated on such adsorbents and assumed to be chlorophylls *a* and *b* should be checked by a sensitive chromatographic method causing little or no damage to the pigments. Rapid working with thin-layer chromatography on cellulose is suitable for this. The formation of altered chlorophylls may lead not only to errors of interpretation, particularly when these pigments are

made more obvious by chromatography with a second solvent, but also to errors in quantitative analysis due, for example, to differences in molar extinction coefficients. Bleaching of the chlorophylls may also occur and affect recoveries. Carotenoids as well as chlorophylls can be damaged on some inorganic adsorbents (Strain, Sherma, Benton & Katz, 1965).

I am indebted to Mr D. Wilson for his assistance with this work, and to the Dow Chemical Company (U.K.) Ltd. for gifts of polyethylene powder.

Bacon, M. F. (1965). *J. Chromat.* **17**, 322.

Bacon, M. F. & Holden, M. (1966). *Phytochemistry*, (in the Press).

Broyde, S. B. & Brody, S. S. (1965). *Biochem. biophys. Res. Commun.* **19**, 444.

Holden, M. (1965). In *Chemistry and Biochemistry of Plant Pigments*, p. 461. Ed. by Goodwin, T. W. London and New York: Academic Press Inc.

Holt, A. S. (1958). *Canad. J. Biochem. Physiol.* **36**, 439.

Michel-Wolwertz, M.-R. & Sironval, C. (1965). *Biochim. biophys. Acta*, **94**, 330.

Schanderl, S. H. & Lynn, D. Y. C. (1966). *J. Fd. Sci.* **31**, 141.
Smith, J. H. C. & Benitez, A. (1955). In *Modern Methods of Plant Analysis*, vol. 4, p. 142. Ed. by Paech, K. & Tracey, M. V. Berlin: Springer-Verlag.

Strain, H. H., Sherma, J., Benton, F. L. & Katz, J. J. (1965). *Biochim. biophys. Acta*, **109**, 23.

Biochem. J. (1966) **101**, 36c

The Location of Chitin in the Yeast Cell Wall

By J. S. D. BACON, ELIZABETH D. DAVIDSON, D. JONES and IRENE F. TAYLOR
Macaulay Institute for Soil Research, Aberdeen

(Received 23 August 1966)

Although chitin was known to be present in the cell walls of many fungi its occurrence in yeasts was not conclusively demonstrated until Houwink & Kreger (1953) showed that the cell walls of *Saccharomyces cerevisiae* after successive acid and alkali extractions yielded a small residue, granular when examined in the electron microscope, that gave an X-ray diagram characteristic of chitin; this confirmed a tentative chemical identification by Roelofsen & Hoette (1951). The total amount of amino sugar in the wall is small (perhaps as little as 0.8%; Eddy, 1958), and Korn & Northcote (1960) have suggested that only about one-tenth of it can be present as chitin.

The chief difficulty in identifying chitin was the

presence of much larger amounts of glucan, hitherto regarded as 'extremely insoluble' in dilute aqueous alkali (Northcote, 1963) despite its highly branched structure (cf. Manners & Patterson, 1966) and lack of internal hydrogen-bonding (Northcote, 1953a). There appeared to be a contradiction between the failure of aqueous alkali to extract any glucan from whole yeast (Bell & Northcote, 1950) and the undoubted presence of an alkali-soluble fraction of the glucan in cell-wall preparations (Eddy, 1958; Roelofsen, 1953; Falcone & Nickerson, 1956). A possible explanation for this discrepancy could be some degradation by an active laminarinase that we have recently found to be present in cell-wall preparations from baker's yeast; alternatively, the

(b)

(c)

EXPLANATION OF PLATE I

Electron micrographs of bud-scar residues, prepared by solubilization of cell-wall glucan by the procedure described in the text, washed with water and shadowed with nickel-palladium: (a) and (b) refer to preparations from different batches of yeast; (c) is preparation (b) after incubation for 5 days with *Cytophaga* culture fluid, in the presence of tris-HCl buffer, pH 7.5, and 2-mercaptoethanol (cf. Bacon *et al.* 1965). The scale on (a) represents 5 μ and those on (b) and (c) 1 μ .

soluble fraction may be trapped inside the intact wall, as is some of the glycogen, even after repeated alkali extractions (Northcote, 1953b).

Most of this glycogen can be liberated by treatment with 0.5N-acetic acid at 75° (Bell & Northcote, 1950); Dr Bell (personal communication) informs us that this relatively mild treatment was suggested by a similar effect of cold N-HCl reported by McAnally & Smedley-Maclean (1937). We have now found that after such treatment almost all the glucan becomes soluble in 3% (w/v) NaOH. Further, it seems unlikely that the breaking of glycosidic bonds is an essential part of the process, because pre-treatment with sodium acetate buffer, pH 5.0, at 75° for 3 hr. is sufficient. After this treatment almost the whole of the previously alkali-insoluble residue goes into solution.

A typical procedure is as follows. A 200g. sample of freshly-obtained pressed yeast (The Distillers Co. Ltd., Glenochil, Clackmannan) is crumbled into 160ml. of 6% (w/v) NaOH at 75–80° and stirred for 1 hr. The suspension is then poured into 600ml. of water, stirred for a further 1 hr. at room temperature and centrifuged at 16000g. Three further extractions are made in a similar manner, beginning with suspension of the residue in 320ml. of 3% NaOH. The final residue is suspended in 750ml. of water and the pH adjusted to 7.0 with acetic acid. Then 100ml. of 0.5M-sodium acetate buffer, pH 5.0, is added, the pH adjusted to pH 5.0 and the suspension stirred for 3 hr. at 75°. The water-clear supernatant is discarded and the residue suspended in 300ml. of 3% NaOH and stirred for 1 hr. at 75°. On centrifuging at 16000g at 1° an orange material is left floating on the surface of a dark viscous fluid; at the bottom is a compact precipitate (unchanged by further alkali extractions), which on examination with an electron microscope (A.E.I. Ltd., Manchester EM 6) is seen to consist almost exclusively of the budscars of the original walls (Plate 1a and 1b). The yield of dry matter is about 130mg., and on hydrolysis the only sugars detectable are glucosamine and glucose; there are traces of amino acids. The infrared spectrum of this material shows the presence of much chitin, with some admixture of another polysaccharide. A carbohydrate determination by anthrone suggested a glucan content of 30–35%, and the nitrogen content (3.6%) would allow for 50% of chitin, which, assuming the dry weight of the original yeast to be 25–30% and 15% of this to be cell wall (Northcote & Horne, 1952), would correspond to 1% of chitin in the wall. After incubation with a culture fluid of *Cytophaga johnsonii* grown on yeast cell walls (Bacon, Milne, Taylor & Webley, 1965) the structures showed less relief (Plate 1c). Paper chromatography of the supernatant obtained on centrifuging at the end of the incubation period showed the formation of oligosaccharides similar to

those produced from the cell-wall glucan, but no signs of N-acetylglucosamine or related oligosaccharides; the infrared spectrum of the residual bud scars now differed very little from that of pure chitin.

Some difficulty was experienced in determining the chitin contents of whole yeast, of alkali-extracted yeast and of the bud-scar residues by measurements of the glucosamine liberated on acid hydrolysis (Boas, 1953); similar difficulties with *Penicillium griseofulvum* mycelium are described by Smithies (1952). Our measurements indicate that at least four-fifths of the glucosamine of whole yeast is lost during preliminary extractions, which convert it into the 'alkali-insoluble glucan', but that the remaining fifth is preserved in the bud-scar residues.

It may be tentatively concluded from these results that most of the chitin of the yeast cell wall is located in the bud-scar region, although its exact relation to the complete bud-scar structure remains to be established. Houwink & Kreger (1953) considered that granular material in the bud-scar region of acid-treated *S. cerevisiae* walls was chitin, but the interpretation of their results was made difficult by the undoubted hydrolytic degradation and the possible formation of artifacts during the acid treatment.

We can offer no explanation for the change in solubility of the glucan fraction resulting from heating at pH 5.0, but it may be recalled that Zechmeister & T6th (1936) referred to the results of applying an incorrect sequence of operations ('unrichtiger Reihenfolge der Operationen') to pressed yeast. Repeated boiling with water, dehydration with hot ethanol and extraction with ether in a Soxhlet apparatus decreased the dry matter to a half; the product practically all dissolved when heated in 3% NaOH. They ascribed this result to the removal of protective lipid, and seemed prepared to accept the contention of Sevag, Cattaneo & Maiweg (1935) that the yeast polysaccharides could be solubilized by prolonged extraction with water in the presence of various immiscible organic solvents. Little attention seems to have been paid to these observations by later workers.

The effect of heating at pH 5.0 cannot be explained simply as increasing the permeability of the wall residue, because wall preparations behave similarly, and mechanical breaking of the glucan preparation (with glass beads in a Mickle shaker) does not render it soluble in alkali. It does, however, cause it to swell when heated in water, recalling the observations of Barry & Dillon (1943), which may have been the result of mechanical damage during the powdering of their preparation.

The procedure we describe offers the opportunity of examining more highly purified specimens of the wall glucan; it should also help to elucidate the

action of microbial enzymes on fungal walls, interest in which was the starting point of the present investigation.

We thank Miss J. Normington for technical assistance and Dr V. C. Farmer and Dr J. D. Russell for the infrared spectra and their interpretation.

- Bacon, J. S. D., Milne, B. D., Taylor, I. F. & Webley, D. M. (1965). *Biochem. J.* **95**, 28c.
Barry, V. C. & Dillon, T. (1943). *Proc. R. Ir. Acad.* **49**, 177.
Bell, D. J. & Northcote, D. H. (1950). *J. chem. Soc.* p. 1944.
Boas, N. F. (1953). *J. biol. Chem.* **204**, 553.
Eddy, A. A. (1958). *Proc. R. Soc. B*, **149**, 425.
Falcone, G. & Nickerson, W. J. (1956). *Science*, **124**, 272.
Houwink, A. L. & Kreger, D. R. (1953). *Leeuwenhoek ned. Tijdschr.* **19**, 1.

- Korn, E. D. & Northcote, D. H. (1960). *Biochem. J.* **75**, 12.
McAnally, R. A. & Smedley-Maclean, I. (1937). *Biochem. J.* **31**, 72.
Manners, D. J. & Patterson, J. C. (1966). *Biochem. J.* **98**, 19c.
Northcote, D. H. (1953a). *Biochim. biophys. Acta*, **11**, 471.
Northcote, D. H. (1953b). *Biochem. J.* **53**, 348.
Northcote, D. H. (1963). *Pure & appl. Chem.* **7**, 669.
Northcote, D. H. & Horne, R. W. (1952). *Biochem. J.* **51**, 232.
Roelofsen, P. A. (1953). *Biochim. biophys. Acta*, **10**, 477.
Roelofsen, P. A. & Hoette, I. (1951). *Leeuwenhoek ned. Tijdschr.* **17**, 297.
Sevag, M. G., Cattaneo, C. & Maiweg, L. (1935). *Liebigs Ann.* **519**, 111.
Smithies, W. R. (1952). *Biochem. J.* **51**, 259.
Zechmeister, L. & Tóth, G. (1936). *Biochem. J.* **284**, 133.