The solubilization of a glucuronyltransferase involved in pea (*Pisum sativum* var. Alaska) glucuronoxylan synthesis

Keith W. WALDRON,* Elias A. H. BAYDOUN† and Christopher T. BRETT‡§

* AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA, U.K., † Department of Biology, American University of Beirut, Beirut, Lebanon, and ‡ Plant Molecular Science Group, Department of Botany, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

A glucuronyltransferase involved in glucuronoxylan biosynthesis was obtained from the epicotyls of 1-week-old etiolated pea (*Pisum sativum* var. Alaska) seedlings and was solubilized in Triton X-100, a non-ionic detergent. The enzyme was inactivated by SDS and inhibited by Derriphat 160 and cholic acid. The enzyme was active in the presence of *NN*-dimethyldodecylammonium-*N*-oxide, but was not solubilized by it. The stimulatory effect of UDP-D-xylose on the particulate and solubilized enzymes was the same, but the optimum Mn^{2+} concentration was lower for the solubilized enzyme, and the product formed by the solubilized enzyme has altered structure and solubility properties. Gel filtration of the solubilized enzyme on Sepharose CL-6B permitted partial separation of the stimulatory effect of UDP-D-xylose from the activity in the absence of UDP-D-xylose. The solubilized enzyme was more stable than the particulate enzyme and could be stored for 2 weeks at $-20^{\circ}C$ without loss of activity.

INTRODUCTION

Most plant cell-wall polysaccharides are heteropolysaccharides, and their synthesis involves the interaction of two or more synthase enzymes (Waldron & Brett, 1985). The specificity and control of such interactions will, in part, determine the eventual structure of the polysaccharides and thus the physical and chemical properties of the cell wall. The study *in vitro* of enzymes involved in cell-wall heteropolysaccharide synthesis has usually involved the incubation of an impure particulate enzyme with suitable radioactively labelled sugar nucleotides. Further progress in understanding the properties and interactions of the enzymes is limited by the impurity of the enzyme preparations, and hence it is important to discover methods for their purification.

Since the heteropolysaccharide synthases are integral membrane proteins, their purification depends on solubilization in detergent solutions. This has been attempted in the case of one cell-wall heteropolysaccharide synthase system, namely that involved in glucomannan biosynthesis (Heller & Vilemez, 1972*a,b*). However, in this case the solubilized enzymes were relatively unstable, losing most of their activity within 24 h at $-20^{\circ}C$ and 5 h at $0^{\circ}C$. Gel filtration resulted in almost total inactivation of one transferase.

Previously, a glucuronyltransferase (GT) involved in the synthesis of the hemicellulose glucuronoxylan was identified in a particulate enzyme preparation from pea (*Pisum sativum*) epicotyls (Waldron & Brett, 1983). It was subsequently shown to be involved in secondary-cell-wall biosynthesis (Waldron & Brett, 1984). The activity of the enzyme system was prolonged by the presence of UDP-D-xylose, a known substrate for xylan biosynthesis (Bailey & Hassid, 1966; Dalessandro & Northcote, 1981), indicating an interaction between GT

and xylosyltransferase. We have recently shown that the glucuronoxylan is formed by transfer of glucuronic acid on to a nascent xylan chain rather than a preformed one (Baydoun *et al.*, 1989). In the present paper we report the results of experiments *in vitro* concerning the detergent solubilization of GT and its partial purification. The solubilized enzyme has similar characteristics to the particulate GT, although the optimal Mn^{2+} concentration is lower, and the structure of the product appears to be altered.

EXPERIMENTAL

Chemicals

UDP-D-[U- ^{14}C]glucuronic acid ($9.77\text{ GBq}\cdot\text{mmol}^{-1}$) was purchased from Amersham International (Amersham, Bucks., U.K.). UDP-D-xylose was purchased from Sigma (Poole, Dorset, U.K.). Peas (*Pisum sativum*, var. Alaska) were supplied by Sinclair McGill (Ayr, Scotland, U.K.) and arrived pre-dressed in 'Furnasan Slurry'. Except for Triton X-100 (which was obtained from Koch-Light Laboratories, Colnbrook, Slough, Berks., U.K.), all detergents used were kindly given by Professor R. J. Cogdell of the Department of Botany, University of Glasgow. These comprised Derriphat 160, Nonidet P40 (NP40), cholic acid, deoxycholic acid, SDS and *NN*-dimethyldodecylammonium-*N*-oxide (LDAO).

Particulate-enzyme preparation

Peas were grown as described previously (Waldron & Brett, 1983). The etiolated seedlings were harvested, the hook and cotyledons removed, and the epicotyls cut into 2 cm lengths. All further operations in the preparation of the enzyme were carried out at between 0 and $4^{\circ}C$.

Epicotyl tissue (between 60 and 100 g) was homo-

Abbreviations used: GT, glucuronyltransferase; LDAO, *NN*-dimethyldodecylammonium-*N*-oxide; NP40, Nonidet P40; PAGE, polyacrylamide-gel electrophoresis.

§ To whom correspondence and reprint requests should be sent.

genized (two bursts of 5 s at speed 5 on a Polytron blender) in approx. 200 ml of Tris (10 mM)/Mes (10 mM) adjusted to pH 6.0 with HCl. The homogenate was then strained through four crossed layers of muslin. The filtrate was centrifuged at 97 000 g_{av} for 30 min in a Sorvall OTD-65B ultracentrifuge with a Sorvall AH627 swing-out rotor. The resulting membrane pellets were then resuspended in fresh homogenization buffer to adjust the final volume to 50 ml per g of epicotyl tissue.

Solubilization of the enzyme in Triton X-100

The 97 000 g membrane pellets used to make particulate enzyme (described above) were resuspended in fresh homogenization buffer to adjust the final volume to 25 μ l per g of epicotyl tissue. This preparation was then diluted with an equal volume of homogenization buffer containing Triton X-100 at 20% (w/v). After mixing thoroughly with a glass tissue homogenizer, the mixture was centrifuged at 230 000 g_{av} for 2 h in a Sorvall OTD-65B ultracentrifuge with a Sorvall T 865.1 fixed-angle rotor. The resulting supernatant was used as the solubilized material. To measure enzyme activity in the non-solubilized material, the 230 000 g pellet was resuspended in homogenization buffer containing Triton X-100 at 10% (w/v).

Incubation

The standard incubation mixture (variations are explained in the Results section) consisted of 50 μ l of enzyme preparation (particulate or solubilized), $MnCl_2$ (1 mM) and UDP-D-[U- ^{14}C]glucuronic acid (0.5 μ M; 27 750 d.p.m.) in a total volume of 100 μ l. Incubations were performed at 25 °C for 4 h and were terminated by the addition of 1 ml of 70% (v/v) ethanol. During the preparation of radioactively labelled hemicellulose or high-molecular-mass pectin for analysis, the incubation medium was scaled up by a factor of 10.

Measurement of incorporation of radioactivity into polysaccharide

Incorporation of radioactivity into hemicellulose was measured as described previously (Waldron & Brett, 1983), except that, in the case of solubilized-enzyme incubations, a carrier such as boiled particulate enzyme or cellulose powder was added after termination to facilitate extraction of hemicellulose. During the preparation of radioactively labelled hemicellulose or high-molecular-mass pectin for chemical analysis, no such carrier was added.

A 'pectin' fraction was extracted from terminated incubations as described by Waldron & Brett (1983). Incorporation of radioactivity into high-molecular-mass pectin was measured as described previously (Waldron & Brett, 1983), after separating polysaccharides in the EDTA extracts from low-molecular-mass contaminants by gel filtration.

Gel filtration

High-molecular-mass pectin was separated from low-molecular-mass contaminants by gel filtration through a 10 ml column of Sephadex G-100 (8 mm internal diameter) in pectin-extraction buffer (Waldron & Brett, 1983) (Fig. 1). Triton X-100-solubilized enzyme was analysed by gel filtration on similar-sized columns containing Sepharose CL-6B or CL-2B. The elution buffer in

Fig. 1. Gel filtration on Sephadex G-100 of radioactive material in the pectin extract

The solubilized-enzyme preparation was incubated with UDP-D-[U- ^{14}C]glucuronic acid in the presence of UDP-D-xylose (1 mM). Pectin was extracted from the incubation medium and passed through a column (volume 10 ml, internal diameter 8 mm) of Sephadex G-100. Eluent fractions (0.5 ml each) were collected and their radioactivity was measured.

this case consisted of homogenization buffer containing Triton X-100 (10%, w/v).

Gel electrophoresis

SDS/polyacrylamide-gel electrophoresis (SDS/PAGE) was carried out by the method of Laemmli (1970) with an LKB 2001 vertical electrophoresis unit. After running, gels were stained in Coomassie Blue G250, photographed, stained in silver by the method of Oakley *et al.* (1980) and re-photographed.

Protein determination

Protein was assayed by the methods of Mejbaum-Katzenellenbogen & Drobyszycka (1959) and of Peterson (1977).

All other procedures were as described by Waldron & Brett (1983). Except where stated, all incubations were performed in triplicate. Results are shown as means $\pm$ S.D.

RESULTS

Effect of detergents at various concentrations on the incorporation of radioactivity from UDP-D-[U- ^{14}C]glucuronic acid into hemicellulose

Incubations were performed in the presence or absence of UDP-D-xylose (1 mM) to assess the effects of different detergents at various concentrations on GT activity in the particulate-enzyme preparation (Fig. 2). Because preliminary experiments had indicated that detergents lowered the optimum concentration of Mn^{2+} , Mn^{2+} was included in the incubations at 5 mM instead of 10 mM (Waldron & Brett, 1983). Incorporation of radioactivity into hemicellulose was measured and, except for SDS, none of the detergents abolished the stimulation of incorporation by UDP-D-xylose, although certain detergents allowed greater incorporation than others. Derriphat 160 and cholic acid (results not shown) caused

Fig. 2. Effect of detergents on the incorporation of radioactivity from UDP-D-[U¹⁴C]glucuronic acid into hemicellulose. Open symbols, no UDP-D-xylose present; closed symbols, UDP-D-xylose (1 mM) present. □, ■, LDAO; ○, ●, Triton X-100; △, ▲, NP40; ----●, SDS.

marked inhibition at concentrations above 0.1 % (w/w). At low concentrations, some detergents stimulated incorporation. This, however, occurred only in the presence of UDP-D-xylose and was not evident at higher detergent concentrations. The effects of LDAO and Triton X-100 were investigated further (Fig. 3). Although changing the concentration of LDAO was shown to affect the incorporation dramatically, the concentration of Triton X-100 was not critical above 1 % (w/v). Subsequently, the solubilization of GT in 10 % (w/v) Triton X-100 was attempted, giving a final concentration of 5 % (w/v) in the subsequent incubations with UDP-D-[¹⁴C]glucuronic acid.

Solubilization of GT in Triton X-100

Solubilization was performed as described in the Experimental section. The final concentration of Triton X-100 in the incubation was 5 % (w/v) and, because increases in the concentration of Triton X-100 had little effect upon enzyme activity above 1 % (w/v) (Fig. 3a), the detergent/protein ratio was not maintained at a fixed value.

Initial solubilization experiments employed centrifugation at 314000 *g* for 2 h to sediment non-dissolved material. Analysis of the clear supernatant, however, indicated a slight gradation of active enzyme within the centrifuge tube, probably as a result of Triton X-100

Fig. 3. Effect of different concentrations of Triton X-100 (a) and LDAO (b) on incorporation of radioactivity from UDP-D-[U¹⁴C]glucuronic acid into hemicellulose.

UDP-D-xylose (1 mM) was present (●) or absent (○).

Table 1. Distribution of GT activity between Triton X-100-soluble and -insoluble material

The particulate enzyme was dissolved in 10 % (w/v) Triton X-100 and centrifuged at 230000 *g* for 2 h. Incubations were performed in the presence or absence of UDP-D-xylose (1 mM) and the incorporation of radioactivity into hemicellulose was measured.

Material	Proportion of enzyme activity (%)	
	No UDP-D-xylose present	UDP-D-xylose present
Supernatant	75.4	72.6
Pellet	24.6	27.4

micelle movement. As a result, subsequent solubilization involved centrifugation at 230000 *g*. The supernatant was clear and colourless at both *g* values and contained approx. 70 % of the recoverable enzyme activity (Table 1). All remaining activity was present in the pellet. In a

Fig. 4. Gel filtration of Triton-X-100-solubilized GT on Sepharose CL-2B (a) and Sepharose CL-6B (b)

Eluent fractions were assayed for the ability to incorporate radioactivity from UDP-D-[U-¹⁴C]glucuronic acid into hemicellulose in the presence (●) or absence (○) of UDP-D-xylose (1 mM). Abbreviations: BD, Blue Dextran; Dnp-Lys, dinitrophenyl-lysine.

Fig. 5. SDS/PAGE of particulate and Triton X-100-solubilized enzyme preparations

Explanation of lanes: M, markers; 1, particulate enzyme prepared from 97000 g membrane pellet; 2, 400000 g pellet (containing no GT); 3–11, fractions 6, 8, 10, 12, 14, 16, 18, 20 and 22 from the gel filtration of the 400000 g supernatant (as shown in Fig. 4b). Note that GT was active in fractions 14–16 only. In order that direct comparisons of protein content could be made, equal volumes of the 0.5 ml gel-filtration fractions were subjected to SDS/PAGE.

parallel experiment with 1%, rather than 10%, Triton X-100, only 0.5% of the enzyme activity was solubilized, and so 10% Triton X-100 was used in all subsequent experiments.

In order to check that GT was fully solubilized, the

230000 g supernatant was analysed by gel filtration through Sepharose CL-6B and CL-2B. The results (Fig. 4) show that GT was fully included in Sepharose CL-2B and partially included in Sepharose CL-6B. They also show that stimulation by UDP-D-xylose was still

Fig. 6. Effect of Mn^{2+} on the incorporation of radioactivity from UDP-D-[^{14}C]glucuronic acid into hemicellulose (a) and pectin (b) by Triton-X-100-solubilized (●) and particulate GT (○)

Incubations were performed in the presence of UDP-D-xylose (1 mM).

possible. From these results, it can be concluded that GT had been fully solubilized and was present in micelles with sizes in the region of 4 MDa.

The solubilization and gel filtration of the enzyme activity resulted in partial purification of the enzyme, as judged by SDS/PAGE (Fig. 5). The active fractions from the gel-filtration columns showed two major protein bands corresponding to about 22 and 23 kDa, and a number of minor bands. Protein estimation showed that the specific activity of glucuronic acid transfer increased from 0.43 pmol/h per mg of protein in the 97000 *g* pellet to 3.39 pmol/h per mg of protein in the pooled active fractions from the gel-filtration column. Triton X-100 (10%, w/v) was found to solubilize only 18% of the protein in the particulate preparation, which implies that most of the purification was achieved at the solubilization step rather than the gel-filtration step.

The elution profile of GT on Sepharose CL-6B (Fig. 4b) shows that the activities of GT in the presence or absence of UDP-D-xylose can be partially separated. This indicates that GT can be subjected to some degree of purification without inactivation.

Fig. 7. Time courses of incorporation of radioactivity from UDP-D-[^{14}C]glucuronic acid into hemicellulose (a) and pectin (b) by Triton-X-100-solubilized GT

Incubations were performed in the presence (●) and absence (○) of UDP-D-xylose (1 mM).

Effect of Mn^{2+} on Triton X-100-solubilized GT activity

Preliminary experiments had shown that the optimum Mn^{2+} concentration for Triton-X-100-solubilized GT was less than 10 mM. Subsequently the effect of Mn^{2+} concentration was investigated more closely. The effect of Mn^{2+} on the incorporation of radioactivity from UDP-D-[^{14}C]glucuronic acid into hemicellulose and high-molecular-mass pectin in the presence of UDP-D-xylose can be seen to be very different for solubilized enzyme when compared with non-solubilized enzyme (Fig. 6). Solubilization in Triton X-100 causes a decrease in the optimum concentration of Mn^{2+} from approx. 10 mM to below 2 mM. Solubilization also results in a large increase in the ratio of high-molecular-mass pectin to hemicellulose. By measuring the total incorporation of glucuronic acid into both hemicellulose and high-molecular-mass pectin at the optimum Mn^{2+} concentrations, it was found that solubilization resulted in the loss of between 0 and 30% of the original activity.

Fig. 8. Paper-chromatographic analysis of partial acid hydrolysates of high-molecular-mass pectin (a) and hemicellulose (b) by Triton-X-100-solubilized GT

Incubations were carried out in the presence of UDP-D-xylose (1 mM).

Effect of UDP-D-xylose on the incorporation of radioactivity from UDP-D-[U-¹⁴C]glucuronic acid into hemicellulose and high-molecular-mass pectin by Triton X-100-solubilized GT

A time course of incorporation of radioactivity from UDP-D-[U-¹⁴C]glucuronic acid into hemicellulose and high-molecular-mass pectin in the presence or absence of UDP-D-xylose (1 mM) is shown in Fig. 7. The effect of UDP-D-xylose is the same for solubilized GT activity as for particulate GT (Waldron & Brett, 1983). This indicates that solubilized GT interacts with the xylosyltransferase in a similar manner to particulate GT, and incubations of the solubilized enzyme with UDP-D-[U-¹⁴C]xylose (1 mM) under the conditions described by Baydoun *et al.* (1989) confirmed that xylosyltransferase activity was present. Although the incorporation of high-molecular-mass pectin follows the same pattern as the incorporation into hemicellulose, there is far more incorporation into the high-molecular-mass pectin fraction.

Effect of freezing on the activity of Triton X-100 solubilized GT

Freezing the Triton X-100-solubilized enzyme to -20°C for up to 2 weeks was found to have little effect on the activity, thus allowing large preparations of solubilized enzyme to be stored at -20°C before use. This contrasts with particulate enzyme, which loses 50% of its activity in 24 h when frozen (Waldron & Brett, 1983).

Solubilization of GT in LDAO

By using a similar procedure to that used in the case of Triton X-100, solubilization of GT in LDAO was attempted. Because the concentration of LDAO was critical (Fig. 2), the ratio of protein to LDAO was taken into account. However, no GT activity was found in the 230 000 g supernatant, although some was found in the non-solubilized pellet.

Analysis of the pectic and hemicellulosic products of Triton X-100-solubilized-enzyme activity

Hemicellulose and high-molecular-mass pectin labelled with ¹⁴C from UDP-D-[U-¹⁴C]glucuronic acid were synthesized by large-scale incubations in the presence of UDP-D-xylose (1 mM) by Triton X-100-solubilized GT. The products were analysed by partial acid hydrolysis, followed by descending paper chromatography in ethyl acetate/formic acid/acetic acid/water (18:1:3:4, by vol.) (Waldron & Brett, 1983). The results of the analysis (Fig. 8) show that, in each case, the major product is an oligosaccharide which has a similar R_{Xyl} value (approx. 0.125) to one identified as GlcA1 $\rightarrow$ 2Xyl1 $\rightarrow$ 4Xyl. When the hemicellulosic products were incubated with xylanase as described by Baydoun *et al.* (1989), 48% of the radioactivity was solubilized, indicating that at least this proportion of the product was glucuronoxylan. The solubilized material remained at the origin when analysed by descending paper chromatography in ethyl acetate/formic acid/acetic acid/water (18:1:3:4, by vol.) (Kauss & Hassid, 1967), indicating that it consisted of large oligosaccharides. The portion of the radioactive hemicellulose which was not degraded by xylanase may have been inaccessible to the enzyme; the corresponding product of the particulate enzyme, already identified as glucuronoxylan (Waldron & Brett, 1983), was only 30% solubilized on treatment with xylanase.

DISCUSSION

The results show that the GT involved in glucuronoxylan synthesis in pea was solubilized in a non-ionic detergent, namely Triton X-100. The main evidence for the nature of the product is the maintenance of the UDP-D-xylose stimulatory effect, which was identified previously (Waldron & Brett, 1983), and the partial degradation of the product by xylanase. Attempts at partial purification of the enzyme system have been encouraging, as shown in Fig. 4, and the partial purification procedure did not inhibit enzyme activity. Previous attempts to purify enzymes involved in cell-wall heteropolysaccharide synthesis have been unsuccessful. Heller & Villemez (1972a,b) solubilized the glycosyl- and mannosyltransferases associated with glucomannan biosynthesis, but gel filtration of the solubilized enzymes resulted in almost complete inactivation of the mannosyltransferase.

In addition, the enzymes, though quite stable in a particulate preparation, were destabilized by solubilization, losing 85% of their activity in 48 h at 20 °C and in 5 h at 0 °C. The solubilized GT is, however, much more stable, and can be stored for long periods at -20 °C without significant loss of activity.

The stimulation of GT by very low concentrations of detergents (Fig. 2) may be due to slight disruption of the Golgi apparatus in which GT is found (Waldron & Brett, 1987), thus allowing greater access of the enzyme to the sugar-nucleotide substrates. The decrease in the optimum Mn^{2+} concentration upon solubilization can be explained in a similar manner. Upon solubilization of GT, it is probable that the effects of the membrane environment on access of Mn^{2+} to the enzyme will be diminished, thus lowering the concentration at which it is most effective.

The solubilized enzyme is stable at -20 °C, unlike its particulate counterpart. The instability of particulate GT under such conditions may be due to the disruption of membranes during freezing. This would be eliminated upon solubilization.

The stimulation of solubilized GT by UDP-xylose, and the incorporation of xylose from UDP-xylose into hemicellulose by the solubilized membranes, suggest that the xylosyltransferase involved in glucuronoxylan synthesis has also been solubilized. However, the gel filtration in Sepharose CL-6B appears partially to separate the activity from the stimulatory effect, suggesting that the enzymes can be separated and do not necessarily interact in the form of an enzyme complex.

The stimulation by UDP-D-xylose indicates full solubilization of the GT system. However, chemical analysis revealed that solubilized GT produced a product of altered structure compared with the product of particulate GT, perhaps due to the loss of a regulatory component during solubilization. Partial acid hydrolysis produced only one major oligosaccharide, rather than the two obtained under the same conditions from the product of particulate GT. This oligosaccharide had an R_{xyl} similar to that of $GlcA1 \rightarrow 2Xyl \rightarrow 4Xyl$, one of the two oligosaccharides obtained from the particulate-GT product. However, it is unlikely to be identical with this trisaccharide, since the latter would be expected to break down in turn to $GlcA1 \rightarrow 2Xyl$ (Waldron & Brett, 1983), and no trace of this disaccharide was seen. Hence the oligosaccharide obtained from the solubilized-GT product must have been relatively acid-stable. One possible structure for it is $GlcA1 \rightarrow 2Xyl \rightarrow 4(GlcA1 \rightarrow 2)Xyl$, which has been identified in partial acid hydrolysates of larch (*Larix leptolepis*) arabinoglucuronoxylan (Shimizu *et al.*, 1978); this might be produced if solubilized GT transferred glucuronic acid preferentially to adjacent xylose residues. Possibilities involving a change in the linkage formed by GT include $GlcA1 \rightarrow 3Xyl$ and $GlcA1 \rightarrow 2(GlcA1 \rightarrow 3)Xyl$ [since the $GlcA1 \rightarrow 3Xyl$ linkage has been reported in the wood of Monterey pine (*Pinus radiata*) by Brasche & Jones (1956a,b)] and $GlcA1 \rightarrow 4GlcA1 \rightarrow 2Xyl$ (since the 4-position of the glucuronic acid will be unmethylated).

Glucosyltransferases may require a primer in order to

initiate polysaccharide synthesis, and there is preliminary evidence that GT may require a protein primer (Waldron & Brett, 1985). If such a primer is necessary for GT, then the results show that it has not been lost during solubilization. There are two possible explanations for this: (1) the primer may be covalently attached to GT, or (2) the primer may have been solubilized along with GT either in aqueous solution or as a component of a Triton X-100/protein/lipid micelle. It is unlikely that any primer was free in solution, because addition of detergent would be expected to result in its dilution and a subsequent decrease in the activity of GT. This is not the case; the apparent lowering of GT activity upon addition of detergent to a particulate enzyme preparation (Figs. 2 and 3) can be explained by a change in the optimum Mn^{2+} concentration (Fig. 6). Any primers would, therefore, have to be covalently bound to, or present in, the same micelles as GT.

Solubilization of GT caused more of the product to be extracted in the pectin fraction than in the hemicellulose fraction. Since incorporation into the two fractions appeared to be identical in terms of the time course, the Mn^{2+} optimum and the linkages in the product, it is likely that the radioactive products extracted in the pectin and hemicellulose fractions are both glucuronoxylan, and that they differ only in chain length or attachment to a water-soluble primer or both. The change in the ratio of 'pectin' to 'hemicellulose' formed that occurs upon solubilization may be due to a corresponding change in chain length or degree of detachment from the primer.

We thank the Science and Engineering Research Council for a Research Studentship awarded to K.W.W.

REFERENCES

- Bailey, R. W. & Hassid, W. Z. (1966) *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1586-1593
- Baydoun, E. A. H., Waldron, K. W. & Brett, C. T. (1989) *Biochem. J.* **257**, 853-858
- Brasche, D. J. & Jones, J. K. N. (1956a) *Tappi* **39**, 581-588
- Brasche, D. J. & Jones, J. K. N. (1956b) *Tappi* **39**, 768-774
- Dalessandro, G. & Northcote, D. H. (1981) *Planta* **151**, 53-60
- Heller, J. S. & Villemez, C. L. (1972a) *Biochem. J.* **128**, 243-252
- Heller, J. S. & Villemez, C. L. (1972b) *Biochem. J.* **129**, 645-655
- Kauss, H. & Hassid, W. Z. (1967) *J. Biol. Chem.* **242**, 1680-1684
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685
- Mejbaum-Katzenellenbogen, W. & Drobyszycka, W. M. (1959) *Clin. Chim. Acta* **4**, 515-522
- Oakley, B. R., Kirsch, D. R. & Morris, N. R. (1980) *Anal. Biochem.* **105**, 361-363
- Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346-356
- Shimizu, K., Hashi, M. & Sakurai, K. (1978) *Carbohydr. Res.* **62**, 117-126
- Waldron, K. W. & Brett, C. T. (1983) *Biochem. J.* **213**, 115-122
- Waldron, K. W. & Brett, C. T. (1984) *Biochem. Soc. Trans.* **12**, 1189-1190
- Waldron, K. W. & Brett, C. T. (1985) *Soc. Exp. Biol. Semin. Ser.* **28**, 79-97
- Waldron, K. W. & Brett, C. T. (1987) *Plant Sci.* **49**, 1-8