activity in the final supernatant fraction. This is probably due to the action of these detergents on releasing the enzyme which are suggested to be adsorbed on the cell wall[5]. PVP had two disadvantages in enzyme extraction: it contains nitrogen, which interferes with enzyme activity determination on protein–nitrogen basis, and it is insoluble in acetone where the excess cannot be washed away. Gelatine did not give a high enzyme activity preparation. This was probably due to the large molecular size which prevented infiltration of the gelatine into the tannin cell. Gelatine or inert protein also has the disadvantages which are associated with PVP (ref. 2).

PEG showed promising results. It does dissolve easily in water and acetone, and does not interfere in deriving the enzyme activity on protein–nitrogen basis.

At present we are investigating the isolation of mitochondria and other enzymes from plant tissues containing polyphenols of high molecular weight.

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Reactivity of the Tryptophan Residues in Lysozyme

REPORTS have recently been published on the nature of the tryptophan residues in hen's egg white lysozyme and the relation of these residues to enzymatic activity. Hartdegen and Rupley[1] showed that the oxidation of a single tryptophan residue with iodine results in the loss of enzymatic activity. Hachimori *et al.*[2] have oxidized the tryptophan residues of lysozyme with hydrogen peroxide, concluding that all the tryptophans in lysozyme are relatively refractory toward oxidation and that the destruction of 1·4 mole of this amino-acid per mole of the enzyme results in the loss of 95 per cent of the lytic activity. Hamiguchi and Kurono[3] have examined the effect of 2-chloroethanol on the secondary structure of lysozyme; they reported that when the enzyme is treated with solvents varying in composition from 0 to 32 per cent 2-chloroethanol (v/v), there are spectral changes indicating the disruption of hydrophobic regions and the exposure of buried tryptophan residues, although the optical rotatory dispersion investigations show no alteration of helix content. Only at higher concentrations of 2-chloroethanol does the helix content increase.

We have investigated the chemical reactivity of the six tryptophan residues in lysozyme[4,5] with increasing concentrations of 2-chloroethanol, using the tryptophan-specific reagent (2-hydroxy-5-nitro-benzylbromide) recently reported by Koshland *et al.*[6]. The lysozyme used was obtained from Mann Research Laboratory, New York, lot No. *L*1087. The 2-chloroethanol (Aldrich Chemical Co., Milwaukee, Wisconsin, $n_D^{20} = 1{\cdot}4412$) and the 2-hydroxy-5-nitro-benzylbromide (Cyclo Chemical Corporation, Los Angeles, Calif.) were used without further purification.

The solvents were made up by mixing appropriate amounts of 2-chloroethanol with 0·001 M hydrochloric acid. 10 mg of the enzyme were dissolved in 5 ml. of the solvent, and the solution was allowed to stand for 1 h at 23°–24° C; a 50 molar excess of the Koshland reagent was added, and the mixture was allowed to react for 45 min. The excess reagent was removed on a 'Sephadex *G*-25' column using *p*H 7·5 ammonium acetate as the eluant; the eluted protein was dialysed against distilled water and lyophilized. The number of tryptophan residues reacting with the Koshland reagent was calculated using a molar extinction coefficient[6] of 18,900, from the optical density at 412 mμ of lyophilized products dissolved in 0·1 N potassium hydroxide. The activity of the lysozyme derivatives was determined turbidimetrically at room temperature from its lytic effect on a suspension of *Micrococcus lysodeikticus* in 0·1 M ammonium acetate at *p*H 7·0, as described by Léonis[7].

Fig. 1 shows the results of these investigations. In the absence of 2-chloroethanol, less than one tryptophan residue is modified, indicating that on the average all these residues are buried or strongly bound in hydrophobic regions. As the composition of the perturbing solvent becomes less polar, the six tryptophan residues in the enzyme molecule are completely exposed. These findings are in agreement with the refractory nature of these residues with respect to oxidation[1,2] and the structural transitions as indicated[3] for these concentrations of perturbing solvent. From the activity results it would seem that nearly 2 moles of tryptophan can be allowed to react with the Koshland reagent without loss of more than 10 per cent of the lytic activity.

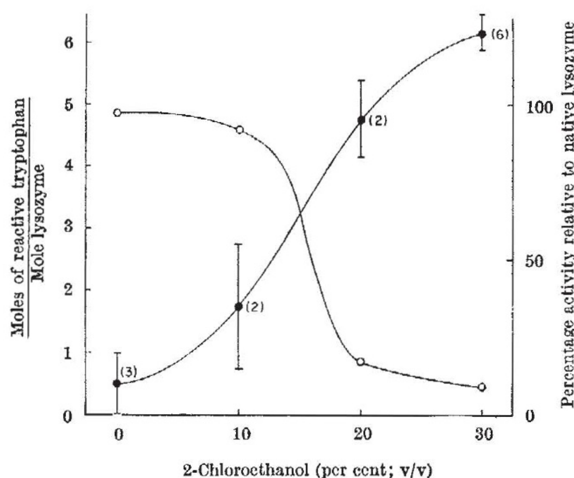

Fig. 1. ●, Uptake of 2-hydroxy-5-nitro-benzylbromide (indicative of reactive tryptophan residues) by lysozyme with increasing concentration of 2-chloroethanol. Each point indicates the mean and standard deviation for separate preparations. The figures in parentheses indicate the number of preparations tested. ○, Lytic activity of the derivatives relative to untreated lysozyme measured under the same conditions. Each point represents a single determination

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