basic protein that reacts with lymphocytes from patients with cancer was shown to be identical with the encephalitogenic determinant Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg (Field *et al.*, 1971; Westall *et al.*, 1971; Lennon *et al.*, 1970; Carnegie, 1971). By treatment of the tumour antigen with 2-hydroxy-5-nitrobenzyl bromide it was shown that tryptophan was an essential residue in the tumour antigen. Studies with synthetic peptides (P. R. Carnegie, E. A. Caspary, F. C. Westall & E. J. Field, unpublished work) have shown that an insertion or substitution of a single amino acid residue can markedly alter the antigenic activity of the peptide.

We suggest that the difference between the normal protein and that from tumour which alters the antigenicity is the result of a slight change in the amino acid sequence around the sole tryptophan residue in the protein.

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Purification of Concanavalin A Receptor from Pig Lymphocyte Plasma Membrane

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It was proposed recently (Crumpton, 1971) that affinity chromatography is applicable to the purification of membrane receptors solubilized in 1% sodium deoxycholate. The validity of this proposal has been evaluated by using an insoluble form of the mitogenic plant agglutinin concanavalin A to separate the homologous receptor(s) from the pig lymphocyte plasma membrane.

The method described by Lloyd (1970), with minor modifications, was used to covalently attach concanavalin A (100mg of 3×crystallized in 10ml of 0.1M-NaHCO₃, pH8.5) to Sepharose 4B (settled volume 10ml) that had been activated with cyanogen

bromide (200mg). After being stirred at 2°C for 24h the product was packed into a column, washed successively with 0.1M-NaHCO₃, 1M-NaCl, water and 5% methyl α -D-glucopyranoside in 1% sodium deoxycholate until no further 280nm-absorbing material was eluted, and then equilibrated with 1% sodium deoxycholate. Approx. 90% of the concanavalin was bound as judged from the E_{280} value of the washings. Pig lymphocyte plasma membrane (20mg of protein) was extracted with 5ml of 1% sodium deoxycholate as described by Allan & Crumpton (1971); 85% of the membrane protein dissolved. The soluble fraction was added to the column and eluted with 1% sodium deoxycholate until the E_{280} value of the effluent returned to the base-line. The unretarded fraction contained approx. 75% of the added protein.

If interaction with concanavalin A persisted in 1% sodium deoxycholate then, in view of its known specificity (Poretz & Goldstein, 1970), membrane components with terminal α -D-glucopyranosyl, α -D-mannopyranosyl or sterically related sugar residues should have been retained. Elution of the column with 2% methyl α -D-glucopyranoside in 1% sodium deoxycholate gave a peak of 280nm-absorbing material. This material was precipitated by adding 0.1 vol. of 2% (v/v) acetic acid and was extracted three times with 10ml of ethanol to remove deoxycholate and methyl glucoside. The residue represented 5% of the protein added to the column. Examination by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate (Allan & Crumpton, 1971) revealed about six bands that stained for protein and carbohydrate; these bands corresponded to the most pronounced glycoprotein bands of the whole membrane. A comparison of the capacities of the lymphocyte membrane and of the residue to inhibit the transformation of pig lymphocytes by concanavalin A showed that the eluted glycoproteins were 20 times as effective as membrane.

The results indicate that membrane receptors can be purified by affinity chromatography and suggest that membrane glycoproteins may be fractionated by using insoluble plant agglutinins with different specificities.

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