Distribution of receptor sites for concanavalin A on epiglycanin

An addendum to 'Distribution of receptor sites on large glycoprotein molecules by electron microscopy: application to epiglycanin'*

Henry S. SLAYTER and John F. CODINGTON

Sidney Farber Cancer Institute, and the Department of Physiology, Harvard Medical School, Boston, MA 02115, and the Laboratory for Carbohydrate Research, Massachusetts General Hospital, and Department of Biological Chemistry, Harvard Medical School, Boston, MA 02114, U.S.A.

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In a recent paper (Slayter & Codington, 1981) we described a method for the visualization by electron microscopy of the locations of receptor sites on large glycoprotein molecules. Mapping of the receptor sites for two lectins, *Ricinus communis* agglutinin and concanavalin A, on epiglycanin, an endogenous cell-surface glycoprotein of the TA3-Ha mammary carcinoma ascites cell of the strain A mouse, was described.

Affinity-purified epiglycanin was incubated with concanavalin A at 0–4°C in 0.10 M-sodium acetate (pH 6.5) containing Ca^{2+} , Mg^{2+} and Mn^{2+} (1.0 mM) for 5 h, as previously described (Slayter & Codington, 1981). After dialysis against 0.15 M-ammonium acetate, complexes were purified by gel-filtration chromatography and prepared for electron microscopy, as described previously (Slayter & Codington, 1981). When visualized by electron microscopy, complexes appeared rather uniformly to possess a single lectin-sized particle very close to one end of the extended epiglycanin molecule. The lectin appeared to bind to about half of the glycoprotein molecules. Plate 1 shows electron micrographs of a field of epiglycanin–concanavalin A complexes (upper half) and of individual complexes at a high magnification (lower half). Of several hundred complexes examined, no epiglycanin molecule appeared to possess more than one bound lectin particle.

The demonstration by electron microscopy of one receptor for concanavalin A per molecule of epiglycanin is consistent with previous chemical and agglutination-inhibition studies (Matsumoto *et al.*, 1980). The failure of each molecule to bind this lectin does not indicate the absence of asparagine-linked mannose-containing chains (Baenziger & Kiete, 1979; Narasimhan *et al.*, 1979), but suggests the possibility of minor differences in structure.

The complexes of *Ricinus communis* toxin and epiglycanin previously described (Slayter & Codington, 1981), which suggest the locations of certain O-glycosyl-linked chains, indicate a significant degree of heterogeneity in epiglycanin, at least with respect to the locations of these chains. The concanavalin A–epiglycanin complexes, as shown in Plate 1, however, suggest that the asparagine-linked chain containing the concanavalin A receptor is homogeneous with regard to its location on the epiglycanin molecule. The observation from electron microscopy that only approx. 50% of epiglycanin molecules possess demonstrable receptor sites for concanavalin A, however, suggests either that there is minor heterogeneity in the structure of the asparagine-linked chain containing the concanavalin A receptor or that this chain is absent from some of the epiglycanin molecules.

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EXPLANATION OF PLATE 1

Electron micrograph of epiglycanin complexed with concanavalin A
Magnification $\times 38\,000$. Insets are selected complexes at $\times 90\,000$.