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Binding Evaluation of Isoform 1 from *Cratylia mollis* Lectin to Human Mammary Tissues

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ABSTRACT

The phenomenon of altered carbohydrates in transformed cell surfaces has been studied through histochemical techniques using lectins. Specific binding patterns to normal and transformed mammary tissues were evaluated by Isoform 1 from *Cratylia mollis* lectin (Cra Iso 1). Protocols using a direct method, incubation of Cra Iso I conjugated to peroxidase (Cra Iso 1-Per) with mammary tissues, followed by diaminobenzidine and hydrogen peroxidase interaction, were performed. Neoplastic tissues, marked by Cra Iso 1, showed a higher intensity of staining than normal ones, in comparison with *Canavalia ensiformis* lectin, Concanavalin A (Con A), conjugated to peroxidase (Con A-Per). The assay with Cra Iso 1 also indicated a possible utilization of this lectin to characterize normal and transformed mammary cells.

Index Entries: Lectin; isoform; human mammary tissue; infiltrating duct carcinoma; fibrocystic disease; fibroadenoma.

INTRODUCTION

All cells carry a saccharide coat that consists, for the most part, of complex carbohydrates, glycoproteins, and glycolipids. This repertoire of surface structures on a cell changes characteristically as it develops, differentiates, or sickens; the array of carbohydrates on cancer cells is strikingly

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different from that on normal ones *(1)*. Qualitative and quantitative changes in the glycosylated components of the cell membrane could be highly significant in the development and progression of the neoplastic process *(2,3)*.

Lectins, ubiquitous natural proteins that noncovalently bind carbohydrates with characteristic specificities *(4)*, have shown to agglutinate, preferentially, mammalian tissue cells that have been transformed spontaneously by oncoviruses or by chemical carcinogens *(5)*. However, these proteins are potentially useful tools in histopathology for the identification of carbohydrates, in addition to distinguishing cells according to their type, differentiation, or function *(6,7)*. Recent etiologic hypotheses concerning breast cancer have received renewed attention within the last decade. Despite the number of studies and the difficulty of measuring several exposures, including electromagnetic fields, psychological factors, prenatal factors, and breast implants, the plausible biologic mechanisms indicate that more research is warranted *(8)*. However, one point is outlined, and its occurrence increases worldwide *(9)*.

Breast cancer is one of the most common cancer in Brazilian women. In the Cancer Hospital (Recife city) of the state of Permambuco, in the northeast region of Brazil, in 19,190 breast biopsies, from 1977 to 1994, 5884 cases (30.8%) were diagnosed as malignant breast tumors.

Concanavalin A (Con A), peanut agglutinin (PNA), *Helix pomatia* agglutinin (HPA), and many other lectins have been used to investigate breast lesions *(10–13)*.

In this work, we have evaluated the binding pattern of a seed lectin (Isoform 1, Iso 1) from *Cratylia mollis* (Cra Iso 1); the legume is a native forage from the semiarid region of the state of Pernambuco *(14,15)*. Con A binding patterns in human breast tissues were used for comparison; Con A and Cra Iso 1 are obtained from related botanical FABACEAE seeds with the same carbohydrate specificity (glucose/mannose), but different structural characteristics and in vitro distinct recognition *(16)*.

MATERIALS AND METHODS

Tissue Samples

Thirty-two formalin-fixed, paraffin-embedded biopsies of human breast lesions, including 5 cases of fibrocystic disease characterized by adenosis and sclerosis, 30 cases of fibroadenoma, and 40 cases of infiltrating duct carcinoma, were obtained from the Pathology Department of The Cancer Hospital, Recife. Normal human breast tissue was obtained from the Obit Identification Service at Federal University of Pernambuco, UFPE, Recife, Brazil.

The age range of patients at the time of diagnosis was 32–82 yr (mean 54) to infiltrating duct carcinoma, 44–68 yr (mean 48) to fibrocystic disease, and 14–50 years (mean 23) to fibroadenoma. The normal tissue sample was biopsied from a 26 year-old patient.

Lectins

Cra Iso 1 was purified *(15)* and conjugated to peroxidase *(17);* Con A conjugated to peroxidase (Con A-Per) was supplied by Sigma Chemical Company. Stock solutions were stored in small aliquots and refrigerated until use. Lectin binding inhibition assays to the studied tissues were performed using D-mannose, D-glucose, and methyl-α-D-mannoside, at concentrations varying from 10 to 30 m*M*.

Electrophoresis in Polyacrylamide Gel

Cra Iso 1 and Cra Iso 1-Per were electrophoresed according to Reisfeld et al. *(18).*

Slide Preparation and Staining Procedure

The slides were washed with 2.5% (v/v) extran detergent, placed in a staining rack, and washed in acetone. They were allowed to drain and immersed, 4 times, 10 dips, in 2% (v/v) aminopropyltriethoxysilane (APES) in acetone. Then the slides were drained, washed in distilled water, and dried at 60°C for 10 min *(19).*

Four-micrometer paraffin sections were deparaffinized in xylene and hydrated through graded alcohol. Tissue sections were treated with a 0.1% (w/v) trypsin solution at 37°C for 2 min, followed by 0.3% (v/v) methanolic hydrogen peroxide for 20 min. The sections were incubated with conjugated lectins (Cra Iso 1-Per, 15 μg/mL; Con A-Per, 12 μg/mL) at 4°C overnight. After each step, the slides were immersed in 10 m*M* phosphate buffer, containing 0.15 *M* NaCl (PBS), pH 7.2 for two 5-min washes. Per was visualized by incubation for 5–8 min in PBS containing diaminobenzidine (DAB) and hydrogen peroxide *(20).* The slices were counterstained with hematoxylin and mounted with Canadian Balsam.

Optic Microscopy

Tissue sections were examined by a Olympus BH-2 microscope, and intensity of lectin binding was evaluated for each studied breast tissue. The cell staining was determined at lectin concentration during which cell boundaring was observed clearly as intense, moderate, or weak. This procedure was used for each lectin (Cra Iso-1 and Con A). The positive lectin binding and the negative sugar-inhibited binding controls were performed

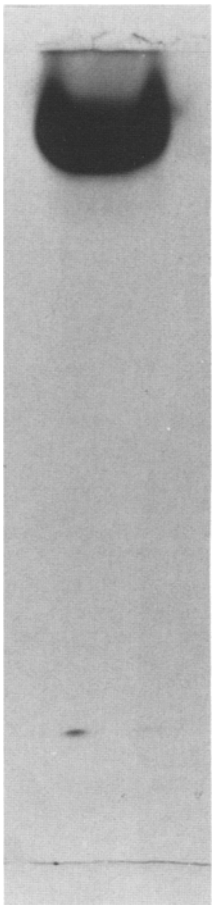

Fig. 1. PAGE to native and basic proteins, pH 4.3. Sample of Cra Iso 1-Per containing 100 μg of protein was stained with Amido black according to Reisfield *(18)*.

in each staining protocols with all normal and transformed tissue samples. The intensity scale was determined as the pattern observed in the majority of the cells in the tissue slice.

RESULTS

Polyacrylamide gel electrophoresis (PAGE) to native and basic proteins resolved Cra Iso 1-Per with a short migration (Fig. 1), different from the non-conjugated lectin. Cra Iso 1 developed a longer run as a basic protein *(15)*.

The staining of hydrated, formalin-fixed paraffin sections of breast lesions with lectins conjugated to Per (Cra Iso 1-Per and Con A-Per) revealed a distinct pattern among the studied cases.

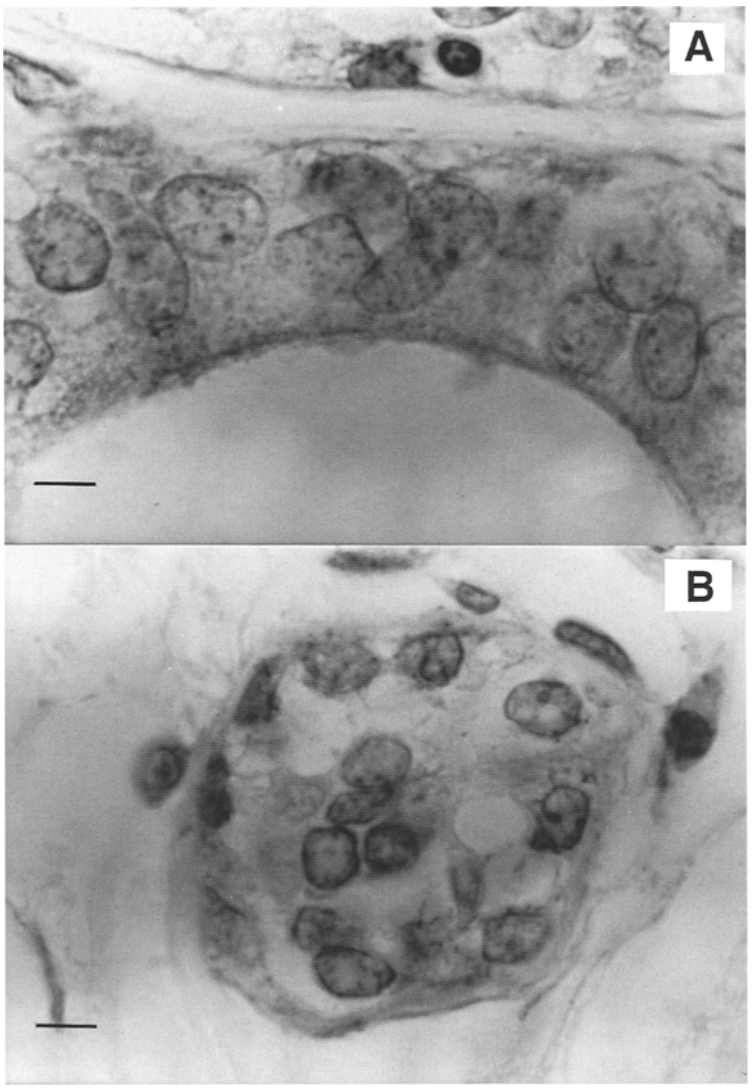

Fig. 2. Normal mammary tissue weakly stained by Cra Iso 1 **(A)** and Con A **(B)** (Magnification ×500, bar = 10 μm.)

The normal tissue showed a discrete staining of epithelial cells to Con A, as well as to Cra Iso 1 (Fig. 2); fibrocystic disease, fibroadenomas, and infiltrating duct carcinoma varied from moderate to intense staining. Fibrocystic tissue presented a moderate staining to cells of ducts and acini to both lectins. Cases diagnosed as fibroadenoma showed duct epithelium moderately stained with Con A and Cra Iso 1 (Fig. 3). Infiltrating duct carcinoma cases were characterized by intense and diffusely stained patterns to both lectins (Fig. 4). A characteristically limited membrane cell staining

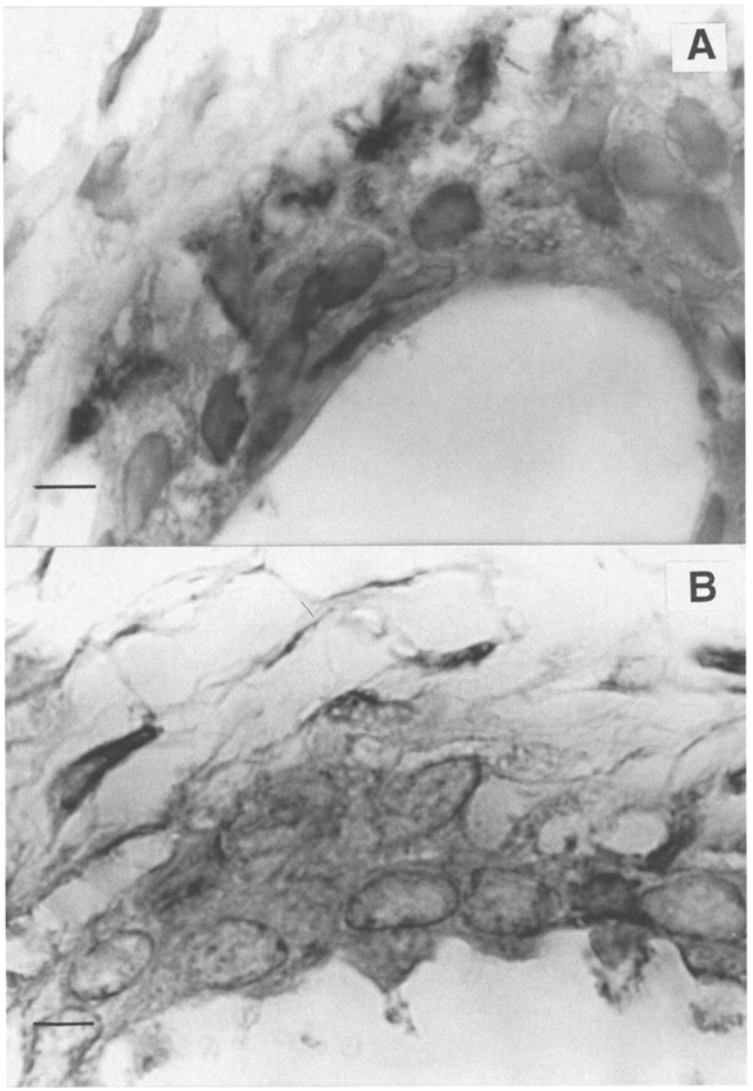

Fig. 3. Fibroadenoma moderately marked by Cra Iso 1 **(A)** and Con A **(B)**. (Magnification ×500, bar = 10 μm.)

was observed. Table 1 shows the binding pattern of the used lectins to the evaluated breast lesions.

In both benign and malignant tissues, Cra Iso 1 and Con A showed variable binding to the stromal tissue.

The best inhibition to normal mammary tissue as well as to transformed ones was obtained with methyl-α-D-mannoside, at 20 m*M* (Fig. 5). The counterstaining with hematoxylin was responsible for the possible

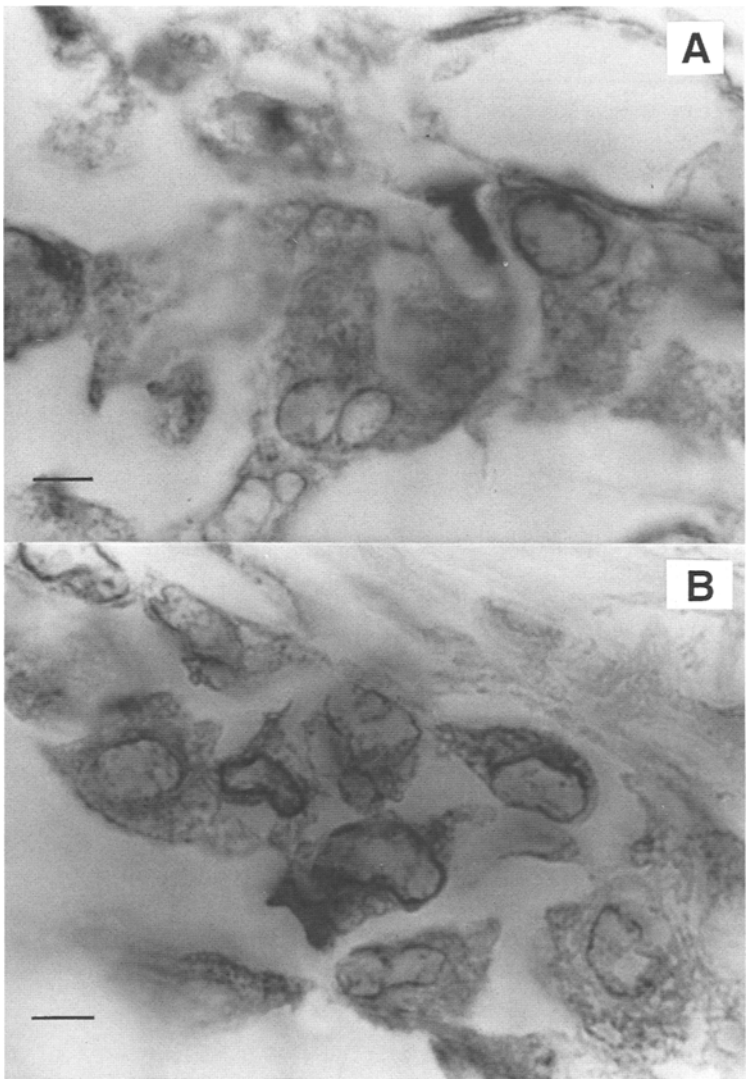

Fig. 4. Cra Iso 1 **(A)** and Con A **(B)** lectins bound to infiltrating duct carcinoma rising an intense staining of the cells. (Magnification ×500, bar = 10 μm.)

higher nucleus staining; the cell boundary was not defined by the lectin, since its binding was inhibited by the sugar.

DISCUSSION

In sections of normal and transformed tissues, Cra Iso 1, as well as Con A, recognized glycoconjugates bearing glucose (Glc) and/or mannose (Man) residues.

Table 1
Lectin Binding Patterns to Breast Lesions

Lectins	Normal	IDC[a]	Fibroadenoma	FD[b]
Con A	+ 1/1	+ 4/40	+ 11/30	−
	−	++ 11/40	++ 17/30	++ 5/5
	−	+++ 25/40	+++ 2/30	−
Cra Iso I	+ 1/1	+ 8/40	+ 10/30	−
	−	++ 10/40	++ 16/30	++ 5/5
	−	+++ 22/40	+++ 4/30	−

[a]Infiltrating duct carcinoma.
[b]Fibrocystic disease.
(+) Weak staining, (++) moderate staining, (+++) intense staining.

The higher binding pattern in transformed cells suggests a dearrangement of the secretory mechanisms observed in normal breast tissue *(21)*. In cell transformation and progression to malignancy, changes in glycosylation, increase in the content, and/or availability of binding carbohydrate residues are well documented *(22–25)*.

The observation that in general, the more anaplastic the cell becomes, more intense is its staining seems to indicate that the site and nature of cell-surface glycoconjugates are altered *(25,26)*. The combined activities of cellular glycosyltransferases and glycosidases determine the final composition of cellular glycoconjugates. In addition to that, tissue factors in the tissue surroundings of the primary tumor may influence and induce differentiation/dedifferentiation reflected in the different lectin binding patterns *(27)*. Our findings show that Cra Iso 1 and Con A binding intensity increases with poorly differentiated tissue cells.

In normal mammary tissue, the binding of Cra Iso 1 and Con A at the luminal surface of ductal epithelial cells was evidenced by its staining. In the neighborhood of transformed cells, a similar result was obtained.

With the considered lectins, there was staining evidence of duct and acinar epithelium to varying degrees, among studied cases. The difference in the cellular site of staining showed that unlike normal, transformed cells presented an extent of reactivity paralleling the degree of histological differentiation.

In both benign and malignant lesions, a mild degree of staining of the stroma had been observed to the studied lectins. *Ulex europeus* agglutinin (UEA I) and *Ricinus communis* agglutinin (RCA) have also been observed to bind to stroma of tumors *(28)*.

The current study demonstrated that Cra Iso 1 binding pattern is associated with transformation of breast epithelium. The isoform behaved

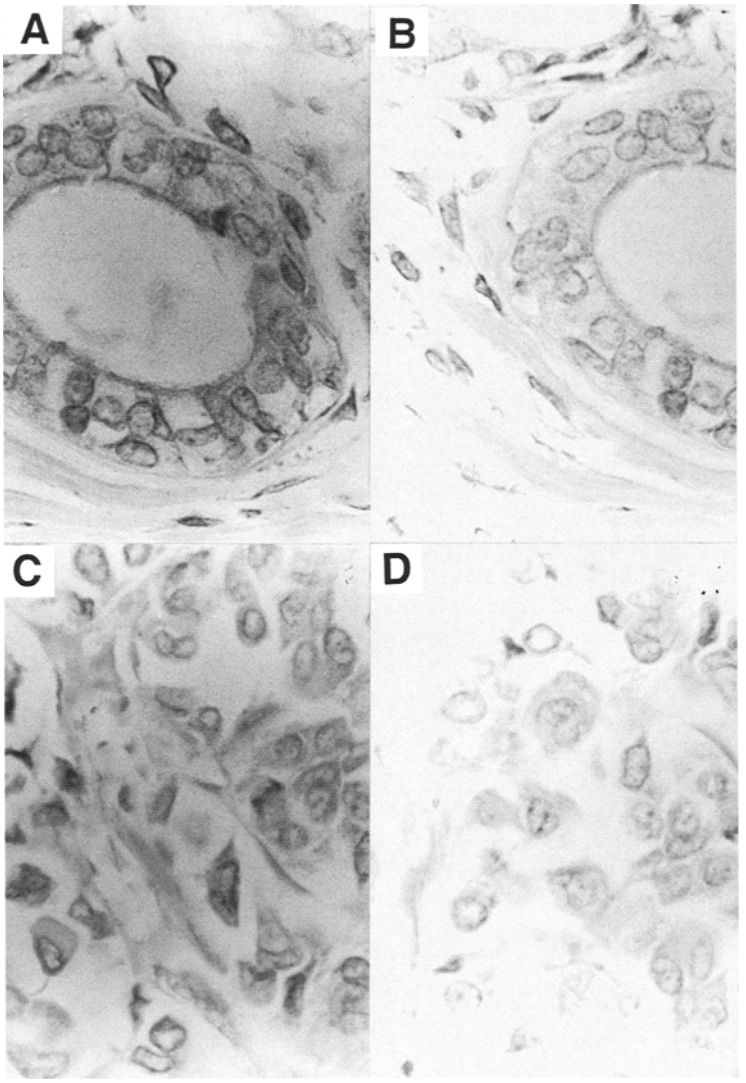

Fig. 5. Cra Iso 1 binding **(A)** and methyl-α-D-mannoside inhibited Cra Iso 1 binding **(B)** to normal tissue; infiltrating duct carcinoma non-inhibited **(C)** and inhibited **(D)** Cra Iso 1 binding. (Magnification ×200.)

similarly to Con A in the binding pattern to breast lesions, and it could be used as an alternative tool in lectin histochemistry protocols.

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