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EFFECTS OF COMMON RADIOIODINATION PROCEDURES ON THE BINDING OF GLYCOPROTEINS TO IMMOBILIZED LECTINS

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Representative glycoproteins including fetuin, protein A, ovalbumin, α_1 acid glycoprotein, and the major glycoprotein of equine infectious anemia virus were labelled with ^{125}I by the chloramine-T or Bolton-Hunter procedure and their binding to immobilized Con A or lentil lectin compared to untreated samples of each glycoprotein. Glycoprotein modification was no greater than one substituted residue per protein molecule. Yet the radioiodinated glycoproteins typically displayed only 0-50% of the lectin binding observed with untreated samples. These results indicate that lectin glycoprotein binding can be markedly altered by minor modifications in protein structure.

Lectin affinity chromatography using concanavalin A (Con A) or Lens culinaris (lentil) lectin bound to Sepharose or agarose beads is frequently employed to specifically isolate detergent solubilized membrane glycoproteins containing α-D-glucopyrannosyl or α-D-mannopyrannosyl residues (1,2). These membrane components are often radiolabeled by one of several iodination procedures for use in a variety of biochemical analyses. Common radioiodination procedures include the modification of tyrosine residues mediated by chloramine-T or lactoperoxidase or the acylation of lysine amino groups by the radioiodinated Bolton-Hunter reagent (3-5). Each of these procedures can be used to specfically label proteins located on the outer surface of intact membranes or to label isolated proteins (5-7). Thus radioiodination and lectin affinity chromatography appear to offer complementary approaches to investigating membrane structure and function. However, we present evidence here which indicates that common radioiodination procedures can dramatically alter glycoprotein-lectin binding. This unexpected alteration does not appear to have been recognized previously, although all glycoproteins tested were affected adversely.

Abbreviations: Con A, concanavalin A; DOC, sodium deoxycholate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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METHODS

Proteins and reagents. Representative glycoproteins including fetuin, α_1 acid glycoprotein, S. aureus protein A and ovalbumin were obtained from Sigma Chemical Co. (St. Louis, MO). The major glycoprotein of equine infectious anemia virus (EIAV gp90) was purifed by affinity chromatography on Sepharose-bound lentil lectin and preparative SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described (8). Na ^{125}I and ^{125}I-Bolton-Hunter reagent were obtained from New England Nuclear (Boston, Mass). Sepharose-bound concanavalin A and lentil lectin were purchased from Pharmacia Chemicals (Piscataway, NJ).

Radiolabelling procedures. Protein samples were labelled using ^{125}I and chloramine-T (4) or Bolton-Hunter reagent (5) according to published procedures. Analysis of each labelled protein preparation by SDS-PAGE revealed only a single radioactive band at the expected molecular weight position and thus demonstrated the integrity and homogeneity of each glycoprotein preparation. Radioactive proteins were at least 95% precipitable in 10% trichloroacetic acid, indicating negligible noncovalently bound iodide.

Lectin affinity chromatography. Protein samples were dissolved to a concentration of 0.5 mg/ml or 10^6 cpm/ml in buffer A (0.02 M Tris, 0.1 M NaCl, pH 8.3) containing 0.5% sodium deoxycholate (DOC) and subjected to centrifugation at 10,000 xg to remove any insoluble material. Protein samples were always less than 10 nmoles per ml of lectin-Sepharose, a protein load significantly less than the estimated binding capacities of Con A - Sepharose (12 nmoles/ml) or lentil lectin-Sepharose (22 nmoles/ml) determined with porcine thyroglobulin (9). The protein samples were applied to columns (0.8 x 8 cm) of either Con A-Sepharose or lentil lectin-Sepharose equilibrated and eluted with buffer A containing 0.1% DOC at a flow rate of about 10 ml/hr. After about one hour of elution to remove nonbinding proteins, the column was eluted with about 20 ml of buffer A containing 0.1% DOC and 0.5 M α-methylglucoside to remove bound glycoproteins. Fractions were collected directly from the column and their protein content determined by assaying for radioactivity (10) or by the Lowry procedure (11).

RESULTS

The relative affinities of native, chloramine-T-labelled and Bolton-Hunter-labelled glycoproteins were measured by chromatography on columns of Sepaharose-bound Con A or lentil lectin. The chromatography conditions employed (column size, elution buffers and flow rates) represent routine procedures which have been proven to be effective for a wide variety of glycoproteins (2); no attempts were made to optimize binding for each glycoprotein sample. Thus the absolute binding level may not be maximized, but the significant degree of binding permits direct comparisons under uniform chromatography conditions.

Figure 1 contains representative chromatograms obtained with fetuin on Con A columns. The data demonstrates that 35% of the native glycoprotein sample bound by sugar reversible interactions to the lectin column under the chromatography conditions employed. In contrast, less than 5% of the fetuin samples labelled by

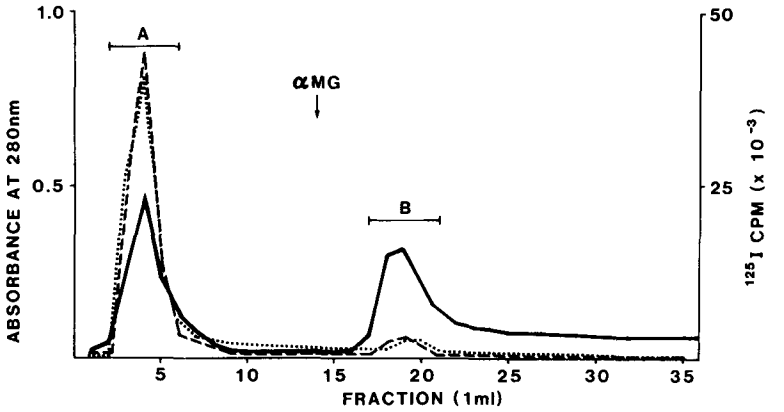

Figure 1. Binding properties of native (———), chloramine-T-labelled (------) and Bolton-Hunter-labelled (.....) fetuin to Con A - Sepharose. Conditions for sample preparation and chromatography are described in Methods. Pools A and B indicate the fractions collected for the determination of percents of nonbinding and binding fetuin, respectively (Table 1). αMG indicates the start of elution with buffer containing 0.5M α-methylglucoside.

the chloramine-T or Bolton-Hunter procedure bound to the Con A matrix under identical conditions. The results indicate that these common radioiodination procedures can greatly diminish the affinity of the fetuin glycoprotein for Con A. To test whether the effect was observed with other lectins, identical samples of native and radioiodinated fetuin were assayed with Sepharose-bound lentil lectin. The results indicate that about 30% of the native glycoprotein bound to the lentil lectin matrix; less than 2% of the fetuin samples labelled by the chloramine-T procedure or Bolton-Hunter reagent bound to the affinity column (Table 1). Thus it would appear that the substituted glycoprotein displays reduced affinities for both Con A and lentil lectin.

The affects of radioiodination on other sample glycoproteins are summarized in Table 1. The α_1 acid glycoprotein and protein A represent samples displaying moderate Con A affinity, while ovalbumin and EIAV gp90 display strong affinities for Con A. However, the results demonstrate that with all the glycoproteins examined, labelling by chloramine-T reduced Con A binding to less than one fourth of the level observed with native glycoprotein samples. The α_1 acid glycoprotein and EIAV gp90, which bound to significant levels on lentil lectin columns, also displayed reduced binding levels to that lectin after chloramine-T-mediated iodination. Labelling with Bolton-Hunter reagent diminished the binding of

TABLE 1
Lectin Binding Properties of
Native and Radiolabelled Glycoproteins

Protein Sample (M)	(a) Specific Activity[b]	Substitution Density[c]	Con A-Sepharose[d] Nonbinding	Con A-Sepharose[d] Binding	Lentil lectin-Sepharose[d] Nonbinding	Lentil lectin-Sepharose[d] Binding
Fetuin (46,000):						
native	---	--	65	35	70	30
CT	5.1	0.11	95	5	100	0
BH	1.0	0.02	95	5	98	2
$\alpha_1$acid glycoprotein (44,000):						
native	---	--	65	35	70	30
CT	5.0	0.10	95	5	100	0
BH	0.5	0.01	95	5	100	0
Protein A (42,000):						
native	---	--	75	25	99	1
CT	3.9	0.08	100	0	100	0
BH	0.8	0.01	100	0	ND	ND
Ovalbumin (43,000):						
native	---	--	25	75	100	0
CT	3.4	0.07	90	10	ND	ND
BH	ND[e]	--	ND	ND	ND	ND
EIAV gp90(90,000):						
native	---	--	10	90	15	85
CT	6.5	0.27	80	20	70	30
BH	0.4	0.02	25	75	10	90

(a) Proteins samples, with molecular weights (M) indicated, were radioiodinated by either the chloramine-T (CT) or Bolton-Hunter (BH) procedure as described in Methods.

(b) Expressed as μCi ^{125}I per μg of protein and calculated for covalently bound label as determined by precipitation with trichloroacetic acid.

(c) Expressed as modified residues per protein molecule and calculated from the formula: D=AM/S where D is the substitution density, A is the specific activity of the labelled protein ($\mu Ci/\mu g$), M is the protein molecular weight and S is the specific activity of sodium ^{125}I and the Bolton-Hunter reagent (both 2.1×10^6 $\mu Ci/\mu g$).

(d) Nonbinding and binding fractions represent the respective percents of the total recovered protein and are an average of several experiments. Values varied less than 5% between multiple experiments.

(e) ND, not done.

α_1 acid glycoprotein, protein A and ovalbumin to the test lectins, but had little effect on EIAV gp90 binding to Con A or lentil lectin.

DISCUSSION

The data reported here demonstrate that the lectin binding properties of a variety of glycoproteins are significantly altered by commonly employed radioiodination procedures. The adverse effects are observed with both lentil lectin and Con A and occur regardless of the native binding affinities of the

test glycoproteins. This alteration in lectin binding affinity by radioiodination does not appear to have been recognized previously.

The reason for the reduction of lectin affinity is not clear at this time. In this regard it has been reported that protein A when modified to a density of four tyrosyl residues per molecule looses its affinity for immunoglobulins (12). Under the reaction condition employed here, however, the labelled glycoproteins contained no more than one substituted residue per molecule (Table 1). Thus the degree of modification is minimal. Moreover test glycoproteins treated with chloramine-T in the absence of ^{125}I displayed no reduction in lectin affinity. These observations suggest that the reduction in lectin affinity must be attributed to the iodination of tyrosyl residues and not to any side reactions possibly mediated by chloramine-T, such as the oxidation of amino acid or oligosaccharide residues. The fact that lectin affinity is also reduced by labelling with the Bolton-Hunter reagent, which reacts with lysine amino groups, argues that tyrosine iodination is not the only cause of altered lectin affinity. Instead the results indicate that glycoprotein-lectin interactions are remarkably sensitive to modification of protein residues, and this sensitivity must be recognized and taken into account when planning or interpreting protocols with lectins and chemically modified glycoproteins.

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REFERENCES

1. Kristiansen, T. (1974) Methods Enzymol 34: 331-341.
2. Lotan, R. and Nicolson, G. L. (1979) Biochim. Biophys. Acta 559: 323-376.
3. Phillips, D. and Morrison, M. (1971) Biochem. 10: 1766-1771.
4. Greenwood, F. and Hunter, W. (1963) Biochem. J. 89: 114-123.
5. Bolton, A. and Hunter, W. (1973) Biochem. J. 133: 529-539.
6. Montelaro, R. and Rueckert, R. (1975) J. Gen. Virol. 29: 127-131.
7. Montelaro, R. and Rueckert, R. (1977) Arch. Biochem. Biophys. 178: 555-564.
8. Montelaro, R., West, M. and Issel, C. (1983) J. Virol. Methods, in press.
9. Affinity Chromatography: Principles and Methods (1979) Pharmacia Fine Chemicals, Uppsala, Sweden, pp. 52-60.
10. Montelaro, R., Sullivan, S. and Bolognesi, D. (1978) Virology 84: 19-31.
11. Lowry, O., Rosebrough, N., Farr, A. and Randall, R. (1951) J. Biol. Chem. 193: 265-275.
12. Sjoquist, F., Meloun, B. and Hjelm, H. (1972) Eur. J. Biochem 29: 572-577.