

Lectin Coprecipitative Isolation from Crudes by Little Rock Orange Ligand

Charles W. Wu,* Rex Lovrien,* and Daumantas Matulis*†

*Biochemistry Department, 1479 Gortner Avenue, University of Minnesota, St. Paul, Minnesota 55108;
and †Institute of Biochemistry, Vilnius, Lithuania

Received August 25, 1997

Matrix ligands are intended for upstream use with dilute crudes on a large scale, splitting out sought-for proteins by coprecipitating them as dense, protected aggregates. Matrix ligand coprecipitation is rapidly, quantitatively reversible, by pH shifting and trapping matrix ligands on ion exchange resin, releasing the sought-for protein. Four lectins, wheat germ agglutinin, peanut lectin, concanavalin A, and *Phaseolus vulgaris* (red kidney bean) lectins, were coprecipitated from crude extracts, 0.05 to 0.4% crude protein, in a single step using Little Rock Orange matrix ligand. All were compared in specific activities (erythrocyte agglutination) and in SDS-PAGE analysis with the four corresponding commercial lectins purified by affinity chromatography. All four matrix-coprecipitated ligands were specifically active within range of the corresponding vendor (Sigma Co.) affinity chromatography-purified lectins. The matrix ligand coprecipitative technique requires optimization of ligand-protein (crude) ratios, denoted γ , and determination of suitable pH ranges for coprecipitation relative to lectin isoelectric pH. These parameters control electrostatic ion pair association: ligand head anion binding to cationic target proteins. The coprecipitative and protective powers of new ligands like Little Rock Orange, their ability to scavenge sought-for lectins from dilute crudes, depend on ligand organic tail-tail association. After the strong anion heads of ligands bind to cationic proteins, their organic tails stack and draw the ligand-protein complexes together as aggregated coprecipitates. © 1998 Academic Press

Key Words: protein isolation; protein crudes; lectins; coprecipitative methods; precipitation of proteins; upstream separations; selectivity; matrix ligands; matrix coprecipitation; separation methods; hemagglutinating lectins; beans; peanut.

Matrix coprecipitation ligands are organic sulfonate anions for helping isolate proteins by coprecipitating proteins to which the ligands bind. Design of matrix ligands and their structure-function features have been described at length (1, 2). Using one such ligand, Little Rock Orange (LRO),¹ we illustrate its use with crude extracts ("upstream" isolation) for the capture of four lectins from seeds: wheat germ agglutinin (WGA), peanut lectin, concanavalin A from jack bean, and *Phaseolus vulgaris* lectin from red kidney bean. Matrix ligands are able, useful agents for both upstream and downstream isolation of proteins. Currently, and for a long time, research in protein isolation has been concentrated on downstream techniques, chromatography of diverse kinds, able to separate proteins downstream on an analytical or quite small preparative scale. However, the large, dilute volumes and crudity of many upstream inputs, used straightaway overwhelm chromatographic systems. The matrix ligand coprecipitation technique described here is but a single step. It cannot be expected to give purification factors and resolving powers of which chromatography is capable. However, within its limitations, matrix ligand coprecipitation has some notably useful features. It has recently been compared with several precipitative and coprecipitative methods, ammonium sulfate salting out, isoionic precipitation, divalent metal ion coprecipitation, and others (3). Rothstein has reviewed protein precipitation in its molecular structure-functional and biophysical aspects (4).

Matrix ligands such as Little Rock Orange are first signaled to bind to cationic proteins by electrostatic forces. Accordingly, the pH is an important parameter, sharply so, in use of such ligands. The structure of Little Rock Orange is shown in Fig. 1. Organic anions

¹ Abbreviations used: LRO, Little Rock Orange; WGA, wheat germ agglutinin; Con A, concanavalin A; PHA, phytohemagglutinin; PNA, peanut lectin agglutinin.

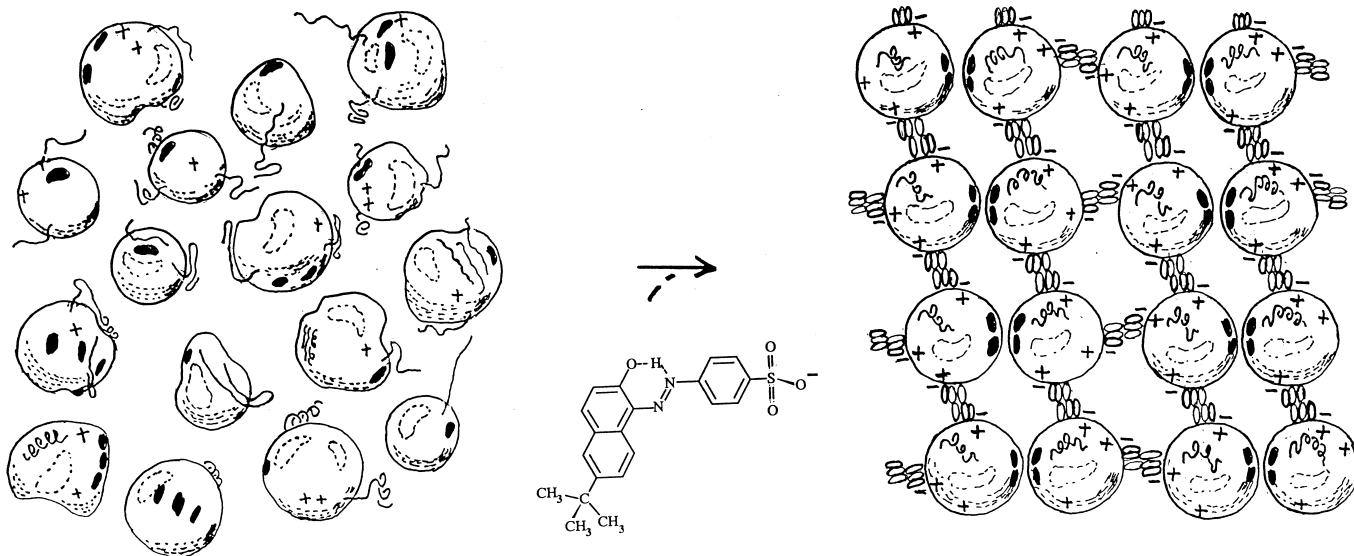


FIG. 1. Little Rock Orange (LRO) matrix ligand, upon binding to conformationally motile, cationic proteins, tightens conformation and narrows their conformational states (2, 6). The sulfonate anion head of LRO initiates binding to protein cationic side chains. The hydrophobic tails of LRO stack, form a tail-tail matrix, drawing ligand-protein complexes together, going on to coprecipitate.

like LRO offer considerable polarizability and water displacement capacity in addition to a coulombic charge. The sulfonate group (the "head") reliably remains anionic throughout most of the biological pH range. The net cation charge of proteins Z_{H^+} is adjustable through the relation of side chain composition, solution pH, and protein isoelectric pH. The organic moiety of matrix ligands are azoaromatics with substituent alkane group(s). In case of LRO, the substituent alkane is *tert*-butyl. Azoaromatic dyes such as LRO have a constitutive coplanarity from their aromatic resonance and diazo conjugation, reinforced by H-atom tautomerization from a hydroxyl group *ortho* to the diazo link (5). The azoaromatic tail groups of these ligands strongly stack and associate, drawing together protein-ligand complexes on the way to aggregation. Such stacking association between ligand tail pairs is further reinforced by the bulky alkane substituents, hydrophobic reinforcement, driving agglomeration between complexes, thence coprecipitation (6). Coprecipitates form with a number of matrix ligands bound per protein molecule, v , within the range of the global net cationic charge Z_{H^+} on the protein molecule, sometimes equaling it. However, large excesses of matrix ligands are not advantageous to add to mixtures which simply saturate all available proteins. Being rather powerful coprecipitating agents, excess ligands coprecipitate diverse proteins, losing selectivity and wasting ligand. Optimal ranges for y_{LRO} in lectin coprecipitation are presented below. Suitable overall protein concentrations in particle-free crudes, ready for ligand addition, range between 0.01 and 0.40% total protein. In general

the optimal pH for coprecipitation is ca. 1 to 4 units lower than the isoelectric pH.

METHODS

Coprecipitation General Procedure: *Crude Starting Material*

The starting material is raw bean (or peanut) meal, finely ground, Soxhlet (petroleum ether) or cold acetone treated to remove lipids. The extraction buffer for all four lectins was 0.015 M phosphate buffer, pH 7.2, 0.15 M in NaCl. Extraction was carried out for 1 h at room temperature with stirring: 250 ml buffer/25 g of dry crude bean (peanut) powder. A first centrifugation removes bulk particles (the sediment in Step 2, Fig. 2). The pH is shifted downward to optimal pH for coprecipitation (Table 1), and the solution is left in the cold room overnight. More particulates may appear which are completely removed by a combination of centrifugation, and (if necessary) filtration on a Buchner funnel with a pad of Hiflo Supercel (Filteraid) on Whatman No. 1 paper. This provides a clear solution, adjusted to optimal pH, ready for ligand addition. A total protein assay is needed here, to adjust the molar ligand/total protein ratio to its optimal value for maximizing yield, achieving best specific activity. A rough approximation, that all four lectins have $M_r \sim 60,000$ Da was used (all their monomers are in fact close to 30,000), for estimating moles protein. Figure 3 shows an example, based on many experiments, varying the y parameter, for determining optimal y_{LRO} . This ratio $y = \text{moles LRO/nominal mole protein}$. Optimal y_{LRO} is

approximately 25 for 3 of the lectins, but it is about 125 in the case of peanut lectin (Fig. 3). The LRO ligand is very water soluble, added in a small, calculated volume as a 60 mM solution with stirring in Step 3, to each awaiting crude.

Coprecipitation usually starts quickly (room temperature), comes essentially to completion in ca. 1/2 h in most cases, especially if total protein concentration is ca. 0.1% or greater. Coprecipitation still can be completed in considerably lower protein concentrations if necessary, in somewhat longer times (1). Matrix coprecipitates are physically dense, and intensely colored by LRO. They are centrifuged down and either stored as wet coprecipitates (well protected in matrix form), or resuspended in reversing buffer (Step 4, Fig. 2).

Reversal (Redissolving Lectin: Trapping LRO Ligand)

Reversing Tris buffers titrate the coprecipitates to pH ~8, 3 to 4 pH units above coprecipitation pH, to enable the ligand to dissociate from its protein. Anion exchanger resin beads, in Cl⁻ form, Dowex or Amberlite (e.g., Dowex 1 × 8, 200 to 400 mesh, 8% crosslink) are suspended in the reversing buffer, ca. 1.5 ml wet resin beads/ml volume of added 60 mM LRO ligand used in the coprecipitation step. This amount of resin provides about 30-fold excess of exchanger equivalents to adsorb the ligand, trapping it. Exchanger resins should be treated, cycled through the OH⁻ form, recycled into Cl⁻ form, and washed, to remove organic fragments that may be leached out of the resin, especially if new (3). Reversal is visible in seconds and completes in 10–30 min. Freedom from ligand in the released protein is seen in two ways: by complete absence of ligand color in solution, via spectrophotometry, and by released lectin activity. Matrix ligands are inhibitors of bioactivity in general, even though they are protective. The regaining of full lectin activity is good evidence that the ligand was thoroughly stripped out and trapped.

If lyophilized freeze-dried lectins are wanted, their solutions are dialyzed first against 0.4 M acetic acid to remove buffer salts, producing grainy products allowing convenient removal from glass.

Little Rock Orange Ligand

Synthesis of Little Rock Orange was described previously (2). LRO has a molar absorption coefficient, $\epsilon = 1.9 \times 10^4 M^{-1} \text{ cm}^{-1}$ in acid, M_w 406.4, sodium salt.

Total Protein Measurements

Total protein measurements were made by the Har-tree–Lowry method (H–L method) (7).

Gel Electrophoresis (SDS–PAGE)

Denaturing gel electrophoresis was carried out as previously described in glycophorin research (8), except that a 15% crosslink density was used in the polyacrylamide gels. Coomassie Brilliant Blue R stain was used for visualization.

Hemagglutination (Lectin Activity) Assays

Lectin activity assays were carried out in microtiter plates, with twofold serial dilutions between wells, in washed human red cells for final assay, having concentrations in all wells of 2×10^8 red cells/ml, 150 mM Tris–saline isotonic buffer, pH 7.4 (9). For peanut, Con A, and kidney bean (PHA) lectins, red cells were pretreated with 0.2 units of Sigma Co. N-2876 neuraminidase enzyme per 1×10^9 washed red cells, 1 h, 37°C. The threshold or agglutination titer end point between (+),(–) agglutination marks the concentration and amount of total protein added as lectin, the level of total protein minimally necessary for visible strong agglutination. This amount of added protein, in milligrams, is the amount of lectin (protein) needed to achieve one agglutination under standard conditions of red cell suspension concentration. The corresponding reciprocal is the specific activity, the number of such agglutinations at (+),(–) threshold, producible by 1.0 mg of the lectin in a total volume of 80 μl with 2×10^8 RBC/ml. Well-purified agglutinating lectins produce several thousand such units (agglutinations/mg), specific activities, in analog to the international units of other kinds of bioactivities on a per milligram of protein basis.

RESULTS

Table 1 lists methods, conditions, in the main steps outlined by Fig. 2 for each lectin. All preparations listed in Table 1 were repeated in their optimized form a minimum of three to six times based on 25 g (bean, peanut) crude starting material. Average values of specific activities gotten after reversal (Step 4, redissolving coprecipitates, trapping LRO ligand) are listed in Table 2. They are compared in Table 2 with our measurements of specific activities of the same four lectins bought from Sigma Co., described by them as purified, affinity-chromatographed, quite pure lectins.

Table 3 reports protein yields for our four lectins per 25 g of raw material (beans, peanut), obtained from LRO coprecipitation. The table also compares lectin protein yields reported in five principal papers scaled to 25 g of raw material (10, 11, 13–15). Lectin isolations in these papers used ammonium sulfate precipitation and affinity chromatography appropriate to each lectin, to isolate the four lectins with which we are comparing LRO coprecipitation. Except for wheat germ

TABLE 1

Isolation Steps, Seed Meal Raw-Crudes, Upstream Little Rock Orange Matrix Coprecipitation

	Concanavalin A	Wheat germ agglutinin	Peanut lectin	<i>Phaseolus vulgaris</i> lectin
Step 1	25 g Soxhlet extracted jack bean meal, 250 ml 0.15 M NaCl, 0.015 M P _i buffer, pH 7.3, 1 h Centrifuge, discard sediment	30 g wheat germ flour, 200 ml 0.2 M HCl, 1 h Centrifuge, discard sediment. Store supernate overnight, 4°C	25 g ice-cold acetone-treated Virginia raw peanut meal: extract with 150 ml 0.15 M NaCl, 0.015 M P _i , pH 7.3 buffer, 1 h Centrifuge, discard sediment	25 g red kidney bean raw meal, 200 ml 0.15 M NaCl, 0.015 M P _i buffer; pH 7.3, stir 1 h Centrifuge, discard sediment
Step 2	Adjust supernate to pH 5.0, with 0.4 M HAc, store overnight 4°C Centrifuge, discard additional particulate	Centrifuge again, discard additional particulate. Dialyze supernate vs 20 mM NaCl Centrifuge, discard particulate or filter, Hy-flo Supercel	Dialyze supernate vs 0.02 M NaCl, centrifuge, discard particulates. Adjust supernate to pH 4.9, with 0.4 M HAc, store overnight 4°C Centrifuge, discard additional particulate	Adjust supernate to pH 4.0 with 0.4 M HAc, store overnight at 4°C Centrifuge, discard additional particulate or filter with filteraid, Hy-flo Supercel
Step 3	Shift pH to 5.5 with 0.2 M P _i , pH 7.3, buffer, take H-L total protein. Add LRO to y = 25, allow 10 min for matrix coprecipitation Centrifuge, discard supernate. Retain LRO-Con A coprecipitate	Shift pH to 4.7, 0.2 M P _i buffer, pH 7.3. Take H-L total protein. Add LRO to y = 23, allow 15 min for matrix coprecipitation Centrifuge, discard supernate. Retain LRO-WGA coprecipitate	Take H-L total protein meas. of supernate. Add LRO at y = 95-115, allow 30 min for matrix coprecipitation Centrifuge, discard supernate. Retain LRO-PNA coprecipitate	Shift pH to 6.4 with 0.2 M P _i buffer, pH 7.3. Take H-L total protein. Add LRO, to y = 25, allow 30 min for matrix coprecipitation Centrifuge, discard supernate; Retain LRO-PHA coprecipitate
Step 4	Reversal: Add a volume of 7 mM Tris buffer, pH 8, to the LRO-lectin coprecipitate, equal to the volume of the coprecipitate's supernate. Add 1.5 ml wet anion exchanger beads, Dowex 1×8-400 Cl ⁻ per ml volume of the 60 mM LRO originally added to achieve the stated y value. Stir (a few minutes) until all color (LRO) transfers to the beads. Centrifuge, dialyze supernate vs 0.4 M HAc for WGA, PNA, PHA, vs 1 M HAc for Con A, then against quality water. Freeze-dry.			

agglutinin, where LRO coprecipitation (optimized as in Table 2) yielded considerably more lectin protein than indicated by Refs. (10) and (11) for 25 g of raw material, the two sets of lectin protein yields data are comparable within a factor of about 2, for the three lectins other than WGA. It is likely that part of such differences between various authors, and between our own results and these others, originate in how total protein was analyzed. The majority of total protein analyses in other laboratories as well as our own are based on cupric ion-reducing reactions, Lowry, and Lowry-based assays. Plant materials, beans, etc. are laden with reducing sugars which incite overestimation of total protein. It is desirable to use more than one strategy for total protein assays when interference of this kind occurs.

Figure 3 shows an example of a search for the optimal LRO ligand level, the y_{LRO} value, for reaching the best available specific activities of a lectin, peanut lectin in this case, produced from crude extract. In the example of Fig. 3, four series of experiments were carried out, involving 27 separate preparations in all. The plot shows that there is a range for LRO addition to crude peanut extract centered around $y_{LRO} \cong 100$ that somewhat roughly but definitively marks the op-

timum y value for achieving good specific activity for PNA lectin. This behavior also occurs for the other three lectins, but their optimal y values for peak specific activity production are considerably lower. These are listed in Table 2, where Con A, *P. vulgaris*, and WGA lectins optimize near $y_{LRO} \cong 25$. Table 2 compares the LRO-coprecipitated lectins, their specific activities, with the specific activities we measured on the four Sigma Co. affinity-chromatographed lectins. In most cases, our specific activities (in human red cell agglutination) are quite comparable, averaging over three to six repeats (Table 2), with the affinity-chromatographed lectins. Our LRO-derived activities for WGA express about half the specific activity of the Sigma product, however. We found that the Sigma Co. lectin-specific activities, on the basis of our agglutination assays, produce specific activities within a factor of $\pm 50\%$ of their quoted values.

Figure 4 reproduces SDS-PAGE denaturing gels for our active LRO-coprecipitated peanut, and Con A lectins, simultaneously comparing them with the Sigma Co. chromatographed lectins in similar gel input loading, as indicated. The matrix-coprecipitated lectins clearly are much improved over the crudes from whence they came, by SDS-PAGE criteria, consistent

25 gm. Solvent extracted bean (peanut) meal

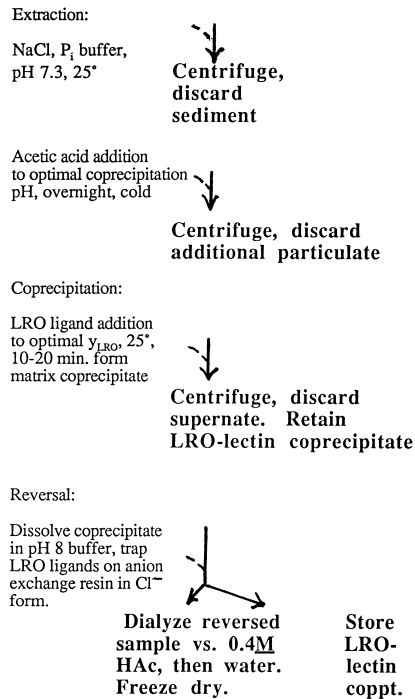


FIG. 2. Outline of four main steps starting from raw bean, peanut meal, extract, LRO coprecipitate, and reversal for isolation of four lectins, Con A, WGA, PNA, and PHA.

with the specific activity comparisons; LRO-coprecipitated lectins with Sigma affinity-chromatographed lectins, shown in Table 2. The crudes were SDS-PAGE electrophoresed using relatively heavy gel input loadings because they contain a large diversity of proteins, in contrast to the coprecipitated products from Step 4. The kidney bean lectin (PHA) from LRO coprecipitation shows equally high-quality SDS-PAGE appearance, a minor foreign band, and a heavy (10 μ g input) single band in excellent register with the authentic (Sigma) affinity PHA. Our WGA electrophoresis on SDS-PAGE also showed full contrast between the

TABLE 3

Yield (Weight) of Four Lectins from 25 g Raw Meals

Lectin	LRO coprecipitation yield, step 3	Lit. quoted yield
Concanavalin A (Con A)	375mg	600 mg (13)
Wheat germ agglutinin (WGA)	80mg	2.2 mg (10) 12 mg (11)
Peanut lectin (PNA)	20mg	21 mg (14)
<i>Phaseolus vulgaris</i> (red kidney bean, PHA)	84mg	50 mg (15)

WGA crude and WGA-LRO-coprecipitated product, with a strong band in good alignment with the authentic (Sigma) product. However, our WGA electrophoresis showed five closely spaced bands, following the main band, consistent with the multiple forms of hybridized isolectins showing heterogeneous SDS-PAGE bands that WGA usually expresses (12). All four lectins evinced profound "cleanup" of crudes, in one LRO coprecipitation, both by SDS-PAGE gel criteria with strong input loading (Fig. 4) and on comparison of specific agglutination activities, LRO coprecipitates and Sigma commercial affinity-chromatographed lectins (Table 2).

An interesting, indicative result ensued concerning the metallolectin, concanavalin A. The LRO-coprecipitated Con A required no addition of divalent metal ion (Ca^{2+} , Zn^{2+}) to express full activity, whereas affinity chromatography prepared Con A frequently (usually) does so require. Such occurs because chromatography media exert cation-anion exchange capacity. In large part, many chromatographic supports in effect are exchangers, capable of withdrawing the rather mobile divalent metal ions from apolectins such as Con A. Ligands such as LRO, anions binding to lectin proteins, may stabilize the needed metal cation in the protein, therefore not require exogenous addition of metal ions later on. This behavior likely extends to other metalloproteins (2).

TABLE 2

Comparison of Specific Activities, LRO-Coprecipitated Lectins from Crude, Reversal (Step 3, Step 4), and Affinity Chromatographed Commercial, Sigma Co. Lectins (Optimal y Values in LRO Coprecipitation)

Lectin	Optimal y , LRO/protein	Specific activities, red cell agglutination in units/mg	
		LRO coprecipitation (n repeats)	Sigma Co. lectin (Cat. No.)
Concanavalin A ^a (Con A)	25	11,000-17,000 (3)	10,000 (C-2010)
Wheat germ agglutinin (WGA)	23	1,300-2,560 (6)	3,200 (L-9640)
Peanut lectin ^a (PNA)	95-115	75,000-133,000 (6)	160,000 (L-0881)
<i>Phaseolus vulgaris</i> ^a (red kidney bean, PHA)	25	75,000-128,000 (3)	76,000 (L-8629)

^a Neuraminidase-treated (desialylated) erythrocytes

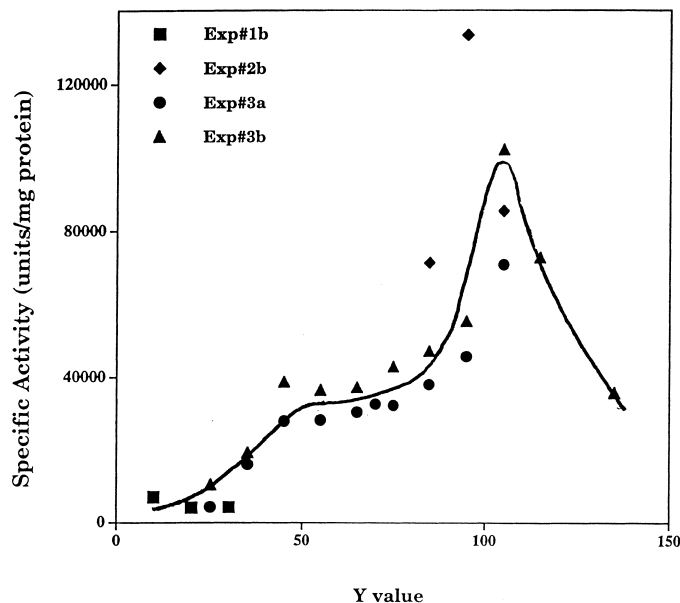


FIG. 3. Optimization of y_{LRO} , moles LRO ligand added per nominal mole of total protein in crudes from Step 3, to maximize peanut lectin agglutinin (PNA) specific activity: Human red cell agglutination titers/mg protein; neuraminidase-treated (desialyated) cells. Coprecipitation pH 5.0, 25°C; data are shown for isolation of four independent preparations from the crude.

DISCUSSION

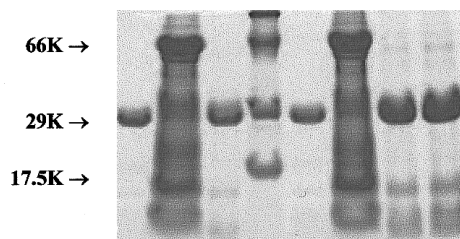
When matrix-coprecipitating ligands related to Little Rock Orange are used for upstream (crude) proteins isolation, two requirements need be met, as in other precipitative techniques. (i) Conditions during coprecipitation must be adjusted to achieve at least some selectivity while concentrating proteins, maintaining activity yields, protecting sought-for proteins. (ii) Coprecipitation needs be rapidly, conveniently reversible, to release sought-for proteins. The second practical criteria, reversibility, generally is easily fulfilled. There has been little trouble releasing some 15 proteins, mostly enzymes in good activity yields that were coprecipitated by matrix ligands related to Little Rock Orange (1, 2).

Selectivity of matrix ligand coprecipitation might first be thought to be determined by ligand organic tail structure, or by how many ligands bind to sought-for versus unwanted proteins. Thence selectivity is partly dependent on how pH, for example, affects ligand binding to target versus unwanted protein molecules. Additional contributors to selectivity are steric in nature. Steric factors are the three-dimensional geometric properties that govern how protein–ligand complexes orient themselves, in drawing the complexes together. Taken further, associative reactions in solution go on to overtly coprecipitate. Protein molecules have various individual association and packing properties dependent on surface structure, where charges, peptide

groups, etc. are placed. In addition to homogeneous (self) association, mixed proteins have the option of heterogenous (differing proteins) association. Homogeneous association to achieve selection for only one or a very few proteins is enhanced by foreign ligands which build complexes, unique to each protein. Little Rock Orange at a typical y_{LRO} level of about 25 ligands per average protein, generates of the order of 2000 to 2500 Å² of organic ligand surface layered over a protein

Peanut (*A. hypogaea*) Lectin Little Rock Orange Coprecipitates From Crude

	Sigma LRO	Crude Y=95	M.W. Markers	Sigma LRO	Crude Y=95	Y=95	Y=95
Specific Activity $\times 10^3$	160	8	133	—	160	8	133
Protein load in μg	10	250	13	30	10	250	26



Concanavalin A Little Rock Orange Coprecipitated Crude

	M.W. Markers	Y-25 LRO 8,000	Crude 500	Sigma 10,000
Specific Activity U/mg dry wt.				
Protein Load in μg	30	10	86	10

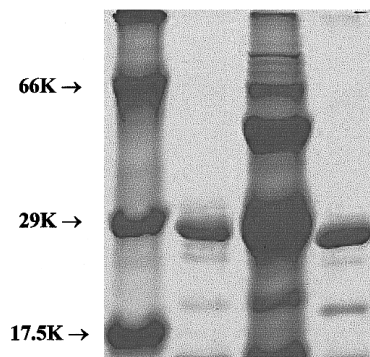


FIG. 4. Electrophoresis of crude (Step 2) and LRO coprecipitate-reversed (Steps 3 and 4) lectins, compared with Sigma Co. affinity-chromatographed lectins in SDS–PAGE gel migration and in their gel input specific activities.

molecule's surface. Protein molecules of 20,000 to 60,000 M_r with radii ca. 20–30 Å, if they were spheres have surfaces of roughly 5000 to 10,000 Å². Hence in the y_{LRO} levels used here, with well over half of the LRO anions bound, from 20 to 50% of protein molecular surfaces are covered with organic ligand, ample to strongly associate by tail–tail stacking and binding. Even from a crude mixture such as the raw extracts are, rather homogenous self-association between ligand–lectin complexes is selected for. In previous papers (1, 2) it was shown from the binding isotherms of Little Rock Orange coprecipitates that such coprecipitates are strongly “pulled” in the thermodynamics of the coprecipitation reaction. That is, such coprecipitates form because the coprecipitate is very stabilized, of lowered chemical potential. The protein molecules are not “pushed” out of solution by raising their chemical potential in a positive direction.

Crude mixtures of proteins are complicated and unknown in their details. Selectivity is hard to design for, in tangles of unknown proteins. However, in practice one may vary parameters, especially y values as in Fig. 3, the pH, and the kind of matrix ligand (the kind of tail) to seek windows or intervals of conditions to in fact achieve selectivity, even from a crude. Capacity to do that is shown by the SDS–PAGE gel results as in Fig. 4. Windows or ranges of conditions for optimizing yields, achieving respectable selectivity, sometimes are rather narrow and require searching. The selectivity of the matrix coprecipitation technique ultimately is due to the cooperative nature, and strong forces stabilizing matrix coprecipitates, when the ligand, the y level, pH etc. are properly “tuned.”

Matrix ligands are quite protective of proteins in coprecipitates (2). Accordingly, they have good prospects for allowing use of higher temperatures, thence more rapid upstream operation. Matrix ligand isolation of some enzymes and proteins from crudes sometimes is best carried out with an opposite strategy than that used with the four lectins here. Namely, use a particularly powerful ligand such as dianion ligands, Razorback Red or Jurga's Red (2). Such very strongly coprecipitating ligands may first bring down most of the unwanted foreign proteins, at a selected pH. In case of horseradish peroxidase crude (including about

10 foreign proteins), the sought for peroxidase was left in solution, nearly pure and highly active (2), with good removal of nearly all else.

Matrix ligand technique is not simply coprecipitation. Coprecipitation per se usually is not a severe problem. Rather, adjusting the system, especially in a crude, to complete the whole cycle, including reversal and release, is key. Little Rock Orange serves well in both sectors of the cycle for lectin crudes.

ACKNOWLEDGMENT

This research was supported by the Minnesota Agricultural Research Experiment Station, funded by the USDA.

REFERENCES

1. Conroy, M. J., and Lovrien, R. E. (1992) *J. Crystal Growth* **122**, 213–222.
2. Matulis, D., Lovrien, R., and Richardson, T. I. (1996) *J. Mol. Recogn.* **9**, 433–443.
3. Lovrien, R. E., and Matulis, D. (1997) in *Current Protocols in Protein Science* (Coligan, J., Dunn, B. H., Ploegh, H. L., Speicher, D. W., and Wingfield, P. T., Eds.), Vol. 1, pp. 4.5.1–4.5.35. Wiley, New York.
4. Rothstein, F. (1994) in *Protein Purification Process Engineering* (Harrison, R. G., Ed.), pp. 115–208. Dekker, New York.
5. Olivieri, A. C., Wilson, R. B., Paul, I. C., and Curtin, D. Y. (1989) *J. Am. Chem. Soc.* **111**, 5525–5532.
6. Lovrien, R. E., Conroy, M. J., and Richardson, T. I. (1995) in *Protein–Solvent Interactions* (Gregory, R. B., Ed.), pp. 521–553. Dekker, New York.
7. Hartree, E. F. (1972) *Anal. Biochem.* **48**, 422–427.
8. Anderson, R. A., and Lovrien, R. E. (1984) *Nature* **307**, 655–658.
9. Anderson, R. A., and Lovrien, R. E. (1980) *J. Cell Biol.* **85**, 534–548.
10. Allen, A. K., Neuberger, A., and Sharon, N. (1973) *Biochem. J.* **131**, 163–171.
11. Nagata, Y., and Burger, M. M. (1974) *J. Biol. Chem.* **249**, 3116–3122.
12. Rice, R. H., and Etzler, M. E. (1975) *Biochemistry* **14**, 4093–4099.
13. Goldstein, I. J., and Agrawal, B. B. (1972) *Methods Enzymol.* **28**, 313–318.
14. Terao, T., Irimura, T., and Osawa, T. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 1685–1692.
15. Rigas, D. A., and Osgood, E. E. (1955) *J. Biol. Chem.* **212**, 607–615.