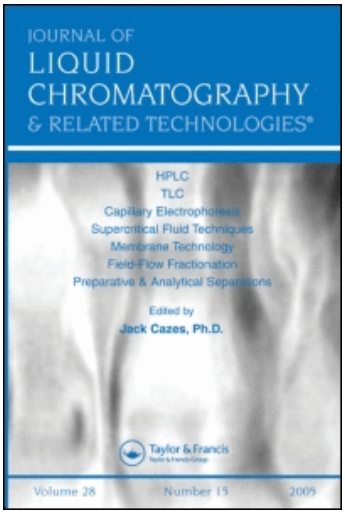

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Simple Method to Optimize Elution from Affinity Chromatography

Mordechai Weiss[ab]; Zemach Eisenstein[ab]
[a] Department of Medicine, The Chaim Sheba Medical Center, -hashomer [b] Sackler School of Medicine Tel-Aviv University, -aviv, Israel

To cite this Article Weiss, Mordechai and Eisenstein, Zemach(1987) 'A Simple Method to Optimize Elution from Affinity Chromatography', Journal of Liquid Chromatography & Related Technologies, 10: 13, 2815 — 2824
To link to this Article: DOI: 10.1080/01483918708066829
URL: http://dx.doi.org/10.1080/01483918708066829

A SIMPLE METHOD TO OPTIMIZE ELUTION FROM AFFINITY CHROMATOGRAPHY

Mordechai Weiss and Zemach Eisenstein
Department of Medicine
The Chaim Sheba Medical Center
Tel-Hashomer 52621
and
Sackler School of Medicine
Tel-Aviv University
Tel-Aviv, Israel

ABSTRACT

A rapid and convenient screening method to optimize elution from affinity chromatography is described. An immunoaffinity system employing antibody bound human thyroglobulin (hTg) served as a model. Anti-hTg was complexed to polystyrene microplates adsorbed hTg. Immune complex (IC) dissociation and elution of antigen by various agents was determined by enzyme linked immunosorbent assay (ELISA). The method permits simultaneous monitoring of residual antigenicity and of possible desorption of antigen from its solid support.

INTRODUCTION

Affinity chromatography is a widely used purification method in which a solid phase covalently bound ligand or

acceptor is used to trap the complementary compound of interest. Subsequently, an eluent is applied to dissociate the complex and solubilize that compound (1).

In order to select the appropriate eluent for any particular system, several dissociating solutions are usually screened for both the efficiency of dissociation and the possible damage incurred to the components of the complex. This may involve many successive chromatographies followed by analyses of these components (2).

In this report we describe a rapid and convenient method to monitor and optimize elution conditions which has the advantage of simultaneous assessment of residual immunogenicity. As a model we used an immunoaffinity system of hTg-anti-hTg.

MATERIALS AND METHODS

Purification and Labelling of hTg

hTg was extracted from a pool of nodular thyroid glands obtained at surgery and purified by a modification of previously described methods (3,4). Briefly, the 15,000 g supernate of the tissue homogenate in PBS was salted out by 45% $(NH_4)_2SO_4$ and gel filtered through Sepharose 4B CL and Sephacryl S200 (Pharmacia, Sweden). hTg migrated as the main protein peak of the first and in the void volume of the

second column. The final product migrated as a single peak in both cellulose nitrate electrophoresis and SDS-PAGE (5). 125-I-hTg with specific activity of 8.5 Ci/g was also prepared (6).

Preparation and Quantitation of Immunobilized hTg-anti-hTg Immune Complex (IC)

hTg was covalently linked to CNBr activated Sephrose 4B beads or adsorbed to polystyrene microtiter plates in wells of 0.3 ml (Nunc, Denmark). Conjugation to Sepharose was carried out according to manufacturer's instructions. Adsorption to polystyrene was achieved by incubation with 10 ng/ml hTg in 0.05M carbonate buffer pH 9.6 at 4°C for 18h. Excess hTg was washed thrice with 0.05% Tween-20 in PBS.

The following protocol was adopted after preliminary experiments: hTg immobilized by either method was incubated in triplicate at 4°C over night with 1:1000 dilution in Tween-PBS of normal human serum or serum obtained from a patient with Hashimoto's thyroiditis containing 1:64,000 titre of anti-Tg antibodies (Fujirebio, Japan). Incubation was followed by three Tween-PBS washings. IC formation was quantified by ELISA using alkaline phosphatase conjugated goat anti-human IgG antibody in 1:1,000 dilution. Enzyme activity was measured as O.D. 405 after adding paranitrophenyl phosphate (Sigma). The non specific binding of normal human serum was always subtracted.

Dissociation of hTg-anti-hTg IC

The following agents were tested for potential dissociating activity at recommended concentrations: Citrate buffer Ø.2M pH 2.2, $MgCl_2$ 7M, acetic acid Ø.5N and KI 4M (7,8,9,1Ø). Since such agents could possibly affect the binding of hTg to the solid phase or alter its antigenicity rendering the ELISA invalid, the following control experiments were always performed: Immobilized stable or 125-I labelled hTg were incubated in triplicate with or without the dissociant for 1h at room temperature and washed. The residual radioactivity was counted while the residual hTg antigenicity was assessed by the ELISA. IC dissociation by the various agents was evaluated by the ELISA following exposure of 1 h at room temperature and three washings.

RESULTS

Immobilized hTg-anti-hTg

Sepharose was efficient as a solid phase support for hTg that interacted in a dose dependent manner with the anti-Tg serum as assayed by the ELISA. However, the non specific binding measured in the presence of normal human serum was substantial and up to 50% in undiluted serum. This was probably due to non specific trapping by Sepharose of the goat anti-human IgG antibody. We abandoned, therefore, further use of the Sepharose and turned to the polystyrene

wells in which the non specific binding was less than 25%. A typical dilution curve is shown in Fig. 1.

Selection of Dissociant

Results of a typical experiment are shown in Table 1. All agents displayed a marginal effect on either the binding of hTg to polystyrene or on its antigenicity, except for KI. However, the degree of dissociation of hTg-anti-hTg IC by the various agnets, each at the recommended single concentration, was markedly varialbe. Acetic acid was the

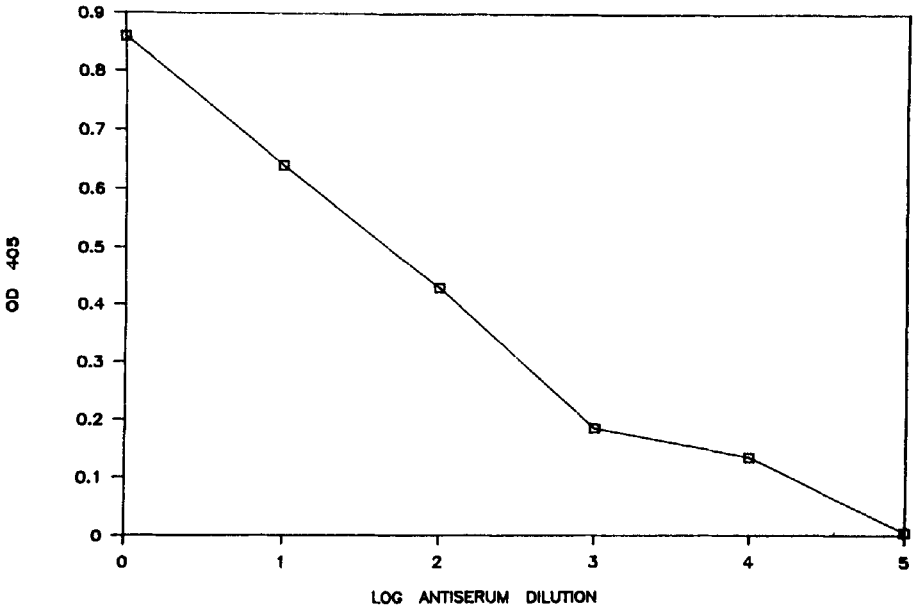

FIGURE 1

Dilution Curve of hTg-anti-hTg IC Formation on Polystyrene Support as Measured by ELISA.

TABLE 1

Characteristics of Various Dissociants

Dissociant	hTg Residual binding (%)	hTg Residual antigenicity (%)	hTg-anti-hTg dissociation (%)
Citrate buffer Ø.2N pH 2.2	95	93	53
MgCl 7M	97	95	4
Acetic acid Ø.5N	96	1Ø8	76
KI 4M	78	97	13

most efficient agnet exerting 76% dissociation and was, therefore, selected for further characterization.

IC Dissociation by Acetic Acid

Dissociation by acetic acid was dose dependent reaching a plateau of 95% at 1N concentration (Fig. 2). This highly efficient dissociation was achieved with a negligible effect on hTg adsorption to the polystyrene or on hTg antigenicity (Table 2).

DISCUSSION

The current practice in selecting the optimal eluent for immunoaffinity chromatography may involve the testing of many solutions (1). The monitoring of eluent efficiency in

TABLE 2

Effect of Acetic Acid on Polystyrene Bound hTg.

Concentration (N)	Residual binding (%)	Residual antigenicity (%)
Ø.25	95	111
Ø.5	98	92
Ø.1	93	110
2.Ø	97	95

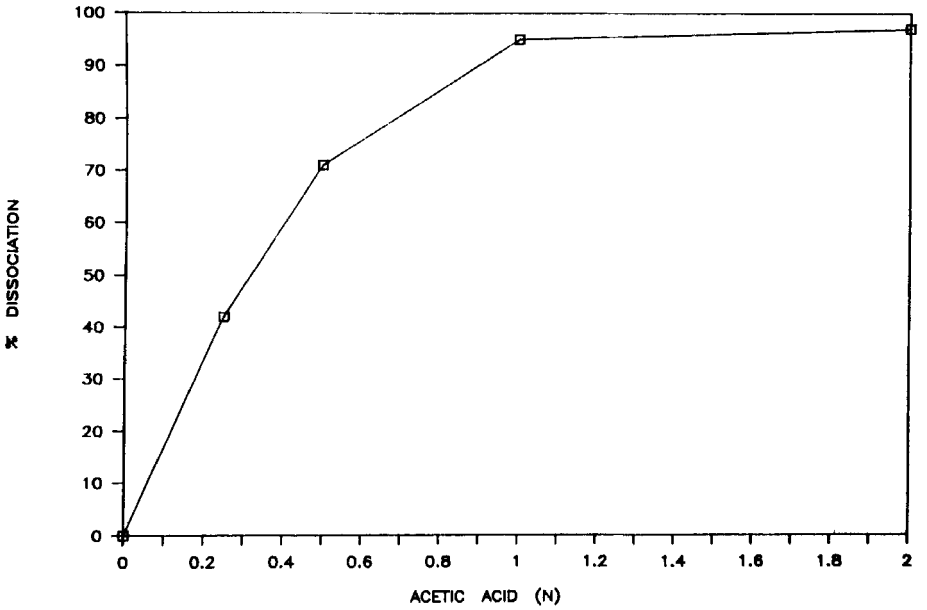

FIGURE 2

Dose Response Dissociation of hTg-anti-hTg IC by Acetic Acid.

standard chromatography (e.g. column chromatography) renders the procedure laborious and time consuming (2).

The method presented here is rapid, convenient and economical. It involves IC generation in immobilized form on microplates enabling the monitoring of dissociation by ELISA. This microtiter method allows simultaneous screening of several agents and, moreover, the residual immunogenicity is readily evaluated.

To demonstrate the feasibility of the method, hTg-anti-hTg IC served as a model in which eluting conditions were optimized. The first step involved the screening of potential solutions followed by dose titration of the selected eluent to be used in standard immunoaffinity systems. The residual function of the antigen rather than the antibody was tested in this study. Testing the latter could easily be accomplished in a reverse fashion by using a solid phase bound antibody.

Our method is probably applicable not only to ICs, but to other ligand systems as well, including receptors (11), enzymes (12) and lectins (13).

REFERENCES

1) Hoffman-Ostenhof, O., Breitenbach, M., Koller, F., Kraft, D., and Scheiner, O., Affinity Chromatography: "Biospecific Sorption," Pergamon Press, Oxford, 1978.

2) Janatova, J., and Gobel, R.J., Rapid Optimization of Immunoadsorbent Characteristics, Biochem. J., 221, 113, 1984.

3) Van Herle, A.J., Uller, R.P., Mattews, N.L., and Brown, J., Radioimmunoassay for Measurement of Thyroglobulin in Human Serum, J. Clin. Invest., 52, 1320, 1973.

4) Schneider, A.B., and Pervos, R., Radioimmunoassay of Human Thyroglobulin: Effect of Antithyroglobulin Autoantibodies, J. Clin. Endocrinol. Metab., 47, 126, 1978.

5) Laemmli, U.K., Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T_4., Nature, 227, 680, 1970.

6) Ratcliffe, J.G., Ayoub, L.A.W., and Pearson, D., The Measurement of Serum Thyroglobulin in the Presence of Thyroglobulin Antibodies, Clin. Endocrinol., 15, 507, 1981.

7) Van Eijk, H.G., and Noort, W.L., Isolation of Rat Tranferrin Using CNBr Activated Sepharose 4B, Chem. Clin. Biochem., 14, 475, 1976.

8) Mains, R.E., and Eipper, B.A., Biosynthesis of Adrenocorticotropic Hormone in Mouse Pituitary Tumor Cells, J. Biol. Chem., 251, 4115, 1976.

9) Chenais, F., Virella, G., and Patrick, C.C., Isolation of Soluble Immune Complexes by Affinity Chromatography Using Staphylococcal Protein A Sepharose as Substrate, J. Immunol. Methods, 18, 183, 1977.

10) Avrameas, S., and Ternynck, T., Use of Iodide Salts in the Isolation of Antibodies and the Dissociation of Specific Immune Precipitates, Biochem. J., 102, 37C, 1967.

11) Lindstrom, J., Einarson, B., and Tzartos, S., Acetylcholine Receptor from Torpedo and Electrophorus have Similar Subunit Structure. Biochemistry, 19, 4791, 1980c.

12) Kaufman, B.T., and Pierce, J.V., Purification of Dihydrofolic Reductase from Chicken Liver by Affinity Chromatography, Biochem. Biophys. Res. Commun., 44, 608, 1971.

13) Van Etten, R.L., and Saini, M.S., Preparation of Homogenous Human Prostatic Acid Phosphatase Using Concanavalin A Sepharose 4B, Biochim. Biophys. Acta, 484, 487, 1977.