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Use of monoclonal antibodies for weak affinity chromatography

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Abstract

Weak monoclonal antibodies were used as ligands in a high-performance liquid affinity chromatography system. Isocratic weak affinity chromatography was achieved when similar carbohydrate antigens were separated according to their weak binding to the immobilized monoclonal antibody. These chromatographic systems were studied in detail in terms of affinity, specificity and efficiency. The influence of the physico-chemical factors of temperature, pH, ionic strength and organic solvents was also evaluated. The issue of specificity was specially considered, as non-specific interactions are prevalent and usually of a weak affinity nature. This study clearly demonstrates the potential to use weak affinity biological interactions as the basis of chromatographic analysis and separation.

Keywords: Affinity chromatography; Stationary phases, LC; Monoclonal antibodies; Antigens; Carbohydrates

1. Introduction

The processes whereby biomolecules interact and form various forms of molecular entities are fundamental to any biological system, whether they relate to intercellular or subcellular activities. These processes are complex and there is a need for a more detailed understanding of them.

The current model used to interpret complex biological interactions is usually rather simplified and is frequently considered only under high affinity (avidity) conditions and at equilibrium. However, there is increasing evidence that many, if not most, recognition phenomena are driven by a multitude of weak interactions (approximately $K<10^5\ M^{-1}$), where the kinetics and the multiplicity of weak bindings rather than the equilibrium state determine the final outcome of the reaction. One recent example is the study on T-cell receptor recognition where it has been suggested that the T-cell receptor shows weak interactions toward its ligand, the peptide–MHC (major histocompatibility complex) complex on the antigen-presenting cell [1].

In recent years, we introduced the concept of using weakly interacting biomolecules as the basis for chromatographic separation: weak affinity chromatography (WAC) [2–6]. The use of weak interactions as the basis for ligand–ligate interactions has changed affinity chromatography from being basically an adsorption/desorption technique to a truly chromatographic procedure. During this process, several reports have indicated [7–9] that a new tool based on WAC has emerged for efficient characterization and exploitation of weak biological interactions.

The purpose of this paper is to provide a solid

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base for WAC using immobilized weak monoclonal antibodies as a model for affinity chromatography. A weak antibody–antigen system [10] was selected using monoclonal antibodies directed towards specified carbohydrate antigens.

2. Materials and methods

2.1. Chemicals

Glucose (Glc), isomaltose (Glcα1–6Glc), cellobiose (Glcβ1–4Glc), maltose (Glcα1–4Glc), maltotriose (Glcα1–4Glcα1–4Glc), maltotetraose (Glcα1–4Glcα1–4Glcα1–4Glc), panose (Glcα1–6Glcα1–4Glc), *p*-nitrophenyl (PNP) and *o*-nitrophenyl (ONP) glucopyranoside and PNP–maltoside were obtained from Sigma (St. Louis, MO, USA). All carbohydrates were in the D-configuration. $(Glc)_4$ (Glcα1–6Glcα1–4Glcα1–4Glc) was purified from urine as previously described [11]. $(Glc)_4$ was conjugated to bovine serum albumin (BSA) according to Zopf et al. [12]. All chemicals were of analytical grade and were acquired from commercial sources.

2.2. Monoclonal antibodies

Hybridomas producing murine monoclonal antibodies, designated 39.5 (IgG2b) and 61.1 (IgG3), were developed as described earlier by Lundblad et al. [10]. The monoclonal antibodies were produced in vitro by growing cells in 3-l stir-flasks for 12–14 days in Dulbecco's modified Eagle's medium supplemented with 10% bovine serum (Fetalclone 1), non-essential amino acids (all from HyClone Labs., Logan, UT, USA) and penicillin–streptomycin (Biochrom, Berlin, Germany). The monoclonal antibodies were purified by protein A chromatography (protein A–Sepharose CL 4B, Pharmacia Biotech, Uppsala, Sweden) [13]. The isolated antibodies were essentially pure (>95%) as was evidenced by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), both reduced and non-reduced, and by capillary zone electrophoresis (CZE). A minor contamination of <5%, originating from bovine IgG in the cell culture medium, is expected as it is partly eluted during protein A chromatography. The antigen-binding activity of the antibodies was determined by enzyme linked immunosorbent assay (ELISA, see below). More than 90% of the activity was retained through the purification steps of the antibodies.

2.3. Elisa of monoclonal antibodies

The ELISA assay of 39.5 and 61.1 was carried out essentially as described by Kumlien et al [14]. Briefly, 96-well microtiter plates (Greiner, Frickenhausen, Germany) were coated with $(Glc)_4$–BSA (0.1 μg/well). Samples and controls were incubated overnight (16 h) at 4°C. The plate was washed and incubated with peroxidase-labelled rabbit anti-mouse Ig (Dako, Copenhagen, Denmark). Substrate [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)] and hydrogen peroxide were added to each well and antibody activity was measured by following the enzyme reaction at 405 nm in a Titertek multiscan spectrophotometer (Labsystems, Helsinki, Finland).

2.4. Preparation of the WAC column

Microparticulate silica (10 μm, 300 Å, Macherey-Nagel, Düren, Germany) was silanized with glycidoxypropyltrimethoxysilane (Hüls, Marl, Germany) according to Ohlson et al. [15] to a substitution level of 454 μmol of diol groups/g of silica. The diol-derivatized silica was converted to aldehyde-silica by periodate oxidation [15], which was then promptly used for coupling of the antibodies. Aldehyde–silica (1.1 g) was mixed with 120 mg of antibodies in 17 ml of 0.1 *M* sodium citrate and 0.02 *M* sodium phosphate buffer, pH 6.0. Panose (Glcα1–6Glcα1–4Glc), which is reversibly bound only by the antibodies, was added at a concentration of 7 mg/ml to protect the binding site from coupling to the matrix. Sodium cyanoborohydride was added (5 mg/ml) and the reaction was allowed to continue for 40 h. The antibody silica was then thoroughly washed with 0.5 *M* sodium chloride to remove non-reacted antibodies. The antibody silica was packed in 10×0.5 cm HPLC stainless steel columns with an air-driven liquid pump (Haskel, Burbank, CA, USA) at 300 bar using 0.1 *M* sodium phosphate, pH 6.0, as both slurry and packing solvent. A reference column prepared in an

analogous manner but omitting the antibodies was used for comparison.

2.5. Chromatography

WAC was carried out using a Varian HPLC system (Varian Associates, Walnut Creek, CA, USA) equipped with both a variable UV–Vis detector as well as with a pulsed amperometric detection (PAD) system (ED40, Dionex, Sunnyvale, CA, USA). Tagged carbohydrate (PNP–maltoside, α or β, or PNP–glucopyranoside, α or β) was detected at 300 nm. Non-tagged carbohydrates were detected with PAD, where sodium hydroxide (0.2 *M*) was added to the column eluent in a mixing tee at 1 ml/min to achieve a pH>12.0. All chromatography experiments were thermostatically conducted using a Spark Holland Mistral column oven (Emmen, Netherlands) that was set at selected temperatures. Handling of chromatographic data was performed with an EZchrom chromatography data system (Scientific Software, San Ramon, CA, USA). Unless otherwise noted, standard chromatography conditions were as follows: flow-rate, 1 ml/min; mobile phase, 0.02 *M* sodium phosphate; 0.1 *M* sodium sulphate, pH 6.0; injection volume, 20 μl; temperature, 30°C.

3. Results and discussion

3.1. Characterization of WAC columns

The weak monoclonal antibodies used in this study, i.e., 39.5 and 61.1, exhibit different weak binding activities towards the carbohydrate antigen [10]. In order to detect retention based on weak binding affinities, a maximum amount of monoclonal antibody was immobilized on the matrix. Based on UV measurements at 280 nm, both directly on the gel as well as indirectly by following the decrease in the amount of free antibody present in the supernatant, the amounts of immobilized antibody for 39.5 and 61.1 were 87 mg/g of silica (0.27 m*M*) and 100 mg/g of silica (0.31 m*M*), respectively. Based on the reasonable assumption that each antibody covers a square surface of 150×150 $Å^2$ and that 1 g of silica has a surface area of 105 m^2/g according to manufacturer's specifications, the amount of immobilized antibody corresponds to a high coverage [74% (39.5) and 85% (61.1)] of the available surface area. This is an estimate but it indicates that these affinity gels are carrying a close-to-maximum load of immobilized antibodies.

The monoclonal IgG antibodies are coupled through their lysine residues of the protein backbone onto the aldehyde groups of the silica gel. Thus, the antibodies are heterogeneously distributed on the surface of the silica, which will result in a number of inactive antibodies with no capacity to bind antigen. The oligosaccharide antigens used in this study are small (M_r<1000), which will allow them to penetrate with unrestricted diffusional access to most binding sites within the porous silica particle. To determine the amount of active immobilized antibodies, frontal affinity chromatography was performed. The weak affinity columns were saturated with antigens at different ligate concentrations [16] and an adsorption isotherm was constructed. Estimates of the total amount of active antibodies (Ab_{max}; mg of antibodies/g of silica) and affinity (K; M^{-1}) can thus be obtained. Ab_{max} was determined by frontal chromatography of PNP–α-maltose under standard conditions (see above). It was found that Ab_{max} was 42 mg/g for a 39.5 column and Ab_{max} was 60 mg/g for a 61.1 column. This corresponds to antigen binding activities of the calculated active sites retained of 48 and 60%, respectively, which is in accordance with earlier investigations [2]. It is possible that the presence of a high concentration of a binding carbohydrate protects the active site from coupling onto the matrix, thereby increasing the likelihood for higher retained activities of the antibodies.

3.2. Stability of WAC columns

An important consideration in weak affinity retardation chromatography is column stability as well as the ability to conserve the activity of the biological ligates during chromatography. When affinity columns are extensively used, their performance often diminishes. This is partly the result of loss of ligand through leakage or of inactivation of the ligand's capacity to bind ligate, e.g. by denaturation of an immobilized protein. In traditional high affinity

adsorption/desorption chromatography, where harsh elution conditions are used, significant losses in performance and capacity are often experienced, even after limited use. A distinct advantage of WAC is that conditions can be selected that maintain the integrity of the immobilized ligand as well as preserving the activity of the ligate. For example, in separations involving plasma proteins, physiological conditions can be utilized. Of special concern is the usage of temperature as a means for elution. For example, in terms of using antibodies as ligands, higher temperatures (above 40–45°C) should be avoided, due to possible irreversible denaturation. Tests on column stability of both 39.5 and 61.1 were performed by repeated injections of maltose under standard conditions (see above). After approximately 150 injections over a time period of three months, the estimated loss of retention measured as k' was marginal (<10%), indicating that the selected conditions did not exhibit any significant detrimental effects to the WAC column.

3.3. Isocratic separations on 39.5 and 61.1 WAC columns

Isocratic separations of various oligosaccharides listed in Table 1 are demonstrated in Fig. 1a,b, where the two immobilized monoclonal antibodies showed similar, but different, separation behaviour towards the carbohydrate antigens. The antigens are retarded differently, according to their weak affinity towards the immobilized antibody, which is unlike traditional high affinity chromatography where ligates are bound and eluted by changing the mobile phase conditions drastically. In this system, it should be possible to completely separate approximately five carbohydrate antigens within a reasonable time frame (up to $k'=10$). The performance (measured as plate numbers of retarded peaks) deteriorates rapidly at k' above approximately ten, resulting in poor separations (Table 1). It should be emphasized that the antigens are separated under conditions of a linear adsorption isotherm, resulting in maximum retention and non-skewed peaks. By studying two monoclonal antibodies with varying specificities towards carbohydrate antigens, we have been able to demonstrate the generality of using weak monoclonal antibody interactions in affinity chromatography. This further substantiates earlier, preliminary findings of the use of immobilized 39.5 for WAC (see Ref. [3]).

Table 1
Capacity factors (k') and dissociation constants (K_{diss}) for carbohydrate antigens on 39.5 and 61.1 WAC columns

Carbohydrate	Capacity factor (k')	K_{diss} (mM)
39.5		
Glucose	0.0	
Isomaltose	0.0	
Cellobiose	0.0	
α-Maltose	1.2	0.36
β-Maltose	5.8	0.07
α-Maltotriose	0.4	1.03
β-Maltotriose	0.9	0.50
α-Maltotetraose	0.4	1.03
β-Maltotetraose	0.4	1.03
α-Panose	9.1	0.05
β-Panose	n.d.	
PNP-α-glucopyranoside	0.1	
ONP-β-glucopyranoside	0.1	
PNP-α-maltoside	12.9	0.03
61.1		
Glucose	0.0	
Isomaltose	0.0	
Cellobiose	0.0	
α-Maltose	0.8	0.66
β-Maltose	5.8	0.09
α-Maltotriose	0.3	1.99
β-Maltotriose	0.3	1.99
α-Maltotetraose	0.1	3.65
β-Maltotetraose	0.3	1.57
α-Panose	4.9	0.11
β-Panose	41.9	0.01
PNP-α-glucopyranoside	0.1	
ONP-β-glucopyranoside	0.1	
PNP-α-maltoside	17.8	0.03

Injected amount: 1 μg. Temperature: 30°C. See Section 2 for chromatographic conditions. n.d.=not determined.

3.4. Dependency of physical and chemical parameters on weak affinity separation

We have shown earlier [17] that the monoclonal antibodies used in this study display a significant temperature dependence upon binding of their antigens. Binding strength is increased up to an order of magnitude per 10°C decrease in temperature, which suggests that the use of temperature can be a useful means of elution in WAC. This is demonstrated in Fig. 2a,b, where the retention of the

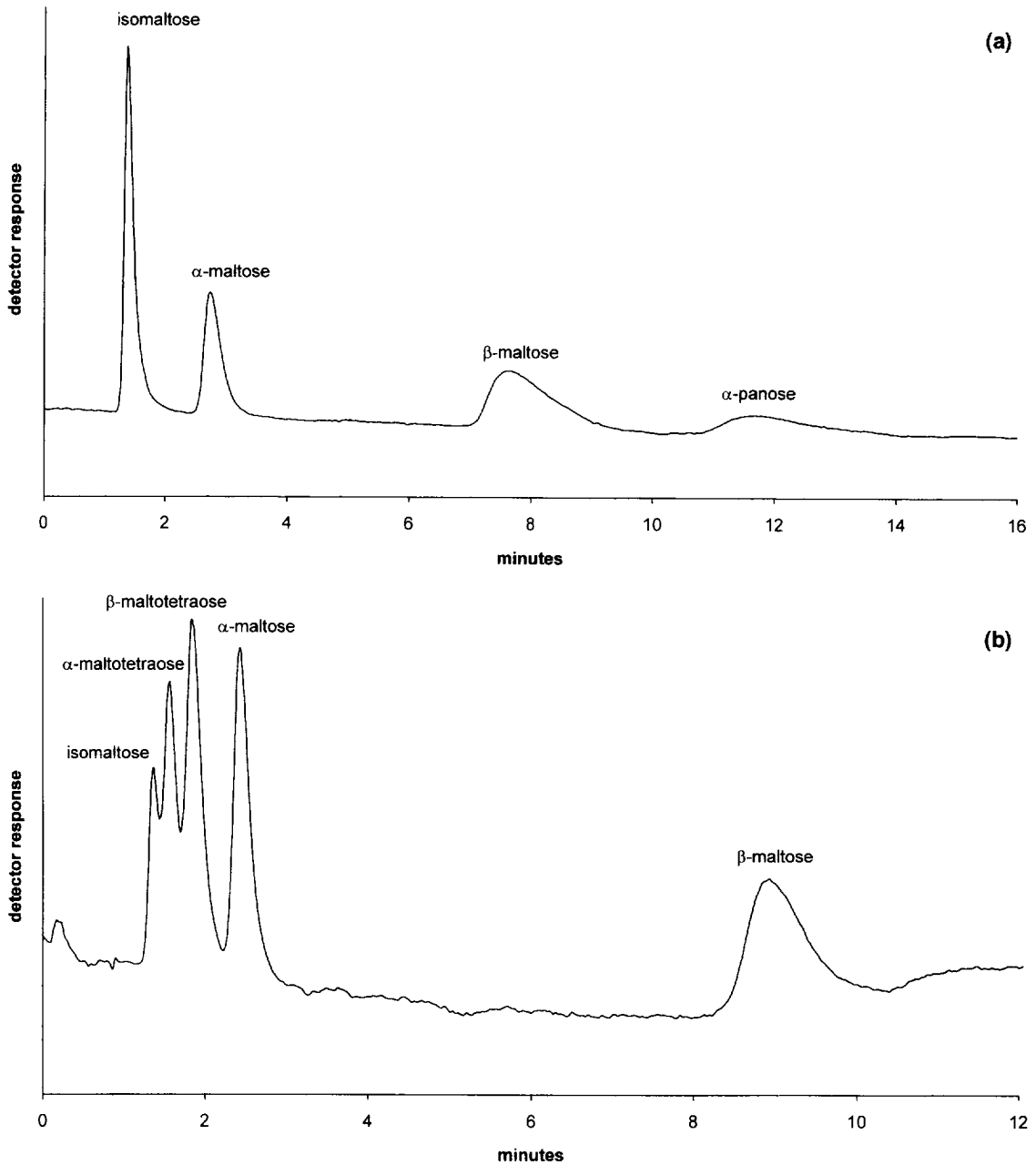

Fig. 1. Isocratic separation of carbohydrate antigens on (a) 39.5 and (b) 61.1 WAC columns. Injected amount=1 μg. Temperature=30°C. See Section 2 for chromatographic conditions.

anomeric forms of maltose is shown as a function of temperature. By changing the temperature within the operational range for the antibodies from approximately 4 to 40°C, retention can be varied several-fold and separation can be optimized according to demands in efficiency and analysis time.

In addition, we have also investigated the chemical parameters of pH, ionic strength and organic

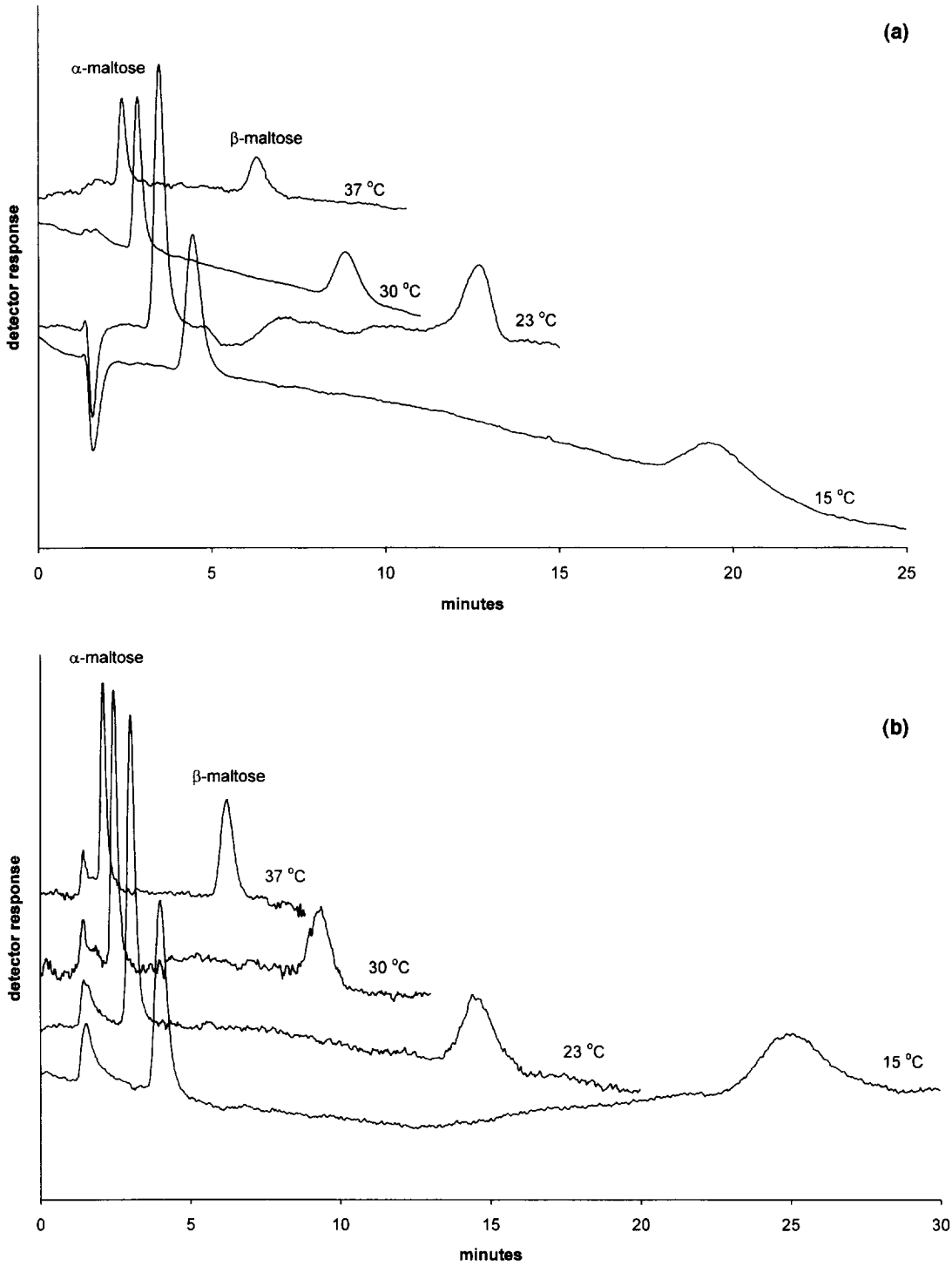

Fig. 2. Isocratic separation of the anomers of maltose at different temperatures on (a) 39.5 and (b) 61.1 WAC columns. Injected amount=0.1–0.2 μg. See Section 2 for chromatographic conditions.

solvents to see if retention can be modulated by changing the mobile phase conditions. As expected, due to the most likely presence of hydrogen bonding and Van der Waals interactions forming the interactions between the antibody binding site and carbohydrate antigen, alterations in pH influence the separation greatly, with a decrease in retention at lower pH (Fig. 3). Isocratic separations at different pH values with the two monoclonal antibodies show a similar pattern. Extremes of pH (pH<3, pH>8) should be avoided, as this has a deteriorating effect on the immobilized antibody, as well as on the support, thereby reducing their ability to bind carbohydrate antigen. The choice of pH can thus be used as an efficient tool to govern retention and should be carefully selected when finding optimum conditions for isocratic separations of various antigens.

The influence of ionic strength was also investigated (data not shown) by changing the sodium sulphate concentration in the mobile phase. When the anomers of maltose were chromatographed on 39.5 and 61.1 columns, a minor decrease (<10%) in k' was seen at higher ionic strength (<0.2 M) in the standard mobile phase (see above).

The effect of solvents, such as alcohols, in the mobile phase is of interest as they can interfere with any hydrophobic binding present between the ligate and the ligand as well as with the matrix. It should be noted that solvents have a denaturing effect on antibodies, both in solution as well as in the immobilized state, and can therefore only be used within a narrow range of concentrations. For example, by adding up to 2% (v/v) methanol to the standard mobile phase (see above), the loss of retention of PNP–α-maltose was small (<10%), indicating that hydrophobic interactions do not play any significant role even with PNP-substituted carbohydrate antigens. It is interesting to note that by tagging the maltose antigen with a PNP-group, retention is increased significantly (see Table 1), whereas PNP-tagged-glucose does not show different binding from the void marker glucose. It seems likely that the PNP group undergoes secondary interactions upon binding of the carbohydrate antigen to the immobilized antibody.

3.5. Studies on affinities between immobilized antibody and antigen in WAC

Earlier work [2] has indicated that the binding

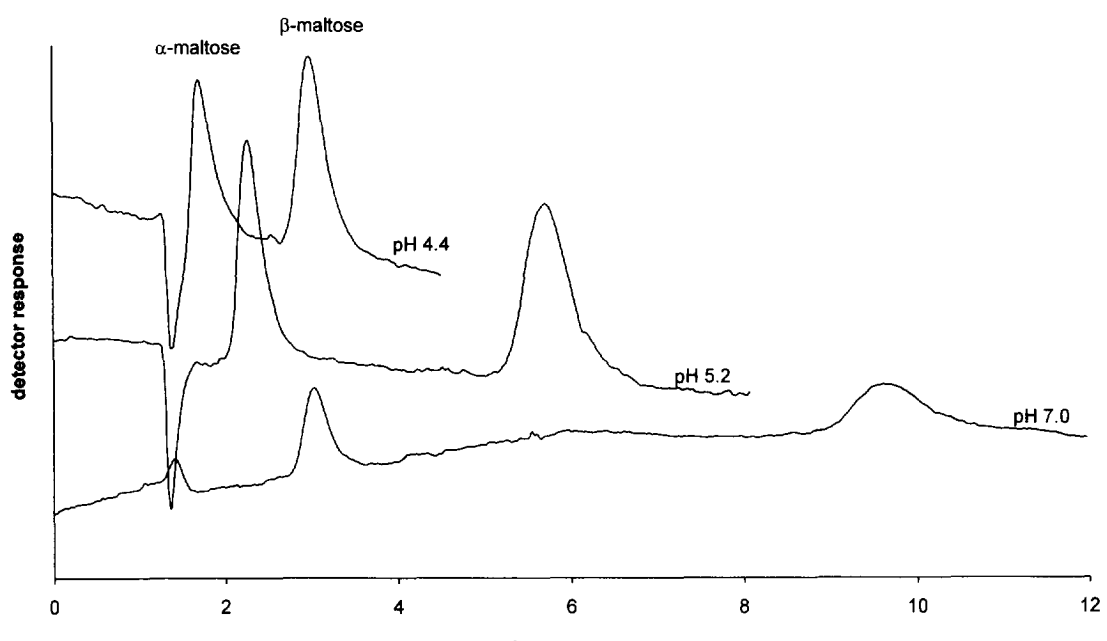

Fig. 3. Isocratic separation of the anomers of maltose at different pH values on a 39.5 WAC column. Injected amount=0.1–0.2 μg. Temperature=30°C. See Section 2 for chromatographic conditions.

between immobilized 39.5 and of the alditol of $(Glc)_4$ is weak. For example, at 37°C, K is $10^3\ M^{-1}$. In order to further substantiate previous data and to extend it to the immobilized 61.1 antibody, we obtained affinity data on a derivatized maltose antigen. A complicating factor is that most of the studied carbohydrate antigens exist in two anomeric forms as a result of mutarotation and these have differing affinities for the monoclonal antibody. Therefore, frontal affinity analysis [16] of these mixtures cannot be evaluated easily. Consequently, we opted to study the PNP–α-maltoside, which is a defined molecular entity and shows significant binding towards both 39.5 and 61.1. The binding constant, K, was determined by frontal affinity chromatography of PNP–α-maltoside by monitoring the absorbance at 300 nm (30°C) with standard mobile phase, as described above. K was determined to be $3.0 \cdot 10^4\ M^{-1}$ for 39.5 and $3.3 \cdot 10^4\ M^{-1}$ for 61.1. According to chromatographic theory [4], $k' = CQ_{max}K$ measured under conditions of a linear adsorption isotherm, which is the case when the concentration of ligate (c_o) is such that the product $Kc_o < 1$. Q_{max} is the maximum number of accessible ligand sites (moles/kg of matrix) and C is a constant (kg of matrix/l of mobile phase) and reflects column characteristics of e.g. void fraction, porosity and density of the stationary phase. Based on frontal analysis data of K, as stated above, and zonal analysis data at conditions of linear adsorption for PNP–α-maltose, we can determine C to be 0.81 for the 39.5 column and 0.73 for the 61.1 column. These figures correlate well with the estimate of C to be 0.70 based on the physical characteristics of the HPLC column. Table 1 gives a summary of the calculation of affinities for various carbohydrate antigens. It is reasonably assumed that K is proportional to k' where C and Q_{max} are constant for the various antigens. In addition, the antigens are run under linear adsorption conditions, as evidenced by achieving constant maximum retention when decreasing amounts are injected. As shown in Table 1, the carbohydrate antigens were retarded under weak affinities within two orders of magnitude from $K = 2.7 \cdot 10^2$ to $3.3 \cdot 10^4\ M^{-1}$.

3.6. Specificity and efficiency of WAC

The potential of WAC to separate ligates is mainly dependent upon (i) its ability to specifically bind the ligates of interest from irrelevant substances present, e.g. in a crude extract of serum or cell culture supernatant and (ii) on its ability to separate ligates according to their weak binding strength towards the immobilized ligand. Specificity is a major concern as weak interactions between ligand and ligate can be more difficult to distinguish from non-specific interactions present, e.g. between solutes and the support with the immobilized ligand. Non-specific interactions per se are frequently weak and will e.g. obscure the non-retarded void fraction. However, sufficient specificity in a WAC system is usually created by parallel or serial binding events between ligand and ligate. In parallel systems, a cooperativity effect is seen where the ligate can weakly interact simultaneously with several ligand sites. In serial systems, multiple one-to-one weak binding events are seen between the ligate and neighbouring ligand molecules. This is very similar to the design that biological systems develop to fine-tune their specificity in determining the right outcome of a biological reaction. The distinction between weak specific reactions from non-specific ones is a matter of quantitative consideration, where the amount of specific or desired reactions versus the amount of non-desired binding events will determine the specificity of the system. In practical terms, this means that in affinity chromatography, all non-specific binding reactions, such as binding of ligate to matrix, have to be minimized, while maintaining an adequate number of specific binding events between ligand and ligate.

In practical work using WAC, the ability to detect a weakly binding ligate in a crude environment is typically beyond a k' of ca. >0.5, where the non-retarded void fraction has been completely eluted. For example, as shown in Table 1, the anomers of maltotetraose with binding constants in the range of $K = 10^2 - 10^3\ M^{-1}$ represent a borderline case, where they are only partly resolved from non-interacting carbohydrates.

Another related issue is that of the concise definition of specificity in affinity chromatography. This is a matter of concern in WAC as we are taking advantage of desired weak biological interactions to promote separations. We are of the opinion that in order to fully demonstrate specificity in affinity chromatography, the ultimate reference system

would be the affinity support itself, where the specific binding sites are non-reactive. In practical terms, this is hard to accomplish for many reasons and we are usually referred to the use of other reference systems, such as irrelevant, but similar, adsorbents or the use of non-binding substances that are similar to the ligates. As the issue of specificity is of crucial importance in WAC, we have examined specificity extensively by running reference columns with no or irrelevant antibodies attached to the support and/or by running 39.5- and 61.1 columns with similar, but non-binding, carbohydrates. In addition, we denatured immobilized 39.5 monoclonal antibodies at high (>10) and low (<2) pH values (data not shown). No non-specific binding could be detected in any of these reference systems. In addition, to conclude this important issue, we were able to show specificity in a series of experiments where PNP–α-maltoside was able to pass unretarded through a 39.5 column when high amounts of maltose (10 mg/ml) were added to the mobile phase (as a blocker of the binding site of the antibody). Given the fact that high amounts of glucose did not have any appreciable effect on the binding of PNP–α-maltoside, this indicates that PNP–α-maltoside shows preferential binding to the antibody binding site. Taken together, these experiments clearly demonstrate that we are exploiting specific weak interactions between the antibody binding site and the antigen and the presence of any non-specific interactions between antigen and antibody/support is negligible. It is important to re-emphasize that in the set-up of a potential WAC system, the issue of specificity should be considered carefully.

Apart from creating sufficient specificity as discussed above, a crucial issue of WAC is what efficiency can be realized. Earlier work (on computer simulation of WAC [18]) studying antibody–antigen interactions has indicated that a peak capacity of approximately ten can be achieved within a reasonable separation time frame of 1 h. Evaluations on chromatographic performance, e.g. in terms of efficiency, are not used in traditional high-affinity chromatography, as this technique is not chromatography as it was originally defined. On the contrary, traditional high-affinity chromatography is rather an adsorption/desorption procedure in a column format. In WAC, however, any considerations of efficiency are relevant since we are dealing with a truly chromatographic system, where ligates are differentially retarded based on their interaction with the immobilized ligand. Efficiency, expressed as number of plates (N) or plate height (H), where $H=L/N$ (L=length of column), is an adequate way to express efficiency in WAC. Approximations of the calculation of N, such as $N=16(t_R^2/t_w^2)$; t_w=the width of the baseline formed by the tangents of the peak intersecting the baseline, are based on the assumption that symmetrical Gaussian peaks are obtained. As this is often not the case due to, e.g., fronting or tailing of peaks, these approximations will give an overestimation of chromatographic efficiency and should be avoided. Therefore, it is more accurate to calculate N according to $N=t_R^2/\delta^2$, where t_R is the retention time (s) expressed as the first central moment [$t_R=\int c(L,t)t\mathrm{d}t/\int c(L,t)\mathrm{d}t$; $c(L,t)$=ligate concentration at t (s) and $L(M)$] and δ^2 is the variance expressed as the second central moment [$\delta^2=\int c(L,t)(t-t_R)^2\mathrm{d}t/\int c(L,t)\mathrm{d}t$)] [19,20]. Based on these models using central moments, studies on the 39.5 column indicate a plate efficiency of N=1200–2000 plates/m for unretarded carbohydrates such as glucose, isomaltose and lactose, which corresponds well with non-sorbed tracers such as ^2H_2O (N=1500 plates/m). It is interesting to note that e.g. α-maltose at N=4500 plates/m shows better performance than the non-retarded peaks, which is due to the absence of tailing compared with tested void substances. When performing affinity chromatography at higher affinities ($K \gg 10^4\ M^{-1}$) with, e.g., PNP–β-maltoside, the performance deteriorates rapidly (N=200 plates/m) and efficient chromatography cannot be obtained. The figures on plate numbers given in this work are generally less than previously reported [2], which reflect our more accurate calculation model that takes into account any skewing of peaks appearing in the chromatogram. The maximum achievable performance in WAC is dependent on the proper design of the matrix as well as on minimization of the presence of extra-column effects such as dead volumes in injection and detection flow tubes. In our studies, we have utilized state-of-the-art HPLAC technology with small sized (d_p=10 μm) microparticulate silica as the support matrix. It is our belief that further optimization of the design of this chromatography system in terms of selection of support, immobilization procedures and ligand design can enhance the overall performance.

4. Conclusions

A basic understanding of weak affinity interactions can be extremely valuable for explaining complex biochemical events. By setting up procedures in the laboratory to study weak biological interactions, tools are provided for the researcher to more adequately evaluate these systems. This work has demonstrated how WAC can be used for the separation of small antigens using immobilized monoclonal antibodies. We feel certain that, on the basis of this work, WAC will fill a role in the identification, separation and characterization of the components of biological weak affinity reactions, which hopefully will lead to a better knowledge of biological processes. The technology can be useful, not only for studies of binding behaviour of weak ligand–ligate pairs, but also for the isolation of presently unknown weak binding receptors. For example, the technology may offer a means to define weak molecular entities on cells that are responsible for communication with other cells or with the surrounding extracellular environment. This can also be of value in drug design, where formulations can be defined based on their weak interactions with the target.

This work has further demonstrated the use of generic ligands of weak monoclonal antibodies for the separation and analysis of similar ligands. We are convinced that this technology of WAC, using monoclonal antibodies as ligands, can be further extended into other types of ligands, such as IgM, that show weak interactions towards various ligates. Of special interest will be the application of the technology for the analysis and separation of larger biological molecules of polysaccharides, proteins and nucleic acids. Results from this study have indicated that the technique can be further refined, both in terms of specificity and efficiency, leading to new avenues for the analysis and separation of biomolecules.

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