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Effect of the detergent Tween-20 on the DNA affinity chromatography of Gal4, C/EBPα, and lac repressor with observations on column regeneration

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Abstract

C/EBPα, Gal4, and lac repressor, representing three different transcription factor homology families, were expressed as fusion proteins and used to characterize the effects of column aging, Mg^{2+}, the nonionic detergent Tween-20, column loading, and bovine serum albumin on DNA-affinity chromatography. When lac-repressor-β-galactosidase fusion protein is loaded onto a new DNA–Sepharose column, less elutes from a new column than one that has been used two or more times. Higher amounts of lac repressor, the Green Fluorescent Protein fusions with CAAT enhancer binding protein (C/EBPα) and Gal4, elute from the columns when 0.1% Tween-20 is added to the mobile phase. The amount of improvement found depends upon the transcription factor studied and the amount of the protein loaded on the column; lac repressor and Gal4 are eluted in higher amounts over a large range of protein loads while C/EBP shows the greatest effect at low protein loads. This detergent effect is seen when either Sepharose or silica is used for the stationary phase. Including bovine serum albumin in the mobile phase gives a similar though lesser improvement to that observed with Tween-20. Mg^{2+} or EDTA in the mobile phase gave similar chromatography for C/EBP; since EDTA protects columns from DNases, its inclusion in the mobile phase is preferred. After extended use, the DNA affinity columns no longer bind transcription factors and this is not due to losses of DNA from the columns. Two simple methods (sodium dodecylsulfate and KSCN) were developed to regenerate such worn out columns.

Keywords: Transcription factors; Affinity chromatography; Tween-20; Surfactants; DNA; Proteins

1. Introduction

DNA affinity chromatography has been used extensively in the purification of transcription factors [1] and other DNA-binding proteins [2]. A common practice in protein purification is to add low amounts of non-ionic detergents and "carrier" proteins to the mobile phase to increase protein stability and recovery. However, the effect of these additives on the purification of multiple transcription factors has never been characterized. It is known from other studies that detergents can dramatically affect DNA binding. For example, mammary gland factor (MGF) bound DNA only poorly in the absence of 0.1% Nonidet p-40, a non-ionic detergent [3]. The purification of c-Rel-p65 required the inclusion of both deoxycholic acid (DOC) and 3-[(cholamidopropyl)dimethylammonio]-propanesulfonate (Chaps), an anionic and zwitterionic detergent, respectively, presumably because these dissociated factors which inhibit DNA binding [4].

Similarly, high concentrations of bovine serum albumin (BSA) or other proteins added during purification can also dramatically increase the yield of active protein. In the purification of cyclic AMP response element binding protein (CREB) and methylated DNA-binding protein (MDBP), adding BSA to concentrations as high as 5 mg/ml increased the yield of active protein from 5- to 20-fold. Interestingly, the addition of a non-ionic detergent increased the yield of CREB but not MDBP [5]. Effects of detergents and carrier proteins on DNA-binding have also been observed in electrophoretic mobility shift assays.

Proteins or detergents could improve recovery by several routes [6,7]. Surfaces in the chromatography (or electrophoresis) apparatus may non-specifically bind small amounts of protein and as proteins become more highly

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purified, this absorption becomes a significant portion of the total. These reagents may also prevent inhibitory interactions with other proteins [3,4,8]. Finally, the natural environment in the cell is protein rich and these conditions may be necessary for the stability of some proteins [7].

A related issue is column lifetime. As DNA affinity columns are used repeatedly, they often lose binding capacity. This may be a related phenomenon since non-specific adsorption or the buildup of binding inhibitors may be adversely affecting column performance.

Here, we have compared the behavior of three different transcription factors—Gal4 (from *Saccharomyces*), C/EBPα (from rat liver), and lac repressor (from *Esherichia coli*) as various additions are made to the mobile phase and as columns age. Each protein was expressed in bacteria as a chimeric fusion protein. Interestingly, each protein and column showed very individual behavior. Detergents and proteins were helpful in some cases but less so for others, column lifetimes also decreased very rapidly in some cases and new strategies had to be developed to regenerate failing columns.

2. Methods

CNBr-preactivated Sepharose was obtained from Sigma (St. Louis, MO, USA). Glycidioloxypropyl-silica (Macrosphere GPC 300 Å, 7 μm) was obtained from Alltech Associates (Deerfield, IL, USA) and was activated with CNBr as previously described [9,10]. Triton X114 and Tween-20 were from Bio-Rad Labs. (Richmond, CA, USA). Igepal-CA630 and deoxycholic acid were from Sigma. All other reagents were of the highest quality commercially available.

2.1. Chromatographic supports

Oligonucleotides were obtained from Integrative DNA Technologies (Coralville, IA, USA). After dissolving in 0.3 ml of TE (10 mM Tris, 1 mM EDTA, pH 7.5), 30 μl of 3 M sodium acetate and 1 ml ethanol were added and the tubes placed in a −85 °C freezer for 1 h. The tubes were then centrifuged at 14,000 × *g* for 10 min., the supernatant aspirated away, and the pellets were washed with 0.5 ml 70% ethanol. The pellets were redissolved in a small volume of TE, the absorption at 260 nm determined, and the concentration adjusted to 1 mM. Just prior to use, the amount required for coupling (50 nmole DNA per ml of support) was again ethanol precipitated and dissolved in coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3). CNBr-preactivated Sepharose was prepared for coupling by swelling for 30 min in 100 ml ice cold 1 mM HCl, filtering, and washing twice with 100 ml ice cold 1 mM HCl. The semi-dry Sepharose was then added directly to the oligonucleotide in coupling buffer and sufficient additional coupling buffer was added to produce an approximate 1:1 slurry. This was mixed on a wheel rotator overnight in a 4 °C cold room. The next day, the support was filtered and washed with blocking buffer (0.1 M Tris, 0.5 M NaCl, pH 8) carefully saving the filtrate. The absorption at 260 nm of the filtrate was used to determine the amount of oligonucleotide that did not couple and, by difference, the amount coupled. After spending overnight in blocking buffer, the support was washed into TE containing 10 mM NaN_3 and stored in the refrigerator until needed.

For preparing the lac operator column, αOp1T6 oligonucleotide (5′-NH_2-ethyl-$(T)_6$AATTGTTATCCGCTCACAATTCCAC) was coupled using 50 nmol/ml of Sepharose of which 22 nmol/ml coupled. A 1 ml plastic syringe column was packed in TE0.1 (10 mM Tris, 1 mM EDTA, 0.1 M NaCl, pH 7.5) and made double stranded by flowing 1 ml TE0.1 containing 33 nmol of the Op1A6 (5′-GTGGAATTGTGAGCGGATAACAATT$(A)_6$) oligonucleotide through the column at 0.1 ml/min. Twenty one nanomole annealed to the column. Similarly, for Gal4 chromatography, 50 nmol/ml GAL-U oligonucleotide (5′-NH_2-ethyl-CGGAGGACAGTCCTCCGG) was coupled yielding 38 nmol/ml Sepharose and was annealed with 57 nmol GAL-D (5′-CGGAGGACTGTCCTCCGG) yielding 28.3 nmol/ml annealed strand. For C/EBP, the self-complimentary oligonucleotide EP18 (5′-NH_2-ethyl-GCAGATTGCGCAATCTGC) was coupled at 50 nmol/ml Sepharose yielding 20.8 nmol/ml DNA–Sepharose. Other columns were also prepared as described in the figure legends.

The lac repressor DNA-silica was prepared as previously described [9,10] and contained 16 nmol duplex DNA per gram silica. Fifty milligrams slurry was packed into a 2 mm × 21 mm stainless steel HPLC column for chromatography.

2.2. Proteins

The lac-repressor-β-galactosidase fusion protein was produced in bacteria as previously described [11] and used as a crude bacterial extract dialysed into TE0.1. The green fluorescent protein (GFP)-C/EBPα protein was produced and affinity purified as described previously [12].

The GFP-Gal4 protein was produced in *Escherichia coli* BL21 DE3 and affinity purified as previously described [13].

3. Results

Fig. 1 illustrates a phenomenon we have observed with different columns and several different transcription factors, in this case the lac repressor. When a new column is first used, less lac repressor elutes than is observed on the second and subsequent uses. In this case, the first use gave only 69% of that found for the second (Fig. 1) and subsequent uses (data not shown). This same effect was also found for the lac repressor and Gal4 (data not shown). This effect was investigated further (data not shown). When columns went

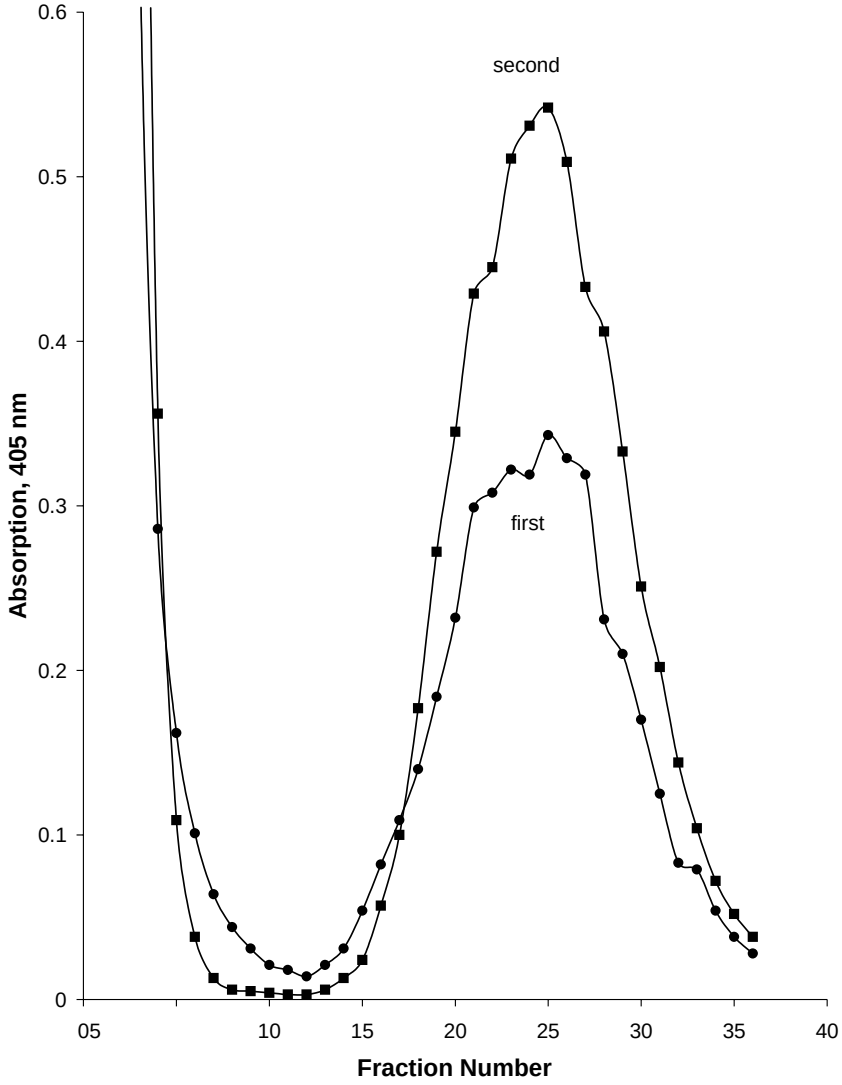

Fig. 1. Initial usage of new columns gives reduced yield of lac repressor. A 1 ml syringe column of the Op1–DNA–Sepharose column was prepared and washed with 20 ml of TE0.1 prior to loading 100 μl of the crude bacterial extract containing the β-galactosidase-lac repressor fusion protein. The column was then eluted with a linear gradient from TE0.1 to TE1.2 (10 mM Tris, 1 mM EDTA, 1.2 M NaCl, pH 7.5) over 50 min followed by 10 min of constant TE1.2. The flow rate was 0.3 ml/min throughout, 0.5 ml fractions collected, and temperature was 4 °C. After re-equilibrating the column with 20 ml TE0.1, the experiment was repeated a second time. For each fraction, 50 μl was assayed for β-galactosidase activity using the *O*-nitrophenyl-β-D-galactopyranoside substrate as previously described [11]. Absorption at 405 nm measures β-galactosidase activity.

unused or were washed extensively, the first use also gave lower elution amounts. We also found that washing new columns briefly with 10 μg/ml bovine serum albumin prior to first use greatly alleviated this effect. A likely explanation of these results is that new columns bind some fraction of protein non-specifically, but that this binding is saturable and BSA can saturate the new columns.

Detergents wet surfaces and decrease protein binding, as well as having other effects. As shown in Fig. 2, when different detergents were added to the electrophoretic mobility shift assay for GFP-C/EBPα, Tween-20, Triton X-100, and Igepal CA-630 (formerly known as NP-40) enhance the amount of the shifted DNA-protein complex while deoxycholic acid and sodium dodecyl sulfate diminish binding. Virtually identical results are obtained when the C/EBPα from rat liver is used in place of the fusion protein (R.A. Moxley, unpublished data). Tween-20 is a non-ionic detergent available in high purity and commonly available in most laboratories. Since it functions as well as any of the tested detergents in promoting DNA-protein complex formation in Fig. 2, we used it for further experiments.

Fig. 3A illustrates both the effect of Tween-20 on the chromatography and a result of prolonged column usage. The same DNA–Sepharose column as that used in Fig. 1 was used for this experiment after it had already been used more than 20 times for other experiments. To eliminate the "new column" effect (Fig. 1), chromatography of lac repressor was performed three times in the absence of Tween-20 and the third run is shown in Fig. 3A. Only a small peak elutes in the absence of Tween-20. When 0.1% Tween-20

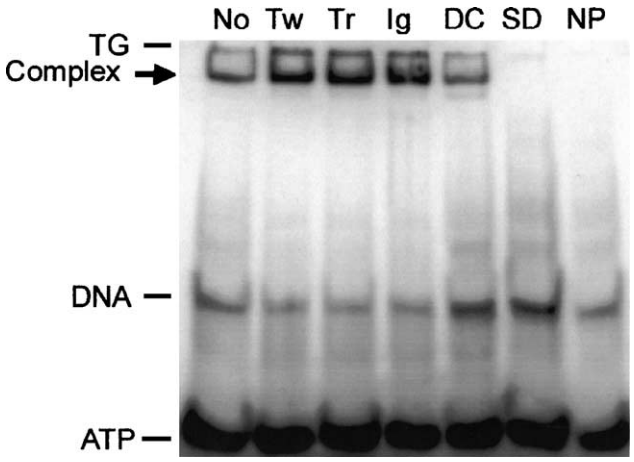

Fig. 2. Detergents affect the DNA binding of C/EBP. Shown is an electrophoretic mobility shift assay of the GFP-C/EBP fusion protein performed as previously described [12] except that the incubation buffer was TE0.1, 2.7 ng of the purified GFP-C/EBP, and 40 fmole of 5′ end labeled DNA was used. The figure shows the effect of including 0.1% of the detergents shown. Abbreviations used: No, no detergent used; Tw, Tween-20; Tr, Triton X-100; Ig, Igepal-CA630; DC, Deoxycholic acid; SD, SDS; NP, no GFP-C/EBP protein added. To the left is shown the position of the top of the gel (TG), the position of the shifted DNA-C/EBP complex (Complex), the unshifted DNA (DNA) and the position of γ-^{32}P-ATP left over from labeling.

is added to the mobile phase, there is a dramatic (a 6.8-fold increase in peak area) improvement and the eluted peak has the same retention time and much greater area as compared to the absence of Tween (Fig. 3A and B) or the first two uses shown in Fig. 1. As larger concentrations of Tween-20 (0.5 and 1%) are used, the peak is shifted to longer retention time and the peak area diminishes (relative to the 0.1% Tween peak, area is decreased to 64 and 39%, respectively). Thus, 0.1% Tween-20 gave the best chromatographic performance.

This effect of detergent is independent of the stationary phase. Recently, we developed a method for the coupling of DNA to silica using CNBr activation [9,10]. For the experiment in Fig. 3B, a small column was prepared by coupling the same DNA used in Fig. 3A to silica (instead of Sepharose) using the new CNBr chemistry. For comparison, an identical control column was constructed using the same CNBr activated silica to which no DNA was coupled. In the absence of DNA, little lac repressor binds. When the column contains DNA, lac repressor binds and elutes but over twice as much elutes when 0.1% Tween-20 is included in the mobile phase. These were the sixth and 11th runs of the column performed on the same day and so do not represent the “new column” effect shown in Fig. 1.

A related phenomenon to non-specific binding and detergent binding enhancement is the effect of protein load. To illustrate this, two other transcription factors, C/EBP and Gal4, were used. For C/EBP (Fig. 4A), when 0.73 μg of protein is loaded, Tween-20 increases the amount eluted 2.4-fold. As the load increases, the benefits of detergent become less pronounced and disappear at 6.6 μg. A somewhat different effect is seen with Gal4 (Fig. 4B). At a low load of 0.13 μg, 1.44-fold more elutes in the presence of Tween-20 as in its absence and virtually the same is seen at 3.5 μg where a 1.51-fold improvement is seen with Tween-20. Thus, while Tween-20 is most beneficial at low loads of C/EBP and less so at high loads, for Gal4 a fairly constant improvement is seen over a wide range of loads.

Since using BSA or Tween-20 can both improve the yield of eluted protein, they were compared in Fig. 5, again using a new column. Protein loaded without either shows only a small peak. Repeated injections would improve the amount eluted as shown in Fig. 1. Next, the same experiment was repeated with 10 μg/ml BSA in the mobile phase throughout. BSA gives a dramatic improvement in the amount eluted (Fig. 5) but at the cost of contaminating eluted proteins with BSA. In the third run on this column, 0.1% Tween-20 was included giving a further increase in the eluted protein recovered. Thus, Tween-20 gave the highest recovery and does so without adding extraneous proteins to the eluent.

In cells, DNA would be complexed with counterions, primarily Mg^{2+}. It is usually impractical to add Mg^{2+} to the mobile phase when purifying crude cellular extracts since DNA hydrolysing enzymes (DNases) would be activated by Mg^{2+} and would degrade the column DNA. However, by using purified fusion proteins we were able to investigate whether Mg^{2+} would improve chromatography. As shown in Fig. 6, chromatography in the presence of Mg^{2+} actually gave somewhat lower binding and recovery. Though the effect is not a large one, it was consistently found.

Another investigated feature of this chromatography was column lifetime and regeneration. We have noticed that some columns such as the ones used for the purification of C/EBP and lac repressor are quite robust and can be used, even with crude tissue extracts for many months before their performance degrades. In other cases, such as the Gal4 columns, we find that performance deteriorates very rapidly and after 10 or fewer uses, even with purified proteins the columns cease to function. It was possible that the conditions we were using depleted DNA from the columns to cause their failure. However, we found that the amount of DNA present in new columns (determined by hydrolysis with $Mg(NO_3)_2$ and measuring total PO_4 as described in [14]) does not significantly decrease during the course of column failure and this was true of each of the columns used in this study (data not shown). Thus, column failure most likely arises from the build-up of some interfering substance during chromatography and column cleaning might be a reasonable approach to regeneration.

The success of this approach for C/EBP is shown in Fig. 7. After using this column for over six months and 84 purifications of C/EBP, column performance had degraded to the point that less than half the recovery of a new column

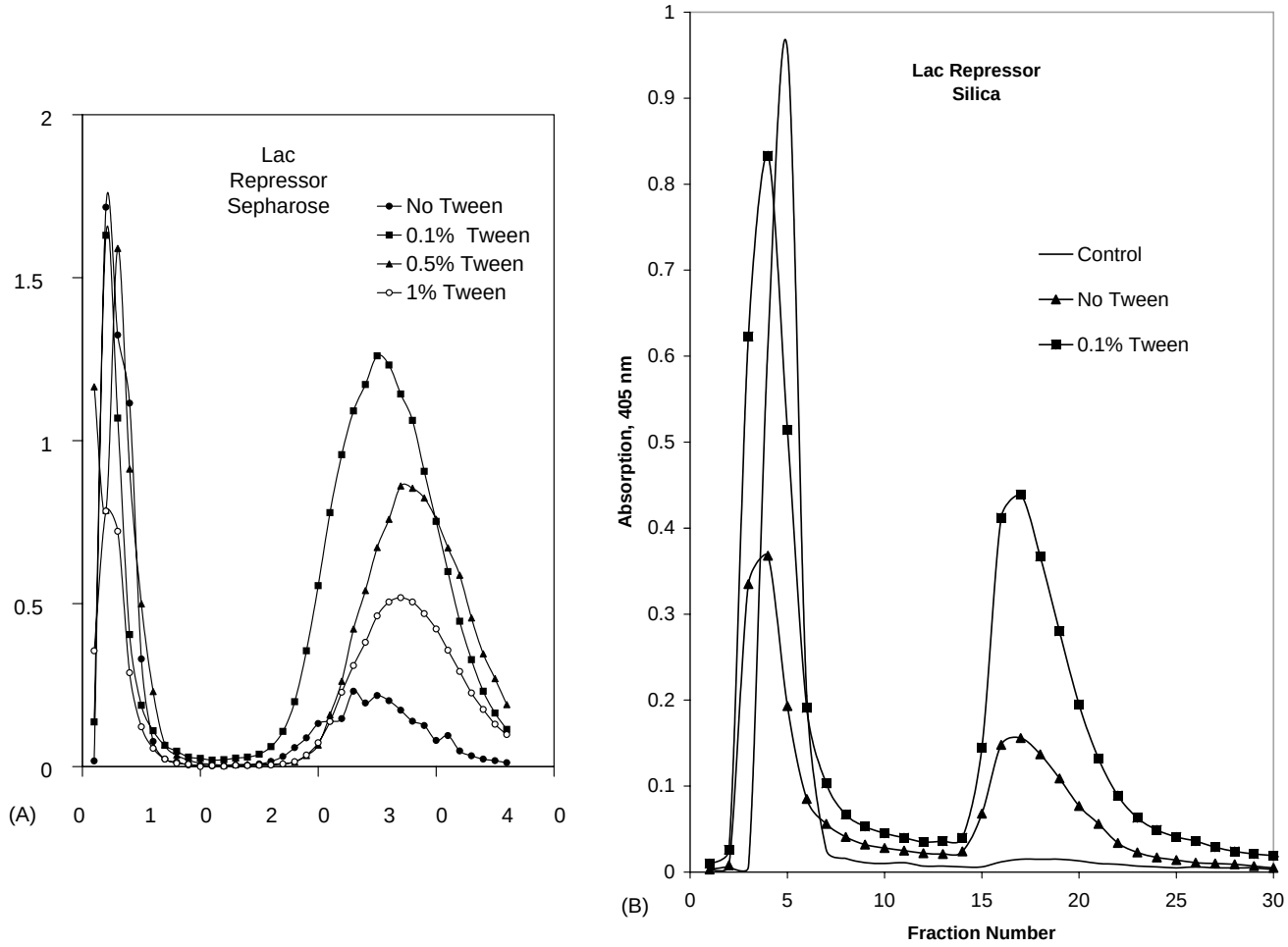

Fig. 3. The best chromatographic performance and yield are obtained at 0.1% Tween. Temperature was 19 °C throughout. (A) After two initial loads of 100 μl lac repressor in the absence of Tween, the chromatography was repeated a third time and this data is shown in the figure. The chromatography was then repeated including 0.1, 0.5, or 1% Tween present in the mobile phases. The column (Op1–DNA–Sepharose) and chromatographic conditions are the same as in Fig. 1. (B) A 2 mm × 23 mm stainless steel column containing Op1–DNA–silica was used with the same chromatographic conditions as in panel A and Fig. 1 except that 10 μl of the crude bacterial extract was injected, the flow rate was 0.5 ml/min, and 250 μl fractions were collected.

was obtained. After washing the column with 0.05% sodium dodecylsulfate (SDS), 0.1% Tween-20, the column performance returned to that of a new column (Fig. 7). This same approach also was successful with the lac repressor columns (data not shown).

The Gal4 column was more problematic. The columns fail rapidly and are not regenerated by the SDS treatment. A new regeneration procedure was developed using the chaotropic salt potassium thiocyanate (KSCN). As shown in Fig. 8, after eight uses, the column bound very little Gal4. After regeneration, the column not only improved markedly but actually had performance that exceeded that of a new column. There are two likely explanations for this improved behavior. Regeneration with high salt (KSCN) would shrink the Sepharose beads and upon return to the lower salt chromatography buffers, the column packing may have improved. Secondly, we suspect that this treatment may have improved the amount of duplex DNA capable of binding Gal4.

4. Discussion

Results for lac repressor, C/EBP, and Gal4 all show beneficial effects of adding Tween-20 to the mobile phase during chromatography. Each of these represent a different family of transcription factor—a helix-turn helix bacterial protein, a basic leucine zipper protein cloned from rat liver, and a zinc cluster motif binding protein cloned from yeast, respectively. Thus, since Tween-20 improved the chromatography of each, these results are probably generally applicable to a wide range of transcription factors.

We have repeatedly observed that the first use of a newly packed column frequently gives poor recovery (Fig. 1). Since transcription factors are usually present in low abundance and the starting materials are often precious, using new columns for each purification seems a wise choice. However, that may not be the case unless the column is preconditioned with a protein-containing mobile phase or unless a detergent such as Tween-20 is used. Moderate amounts of

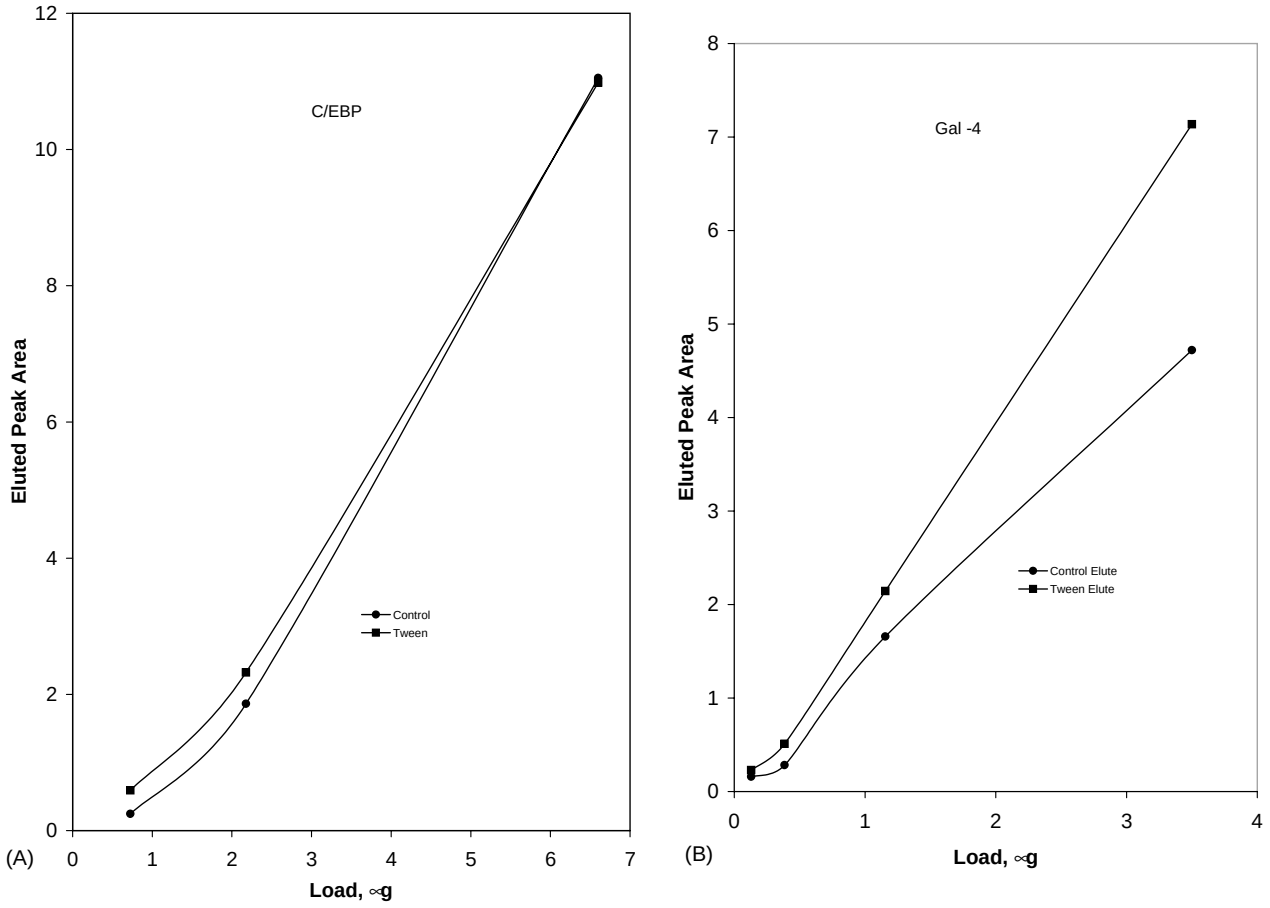

Fig. 4. The effect of Tween is dependent on sample load and is different for C/EBP and Gal4. For all experiments, a 1 ml syringe column packed with the appropriate DNA–Sepharose was used. The columns were equilibrated with 20 ml of TE0.1 and then 0.1 ml of TE0.1 containing different amounts of protein were loaded. The column was then washed with 10 ml TE0.1 and eluted with 10 ml TE1.2. The flow rate was 0.5 ml/min, 1 ml fractions were collected, and the temperature was 19 °C. The proteins (both GFP fusion proteins) were assayed by fluorescence with 395 nm excitation and 515 nm emission. Each protein load was repeated in triplicate for both proteins and the area of the eluted peaks determined and averaged. (A) The results of loading 0.73, 2.2, or 6.6 μg of GFP-C/EBP. (B) The result of loading 0.13, 0.38, 1.16, and 3.5 μg GFP-Gal4.

Tween-20 are commonly used in chromatography, ELISA, and western blotting to prevent non-specific binding and this appears to also be true of DNA affinity chromatography. This is especially true when low amounts of protein are to be eluted from the columns. While the improvement in C/EBP recovery is most pronounced at low loads, Gal4 showed significant improvement over a large range of loaded protein amounts. For all three transcription factors studied here, the effect of Tween-20 was only beneficial and this may be generally true of DNA affinity chromatography. BSA can substitute for Tween-20 to some extent suggesting that one of the effects of Tween is to prevent protein adsorption to column surfaces.

The Mg–DNA complex present in cells is what transcription factors probably bind. However, since transcription factor binding is known to displace counterions upon binding [15,16], it was unclear whether Mg^{2+} would have any effect on the chromatography. For C/EBP, it clearly has little effect and chromatography in the presence of the chelator EDTA was actually higher performance. Since EDTA is a potent inhibitor of DNases, it also helps preserve the column DNA from digestion.

It is interesting to note that the Gal4 protein, which contains tightly bound Zn^{2+}, can be chromatographed in TE buffers containing EDTA. In other experiments (data not shown) we found the chromatography to be little affected by the deletion of EDTA from the buffers and/or the inclusion of 50 μM $ZnSO_4$. Apparently the Zn^{2+} is bound so tightly that it does not dissociate during chromatography. We have also successfully purified Sp1, a Zn^{2+} finger motif protein, from HeLa cells in EDTA containing buffers [17]. Whether chelators like EDTA will adversely affect chromatography will depend on the individual metal-binding transcription factor.

Finally, columns have a limited useful lifetime. For some transcription factor-column combinations this can be quite long. For example, we have used one column for purification of lac repressor from crude bacterial extracts for over six months and over 50 injections (of 0.1–1 ml of crude bacterial extract) with 0.1% Tween showed little loss of perfor-

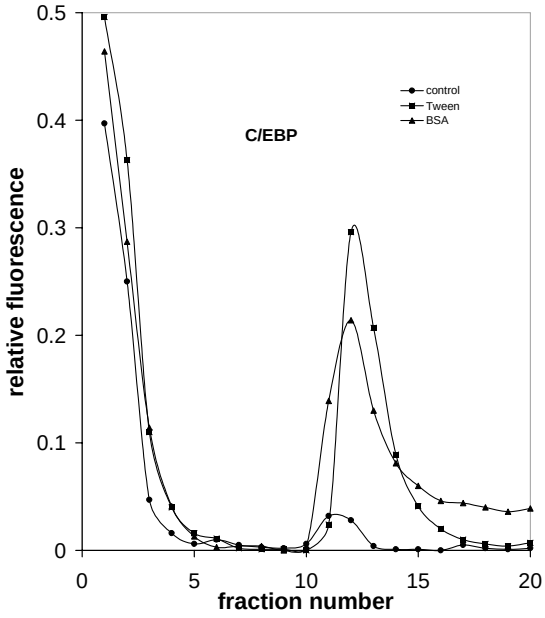

Fig. 5. Bovine serum albumin can also improve yield. A 1 ml DNA–Sepharose column containing 31 nmol of EP18 DNA was loaded with 100 μl of 0.044 mg/ml GFP-C/EBP. After washing with 10 ml TE0.1, the column was eluted with 10 ml TE1.2. The flow rate was 0.5 ml/min, 1 ml fractions were collected, and the temperature was 4 °C. Where indicated, the mobile phases additionally contained either 0.1% Tween or 10 μg/ml bovine serum albumin.

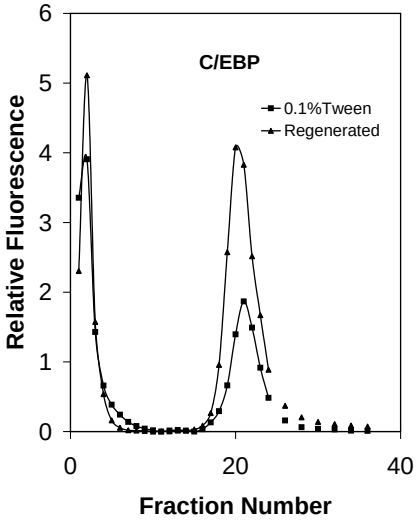

Fig. 7. A simple regeneration procedure for the C/EBP DNA–Sepharose. This 1 ml column contained 18 nmol EP18 DNA and was loaded with 12 μg of GFP-C/EBP. All other chromatography parameters were the same as in Fig. 6. After 84 previous uses of this column, a new chromatogram in 0.1% Tween is shown and indicated greatly diminished performance. The column was regenerated with 20 ml of 0.05% SDS, 0.1% Tween in TE0.1 followed by a 20 ml wash with the same buffer without SDS. The regenerated column was then used to produce the chromatogram shown.

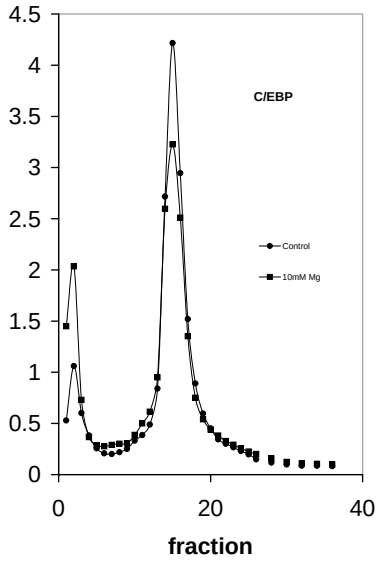

Fig. 6. Mg^{2+} has little effect on the chromatography of C/EBP. A 1 ml DNA–Sepharose column containing 47.4 nmol EP18 was equilibrated in TE0.1 and 18 μg of GFP-C/EBP was loaded. The column was washed with 5.8 ml TE0.1, eluted with a linear gradient of 10 ml to TE2 (10 mM Tris, 1 mM EDTA, 2 M NaCl, pH7.5) followed by a constant TE2 for 2.2 ml. The temperature was 4 °C, the flow rate was 0.3 ml/min and 0.5 ml fractions were collected. The same experiment was then repeated except including 10 mM $MgCl_2$ in the mobile phases.

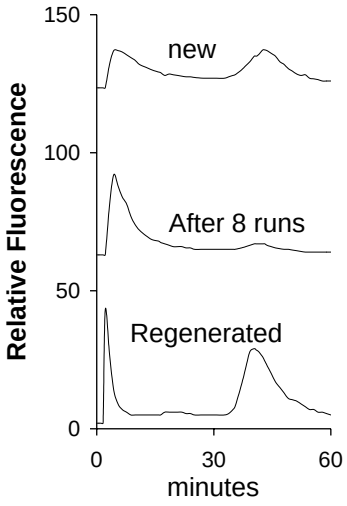

Fig. 8. A different regeneration procedure was required for the Gal4 DNA–Sepharose. A new 1 ml column of Gal4 DNA–Sepharose containing 28 nmol of duplex DNA was used for the upper chromatogram. The eighth chromatogram for this column is also shown demonstrating that the column is no longer performing. The column was then regenerated by washing with 10 ml of 1 M KSCN in TE0.1 and re-equilibrated with 100 ml of TE0.1. Chromatography was then repeated on the regenerated column. For each chromatogram, the column was equilibrated in TE0.1, 214 μg of GFP-Gal4 was loaded, the column was washed with TE0.1 for 10 min., a linear gradient to TE1.2 over 50 min., followed by a constant TE1.2 for 10 min. The flow rate was 0.3 ml/min and detection was with a Shimadzu RF-530 fluorescence detector at excitation 395 nm, emission at 515 nm. The temperature was 4 °C.

mance (data not shown). The similar use of the C/EBP column causes the loss of about 50% capacity (Fig. 7), while even 10 uses of the Gal4 column leads to its inactivity (Fig. 8). Regeneration of columns with the denaturing detergent SDS works for the lac repressor and C/EBP columns but a different procedure using KSCN was needed for the Gal4 column. In either case, it appears that a protein denaturant is required for column regeneration suggesting that it is the build-up of some proteins on the column which cause the loss of performance. In other experiments, we used trypsin to clean columns by digestion; however, we cannot recommend the procedure. Trypsin persists on the column even after extensive washing and we had to finally wash the column with the SDS regeneration medium to restore the column to usefulness.

The data presented suggest that 0.1% Tween-20 and EDTA should be included in most DNA affinity chromatography of transcription factors and that columns can be successfully regenerated (at least five times) by two different protocols using protein denaturants.

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References

[1] H. Gadgil, L.A. Jurado, H.W. Jarrett, Anal. Biochem. 290 (2001) 147.
[2] H. Gadgil, S.A. Oak, H.W. Jarrett, J. Biochem. Biophys. Methods 49 (2001) 607.
[3] H. Wakao, F. Gouilleux, B. Groner, EMBO J. 13 (1994) 2182.
[4] S.K. Hansen, P.A. Baeuerle, F. Blasi, Mol. Cell Biol. 14 (1994) 2593.
[5] X.Y. Zhang, C.K. Asiedu, P.C. Supakar, M. Ehrlich, Anal. Biochem. 201 (1992) 366.
[6] T. Pohl, Methods Enzymol. 182 (1990) 68.
[7] M. Deutscher, Methods Enzymol. 182 (1990) 83.
[8] H. Nakshatri, P. Bhat-Nakshatri, Anal. Biochem. 249 (1997) 103.
[9] L.A. Jurado, J. Mosley, H.W. Jarrett, J. Chromatogr. A 971 (2002) 95.
[10] L.A. Jurado, H.W. Jarrett, US Pat. 6 375 846 (2002).
[11] H. Gadgil, H.W. Jarrett, J. Chromatogr. A 848 (1999) 131.
[12] H.W. Jarrett, W.L. Taylor, J. Chromatogr. A 803 (1998) 131.
[13] H.W. Jarrett, Anal. Biochem. 279 (2000) 209.
[14] L.R. Massom, H.W. Jarrett, J. Chromatogr. 600 (1992) 221.
[15] R.B. Winter, P.H. von Hippel, Biochemistry 20 (1981) 6961.
[16] P.A. Whitson, J.S. Olson, K.S. Mathews, Biochemistry 25 (1986) 3852.
[17] H. Gadgil, W.L. Taylor, H.W. Jarrett, J. Chromatogr. A 917 (2001) 43.