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# Comparative studies on chemically and enzymatically coupled DNA–Sepharose columns for purification of a lac repressor chimeric fusion protein

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**Abstract**

The length of a DNA sequence attached to an affinity chromatography column affects column retention of transcription factors. Even when unrelated sequences such as a poly(A):poly(T) tail are included in a DNA sequence, transcription factors such as the lac repressor are bound more tightly by the column. The position of the additional sequences is also important. To compare coupling procedures, an identical DNA sequence was covalently attached to Sepharose by chemical coupling or produced enzymatically by template driven enzymatic primer extension. These two types of supports, containing the $O_1$ operator sequence bound by lac repressor, were packed into identical columns and compared by purification of a lac repressor–β-galactosidase fusion protein. We found that the purity and yield of proteins eluted from the two columns were similar. Overall, the results suggest that there is no significant advantage to either type of support for the purification of some proteins. The study revealed a potentially important effect of the length of DNA sequences on column selectivity. 

*Keywords:* Affinity adsorbents; Proteins

## 1. Introduction

DNA affinity chromatography is frequently an important step in the purification of transcription factors and other DNA binding proteins. DNA affinity chromatography offers greater selectivity and hence is preferred over other methods. Affinity chromatography involves coupling of a DNA sequence to a solid support such as Sepharose, cellulose or silica. The different methods of coupling have been reviewed previously [1,2].

Our laboratory developed protocols for template-directed enzymatic synthesis of DNA columns using DNA polymerase or reverse transcriptase [3–5]. This procedure involves coupling of the 5′ end of oligomeric $(T)_{18}$ to a solid support, using chemistry which does not result in the modification of thymidine bases. A template sequence containing a 3′-oligoadenylate tail is then hybridized with the bound sequence and the template specified sequence is copied enzymatically.

The only comparison of chemical and enzymati-

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cally produced columns was an unintended consequence of a study originally designed to improve upon the affinity purification of the FADR transcription factor [6]. In that study, we reported that enzymatically prepared columns gave better yield of purified FADR protein than was obtained with a column prepared by conventional chemical coupling. This better yield was attributed to enzymatically synthesized DNA all being accessible to protein binding while chemical coupling may render some of the DNA inactive or inaccessible. In the same study we also reported that the protein eluted from the chemically coupled column appeared to be purer by gel electrophoresis. Enzymatic synthesis requires an oligo-A:oligo-T primer region which was unnecessary and not used for chemical coupling. The increase in length makes enzymatically synthesized columns more complex[1]. This region could have bound the extra proteins contaminating the FADR protein purified on the more complex enzymatically prepared support [6]. Since this issue of complexity is poorly understood, we investigated it here and again addressed the issue of comparing columns produced chemically or enzymatically.

In this report, enzymatic and chemical coupling are compared using the lac operator DNA sequence and a lac repressor–β-galactosidase fusion protein. Lac repressor protein, which regulates the lac operon in *E. coli*, has been well characterized. Here, a lac repressor–β-galactosidase fusion protein [7] facilitated accurate assay of the protein, important for determining purity. This lac repressor–β-galactosidase fusion protein has DNA binding properties comparable to the native protein [8,9]. Differences in DNA complexity were negated by coupling the same DNA sequence using chemical and enzymatic coupling methods. Column dimensions and experimental conditions were also the same.

[1]Complexity and length are related but not identical concepts. A longer DNA sequence is of necessity more complex (i.e., complicated) than a shorter one. However, two sequences of the same length can differ in complexity if one is more repetitive than the other. Since DNA length is increased here by adding homopolymeric sequences (low complexity), the terms length and complexity can be used interchangibly.

## 2. Methods

Unless stated otherwise, chemicals were of the highest purity available from Sigma (St. Louis, MO, USA).

### *2.1. Enzymatic synthesis*

Enzymatic and chemical synthesis is depicted in Fig. 3. Enzymatic synthesis was essentially by the method described previously [6] except that Sequenase 2.0 T7 DNA polymerase (Amersham, Arlington Heights, IL, USA) which lacks 3′,5′-exonuclease activity was used in place of the Klenow large fragment DNA polymerase. Briefly, 10 g of moist, suction dried Sepharose was washed thoroughly with water, 2 g of CNBr was added to it while stirring, and the mixture was maintained at pH 11 by addition of 5 *M* NaOH until the reaction slowed. The activated Sepharose was then rapidly washed under vacuum on a coarse sintered glass funnel with 200 ml ice-cold water and then with 200 ml of 0.1 *M* $NaHCO_3$ pH 8.3, 0.5 M NaCl. A 3-g amount of the activated Sepharose was reconstituted to 5 ml in the last buffer and 150 nmol of 5′-$NH_2$–$CH_2$–$CH_2$–$(T)_{18}$ DNA was added. The mixture was mixed overnight on a tube rotator. The support was washed with 4 ml of the $NaHCO_3$ buffer and then blocked for 2 h with 2 ml of 0.1 *M* Tris–Cl, pH 8. The amount of $(T)_{18}$ coupled (30 nmol per g of Sepharose) was determined by the difference in the ultraviolet absorption of added DNA and that recovered from coupling in the wash fractions.

A 1-g amount of $(T)_{18}$-Sepharose was washed three times with 2 ml of Sequenase buffer (10 m*M* Tris, pH 7.5, 20 m*M* $MgCl_2$, 50 m*M* NaCl) and resuspended in 2 ml of the same buffer containing 45 nmol of Op1-$A_{18}$ [5′GTGGAATTGTGAGCGGATAACAATT$(A)_{18}$]. The mixture was heated to 95°C and allowed to cool slowly to room temperature with mixing. The support was then washed five times with 2-ml portions of Sequenase buffer. Washes included a 5 min incubation on ice with mixing prior to centrifugation. The support was then washed three times with 2 ml of Sequenase reaction mixture [300 μ*M* deoxyribose nucleotide triphosphate (dNTP), 5 m*M* dithiothreitol

(DTT) in Sequenase buffer]. The Sepharose was resuspended in 2 ml of the last buffer, 4 μl (52 units) of Sequenase 2.0 was added and the mixture was incubated at 4°C for 5 min followed by incubation at 37°C for 2 h. The mixture was washed with 5 ml of Sequenase buffer, followed by 10 ml TE0.1 (0.1 *M* NaCl, 10 m*M* Tris, pH 7.5, 1 m*M* EDTA) containing 10 m*M* $NaN_3$ and stored at 4°C until needed. A portion of the support was eluted by washing repeatedly with boiling water and the amount of complementary strand eluted was determined by ultraviolet absorption. Support synthesized in this way had 12 nmol double-stranded DNA in the 0.56 ml bed volume column used in Fig. 4 and Table 1.

### 2.2. Chemical synthesis

Coupling of DNA to Sepharose was by the same protocol used for 5′-$NH_2$–$CH_2$–$CH_2$–$(T)_{18}$, described above, except in this case 50 nmol 5′-$(T)_{18}$αOp1 [5′ $NH_2$–$CH_2$–$CH_2$–$(T)_{18}$–AATTGTTATCCGCTCACAATTCCAC] was used per gram activated Sepharose instead. After blocking with 0.1 *M* Tris, pH 8, the amount of DNA coupled was 33 nmol of $(T)_{18}$-αOp1. After coupling the DNA–Sepharose was washed with 10 ml TE0.1 (10 m*M* Tris, pH 7.5, 1 m*M* EDTA, 0.1 m*M* NaCl) and reconstituted in 2 ml of the last buffer. 50 nmol of Op1-$(A)_{18}$ was added to the mixture and the mixture was heated to 95°C and allowed to cool slowly to room temperature. It was then washed with TE0.1 containing 10 m*M* $NaN_3$ and stored at 4°C until needed. As with the enzymatic column, a portion of the support was eluted with boiling water and the amount of second strand eluted was determined by ultraviolet absorption. Support synthesized in this way had 11.8 nmol double-stranded DNA in the 0.59 ml bed volume column used in Fig. 4 and Table 1. All oligonucleotides were synthesized by standard phosphoramidite chemistry using the university Molecular Resource Center DNA synthesis facility. 5′ aminoethyl-oligonucleotides were synthesized in a similar manner except the last cycle utilizing the amino link reagent (Applied Biosystems) was included.

### 2.3. Production of lac repressor–β-galactosidase fusion protein

Lac repressor–β-galactosidase fusion protein was produced by growing clone BMH-72-19-1 which was a generous gift of Dr. David Levens (Laboratory of Pathology, National Cancer Institute, Bethesda MD, USA). The clones were grown overnight in 2 l superbroth (1.2% bactotryptone, 2.4% yeast extract, 0.5% glycerol, 0.072 *M* $K_2HPO_4$ and 0.028 *M* $KH_2PO_4$) at 37°C and induced for 4 h with 1 m*M* isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were pelleted by centrifugation at 8000 rpm for 30 min in a Sorvall GS-3 rotor. The pelleted cells were resuspended in 40 ml lysis buffer [4 mg/ml lysozyme (Boehringer Mannheim, Indianapolis, IN, USA), 5 m*M* $NaH_2PO_4$, 10 m*M*, $Na_2HPO_4$, 30 m*M* NaCl, 25 m*M* benzamidine, 10 m*M* 2-mercaptoethanol, 10 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride (PMSF) and 0.2% Tween 20]. The cells were lysed by sonication on ice, 30 s on followed by 30 s off, repeated three times at setting 12 using a VirSonic 50 sonicator with a microprobe (Gardiner, NY, USA). Cellular debris was removed

Table 1
Balance sheet for purification on chemically and enzymatically produced DNA–Sepharose columns[a]

| Fraction | Total activity (Units) | Total protein (mg) | Yield (%) | Purification (fold) |
|---|---|---|---|---|
| Crude | 2.3±0.1 | 5.2±1.2 | 100 | 1 |
| Chemical | 0.62±0.33 | 0.014±0.004 | 33±13 | 93±20 |
| Enzymatic | 0.68±0.44 | 0.020±0.014 | 33±13 | 82±23 |
| | | | *P*=0.50 | *P*=0.28 |

[a] Shown are averages±standard deviation for three ($n$=3) different experiments on each column.
*P*=probability that the means for the chemical and enzymatic columns are the same.

by centrifugation at 15 000 rpm for 30 min in a Sorvall SS-34 rotor. The protein was dialyzed against 5 l of TE0.1. This crude protein preparation was stored at −85°C in 1-ml aliquots until needed.

### 2.4. Chromatography

Two columns, one from the chemically coupled support (0.59 ml bed volume) and the other from the enzymatically produced one (0.56 ml bed volume) were packed in 1-ml syringe columns and equilibrated in TE0.1. A 500-μl volume of crude fusion protein was loaded onto the columns. The columns were washed with 15 ml TE0.1 and the proteins were eluted with a 20 ml linear gradient from TE0.1 to TE1.2 (10 m*M* Tris, pH 7.5, 1 m*M* EDTA, 1.2 *M* NaCl). One-ml fractions were collected and the flow-rate was maintained at 0.33 ml/min throughout.

### 2.5. Assay of lac repressor–β-galactosidase fusion protein

Lac repressor–β-galactosidase fusion protein was assayed for galactosidase activity using Buffer O (3 m*M* *o*-nitrophenyl-*O*-β-D-galactopyranoside, 0.1 *M* sodium phosphate, pH 7.5, 1 m*M* $MgCl_2$ and 45 m*M* 2-mercaptoethanol). A 150-μl volume of buffer O was added to 50 μl of each sample to be assayed. The reaction was done in a microtiter plate and absorption at 405 nm monitored continuously at 25°C.

### 2.6. Definition of enzyme Units

One Unit of lac repressor–β-galactosidase fusion protein is defined here as that which causes a change of one absorbance unit per min at 25°C.

### 2.7. Gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was on 7.5% gels by the method of Laemmli [10] and stained with silver using a Bio-Rad Labs. (Richmond, CA, USA) kit.

### 2.8. Protein assay

Protein concentrations were determined by bicinchoninic acid method using the protocol provided by Pierce. Samples were precipitated with ice-cold 10% trichloroacetic acid and reconstituted in a reduced volume of 2% $Na_2CO_3$ in 0.1 *M* NaOH before assay.

## 3. Results

We had hypothesized previously that the complexity of a column attached DNA would affect the purity obtained in transcription factor purification [6]. As a DNA sequence becomes more complex we reasoned that other sequences are produced which may be bound by other cellular proteins. Since a common practice in transcription factor purification is to use long concatemers (produced by ligating oligonucleotides to produce long stretches of DNA) containing multiple copies of the binding site [1], this issue of complexity could be quite important. Fig. 1 shows that indeed DNA complexity does affect transcription factor binding but not quite in the way we envisioned.

The Op1 operator sequence used here is a 25-mer. It is shown in Fig. 3. The complementary strand DNA (i.e., αOp1) was synthesized with a 5′-aminoethyl group using the AminoLink reagent. It was directly coupled to Sepharose using CNBr activation, and annealed to the Op1 strand. Since this DNA lacks an oligoA:oligoT region, we have called it A0 in the figure. The same DNA sequence was also synthesized containing either a six or an eighteen long 3′-oligoadenylic acid ‘‘tail’’ on the Op1 strand (and a complementary 5′-oligothymidylic acid tail on the other strand). These DNAs were also coupled and are called A6 and A18 in the figure. To test columns prepared from these DNA sequences, a crude bacterial extract containing a chimeric fusion protein (laciz) of lac repressor (i.e., lac i) and β-galactosidase (i.e., lac z) was applied to each column and eluted using a salt gradient. The column fractions were then assayed for β-galactosidase activity in an assay which results in an increased absorption at 405 nm for active fractions. While lac repressor would be expected to have high affinity for the Op1 sequence, it should have minimal affinity for the unrelated homopolymeric sequences and yet clearly these sequences do affect retention as shown in Fig. 1. Since the presence of A6 or A18 increases

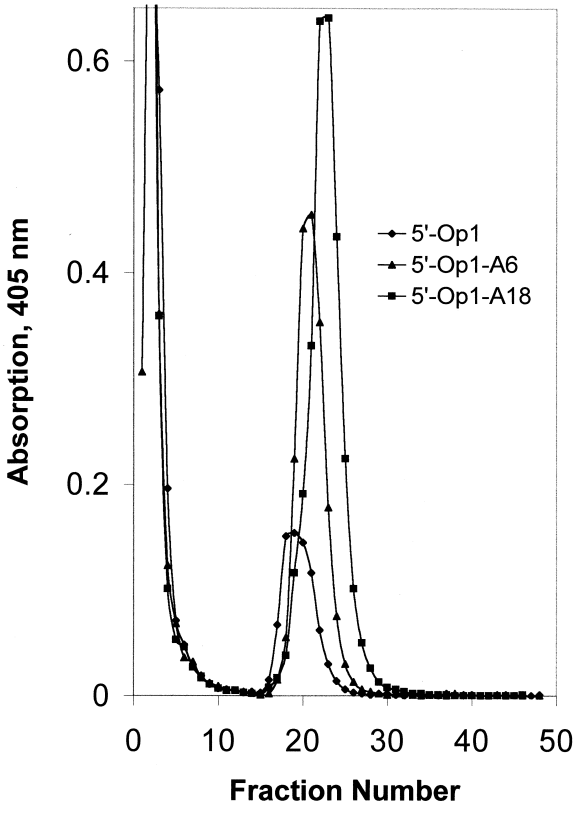


Fig. 1. The length of coupled DNA affects retention of laciz. Each of the three DNAs shown was chemically coupled to CNBr-activated-Sepharose and a 1-ml syringe column packed. Additions in each case were to the 3′ end of the Op1 sequence (see Fig. 3), at the same end of the double-stranded DNA where the 5′-aminoethyl group on the complementary strand would be found. The amount of DNA coupled was 16 nmol/g Sepharose for Op1, and 32 nmol/g for the other two. For each column, 0.5 ml of the crude laciz bacterial extract was loaded. Elution was with 10 ml of constant TE0.1, followed by a linear gradient of 20 ml from TE0.1 to TE1.2, followed by 20 ml of constant TE1.2. One-ml fractions were collected and assayed for β-galactosidase activity (absorption 405 nm).

retention time, this sequence must increase the overall affinity of the repressor for the stationary phase DNA.

Peak heights are also different in Fig. 1 but this is due to differences in the amount of DNA coupled to each column which affects the capacity of the columns for laciz. We have found that the amount coupled, while affecting capacity does not affect retention times for salt gradient elution though (data not included). These differences in retention are due to the DNA complexity and were consistently observed with columns containing different amounts of coupled DNA.

Furthermore, the position and distribution of homopolymeric sequences can also affect retention as shown in Fig. 2. In this experiment, six thymidylate residues were added to one end or the other of the Op1 sequence, or three residues was added to each end. The corresponding number of thymidylates were added to the complement strand. The results show that additions of three residues to each end give lower retention times than is obtained by adding six residues to either end and that, furthermore, additions to the 3′-end of the Op1 sequence give the highest retention time of all.

From these results it is clear that minor differences

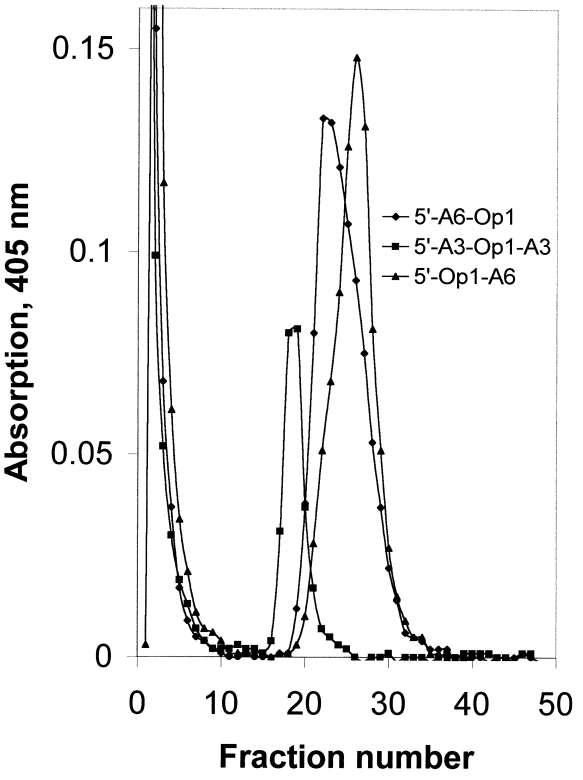


Fig. 2. The position of additional sequences also affects retention. For each of the DNAs, six additional adenylate residues were added, but the position was different as shown in the figure. The columns were all 1 ml and contained 26, 30, or 29 nmol DNA/g Sepharose for 5′-A3-Op1-A3, 5′-A6-Op1, and 5′-Op1-A6, respectively. The columns were loaded and eluted as described for Fig. 1.

in sequence, even with unrelated homopolymeric regions, can affect retention. Thus, to find out if the differences previously observed for chemically and enzymatically produced columns [6] were due to the method of synthesis or to differences in sequence, we prepared columns by both procedures using the same DNA sequence. The scheme used for column synthesis is depicted in Fig. 3. Columns prepared by chemical and enzymatic synthesis depicted in the figure would have identical double-stranded DNA, despite differences in how they were synthesized.

Fig. 4 shows the elution of laciz fusion protein from chemically and enzymatically synthesized columns. Both columns were the same dimensions and contained virtually identical amounts of the same double-stranded DNA sequence; they differ only in how they were produced. It can be seen that both columns have similar properties in terms of amount of fusion protein they bound and eluted. However, we also found that the repressor elutes at slightly lower salt concentration from the enzymatic column. The peak fraction elutes from the enzymatic column at 0.7 *M* NaCl (determined by conductivity) while the peak fraction from the chemical column elutes at 0.8 *M*. This result was reproducible in all our chromatographic runs and with columns made at different times and containing different amount of DNA coupled but its cause is unknown.

Fig. 5 shows an acrylamide gel of fractions obtained from the chemical and enzymatic columns. A protein band corresponding to the expected molecular mass [7] of the lac repressor–β-galactosidase fusion protein ($M_r$ 155 000) can be seen in both fractions and is indicated by the arrow. This band, and two prominent bands just below it (indicated by lines) all stain with an anti-lac repressor antibody (data not shown). Thus, the full length fusion protein and at least two truncated forms of it are bound by and elute from both columns. Only a single, minor band (indicated by an asterisk) is unique to the enzymatic column and the purity of both fractions is comparable.

Table 1 shows a balance sheet for the average purification and yield of protein eluted from the two columns. Yield and purity are virtually identical for the two columns. Since the columns were loaded with an excess of the fusion protein, the yield is a measure of column capacity in this experiment. Thus, column capacity is virtually identical for the two types of columns. The purification was repeated three times with each column and the results were averaged and compared statistically. There is no statistically significant difference between the yield or purity obtained with either column.

## 4. Discussion

In a previous report from our laboratory [4] we had reported that a chemically synthesized column yielded purer FADR protein while more protein could be recovered from the enzymatically synthesized column. However, the DNA sequence on the two types of columns was not the same. The enzymatic synthesis required a $T_{18}$:$A_{18}$ primer region not necessary or used for chemical coupling. Since the operator B sequence bound by FADR in those experiments is a 30-mer, this difference amounted to coupling a 30-mer chemically or copying a 48-mer enzymatically. This makes the enzymatically produced column more complex. If this additional DNA sequence could bind other proteins, this could account for the lower purity. To answer this, here we chemically coupled the same DNA sequence used for enzymatic column production. Under these conditions, there is no difference in the purity obtained. In our studies with the purification lac repressor–β-galactosidase fusion protein, when identical DNA–Sepharose columns are produced by the two methods, both types of columns behaved almost identically and neither of the columns had an advantage in terms of yield or fold purification. The only difference found was that the protein eluted at a slightly lower salt concentration from enzymatic column than from chemical column. We do not understand the mechanism behind this behavior but it does not seem to be important to the chromatography.

While the method of synthesis was not important in this study, the exact sequence and complexity of DNA coupled was shown to be very important. Here, we show that even simple, homopolymeric sequences can have marked effects on column performance and retention times. Why this is so is not known but could arise in at least two ways. Base pairing involves weak forces, primarily hydrogen

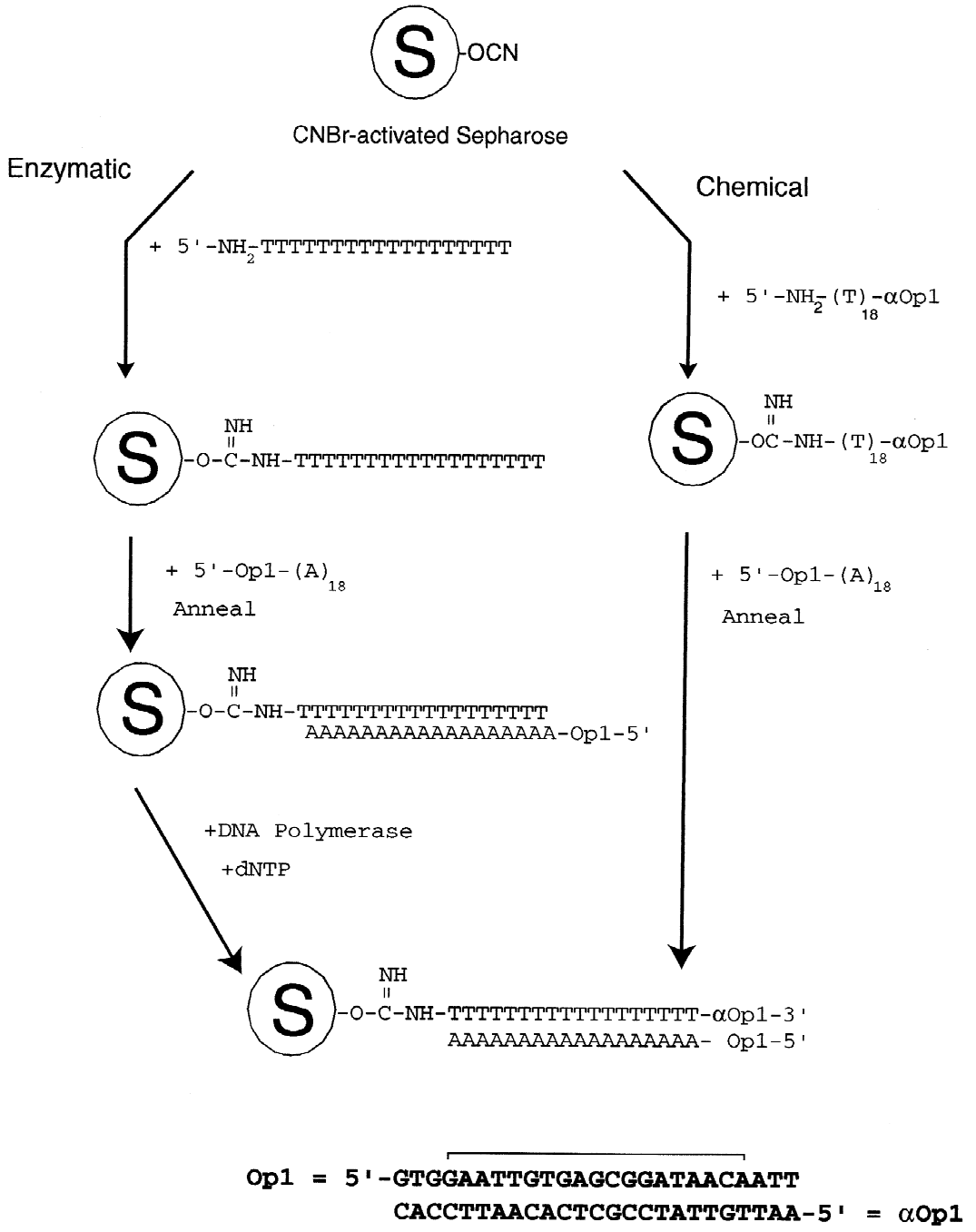


Fig. 3. Schematic representation of enzymatic and chemical DNA–Sepharose synthesis.

bonding, stabilizing the double-stranded DNA structure. As DNA is heated, these weak forces are ultimately insufficient to resist thermal motion and at some temperature, DNA becomes single stranded. This ''melting'' temperature was never exceeded in our experiments. Even at lower temperatures though,

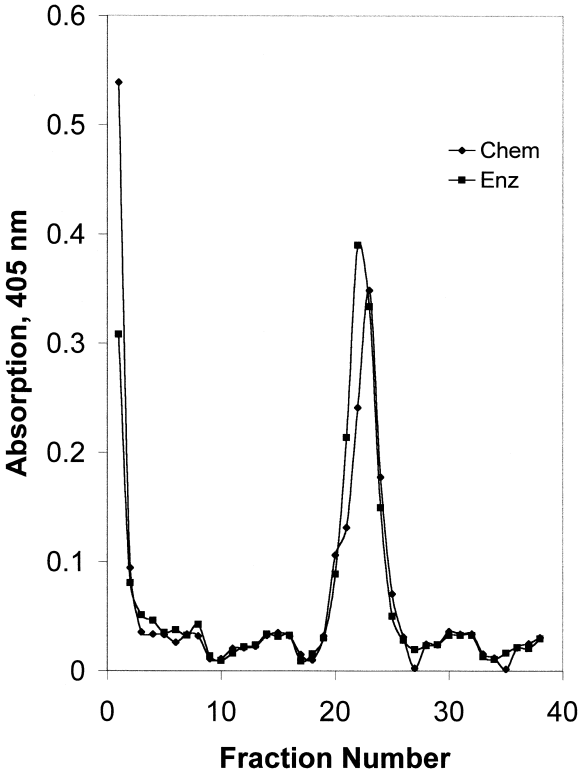


Fig. 4. Enzymatically and chemical synthesized supports show similar chromatography. Synthesis was as depicted in Fig. 3 and described in Methods. The enzymatically prepared column was 0.56 ml bed volume and contained 12 nmol double-stranded DNA [5′-Op1-$(A)_{18}$-3′:5′-aminoethyl-$(T)_{18}$-αOp1-3′]; the chemically prepared column was 0.59 ml and contained 11.8 nmol double-stranded DNA. Flow-rate was 0.3 ml/min and 1-ml fractions were collected. The elution was with a gradient of constant TE0.1 for 90 min, a linear increase to TE2.0 (10 m*M* Tris, pH 7.5, 1 m*M* EDTA, 2 *M* NaCl) at 230 min, followed by 20 min of constant TE2.0.

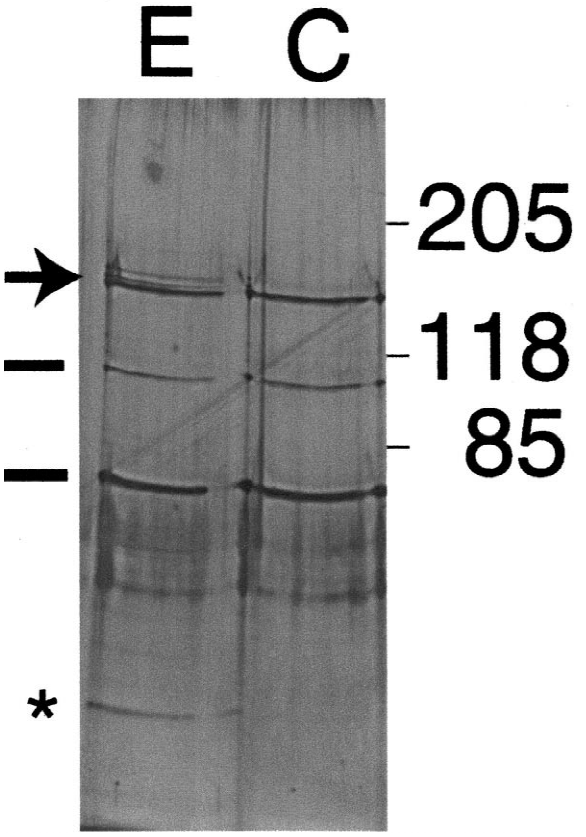


Fig. 5. Electrophoresis demonstrated that purity is similar for proteins purified on the chemical and enzymatic columns. Crude bacterial extract containing the laciz fusion protein was purified on the chemical (C) and enzymatically (E) produced column using the conditions in Fig. 4. The peak eluted fractions were pooled, concentrated, and applied to a 7.5% SDS–polyacrylamide gel [10]. The position of molecular mass markers ($\cdot 10^{-3}$), are shown to the right of the gel. Other symbols are described in the text.

the ends of a double-stranded DNA are not stabilized by as much hydrogen bonding as occurs mid-strand, base stacking is more solvent exposed, and localized ''melting'' or fraying of the ends can occur. If sequences near the end are important to transcription factor binding, this fraying could adversely affect binding. Footprinting with DNAase I [11] shows that lac repressor binds to the DNA shown in Fig. 3 covering the entire sequence shown except for the last three base pairs at each end (indicated in the figure). By adding additional DNA to the ends, the fraying is distanced from the binding site and an intact binding site is maintained. Thus, the homopolymeric sequenced could prevent thermal denaturation of more distal regions of the operator DNA sequence.

The other potential explanation involves what is frequently referred to as ''sliding'' [12]. It has been observed that lac repressor and operator 1 DNA associate at a rate of about 100-fold faster than three dimensional diffusion should allow [13]. Lac repressor also binds ''non-specific'' (i.e., non-operator 1) DNA sequences with relatively high affinity. For example, under low ionic strength conditions, the dissociation constant for operator 1 is about $5 \cdot 10^{-14}$

*M* while the constant for ''non-specific'' [alternating poly(AT) DNA] is about $10^{-9}$ *M*. This led to the hypothesis that the repressor may bind from bulk solution (i.e., three-dimensional diffusion driven) to any non-specific DNA sequence and then slide (i.e., one-dimensional diffusion) along the helix until the operator is encountered and bound [14]. In fact, any of several other mechanisms which restrict the dimensionality of diffusion would accomplish the same enhancement of rate; what is necessary is that binding be a two step process involving ''non-specific'' binding followed by a ''sliding'', ''hopping'', or ''bridging'' step which restricts diffusion to less than three dimensions [12,15]. The dependence of Op1-binding of lac repressor on both salt concentration and DNA length agrees with what would be predicted for such a sliding mechanism [11,15–18]. While long DNA sequences (>70 base pairs, bp) do affect association rates in a way consistent with a sliding mechanism [18], any affect of shorter lengths is unclear and may not be measurable by current binding assay methods. Since lengths as short as 6 bp do affect our chromatography, chromatography may provide an even more sensitive method for measuring limited diffusion affects on protein–DNA binding.

Whether either of these possible explanations (melting stabilization or sliding) accounts for the current results is not currently known but is being investigated. However, it is interesting to note that enhanced lac-repressor binding to poly(AT) DNA had been noted using filter binding assays as far back as 1970 [19], is in agreement with the data presented here.

Another possible way that additions to the ends of DNA sequences could contribute to column performance is by acting as a spacer, distancing the operator sequence from support surfaces which could sterically inhibit binding. This seems unlikely to explain the results here. Cyanogen bromide-activated Sepharose can couple directly to nucleic acid bases, presumably adenine, guanine, or cytosine [20]. Here, we have introduced a 5′-aminoethyl moiety which should provide a favored coupling site but coupling elsewhere would presumably also occur. While this issue has not been directly investigated for CNBr-activated Sepharose, it was shown with activated ester coupling that coupling is predominantly through the aminoethyl but that some base modification also occurs [3]. The DNA strand which was chemically coupled in the present report was always the αOp1 strand containing a 5′-aminoethyl group and any poly(T) sequences necessary to complement poly(A) regions on the complement strand. This was done since T is unreactive for coupling and so the added regions were not providing additional coupling sites. Thus, reaction can occur at the 5′-end or intrachain but not within the homopolymeric sequences introduced. An aminoethyl group would contribute about 5 Å spacing and each base pair in the DNA helix contributes 3.4 Å. The lac repressor binding site (DNAase I footprint) is 3 bp from either end. Thus, aminoethyl coupled Op1 would place the binding site about 15 Å from the Sepharose surface and, for example, $(A)_6$ would add an additional 20 Å. In Fig. 2, the sequence Op1-$A_6$-3′ has the homopolymeric region on the same end of the DNA as the aminoethyl moiety and the spacer length would be about 35 Å; while 5′-$A_6$-Op1 has the same sequence at the opposite end and a spacer length of about 15 Å. Either of these gave greater retention than the $A_3$-Op1-$A_3$ (spacer length about 25 Å). Thus, spacer length does not correlate with retention.

While the mechanism by which DNA complexity affects retention time is at present unclear, the effect is undoubtedly useful in transcription factor purification. Our previous study of FADR showed that purification on columns of different complexity impacted both yield and purity [6]. Others have reported that highly complex DNA, composed of concatemers of ligated oligonucleotides are necessary for effective purification of some transcription factors [1,21]. Since complexity alters retention time (Figs. 1 and 2), it also affects resolution of a specific transcription factor from other cellular DNA-binding proteins. Careful characterization of this complexity effect should greatly benefit our understanding of this affinity chromatography and improve protein purification.

Enzymatic synthesis has an inherent disadvantage in being more cumbersome than chemical coupling. Hence, chemical synthesis seems to be preferable for purification of lac repressor and probably other transcription factors. However, enzymatic synthesis does have other advantages. Techniques are available for producing columns enzymatically from either

RNA [4,5], or DNA templates [3] and the DNA support produced can be directly sequenced [4]. Furthermore, since chemical coupling procedures modify nucleic acid bases while the milder enzymatic synthesis does not, it may prove more useful for the purification of proteins whose DNA binding affinity is more sensitive to chemical modification such as, for example, proteins involved in DNA repair.

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