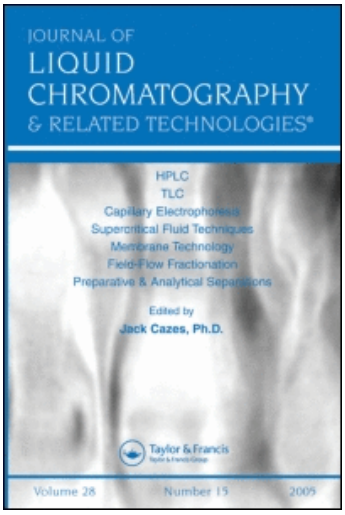

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SOLID PHASE EXTRACTION OF URINARY POLYPEPTIDE GROWTH FACTORS USING METHYL BONDED SILICA IN FREE SUSPENSION

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ABSTRACT

Methyl bonded silica effectively concentrates EGF, TGF-α, and TGF-β from urine. Fractional elution of the sorbent with aqueous acetonitrile released approximately 70 to 80% of the EGF at between 25 and 30% acetonitrile, 80% of TGF-α between 15 and 25% acetonitrile and 70% of TGF-β between 30 and 50% acetonitrile. Enrichment in a single step was 1400 fold for EGF, 200 fold for TGF-α, and 250 fold for TGF-β. Further chromatography on sizing gel, carboxymethyl cellulose, and HPLC provided complete separation of TGF-α from the much more abundant but bioactively similar EGF.

INTRODUCTION

Cancer detection by radioimmunoassay (RIA) of tumor markers in urine is a non-invasive procedure potentially suitable for use in screening programs of high risk cancer-prone individuals. Our laboratory has focused on a family of epidermal growth factor (EGF)-related polypeptides, termed transforming growth factors (TGFs). These molecules compete with EGF for membrane receptor binding, (1) and are capable of promoting a reversible transformation of immortal, but untransformed, normal rat kidney (NRK) cells in semi-solid culture medium (2). This is a measurable phenomenon

which is referred to as clonogenicity in soft agar. One member of the epidermal growth factor family, transforming growth factor α (TGF-α), is produced by tumor cells in culture, and may be a marker for the transformed phenotype (3).

The problems of identifying and quantifying peptide growth factors in urine are related to their biological activity at nanomolar and picomolar concentrations. Consequently, when excreted, these trace peptides occur at concentrations of parts per billion and parts per trillion in urine (4,5). Although a 24 hr urine sample is a dilute solution containing only about 2 or 3 mg of protein per ml by Lowry protein determination, extraneous protein still remains between a million and billion times more abundant than are growth factor molecules. Interference by this large excess of extraneous proteins can cause serious assay problems. Moreover, because EGF and TGF-α react similarly in bioassays for NRK clonogenicity (2) and for EGF receptor-binding activity (1), and because EGF may be as much as a thousand times more abundant than the TGF-α found even in tumor patients (5), TGF-α must be resolved from EGF before it can be quantified by these assays. EGF is slightly larger than TGF-α (53 vs. 50 amino acids) and, although similar in structure (6), the divergence between EGF and TGF-α might be sufficient to allow for different chemical behavior and effective separation.

Prior concentration procedures, used for both conditioned medium from a cell cultures and urine samples, involved laborious acid-ethanol extraction and other precipitation procedures (7). We sought to replace this liquid-liquid extraction method with a solid phase procedure. Methyl bonded (C1) silica has been used successfully to extract the growth factor IL-2 from tissue culture medium (8). Consequently, we investigated the ability of various bonded silicas to adsorb EGF-related growth factors in urine and the ability of selective solvent elution of the adsorbed protein to concentrate and partition growth factors. To determine recovery and resolution of urinary peptide growth factors. To avoid bioassay problems resulting from low concentration, interference,

and cross reactivity, a series of reconstruction experiments were performed by adding ^{125}I-labeled synthetic EGF-related peptides to normal urine.

Because biologically active human TGF-α (hTGF-α) was not originally commercially available, ^{125}I-labeled synthetic rat TGF-α (9) was used. Rat TGF-α (rTGF-α) differs from hTGF-α by amino acid substitutions at only four positions (10). Three of the substitutions are conservative and the fourth is the substitution of a positively charged residue for a negatively charged residue. Consequently, rat and human TGF-α are similar with respect to hydrophobicity and molecular weight, justifying the use of rTGF-α as a substitute tracer for hTGF-α for our initial evaluations.

Recently, human TGF-β (hTGF-β) and hTGF-α became commercially available. TGF-β is an important biologically active molecule that is synergistic with EGF and TGF-α for the growth of NRK fibroblasts in soft agar (2). Although it is a widely distributed peptide, it might also reflect some aspect of malignancy. Therefore, we asked if radiolabeled TGF-β could also be recovered from human urine using C1 silica.

The availability of hTGF-α permitted us to confirm results obtained using rTGF-α.

EXPERIMENTAL

Urine source and radioiodination of growth factors. 24 hr collections of human urine were obtained from the same normal healthy donor. Following the addition of ^{125}I human EGF or rTGF-α (see below), the respective urine sample was adjusted to pH 3 with concentrated trifluoroacetic acid (Pierce Chemical Co., Rockford, IL) and placed at 4°C overnight to precipitate acid-insoluble urinary proteins. After decanting, the urine was frozen for storage at -40°C. In subsequent experiments with ^{125}I TGF-β and human TGF-α, after the addition of growth factor the urine was made 12% in acetonitrile (MeCN) then adjusted to pH 3 with trifluoroacetic acid, and frozen at -40°C. Recombinant human formylmethionine EGF (catalogue No. 05100; Amgen Biologicals, Thousand Oaks, CA), synthetic rTGF-α (9) (Dr. R. L. Pardue and

Peninsula Laboratories, Belmont, CA), human hTGF-ß (R & D Systems of Minneapolis, MN) and recombinant hTGF-α (Creative Biomolecules, Hopkinton, MA) were iodinated by the chloramine T method (11).

Methyl bonded microparticulate silica bead adsorption. Sepralyte C1, a commercially available methyl bonded silica (40-µm, 60-Å pore size; catalogue No. 11021), and other bonded silicas were obtained from Analytichan International, Harbor City, CA. After thawing the frozen urine and decanting the supernatant, any precipitate that settled overnight at 4°C was extracted with 25 ml of 30% MeCN in 2 M acetic acid. Urine and extract, (if not already at 12% MeCN) were made 12% in MeCN. The C1 bonded beads were transferred in 12% MeCN to the batch urine in a ratio of 3 g of beads/g of urinary protein. Free suspension adsorption of select urinary proteins to beads was achieved by circulating beads in urine with a magnetic stirring bar for 30 min. The beads were allowed to settle for at least 30 min, urine was decanted, and the beads were transferred in 12% MeCN to a chromatographic column containing a lower layer "cushion" of beads in ratio of 1 g of cushion beads/3 g of adsorbed beads. Sequential, stepwise batch elution of C1 bonded beads with 12, 15, 20, 25, 30, 35, and 50% MeCN in 0.1 M NaCl and 0.1% trifluoroacetic acid was then carried out. Five ml of the aforementioned percentages of MeCN were used per g of total beads (circulating beads plus cushion beads).

In more recent experiments with hTGF-α, the MeCN eluents were 12, 17, 24, 30, 40, and 50% MeCN in 0.1 M NaCl and 0.1% trifluoroacetic acid. For the free suspension elution method rather than transferring beads to a column, eluent was added directly to the settled beads followed by 30 min vigorous stirring and 45 min settling time before decanting the supernatant at each elution step.

Chromatography. The acetonitrile elutions were desalted by dialysis against 0.1 M acetic acid (Spectraphor tubing, molecular weight cutoff 1,000; Spectrum Medical Industries, Inc., Los Angeles, CA), lyophilized, resuspended in 7.0 ml of 1 M acetic acid, centrifuged at 100,000 x g for 30 min, and then applied to a

column (2.5 x 95 cm; 450-ml bed volume) of Bio-Gel P-10 (200 to 400 mesh; Bio-Rad Laboratories, Richmond, CA) equilibrated with 1 M acetic acid at 22°C. Fractions of 3.5 ml were collected.

Chromatography on CM-C (CM-52; Whatman, Clinton, NJ) was carried out on fractions containing ^{125}I or biologically active regions from the P-10 columns. Fractions were pooled, lyophilized, resuspended in 1 M acetic acid, and dialyzed against 5 mM ammonium acetate (Ph 4.5) overnight at 4°C. Following centrifugation at 100,000 x g for 30 min, the sample was applied to a 1.5- x 3-cm column of CM-C (CM-52; Whatman) equilibrated with 5 mM ammonium acetate, pH 4.5 (starting buffer). Elution was achieved with a linear gradient of starting buffer (200 ml) and limit buffer (200 ml of 0.5 M ammonium acetate, pH 6.8) pumped through a two-chamber mixing device at a flow rate of 80 ml/hr at 22°C. Fractions of 10 ml were adjusted to 1 M with respect to acetic acid and concentrated by lyophilization.

HPLC was performed with a reverse phase C_{18}-μBondapak column (10-μm particle size; 0.39 x 30 cm; Waters Associates, Milford, MA) at a flow rate of 0.7 ml/min at 22°C on a Waters Associates liquid chromatograph system equipped with two Model 6000 M solvent delivery pumps, a Model 660 solvent programmer, and a Model 450 variable wavelength detector.

Anchorage-independent growth assay. Soft-agar clonigenic assays were carried out in Dulbecco's modified Eagle's medium containing 10% calf serum as previously described (12). In addition, purified TGF-β, kindly provided by Dr. Michael B. Sporn, was added at 2 ng/ml. A 0.5% base layer and 1 ml of a 0.3% agar overlay containing the test sample were used. NRK cells, clone SA6 kindly provided by Dr. Joseph E. De Larco, were seeded at 3 x 10^3 cells/35-mm dish, overlaid with medium containing 0.3% agar on day 5, and incubated at 37°C in a humidified 5% CO_2 atmosphere. The number of colonies containing either 20 or 50 cells or more/eight low-power fields was scored on day 14.

Radioimmunoassay. The radioimmunoassay (RIA) for TGF-α was performed with a kit (Biotope, Inc., Seattle, WA, Cat. No. TK 110)

using rabbit antiserum directed to the COOH-terminal 34 to 50 amino acid sequence of rTGF-α. Full-length bioactive synthetic rTGF-α was used as tracer and reference standard. This TGF-α RIA detected hTGF-α and rTGF-α equally well. Half-maximal displacement was obtained with 0.5 to 1.0 ng of reference standard rTGF-α. Sample proteins were denatured (0.1% lithium lauryl sulfate), reduced (40 mM dithiothreitol), and heated (100°C for 1 min) prior to assay.

RESULTS

The usefulness of bonded silica depends first on how efficiently it adsorbs growth factors from large volumes of urine (0.5-40 L), and second, on how selectively those adsorbed growth factors can be eluted. To reduce the amount of sample handling, a free suspension adsorption procedure was designed in which bonded silica sorbants were mixed directly with urine. The capacity of bonded silica to adsorb growth factor polypeptides from urine was tested using ^{125}I EGF as tracer. Methyl (C1), ethyl (C2), octyl (C8), octadecyl (C18), phenyl (Cϕ) or cyclohexyl (CCH) bonded silica was mixed for 30 min with urine and tracer in the proportion of 3 g bonded silica per g urinary protein. The extent of adsorption was determined by subtracting the number of counts remaining in the urine from the amount added. EGF was efficiently adsorbed to all six types of bonded silica. (Table 1).

Of the six bonded silicas, C1 silica was selected for additional testing. The rate and efficiency of adsorption of ^{125}I rTGF-α to C1 silica is demonstrated in the time-course study shown in Figure 1. In this experiment, synthetic ^{125}I-labeled rTGF-α was added to a 24 hr urine sample from a normal human donor. The urine was then brought to 12% MeCN and pH 3 with trifluoroacetic acid. Three g of C1 silica per g of urinary protein was added, followed by sufficient magnetic stirring to keep the silica in circulation. Duplicate 2 ml aliquots of C1 silica in circulation with urine were removed from the specimen at the indicated times. Each time-point sample was centrifuged (3000 rpm x 5 min), the pellet of C1 silica was rinsed with 12% MeCN, and then was eluted with 1 ml of 50% MeCN. After the silica was removed by centrifugation, aliquots

TABLE 1

Adsorption of ^{125}I EGF to Bonded Silicas

Bonded Phase	C1	C2	C8	C18	Cϕ	CCH
Percent Adsorbed	99.4	99.4	98.9	98.9	99.4	98.9

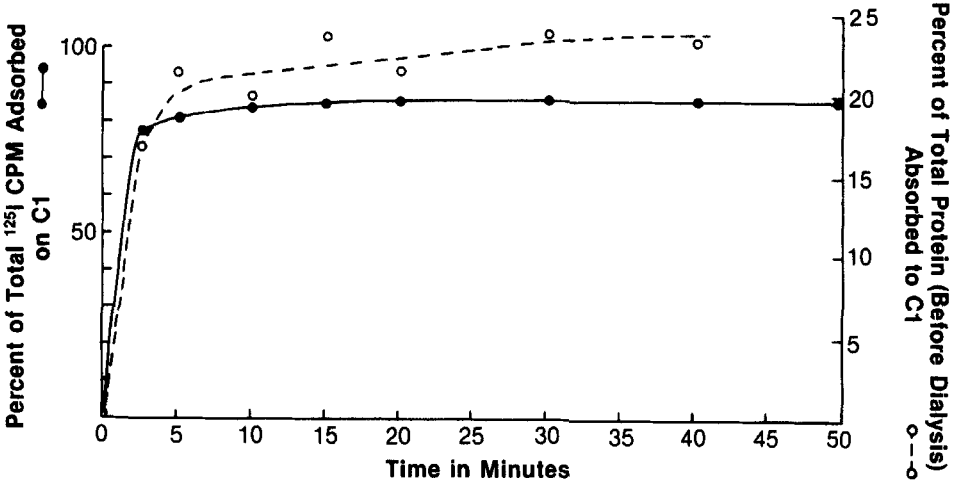

FIGURE 1. Time course adsorption of ^{125}I TGF-α and protein to C1 silica.

were withdrawn either for a determination of radioactivity or were lyophilized for protein assay by the method of Lowry. The results indicate that most of the ^{125}I-labeled rTGF-α was absorbed within 2 min, and that equilibrium was reached at 82% within 15 min. While only 6% of the total urinary protein (by Lowry assay) was absorbed to C1 silica the time-course of protein adsorption from urine parallels that of TGF-α.

After establishing that rTGF-α can be efficiently adsorbed from urine, the question then asked was whether or not TGF-α and

TABLE 2

Comparative Hydrophobicity of rTGF-α, hEGF, and hTGF-β

	rTGF-α (Mr 5600)	hEGF (Mr 6000)	hTGF-β (Mr 25,000)
Percent MeCN required for elution from C18 HPLC column	22%	28%	34%
Percent MeCN required for elution from C1 bonded silica	17-24%	24-30%	30-50%

other EGF related growth factors could be selectively eluted. Unlike smaller organic molecules, proteins do not exchange rapidly between liquid and solid phase during reverse phase chromatography. Consequently, the elution of proteins is much more dependent on small variations in solvent composition than solvent volume. As a result, the solvent composition required to elute a protein from a particular type of reverse phase column can be specified and is an indication of the hydrophobic nature of the protein.

The divergence in hydrophobicity of TGF-α, EGF, and TGF-β as demonstrated by HPLC and MeCN elution from C1 bonded silica is summarized in Table 2. rTGF-α, which eludes sharply from a C18 HPLC column at 22% MeCN, is less hydrophobic than hEGF, which eludes in a broader range between 26 and 28% MeCN. TGF-β, which eludes at 35% MeCN, is more hydrophobic. Application of differential elution to C1 silica is seen in the following experiments.

C1 silica that has adsorbed protein from urine in free suspension as described earlier can be transferred to a column, washed with 5 ml 12% MeCN per g of C1 silica, and eluted using stepwise increases in MeCN starting with 15% MeCN, 0.1 M NaCl and 0.05% trifluoroacetic acid and increasing MeCN in 5% increments up to 35% MeCN, followed by a 50% MeCN wash. The distribution of eluted ^{125}I-labeled hEGF and ^{125}I-labeled rTGF are shown in Table

3. In the first experiment, ^{125}I-labeled hEGF was added to a 1.5 liter urine sample collected over 24 hr containing 6.5 g of Lowry protein. Bonded silica adsorbed 97% of the ^{125}I-labeled hEGF. Release of the ^{125}I-labeled hEGF occurred between 20 and 35% MeCN. The 30% MeCN eluent contained 67.1% of the ^{125}I-labeled hEGF and only 0.33% of the total protein. After dialysis this value fell to .045% providing an overall enrichment of 1400 fold.

In the second elution experiment, 87% of the ^{125}I-labeled rTGF-α added to a normal 24-hr urine sample was absorbed to the C1 silica (Table 3). Of this, 81% was eluted between 15 and 25% MeCN. Of the 8.4 g of protein in the sample, as determined by the Lowry procedure, only 0.4% remained in the 15-25% fraction after dialysis. Enrichment of ^{125}I TGF-α was 200 fold. Although the ability to promote NRK growth in soft agar (clonogenicity), particulary in the presence of TGF-ß, is a characteristic of both EGF and TGF-α, the occurrence of TGF-α in human urine (if present) is insufficient to generate a clonigenic response without concentration. In comparison, EGF is abundant in urine. Therefore, the clonogenicity recorded in Table 3 reflects the distribution of EGF in the MeCN eluent fractions. Notice in Table 3 that there is no clonogenicity in the 20% and little clono genicity in the 25% MeCN fractions in which TGF-α is preferentially eluted. Clonogenicity is most apparent in the 30% MeCN fraction which concentrates EGF.

Additional enrichment of EGF and TGF fractions as well as resolution of TGF-α from the much more abundant EGF was achieved by three more chromatographic steps. The first was based on molecular weight, the second on molecular charge, and the third on hydrophobicity. Molecular sieving on BioGel P-10 enriched the EGF fraction to 11,995 fold and the TGF-α fraction to 3,833 fold (Table 4). Because ^{125}I-labeled rTGF-α chromatographed with an apparent molecular weight of 8000, while ^{125}I-labeled hEGF chromatographed with an apparent molecular weight of 5000, further resolution between the two peptides was obtained in this step.

TABLE 3

Distribution of protein, radioactivity, and clonogenicity during concentration of C1 silica adsorption of iodinated growth factors added to normal human 24-hr samples

Growth factor	Assay procedure	Raw urine	Urine after acidification and filtration	Percent MeCN elution from Sepralyte beads 12%	15%	20%	25%	30%	35%	50%
^{125}I-hEGF	Total protein (mg)	6,650	6,175							
	Before dialysis	224	213	82.5	75	45.1	46.2	22.1	23.4	13
	After dialysis	224	213	4	5	8.6	18	3.2	3.2	1
	Radioactivity	100	99	0	0	0.2	21.4	67.1	7.2	0
	Clonogenicity			0	0	0	4	16	2	0
^{125}I-rTGF-α	Total Protein (mg)									
	Before dialysis	8,408	7,900	198	120	60.8	67.2	52.8	20.8	14
	After dialysis	237	231	3.2	5.8	12.8	21	4.6	2.3	0
	Radioactivity	100	99	0.2	1.9	41.0	40.1	3.0	0.7	0
	Clonogenicity			0	0	0	8	32	0	0

TABLE 4

Recovery and fold enrichment of iodinated growth factor during chromatographic steps

	Growth Factor					
	^{125}I hEGF			^{125}I rTGF		
	Percent Recovery		Fold	Percent Recovery		Fold
	Per step	Cumulative	Enrichment	Per step	Cumulative	Enrichment
Raw urine	100	100	1	100	100	1
Elution from C1 bonded silica (MeCN %)	67.1 (25-30%)	67.1	1,394	81 (15-25%)	81	202
Chromatography Bio-Gel P-10	77	52	11,995	65	52	3,833
Carboxymethyl cellulose	76	40	1.6×10^6	72	37	10,364
C18 HPLC	84	34	N.D.	75	28	117,647

CM-C chromatography was used to take advantage of differences in molecular charge. ^{125}I-labeled hEGF eluted from CM-C at pH 5 and was enriched to 1.6 x 10^6 fold (Table 4), while the more basic ^{125}I-labeled rTGF-α eluted at pH 6 and was enriched to 10,364 fold.

The third chromatography step was reverse phase HPLC on a C18 μ Bondapak column. Radiolabeled hEGF and TGF-α are well resolved in this stage (Fig. 2). The elution of ^{125}I-labeled hEGF (Upper Panel) closely coincides with the elution of the large amount of native hEGF (clonogenicity) which is normally present in human urine. ^{125}I-labeled rTGF-α eludes from a C18 HPLC column at a lower concentration of acetonitrile (Lower Panel). The radioactivity is accompanied by a very small amount of clonogenicity that is probably caused by the tracer rTGF-α rather than endogenous hTGF-α, which we have not observed at this stage of purification in the urine of normal donors. Note that there is no clonogenicity in the TGF-α enriched fraction at the 27 to 28% acetonitrile region where hEGF would be expected. Therefore, ^{125}I-labeled rTGF-α is well separated from native, endogenous hEGF that is abundantly present in urine. Because rTGF-α and hTGF-α are similar molecules, we expect that urine samples treated to enrich hTGF-α in the same manner as was used to enrich ^{125}I-labeled rTGF-α would yield a clinically useful sample for the determination of hTGF-α.

When h-TGF-α became commercially available, we were then able to test its efficiency of adsorption from urine and elution from C1 silica (Figure 3). In addition, important variables in the procedure of elution were evaluated. Two techniques of MeCN desorption from C1 silica were compared. One involved a direct elution with increasing concentrations of MeCN in free suspension, while the second required transfer of the adsorbed bonded silica to a column for elution. Using the column elution method described earlier and adjusting the eluent MeCN concentrations to increase the amount of TGF-α eluting in a single fraction, 92.6% was recovered in the 17-24% MeCN fraction (Figure 3A, and Table 2). Total recovery was quantitative. The importance of NaCl in the elution buffer is seen in Figure 3B. Eliminating NaCl from the

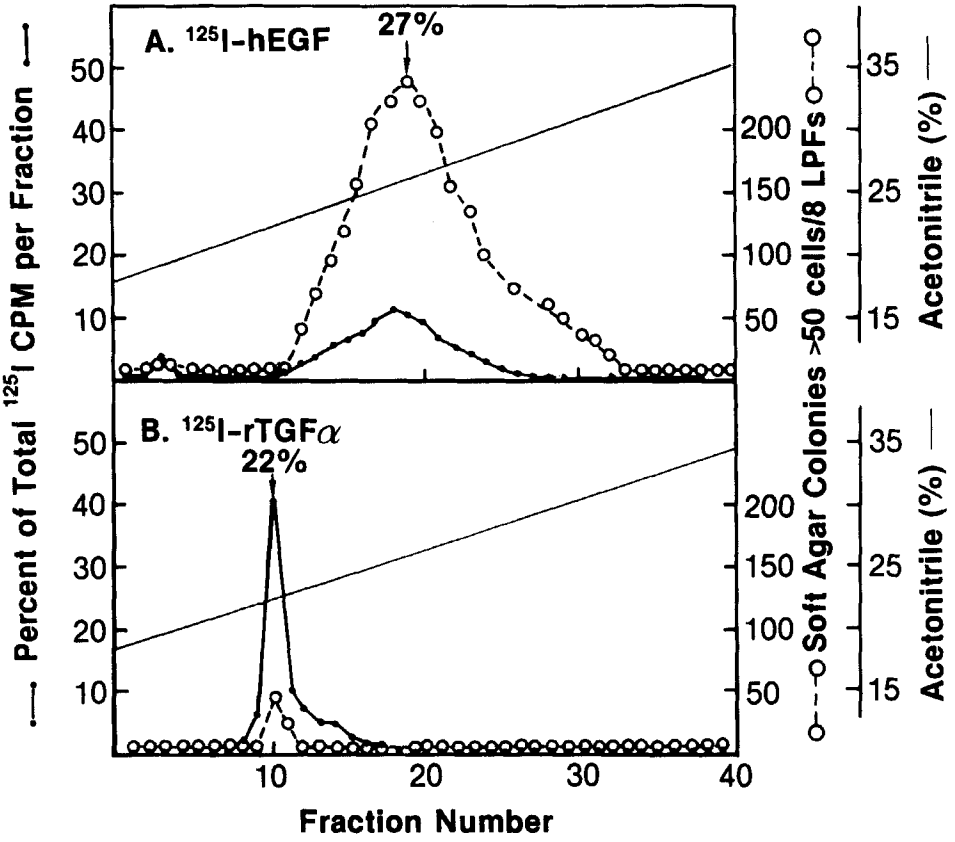

FIGURE 2. Comparison of C18 HPLC chromatography of ^{125}I-hEGF and ^{125}I-rTGF-α.

elution buffer reduced the recovery of ^{125}I TGF-α to 90% and shifted the elution peak to 30% MeCN. In the free suspension elution of ^{125}I TGF-α, rather than transferring adsorbed silica to column, eluate was added directly to the adsorbed silica followed by vigorous stirring for 30 min. After allowing the silica to settle for 45 min, the eluate was decanted and the cycle repeated using the next eluate in sequence. Free suspension elution

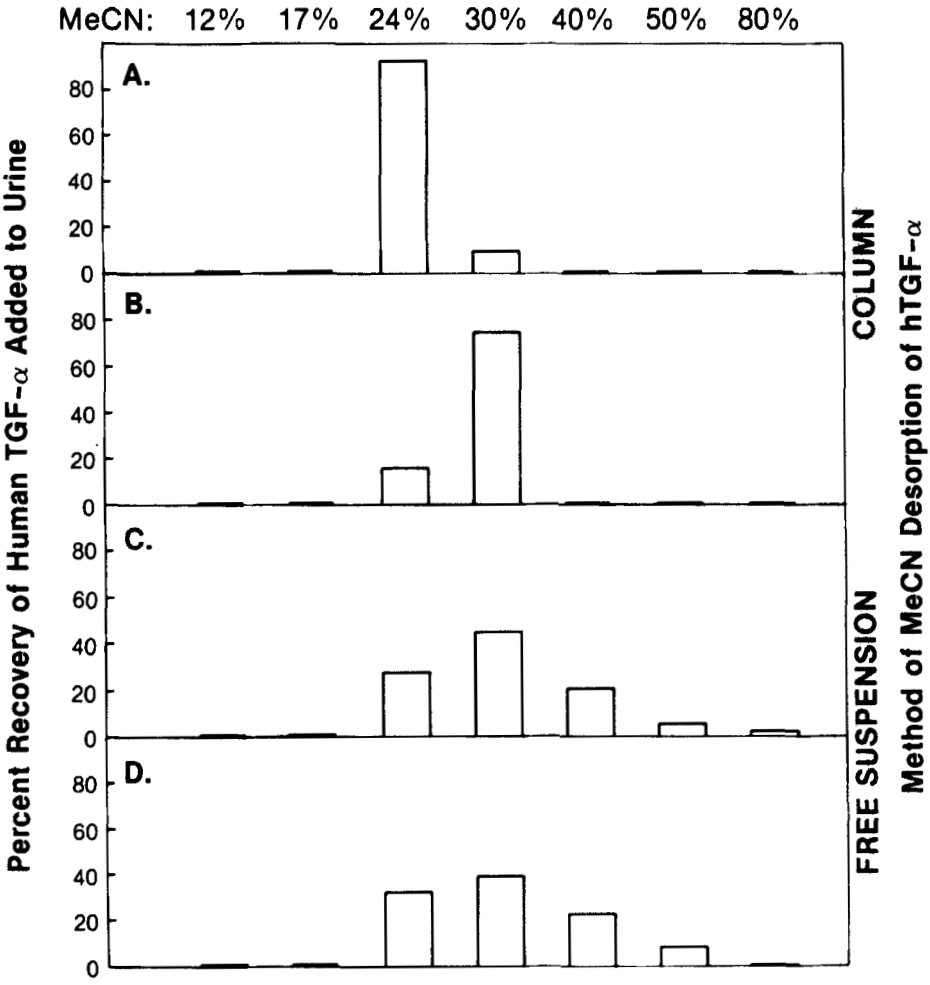

FIGURE 3. Distribution of human TGF-α in the MeCN elute from C1 silica. Bound hTGF-α was eluted from C1 silica using aqueous MeCN 0.1 M NaCl and 0.05% TFA. (A) Column elution of ^{125}I hTGF-α; (B) Column elution of ^{125}I hTGF-α without NaCl; (C) Free suspension elution of ^{125}I hTGF-α; (D) Free suspension elution of non-radioactive hTGF (assayed by RIA).

resulted in a less selective elution profile (Figure 3C), in comparison to column elution, with the greater amount of TGF-α eluting in 30% MeCN. The free suspension elution of added, non-radioactive hTGF (Figure 3D) yielded a total recovery of 97% as determined by RIA and a profile that paralleled that of ^{125}I TGF-α, indicating the validity of using iodinated polypeptide to follow and quantify recovery.

Subsequent dialysis of ^{125}I hTGF-α eluates against 0.5 M acetic acid resulted in a 20% loss of radioactivity from eluted fractions (data not shown). If a final dialysis against 15% MeCN 1 M acetic acid is used, the loss of ^{125}I-hTGF is reduced to less than 10%. Apparently, hTGF binds to dialysis membranes. Consequently, it may be a useful precaution when handling any of the growth factors discussed here to include a small amount of organic solvent in buffers whenever practical.

As Table 2 indicates, TGF-β is more hydrophobic than EGF and might bind strongly to C1 silica. To determine whether TGF-β could be adsorbed from urine and eluted in a simple and rapid manner, a free suspension elution procedure was tested. During the adsorption process in which 12 g of Sepralyte C1 silica were added to a 24 hr urine sample, previously adjusted to pH 3 and 12% MeCN, 85% of added ^{125}I-labeled hTGF-β was adsorbed after 30 min of stirring (Table 5). Selective elution was accomplished by suspending the ^{125}I-labeled hTGF-β adsorbed to C1 bonded silica in 5 ml/g of silica using an aqueous MeCN buffer containing 0.05% trifluoroacetic acid and 0.1 N NaCl. The concentrations of MeCN used for stepwise desorption were 15%, 20%, 25%, 30% and 50%. Of the total ^{125}I-labeled hTGF-β counts added to the urine, 70% eluted from silica at between 30% and 50% MeCN. Although this rapid method, in which protein-adsorbed C1 silica was eluted directly in free suspension by stirring in the elution buffer, differs from the column elution method, previously described for radiolabled hEGF and rTGF-α, a 250-fold enrichment was still achieved.

DISCUSSION

This report confirms and extends a prior one (13) by 1) reporting the binding efficiency of growth factors to additional

TABLE 5

Adsorption and Elution of ^{125}I hTGF-β Added to Human Urine

Treatment of 24 hr Urine Sample	Radioactivity Percent of Total	Protein 5.2 g = 100%
Adsorption to C1 bonded silica	85%	0.125 g (2%)
Elution in 30-50% MeCN	70%	0.014 g (0.27%)
	Fold enrichment - 250	

alkyl bonded silicas, 2) by comparing the efficiency of free suspension versus column desorption of growth factor from its sorbant, 3) by evaluating the additional relevant growth factors hTGF-α and hTGF-β, and 4) by quantifying by RIA as well as by iodination the recovery of exogenously added hTGF-α from urine by adsorption to and free suspension elution from C1 silica.

All six alkyl bonded silicas tested efficiently adsorbed ^{125}I-hEGF from urine. This absence of specificity probably reflects a general affinity of EGF for hydrophobic surfaces. C1 silica, as previously reported (13), quickly adsorbs rTGF-α and can be used to recover this growth factor from urine. Column elution of simultaneously bound EGF and rTGF-α can be performed selectively, according to relative hydrophobicity, so as to concentrate EGF and TGF-α in separate fractions. Fractional elution released 70 to 80% of the EGF between 25 and 30% MeCN, and 80% of rat TGF-α between 15 and 25% MeCN while increasing the specific activity 2400.fold and 200 fold, respectively.

TGF-β, which is structurally unrelated (2) and more hydrophobic than EGF and TGF-α, was also recovered from urine using C1 silica. Employing a free suspension elution method, 70% of TGF-β that had been added to urine was eluted from C1 silica between 30

and 50% MeCN. This method successfully enriched TGF-ß about 250 fold. The free suspension elution distribution of hTGF-α, on the other hand, indicated that this method is less selective than column elution for this peptide but would be useful when the primary objective is to concentrate peptides quickly without regard to their separation.

The MeCN elution of non-radioactive hTGF-α as determined by RIA parallels that of ^{125}I-hTGF-α and totals 96% of added hTGF-α. These results indicate C1 silica adsorption and MeCN elution can provide a sample that is sufficiently free of interfering material to give accurate RIA values without further purification.

The methods described here may not be limited to urine and might be useful for the extraction of these growth factors from large volumes of cell culture medium (8), and perhaps even from serum. Moreover, while the extraction of TGF-α, or other polypeptide growth factors cleared at the renal level, may prove useful in cancer detection (5), the growth factors studied here may find pharmacologic application in clinical treatment (14, 15). In this latter possibility, this method of rapid extraction, concentration and selective elution could be applied to clinical monitoring of circulating and excreted growth factor concentrations.

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