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Gradient Sievorptive Chromatography. A Focusing System for the Separation of Cellular Components†

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ABSTRACT: Chromatographic systems that employ an active combination of molecular sieve and adsorption chromatography are useful for the rapid purification of enzymes. Such systems, called sievorptive chromatography, will separate either strongly interacting macromolecules or similar macromolecules under conditions of maximal resolution. If appropriate gradients are used in sievorptive chromatography, a system is formed that focuses macromolecules within the chromatographic column at positions that depend upon their adsorptive properties. This report introduces focusing chromatography and illustrates its use for the rapid purification of *Escherichia coli* enzymes. Special problems are encountered when salt gradients are used with ion exchange gels due to the formation of inherent gradients. The nature of these gradients and the factors which control them are discussed.

The objective of modern biochemical separation techniques is to fractionate cellular components as rapidly and gently as possible in order to minimize the loss of biological activity. Additionally, it is important to reduce associated manipulations to a minimum. Sievorptive chromatography (Kirkegaard and Agee, 1973), the name for systems that actively combine molecular sieve (gel filtration) and adsorption chromatography, substantially assists in the realization of this objective. This article describes how appropriate gradients expand the usefulness of the two basic mechanisms of sievorptive chromatography. The first mechanism, ion filtration chromatography (Kirkegaard *et al.*, 1972), is a process that is optimized for the separation of macromolecules of similar physical properties. The second, intervent dilution chromatography (Kirkegaard and Agee, 1973), utilizes a solvent boundary to separate strongly interacting macromolecules. These processes, the former using no gradient at all, and the latter a nearly infinite gradient, are the extremes of sievorptive chromatography. Between these extremes is the domain of gradient sievorptive chromatography.

As a brief background to the basic mechanisms of sievorptive chromatography, the process of ion filtration chromatography (Kirkegaard *et al.*, 1972) employs a highly cross-linked ion exchange gel equilibrated at a precisely defined condition of pH and ionic strength such that the enzyme to be purified elutes in the sieving range, *i.e.*, after the peak of noninteracting, totally excluded material but sufficiently before the column liquid volume to permit the removal of low molecular weight substances from the enzyme. The system is adapted for the purification of any desired enzyme by changes in the pH and/or ionic strength of column equilibration or by using an alternate ion exchange gel. A single chromatographic step accomplishes the ion exchange fractionation and dialysis of an enzyme.

Intervent dilution chromatography (Kirkegaard and Agee, 1973) uses the sieving properties of ion exchange gels to repeatedly propel strongly interacting macromolecules across the boundary between a region of concentrated intervents[1] where macromolecules are relatively independent and a region of diluted intervents where macromolecules are separated by adsorptive or ion exchange processes. Under appropriate conditions, this repetitive action will separate ribosomal proteins from ribosomal RNAs, one of the strongest complexes encountered in biochemistry.

Between the extremes of intervent dilution and ion filtration chromatography lie a vast number of chromatographic systems of intermediate properties. A common property of these systems is a gradient of small molecules which extends through the sieving range, hence the description gradient sievorptive chromatography.

When appropriate gradients are used in sievorptive chromatography, a system is formed that focuses macromolecules as they move along the column at positions determined by the chromatographic properties of the molecules. A gradient, composed of small molecules and extending over about one-third of the length of the column, moves along the column at nearly the same rate as the solvent. Macromolecules, on the other hand, are totally excluded and in the absence of adsorption move along the column between two and three times the rate of the solvent. If the direction of the gradient is such that adsorption of a macromolecule is high on the leading edge and absent at the trailing edge, the macromolecule will slow down as it progresses along the gradient. Eventually the macromolecule reaches a level in the gradient that permits it to move along the column at the same rate as the gradient moves.

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[1] Small molecules that reduce or intervene in the attractions between macromolecules are called intervents (Kirkegaard and Agee, 1973).

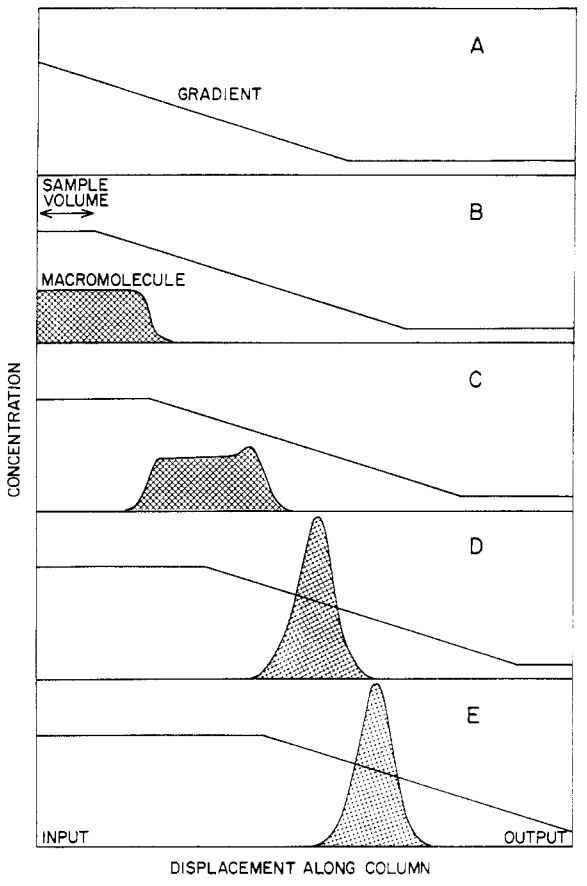

FIGURE 1: Focusing properties of a sieving range gradient. Frames A–D show steps in the development of a sievorptive column utilizing a gradient. The left-hand side of the figure represents the input of the column and the right-hand side the output. Frame A shows the gradient that is applied to the gel column before the addition of sample. The gradient is such that the applied macromolecule binds to the gel at the front of the gradient, but has no interaction with the resin at the back of the gradient. Frame B shows the situation after a sample has been added to the column. In this illustration, the macromolecule travels twice as fast as the sample solvent; hence the macromolecular peak is twice as broad as the sample volume. It is further assumed that aside from the macromolecule, the sample has the same composition as the end point of the gradient. Frame C shows the situation after a volume of eluent equivalent to the sample volume has been added to the column. The leading edge of the macromolecule peak has reached the point in the gradient where adsorptive forces are beginning to slow the macromolecular migration rate to that of the gradient. Frames D and E, respectively, show the situation after three and four sample volumes of the eluent have been added to the column. After concentration the sample peak moves along the column at the same rate as the gradient migrates. The position along the gradient where the concentrated peak and the gradient move at the same rate depends upon the adsorptive properties of the macromolecule and of the gel.

From another viewpoint, the sieving properties of the gel act on a macromolecule as a force to cause it to move rapidly along the column. The adsorption properties of the gel act as a counterforce to retard the movement of the macromolecule. Since the counterforce increases as the macromolecule advances along the gradient, it eventually reaches a point where the two forces are balanced in such a way that the macromolecule moves at the same rate as the gradient moves. A molecule significantly behind the balance point continues to migrate rapidly, and one that is ahead of the point waits until the gradient overtakes it before it continues to move. This action results in a system that focuses a class of macromolecules at a point along the gradient which is determined by the physicochemical properties of the molecules. This focusing action is illustrated in Figure 1. From this viewpoint it is easy to understand why highly cross-linked gels are important for this technique. If a macromolecule partially penetrates the gel, the driving force of the focusing action is greatly reduced. Furthermore, within a population of differing macromolecules, the driving force is not constant. These factors result in a complex system that is too complicated to adapt for a particular separation.

The behavior of gradient sievorptive chromatography is similar to that of other focusing systems, such as electrofocusing enzymes on a pH gradient or centrifuging macromolecules through a sucrose or CsCl gradient. The chromatographic system, however, offers certain advantages in system flexibility and in experimental economy. Gradients of various kinds and shapes can be used on gels having a wide range of adsorption properties. Column preparation and operation are much easier than electrofocusing procedures, and equipment costs are minor compared to ultracentrifuges. Furthermore, chromatographic procedures may be scaled up far beyond the practical limit for electrophoretic or centrifugation techniques.

The ability of a sievorptive column to focus and separate macromolecules depends upon several factors. Most directly, the focusing ability depends upon the slope of the gradient and the position in the gradient where the macromolecule elutes. Clearly, a steep gradient focuses macromolecules more effectively than a gentle one. However, with the increased focus comes less distance between peaks. Diffusion effects are large when a solute is maintained in a very concentrated band. To counteract diffusion, a steep gradient is needed. No matter how steep the gradient, it is impossible to focus compounds beyond their limit of solubility. The amount of focusing depends upon the position of elution because molecules that elute at the excluded volume are not focused at all, whereas molecules that elute late in the sieving range are focused the entire length of the column. As yet there is insufficient data to precisely correlate resolution with the steepness of the gradient. Another factor upon which the focusing ability and resolution depend is the uniformity of the molecular association responsible for retarding the migration rate of the macromolecule. If there exists a wide range in the amount of interaction between the macromolecule and the resin at a given point in the gradient, it is impossible for the sievorptive system to effectively focus and to resolve various species of macromolecules. Probably the limiting factor for the separation of molecular species is the uniformity of interaction. This emphasizes the need for gels with uniform physical properties.

There are two kinds of gradients in sievorptive chromatography, applied gradients and inherent gradients. In the case of applied gradients, the appropriate gradient, spanning about one-third of the column volume, is applied to the column before the addition of sample. Inherent gradients are produced by the small molecules in the applied sample as a result of molecular diffusion and the exclusion properties of the gel.

Experimental Section

Production of Inherent Gradients on DEAE- and CM-Sephadex by a Sudden Increase in Salt Concentration. A 15.8-ml DEAE-Sephadex A-25 column (height 31.8 cm) and a 16.1-ml CM-Sephadex C-25 column (height 32.2 cm) were equilibrated at pH 7.0 with 0.020 M KCl as described previously for ion filtration chromatography (Kirkegaard *et al.*,

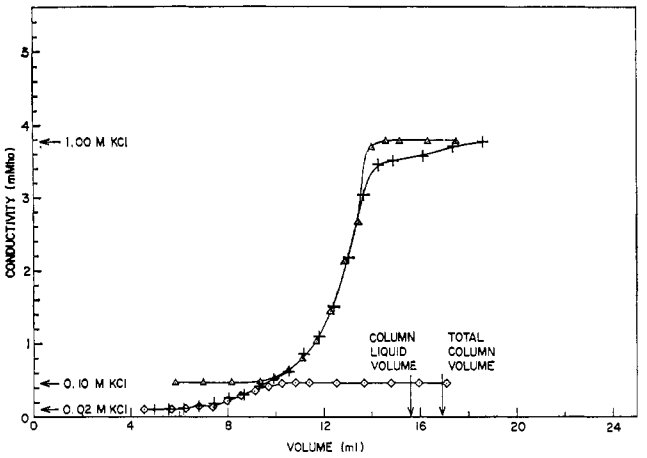

FIGURE 2: Inherent gradients produced on DEAE-Sephadex A-25 by KCl. The curves denoted with pulses, diamonds, and triangles show the gradients produced by an increase in the KCl concentration from 0.020 to 1.0 M, 0.020 to 0.10 M, and 0.10 to 1.0 M, respectively. The rate of column development was 24 ml/hr per cm^2.

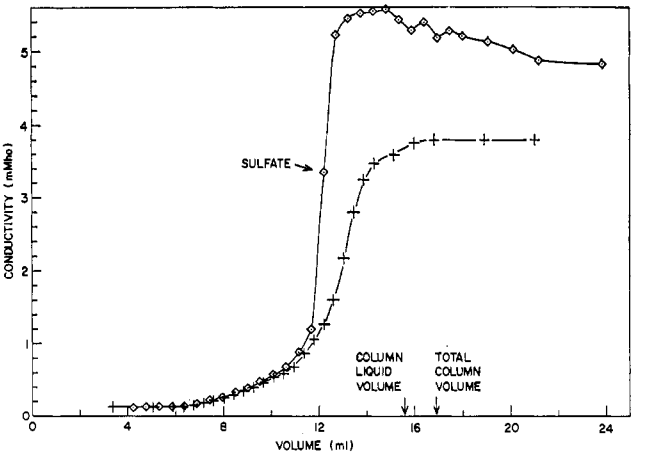

FIGURE 4: Comparison of the inherent gradient produced by KCl and $(NH_4)_2SO_4$ on DEAE-Sephadex. The inherent gradients produced by the addition of 1.0 M KCl and 1.0 M $(NH_4)_2SO_4$ to a DEAE-Sephadex A-25 column equilibrated with 0.02 M KCl are shown by the curves marked with pluses and diamonds, respectively. The rate of column elution was 8 ml/hr per cm^2.

1972). The columns were developed in the reverse direction, *i.e.*, from bottom to top, with the aid of a peristaltic pump at the rate of 12 ml/hr (24 ml/hr per cm^2) and 4 ml/hr as noted in Figures 2–5. At the beginning of the experiment at volume 0, the salt concentration of the solution at the pump inlet was increased to the noted values. The conductivity at 25° of the collected fractions was measured on a Leeds Northrup conductivity bridge, Model 4960. (The specific conductivity may be obtained from the plotted values by multiplying by a cell constant of 25.) To determine the column liquid volume, the columns were again thoroughly equilibrated with 0.020 M KCl and weighed and the contents dried. The difference in the weight of the column packing before and after drying was used to calculate the column liquid volume. The H_2O content of the columns was 14.5 and 15.4 ml in the DEAE and CM columns, respectively. The volumes noted in Figures 2–5 include the 1.1-ml volume of the inlet plus outlet tubing. Analysis of the dried resins for Cl and K gave the following percentages: DEAE, 10.01% Cl, 0.29% K; CM, 0.13%, Cl, 14.99% K. Using these values to calculate the amount of KCl in the dried resin samples, it was found that 2.931 g of anhydrous DEAE-Sephadex A-25 as the chloride salt hydrated to 15.8 ml of tightly packed resin when equilibrated with 0.020 M KCl, and 2.357 g of anhydrous CM-Sephadex C-25 as the potassium salt expanded to 16.1 ml.

Purification of Escherichia coli Enzymes with Gradient Sievorptive Chromatography. Escherichia coli enzymes tRNA-nucleotidyltransferase (EC 2.7.7.25) and polynucleotide phosphorylase (EC 2.7.7.8) were purified by gradient sievorptive chromatography. Three-hundred grams of frozen *E. coli* K-12, strain M72-44, genotype *F$^-$lac$^-$str^{r}trp$^+$sup$^-$*/(T1,T5), was slurried in 1200 ml of 0.1 M phosphate buffer (pH 7.2) (0.050 M KH_2PO_4—0.050 M K_2HPO_4). After the addition of 0.3 g of 3× crystallized egg-white lysozyme, the cells were incubated for 30 min at 37°. Toluene, 300 ml, was added to assist in cell disruption and the highly viscous suspension was centrifuged at 13,000 rpm for 40 min in the GSA, Sorvall rotor. The unsheared DNA in the lysate entraps cellular debris and particles; and therefore most of the DNA and ribosomes are removed from the resultant supernatant. The pH of the 1150 ml of supernatant was raised to 9 by the addition of 42 g (3.65 g/100 ml) of Tris, and the enzymes were fractionated by precipitation at 0° with ammonium sulfate. The lower ammonium sulfate concentration was 17.5 g/100 ml of supernatant; the

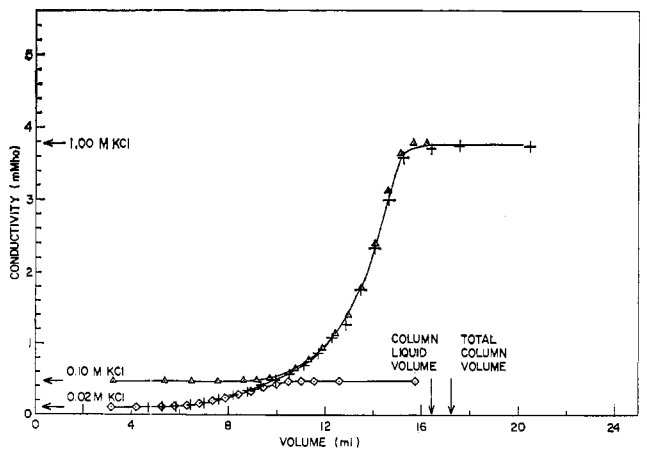

FIGURE 3: Inherent gradients produced on CM-Sephadex C-25 by KCl. Curve symbols and rate of column development are the same as in Figure 2.

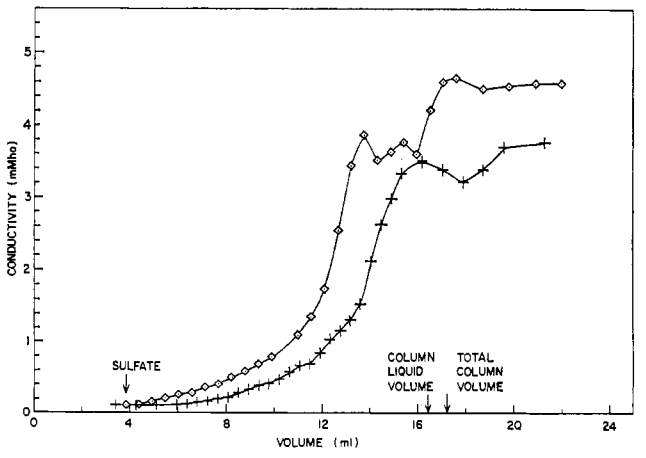

FIGURE 5: Comparison of the inherent gradient produced by KCl and $(NH_4)_2SO_4$ on CM-Sephadex. A CM-Sephadex C-25 column equilibrated with 0.02 M KCl was treated with 1.0 M KCl and $(NH_4)_2SO_4$ as described in Figure 4.

upper concentration was 35 g/100 ml of original supernatant. The precipitate was dissolved in 0.9 M NaCl buffered at pH 7 with 0.1 M imidazole-HCl (0.050 M imidazole–0.050 M imidazole-HCl). The sample was kept as concentrated as possible. During this enzyme preparation, four batches of *E. coli* were processed. Sample volumes ranged from 120 to 140 ml and the absorbance at 260 nm ranged from 125 to 260.

A 2000-ml column 30 cm high of DEAE-Sephadex A-25 was washed with 0.5 M NaOH, then 0.5 M HCl and equilibrated with 14 l. of 0.05 M NaCl in 0.10 M imidazole-HCl (pH 7.0). Before the application of the sample, a linear 600-ml gradient from 0.050 to 0.300 M NaCl in the same imidazole buffer was added to the column. Column development was from bottom to top at a pumped flow rate of 650 ml/hr. After the sample had been applied to the column, about 200 ml of 1 M NaCl in 50% glycerol was added to the column in order to keep a uniform sample band. Finally, 1 M NaCl was applied. Fractions of 9.5 ml were collected each 0.9 min. The uv absorbance at 254 nm was continuously monitored with a Chromatronix UV monitor (Model 200).

tRNA-nucleotidyltransferase activity was measured by the incorporation of radioactive ATP into tRNA missing the terminal adenosine as described elsewhere (manuscript in preparation; Carre *et al.*, 1970). Polynucleotide phosphorylase was measured by the polymerization of tritiated ADP into material that is precipitated by cetyltrimethylammonium bromide or trichloroacetic acid (manuscript in preparation; Williams and Grunberg-Manago, 1964).

Results

The most useful materials for sievorptive chromatography of biological macromolecules are highly cross-linked ion exchange gels. The overriding adsorptive process in these gels is ion exchange. As with conventional ion exchange chromatography, salt gradients are the most useful with these resins. Although the size of the commonly used salt ions is small compared to the exclusion limits of the gel matrix, ions that have the same charge as the gel are partially excluded. The amount of exclusion depends upon the ionic strength around the gel particle and is highest at the lowest ionic strength. When an ion exchange gel column is equilibrated at low ionic strength, these ion exclusion properties result in the formation of an inherent gradient that is projected throughout the sieving range. Figures 2–5 show the inherent gradients that are produced under various conditions. In these figures columns are equilibrated at a low ionic strength and the experiment is started at volume 0 by switching the pump inlet to a solution of higher ionic strength.

Figures 2 and 3 show what happens when the KCl concentration of the solution applied to columns of DEAE-Sephadex A-25 and CM-Sephadex C-25, respectively, is suddenly increased. The conductivity of the column effluent is plotted as a function of the volume eluted from the column after the increase in ionic strength. Three experiments are shown in each figure. In the experiment denoted with pluses, the KCl concentration of a column equilibrated at 0.02 M was increased to 1.0 M KCl. The conductivity of the effluent begins to increase after approximately 0.4 column bed volume has issued from the column. This is about the same point at which nonadsorbed and totally excluded macromolecules begin to elute. The conductivity increases nearly linearly through the sieving range until it begins to curve upward rapidly near the column liquid volume. If the gel column is equilibrated at 0.10 M KCl (curve denoted by triangles) and the sudden transition is made to 1 M KCl as in the first experiment, the conductivity does *not* increase within the sieving range until nearly the same point where the previous column reached 0.1 M KCl, whereupon the conductivity curves of the two experiments become nearly superimposable. In the experiment denoted with diamonds, the column is equilibrated at 0.02 M KCl as in the first column, but the salt level of the eluent is increased to only 0.1 M KCl. In this experiment the conductivity begins to increase at the excluded volume and continues to increase throughout the sieving range until it reaches 0.1 M in a curve that nearly superimposes the early portion of the first experiment.

Figures 4 and 5 show what happens when 1 M ammonium sulfate is applied to the DEAE- and CM-Sephadex columns equilibrated with 0.020 M KCl. The flow rate of these experiments was one-third that of Figures 2 and 3. The experiment denoted with pluses is the control experiment where 1 M KCl was applied to the column. The experiment illustrated with diamonds shows the effect of the ammonium sulfate. The gradient produced by $(NH_4)_2SO_4$ through the sieving range of the DEAE column, Figure 4, is virtually identical with the gradient produced by the KCl. This is because the applied sulfate displaces chloride from the gel to produce NH_4Cl which has a similar behavior to KCl. In the example of CM-Sephadex, Figure 5, the gradient produced in the sieving range by the ammonium sulfate is considerably steeper than that produced when KCl is applied. Sulfate begins to elute after only 0.3 column liquid volume has emerged from the column. The first tube to contain sulfate is noted on the chromatograms. It is also of interest to note that the point of maximum increase in conductivity occurs significantly earlier in the columns where $(NH_4)_2SO_4$ is applied. Although the molecular weights of KCl and $(NH_4)_2SO_4$ are small compared to the exclusion limits of the gels, it is clear that these molecules do not penetrate the gel to the same extent. The column liquid volume as measured by the loss in weight upon drying of the column packing is noted on Figures 2–5. From these figures, it is apparent that even KCl is excluded from some of the interior regions of the gel.

It is important to realize that convection, column channeling, and nonequilibrium between column phases did not contribute to the sieving range gradients shown in Figures 2–5. All of these columns were eluted in the reverse direction so that the dense, concentrated salt solution applied to the column was always below the lighter solution in the column. This precaution rules out convection and channeling as the cause of the sieving range gradients. The KCl controls in Figures 4 and 5 which were eluted at one-third the rate of the experiments in Figures 2 and 3 are superimposable,[2] within the error of volume measurement, on the corresponding curves in the earlier figures. This means that the sieving range gradient was not the result of nonequilibrium between column phases. Any channeling, convection, and nonequilibrium would add their effects to the basic sieving range gradients shown in Figures 2–5.

The gradient that is produced in the sieving range is determined principally by the repulsion properties of the gel and by the electrolyte properties of the ion(s) in the applied sample that has the same charge as the gel. Thus, the gradient in the

[2] Some significant differences are noted between curves when the effluent salt concentration nears 1 M. This results from the shrinkage of the gel beads and the convection caused by the consequent settling of the resin in the columns operated upside down at low flow rates. This phenomenon has no effect on the sieving range gradient.

early sieving range produced on columns equilibrated at low ionic strength is virtually independent of the salt concentration of the sample, since the same gradient is obtained when 0.1 M KCl is applied to the column as when 1 M KCl is applied.

It is of interest to note what happens when a short pulse of concentrated salt solution is added to an ion exchange gel column equilibrated at low ionic strength. This happens when an enzyme sample containing high salt is applied to the column. Under this circumstance, a gradient is formed in the sieving range that is identical with the illustrated gradients up to the point that the amount of salt in the sample will support, whereupon the salt level returns to the starting value. It is important for reproducible sievorptive chromatography to avoid this condition. This is most easily done by following the sample with a solution of equivalent or higher ionic strength so that a continuous gradient is formed.

If an ion exchange gel is equilibrated with KCl at concentrations greater than 0.1 M, no gradient is produced throughout the sieving range simply by the addition of a sample of high ionic strength. A sieving range gradient is obtained in this system by applying a short gradient to the column before the sample is applied. Usually it is satisfactory to apply a gradient spanning the appropriate range over a volume equivalent to 30% of the column bed volume. The same procedure can be used to obtain gradients of nonionic molecules within the sieving range. Finally, a short gradient may be added to an ion exchange gel column to modify the inherent gradient produced by the sample salts.

As with any form of gradient chromatography, the appropriate range for the gradient depends upon the chromatographic properties of the molecules to be purified. As previously described for ion filtration chromatography (Kirkegaad *et al.*, 1972) best results are obtained when the purified enzyme is eluted midway in the sieving range. The procedures used in that reference may be used to precisely adjust the column so that the desired enzyme will be eluted at the optimum position.

Figure 6 shows an example of how gradients may be used to obtain a useful compromise between intervent dilution and ion filtration chromatography. A system is shown which accomplishes the equivalent of high-speed centrifugation, dialysis, and ion exchange fractionation of an enzyme preparation while simultaneously removing nucleic acids from the proteins. The chromatographic parameters of this system were chosen so that the nucleic acids were removed from the proteins by the intervent dilution process resulting from a NaCl boundary produced at the sample front, while proteins were simultaneously fractionated by the focusing gradient within the sieving range. Remarkably, at the scale of this experiment, the chromatographic step required less time than the ammonium sulfate fractionation that preceded it. The enzyme lysate obtained from 300 g of *E. coli* was fractionated by ammonium sulfate precipitation (25–55%) at pH 9, dissolved in buffered 0.9 M NaCl, and applied to a 2-l. column of DEAE-Sephadex A-25. Before the addition of the sample a 600-ml gradient between 0.05 and 0.30 M NaCl was applied to the column. Under these chromatographic conditions, proteins separate on the basis of their ion exchange properties and elute throughout the sieving range. Just before the ionic strength begins to rapidly increase, ribosomes elute followed by polynucleotides and low molecular weight substances that were in the sample. Since the enzymes elute from the column in only 1.5 hr, it was not necessary to develop the column at cold room temperature, hence the use of 25°. The enzyme activity profiles of two enzymes of interest, polynucleotide phosphorylase (· · ·) and tRNA-nucleotidyltransferase (C-C-A enzyme, ---) are shown along with the conductivity of the samples to illustrate the resultant gradient. The activity profile for polynucleotide phosphorylase indicates multiple enzymes. Subsequent purifications have confirmed the presence of at least two enzyme forms.

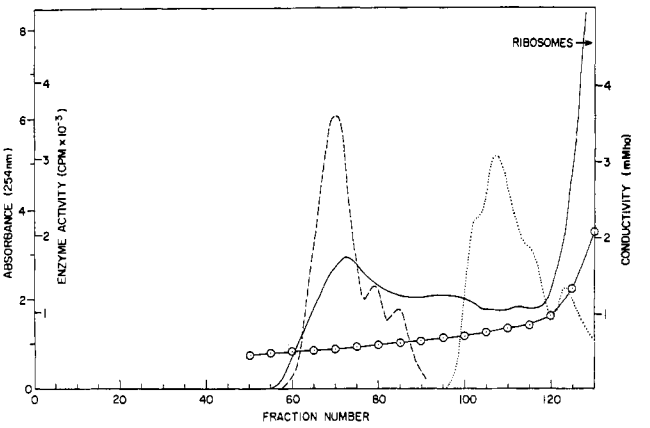

FIGURE 6: Purification of *E. coli* enzymes with gradient sievorptive chromatography. *E. coli* enzymes, tRNA-nucleotidyltransferase (- - -) and polynucleotide phosphorylase (· · ·), were purified by gradient sievorptive chromatography. The uv absorbance at 254 nm is shown by the solid line (—) and the sieving range gradient is illustrated by the conductivity trace (⊙–⊙). The conditions of chromatography and of sample preparation are described in the Experimental Section.

Other examples illustrating the usefulness of gradient sievorptive chromatography with particular emphasis on the development of chromatographic conditions and the measurement of recovered enzyme activity will be presented in a forthcoming paper by T. J. A. Johnson and R. M. Bock.

Discussion

Sievorptive chromatography adds a powerful new dimension to protein purification. The combination of molecular sieve and adsorption chromatography permits the construction of systems that rapidly accomplish separations that are difficult or impossible using either of the component processes alone. With the introduction of gradients in sievorptive chromatography, nearly any compromise between ion filtration and intervent dilution chromatography may be obtained. For example, a gentle, sieving range gradient makes ion filtration chromatography less sensitive to slight environmental changes while retaining excellent separation properties. This work illustrates how a sieving range gradient permits intervent dilution chromatography to resolve proteins while removing nucleic acids from the enzymes.

Before attempting to use gradients in sievorptive chromatography, it is essential to understand the inherent gradients that are produced within the system. When these gradients are understood, it is usually possible to manipulate them to the benefit of the system and to avoid experimental pitfalls. For a given ion exchange gel, the inherent gradient depends upon both the ionic strength of column equilibration and the electrolyte properties of the ions applied to the column. A steeper gradient is produced when ammonium sulfate is applied to a CM-Sephadex column than when potassium chloride is added. A similar result is obtained when magnesium chloride is applied to a DEAE-Sephadex column. Although other ion exchange gels have not been tested, it is expected that with

reference to these salts the inherent gradients will have the same relative relationships, but will be quantitatively different.

Although this study dealt primarily with salt gradients on ion exchange gels, the concept of gradient sievorptive chromatography has much broader scope. Gradients of nonionic substances can also be used on ion exchange gels for specific separations. Alternatively, sieving range gradients can be used on noncharged gels. For example, a gradient of decreasing ammonium sulfate on Sephadex G-100 was used by Porath (1962) in a system called zone precipitation to separate serum proteins. Contrary to the principles of this report, Porath attempted to resolve macromolecules on the basis of size as well as adsorption (solubility) parameters. Although a useful separation was obtained, the combination of both sizing and adsorption parameters results in an empirical process that cannot be systematically adapted to a particular problem. More recently gradients of detergents on agarose gels have been used to obtain separation of proteins in cell membranes (Swanljung, 1971).

Ion exchange gels were recently used to separate nucleosides by ion exclusion chromatography (Singhal and Cohn, 1972; Singhal, 1972). It is imperative to understand the inherent gradients produced by ion exchange gels when using ion exclusion chromatography, because a salt gradient that focuses macromolecules in sievorptive chromatography serves to disperse the molecules in ion exclusion chromatography. For reproducible ion exclusion chromatography, the salt composition of the sample should be as close as possible to that with which the column is equilibrated.

Sievorptive chromatography provides systems with capabilities ranging from the separation of strongly interacting macromolecules to the separation of similar molecules under conditions of maximal resolution. The basic mechanisms of sievorptive chromatography demand that proteins interact only with the surface of the resin particles. In this respect, the methods are consistent with modern principles of high-efficiency chromatography which utilize surface adsorption to achieve rapid equilibration of the solutes between column phases. Chromatographic conditions are carefully defined so that the purified enzymes are never permitted to interact strongly with the resin. This minimizes surface denaturation and avoids cooperative interactions with the resin which ultimately destroy column resolution. While on the column the purified enzymes are continuously subjected to an environment causing fractionation. No time is wasted under conditions where the enzyme is irreversibly adsorbed to the top of the column.

In summary, sievoptive chromatography is rapid and gentle so that enzyme loss is minimized. The procedures are flexible and can be adapted to a wide range of problems. Experimental minipulations associated with enzyme purification are greatly simplified. In this study the time-consuming steps of ribosome removal by high-speed centrifugation and ammonium sulfate removal by dialysis are avoided. Enzymes are minimally diluted during chromatography and subsequent concentration is greatly simplified. Reproducible procedures mean that after the appropriate chromatographic systems are identified, enzyme assays are almost unnecessary. These properties of sievorptive chromatography mean that sequential steps of purification are easily coupled. With optimization it is straightforward to couple three or four stages of purification to yield homogeneous enzyme in less than 24 hr of total processing time.

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