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Series Editors

Sidney P. Colowick and Nathan O. Kaplan

RNA AND PROTEIN SYNTHESIS

Edited by

KIVIE MOLDAVE

RNA *and* PROTEIN SYNTHESIS

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Foreword

The *Methods in Enzymology* series, which was originally published as a four-volume treatise over twenty-five years ago, has now grown to over 80 volumes. It has become more and more difficult for an individual investigator to locate particular methods of interest, especially in rapidly developing fields in which pertinent information now appears in many volumes of the series. Although individual and cumulative indexes are provided, the task of information retrieval is still formidable. We have, therefore, undertaken to provide such investigators and their students with a single volume work in a given area of interest, compiled by selection of the most essential and widely used procedures published in volumes of *Methods in Enzymology* in that particular area. The aim is to permit the individual investigator or student to have conveniently at hand all of the basic methodology in that field at relatively low cost. The articles, which are selected by the editors in that area, will be unabridged. A new Subject Index will be prepared for each volume of "Selected Methods in Enzymology."

It is our intention that one volume of "Selected Methods" will be derived from five to six related volumes of equivalent size in the parent series. This volume, which is the first of the "Selected Methods" series, deals with RNA and Protein Synthesis, and is comprised of articles selected by Dr. Moldave from volumes of the *Methods in Enzymology* series for which he served as an editor. We hope that this experiment in publication proves useful to the broad audience for this new series.

SIDNEY P. COLOWICK
NATHAN O. KAPLAN

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Section I

Transfer RNA

[1] Reversed-Phase Chromatography Systems for Transfer Ribonucleic Acids—Preparatory-Scale Methods[1]

By A. D. KELMERS, H. O. WEEREN, J. F. WEISS, R. L. PEARSON, M. P. STULBERG, and G. DAVID NOVELLI

Reversed-phase chromatography (RPC) is a system in which a water-immiscible organic extractant is present as a film on an inert support and an aqueous solution, passed through the column, develops the chromatogram. Column chromatographic techniques are inherently simple with regard to the apparatus involved (a length of glass pipe) and can readily be scaled up for production applications or scaled down for microanalytical use by changing the column size.

In designing these RPC systems for tRNA separations,[2-4] it was assumed that the tRNA's could be considered as long-chain polyphosphates, and thus an anion-exchange type of column would be needed. Problems of diffusion within conventional anion-exchange resin beads are avoided, since all the exchange sites are on the surface of the inert diatomaceous earth material of relatively high surface area employed as the solid support. The latter is acid washed and then treated with dimethyldichlorosilane to yield a hydrophobic surface of minimum surface activity. This support is then coated with a water-insoluble quaternary ammonium salt of high molecular weight which functions as the active extractant. A variety of quaternary ammonium compounds that meet these fundamental criteria are commercially available, and several different ones have proved useful for the separation of tRNA's.

A simple model of the mechanism of tRNA mobility on these RPC columns is anion exchange controlled by mass action. The tRNA's are applied to the column in the chloride form in a dilute sodium chloride solution and chloride ions bound to the quaternary ammonium extractant exchange for tRNA phosphate anionic sites; the tRNA's are thus retained on the column with essentially zero mobility. At higher sodium

[1]Research sponsored by the National Institute of General Medical Sciences, National Institutes of Health and the U. S. Atomic Energy Commission under contract with Union Carbide Corporation, Nuclear Division.

[2]A. D. Kelmers, G. David Novelli, and M. P. Stulberg, *J. Biol. Chem.* 240, 3979 (1965).

[3]J. F. Weiss and A. D. Kelmers, *Biochemistry* 6, 2507 (1967).

[4]J. F. Weiss, R. L. Pearson, and A. D. Kelmers, *Biochemistry* 7, 3479 (1968).

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chloride concentrations, mass action then favors chloride binding with the quaternary ammonium compound; the tRNA's are thus released from the support to the aqueous phase and eluted from the column. If step elution (loading at a low sodium chloride concentration followed by elution at a high sodium chloride concentration) is employed, the tRNA's are eluted as a group with little separation. However, if the sodium chloride concentration increases in a continuous manner, gradient elution, each tRNA transfers from the immobile quaternary ammonium compound to the mobile aqueous phase at a characteristic sodium chloride concentration determined by the specific chromatographic conditions.

Several factors regulate the separation of individual tRNA's during such gradient elution. Since most tRNA's have similar molecular weights, and thus nearly equivalent numbers of phosphate groups, it is unlikely that differences in size or total number of phosphates is a major factor in determining the elution sequence. The tRNA's possess a considerable degree of secondary and tertiary structure that would restrict the number of phosphates available for interaction. Solution conditions known to affect the structure of tRNA's, such as temperature and magnesium ion concentration, affect the elution position from RPC columns. Further, the order of elution of tRNA's from polyacrylamide gel columns, where the controlling factor is effective size (not molecular weight), is the reverse of the order from reversed-phase columns.[5] These results are consistent with the concept that tRNA elution from reversed-phase columns is controlled by the availability of phosphate groups for interaction with the quaternary ammonium exchange sites. Thus, the more tightly structured tRNA's would be eluted first (at low sodium chloride concentrations), and the more flexible, loosely structured tRNA's would be eluted later (at higher sodium chloride concentrations).

A number of commercially available quaternary ammonium compounds have proved useful in these RPC systems, either deposited as a solid on the surface of the diatomaceous earth or dissolved in a water-immiscible inert diluent. Each of these systems, while in general similar, exhibits certain differences in the elution order, sodium chloride concentration, sharpness of peaks, etc., that give each system certain advantages or disadvantages in specific applications. Three RPC systems are described in this report. They are:

RPC-2, tricaprylylmethylammonium chloride (Aliquat 336, General Mills, Kankakee, Illinois) dissolved in tetrafluorotetrachloropro-

[5] B. Z. Egan, R. W. Rhear, and A. D. Kelmers, *Biochim. Biophys. Acta* 174, 23 (1969).