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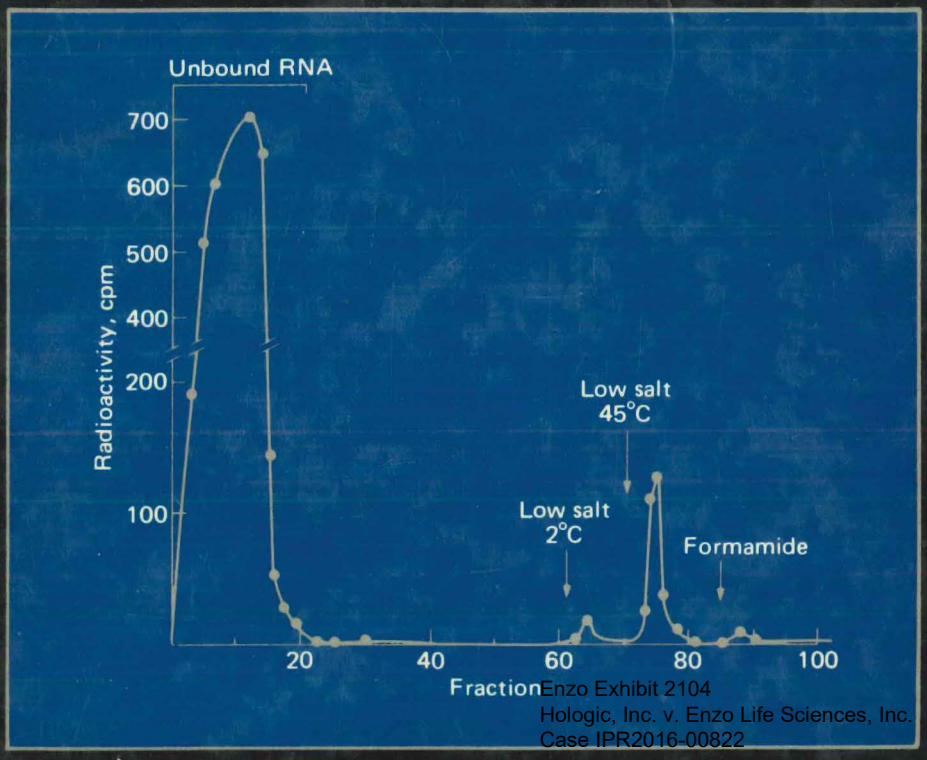
AFFINITY CHROMATOGRAPHY

Template Chromatography of Nucleic Acids and Proteins



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Affi Chromatography

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Contents

III. SPECIAL METHODS FOR THE IMMOBILIZATION OF RNA AND POLYRIBONUCLEOTIDES

The efficiency of immobilization of high-molecular-weight yeast RNA on aminated silica carriers has been studied [449]. More than 90% of the RNA added was bound to the carrier in undegraded form. The procedure allows us to link as much as 2.0 mg of RNA per gram of carrier. During nucleic acid hybridization (65°C, 2 × SSC) in the presence of trace amounts of pronase, chipping off of RNA was observed only during the first 6 hr of incubation. More than half of the immobilized poly(A) RNA remained tightly bound to the carrier. The immobilized messenger and ribosomal yeast RNAs were hybridized with total yeast [^{125}I] DNA. Several methods for the immobilization of polyribonucleotides take advantage of the vicinal diol groups present or potentially present in polyribonucleotides.

A. Immobilization of Oxidized Polyribonucleotides

The free terminal 3'-hydroxyl groups of polyribonucleotides can be oxidized in aqueous solution by periodate as reported in Sec. I.B of Chap. 2 for the oxidation of mononucleotides. The resulting aldehydropolyribonucleotides form Schiff bases with the primary amino groups of AE-cellulose (Scheme 16). The unstable Schiff bases can be converted to stable structures by reduction using $NaBH_4$ [8,103-105]. The binding in these products is thought to arise from the formation of a substituted morpholine ring structure that includes the nitrogen of the AE-cellulose and the five atoms that originally constituted the ribose ring of the terminal nucleoside. The assignment of this linkage is based on the structure of the product obtained from the borohydride reduction of the complex formed between periodate-oxidized AMP and methylamide [106].

The aldehyde-polyribonucleotides may also be added to a hydrazine or a hydrazine-containing support to form a stable hydrazone bond (Scheme 16). For example, a mixture of agar and polyacrylic acid hydrazide has been used in this way to immobilize tRNA [107]. Periodate-oxidized mRNA has been coupled to a linear polymer of acrylic hydrazide entrapped in agar [108]. The reduction with $NaBH_4$ has also been used to stabilize the linkage in a similar complex obtained from periodate-oxidized tRNA and hydrazinyl-Sepharose [109].

The attachment of RNA by its 3' terminus to agarose has been accomplished [110] using alkaline activation of agarose with CNBr,

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