26

Peptides and Proteins

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I. INTRODUCTION

Thin-layer chromatography has found extensive application in protein chemistry. This includes recovery of peptides in microgram and nanogram quantities for further primary structural analysis; identification of peptides in partial hydrolysates, in correlating the chromatographic properties of the intact peptides with those of individual amino acids; peptide mapping to characterize or identify a protein available in very small quantities; resolution of diastereomeric and enantiomeric peptides without any derivatization; fractionation of proteins on the ultramicro scale; testing the optical homogeneity of synthetic peptides; and determination of molecular weights of peptides and proteins. However, TLC has been practiced to a lesser extent for the analysis of peptides, especially the proteins, because several other high-resolution techniques are available, e.g., HPLC; column liquid chromatography involving size exclusion, ion-exchange, and affinity phenomena; SDS-PAGE; capillary electrophoresis; and mass spectrometry as a detector.

Optimization of chromatographic separations of peptides and proteins means a complete resolution of all components in minimum time, on a preparative scale, and with the recovery of bioactivity. Therefore, a knowledge of the behavior of peptides and proteins with both mobile and stationary phases is required; this includes information about kinetics of diffusion, adsorption and desorption, denaturation, and conformational changes. Various principles of liquid chromatography have successfully been applied to TLC resolution of peptides and proteins, e.g., reversed phase, size exclusion, and ion exchange. The thin-layer materials used for the purpose include silica gel, cellulose, mixtures of silica gel and cellulose, hydroxyapatite, and cross-linked dextran gel filtration media such as Sephadex (various grades from Pharmacia, Uppsala, Sweden). The ordinary porous silica-based stationary phases containing chemically bonded alkyl chains of varying lengths have several disadvantages, including low stability at alkaline pH values (pH > 8), secondary equilibria caused by low diffusion kinetics within the pores, and ion-exchange effects due to ionized underivatized silanol groups. Therefore, alternative stationary phases are being developed, e.g., coated silica phases, polymer-based phases, and nonporous materials. The separation and purification of peptides and proteins by an ion-exchange approach offers advantages because the mild separation conditions provide higher recovery of bioactivity.

The various peptides and proteins have been located on thin-layer chromatograms by using ninhydrin, fluorescamine, orthophthaldialdehyde, iodine vapors, or UV. Quantification has been

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