Until relatively recently, only one general protein property, i.e., electrical charge in the case of ion exchange adsorbents, was wittingly employed as a parameter for nonspecific adsorption chromatography of proteins. One of the reasons for the delay of investigations of possible hydrophobic effects probably was the assumption that hydrophobic groups of proteins generally are situated in the interior of the native protein molecule and thus are inaccessible. The occurrence of external hydrophobic groups was looked upon as relatively rare. However, recent observations (Klotz, 1970) indicate that the hydrophobic amino acid side chains, including the largest ones such as those of
phenylalanine and tryptophan, occur much more frequently on the surface of native protein molecules than had been assumed. These findings are in accord with the “extremely wide range of processes in which hydrophobic bonding plays a critical role” (Dunn and Hansch, 1974) and with the observation that in the presence of high salt concentrations many proteins are bound by adsorbents carrying hydrophobic groups (Hofstee, 1973a; Porath et al., 1973; Hjertén, 1973). Thus the suggestion that “the hydrophobic effect is perhaps the most important single factor in the organization of the constituent molecules of living matter into complex structural entities” (Tanford 1973), which refers primarily to the formation of micelles and biological membranes, perhaps should be extended to include the interaction of proteins with such entities as well as the interaction of proteins with each other and with smaller molecules.

The apparent importance of hydrophobicity probably is related to the fact that in an aqueous milieu electrostatic interactions per se are diminished through a quenching effect of water. On the other hand, this quenching effect is counteracted and electrostatic interactions may become very important, e.g., when the pertinent polar groups are shielded by hydrophobic groups (Tanford, 1961; Jencks, 1969; Epstein, 1971). Furthermore, the larger hydrophobic groups also may enter into hydrophobic bond formation. These two distinct aspects of hydrophobicity will be discussed below.

Another reason for the lack of studies on the interaction of proteins with strongly hydrophobic ligands is that such studies are hampered by the inherently low solubility of these ligands in aqueous solutions in the absence of agents that enhance their dissolution. The addition of such agents to the medium is undesirable not only because this diminishes hydrophobic interaction between ligand and protein but also because these agents would tend to destabilize the hydrophobic interior of the protein. Solubilization of the hydrophobic ligand through the introduction of polar (e.g., charged) groups defeats the purpose, since such groups merely would decrease the hydrophobicity of the ligand. However, by virtue of developments in the preparation and substitution of materials suitable for column chromatography (Hjertén, 1964; Axén et al., 1967; Porath et al., 1967; Cuatrecasas and Parikh, 1972), even strongly hydrophobic compounds can be molecularly dispersed in an aqueous milieu in the absence of additions. This can be achieved through the covalent attachment of such a compound to an insoluble but hydrophilic (wettable) matrix such as is provided by beaded agarose. Thus the stage is set for new chromatographic procedures in investigations on the hydrophobicity of proteins and other materials. The present chapter is concerned primarily with the possibility for chromatographic separations of proteins based on differences in their affinities for agaroses substituted with hydrophobic ligands.