THE DISTRIBUTIONS OF CERTAIN PROTEINS, POLYPEPTIDES, AND POLYAMINES IN AN AQUEOUS-PHENOL SYSTEM[1]

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Kirby (1957) has reported on the use of certain salt solutions and phenol in liberating deoxyribonucleic acid (DNA) from mammalian cells. This report has been confirmed and enlarged upon by Colter et al. (1962), who showed that DNA isolated by this method could be obtained relatively free of contamination by polypeptides, polysaccharides, and ribonucleic acid. Shepherd and Petersen (1962), using this technique, extracted tritium-labeled DNA from mammalian cells and separated DNA from phenol by Sephadex gel filtration. During this latter investigation, a question arose as to the fate of the protein and protein-like components of biological systems extracted in this manner. Grassman and Deffner (1953) briefly investigated similar systems and published the distribution values for five proteins in an aqueous-phenol system in the absence of salts. They also discussed the mechanisms of solubilization and denaturation encountered.

Inasmuch as different proteins might be expected to exhibit different behaviors in such a system with respect to their intrinsic and salt-affected solubilities, and inasmuch as such

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differences would be of interest when extracting biological systems with different protein compositions, an investigation was made into the influence of NaCl concentration on the partitioning of certain proteins, polypeptides, and polyamines in an aqueous-phenol system. The polyamines were included on the basis of their reported occurrence in mammalian cells and bacteriophage (Ames et al., 1958).

Methods

Freshly distilled phenol was used throughout. Proteins, polypeptides, and polyamines of the highest available purity were obtained from commercial sources. Whole calf thymus histone was donated by Dr. K. Murray, Stanford University. Tobacco mosaic virus (TMV) protein was prepared by the method of Fraenkel-Conrat (1957) from virus donated by Dr. W. M. Stanley, University of California. Nitrogen was determined by a micro-modification (Murray, 1959) of the method of Belcher and Bhatty (1956). The distribution of nitrogen was assumed to be indicative of the distribution of the nitrogenous solute. Percentage data were based on the summation of nitrogen present in both phases and reported as percentage of total nitrogen, corrected for volume change and nitrogen loss when necessary.

Each of the substances tested was dissolved to a concentration of 1.0 $\pm$ 0.01 mg/ml in water or saline. All experiments were carried out in a water bath at 30 $\pm$ 0.01°C. Equal volumes of water-saturated phenol and the solution to be tested, equilibrated to bath temperature, were mixed at zero time with vigorous mechanical stirring. Aliquots were withdrawn at predetermined intervals until nitrogen analyses indicated that an equilibrium had been reached. In almost all cases, no longer than 10 min

was required. Subsequent analyses were performed upon aliquots removed after 30 min.

Aliquots removed from the system were centrifuged at 2000 RPM for 60 min and the separated phases removed with a syringe. Duplicate aliquots of each phase were pipetted into microkjeldahl flasks and dried in a vacuum oven at 170°C and 2 mm pressure for 12 hr. Both phenol, which interfered with digestion, and water were removed during the drying process. Preliminary experiments with standard solutions of several proteins, both in the presence and absence of phenol, revealed no detectable loss of nitrogen in this step. Dried aliquots were digested and analyzed for nitrogen. The results are illustrated in Fig. 1.

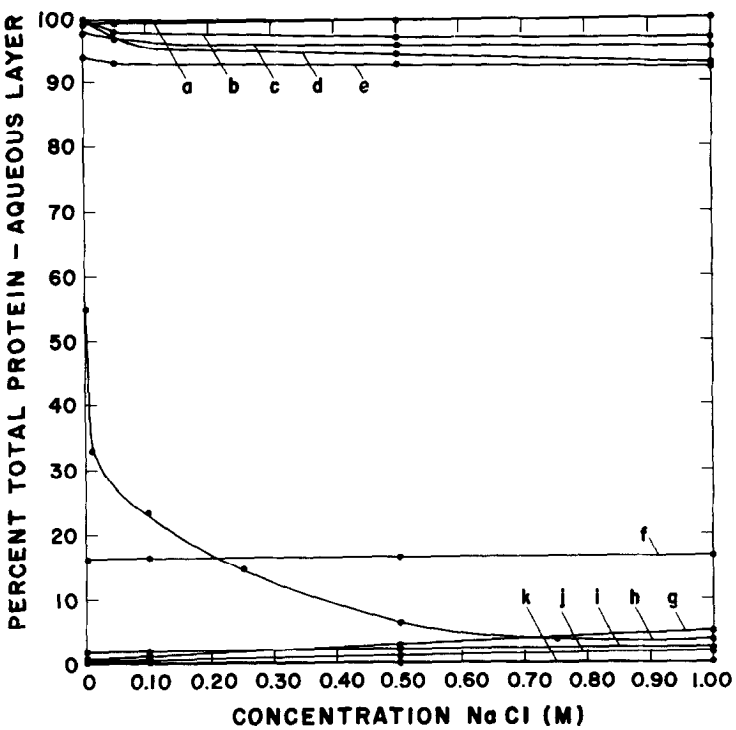

Fig. 1. The effect of NaCl concentration upon the distribution of certain proteins, polypeptides, and polyamines in a two-phase aqueous-phenol system. A = poly-l-lysine; B = poly-l-aspartic acid; C = spermine; D = spermidine; E = myosin; F = urease; G = hemoglobin; H = protamine sulfate; I = cytochrome-c, TMV protein, pepsin; J = myoglobin, histone, trypsin; and K = ribonuclease, α-chymotrypsinogen, ovalbumin.

Results and Discussion

The polyamines spermine and spermidine, of low molecular weight, polar, and basic in nature, were contained almost exclusively in the aqueous phase. The low molecular weight polypeptides poly-l-aspartic acid and poly-l-lysine, respectively acidic and basic in nature, were also retained in the aqueous phase.

It may be seen that not all proteins exhibited the same degrees of partitioning response to salt concentration in a simple water-phenol-salt system. The behavior of protamine sulfate is of interest. A basic protein of low molecular weight, protamine sulfate was equally distributed between the phases in the absence of salt but was almost completely partitioned into the phenol phase as the salt concentration increased to 1 M. No other protein tested exhibited this behavior to the same degree. Ribonuclease, a basic protein of relatively low molecular weight, was completely excluded from the aqueous phase at all salt concentrations. Calf thymus histone, a mixture of basic proteins with higher molecular weights than that of protamine sulfate, remained in the phenol at all concentrations of salt up to 1 M. The behavior of myosin might be attributed in part to the dissociation of the original molecule into its sub-units L-meromyosin and H-meromyosin, both of considerably lower molecular weight.

It may be seen that molecular weights and isoelectric points (as reported in Table I) are not the only parameters influencing the partitioning of proteins in the system described. A strict accounting of their behaviors must take into consideration, for each protein, its tertiary structure, intramolecular bonding, and availability of surface and internal functional groups for the

processes of solubilization and denaturation which involve hydrogen bonding.

TABLE I

Partitioning Behaviors and Physical Properties of Test Substances

	% N in Aqueous Layer		Molecular Weight*	Isoelectric* Point
	Concentration NaCl			
	0 M	1 M	x 10^3	
Poly-l-lysine	99.2	100	210	
Poly-l-aspartic acid	100	97.3	35	
Spermine	97.4	95.6		
Spermidine	100	93.1		
Myosin	93.9	92.4	1,000	6.2 - 6.6
L-Meromyosin			96	
H-Meromyosin			230	
Urease	13.6	14.3	480	5.0 - 5.1
Hemoglobin, human	0	5.0	63	6.8
Cytochrome-c	2.5	2.5	15.6	9.6
TMV Protein	0.6	2.5	4,000	(17.5)
Pepsin	4.9	2.6	35.5	2.8 - 3.0
Myoglobin	2.0	1.6	16.9	7.0
Histone	2.6	1.8		>10.5
Trypsin	0.6	1.7	15.1	5 - 8
Ribonuclease	0	0	12.7	9.5
α-Chymotrypsinogen	0	0	22.0	9.5
Ovalbumin	0	0	44.0	4.6 - 4.8

*Molecular weights and isoelectric points quoted are average values taken from the literature.

The system described is admittedly less complex than those encountered in the extraction of biological materials. In general,

however, it is likely that different proteins will exhibit different partitioning behaviors in biological extracts as well. It is suggested that these differences be taken into account, along with the nature of the predominating protein or proteins in a biological system, when aqueous-phenol is considered for extraction of DNA from such systems.

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