proteins including horse heart and *Pseudomonas* cytochromes C, and the basic polypeptide neurotoxins from cobra and rattlesnake venoms[5]. It should also be noted that all the above mentioned proteins have isoelectric points ~ pH 10.8 or higher.

The small change in exclusion volume occurring after the first filtration is typical and can probably be accounted for by protein binding-induced structural changes in the polyacrylamide gel matrix. The nature of these changes will be reported elsewhere. In view of these findings, it is recommended that caution be exercised in the utilization of highly cross-linked polyacrylamide gels (Bio-Gel P-2) for desalting of water-soluble, basic polypeptides and proteins.

This work was made possible by financial support from the Midwestern University Research Foundation (Grant MU 1536).

Molecular Biology Section, Department of Biology, Midwestern University, Wichita Falls, Texas 76308 (U.S.A.) CARLOS A. BONILLA

1 C. A. BONILLA, *Anal. Biochem.*, 32 (1969) 522.
2 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
3 E. M. JORDAN AND S. RAYMOND, *Anal. Biochem.*, 27 (1969) 205.
4 C. A. BONILLA AND N. V. HORNER, *Toxicon*, in press.
5 C. A. BONILLA AND L. P. FRANK, *II Int. Symp. Animal Plant Toxins, Tel-Aviv, Israel, 1970.*

Received January 2nd, 1970

CHROM. 4559

Gel filtration of lipid–protein complexes on cross-linked polystyrene

Cross-linked polystyrene was first used for lipid separation by RADIN *et al.*[1], with toluene–ethanol–water as eluant in a reversed-phase partition system for the purification of cerebrosides. Later, TIPTON *et al.*[2] separated phospholipids and glycolipids from less polar lipids using benzene as the eluant. BERRY AND KAYE[3] found the latter procedure of "little or no value" in the quantitative analysis of phospholipids, but DAVENPORT[4], using 1% methanol in benzene as eluant, achieved the desired separation of phospholipids from neutral lipids. The applicability of a modification of the method to the isolation of lipid–protein complexes from crude lipid extracts of wheat flour is now reported. The modification, use of chloroform as eluant, permits continuous UV absorption monitoring of the column effluent ("Uvicord", 254 nm), LKB Instruments Ltd.).

The divinylbenzene cross-linked polystyrene* required prolonged washing with the eluant before use, to remove a persistent UV-absorbing impurity (probably the cause of the excessively high yields of BERRY AND KAYE[3]). 3.05 g of the lipid extracted

* BioBeads S-X2, Bio-Rad Laboratories, Richmond, Calif.

from an untreated and unbleached flour by light petroleum was separated by ascending chromatography on a 69 cm × 2.5 cm diameter column using ethanol-free chloroform as eluant into six fractions differing in lipid composition, only one of which (232 mg yield) contained the protein purothionin[5-8], in the form of its lipid complexes ("lipopurothionins"). When re-run on a similar column, this fraction (100 mg) was further purified by removal of other lipid–protein complexes and free lipids accompanying the purothionin, all of which was again found in a single fraction (39 mg) with comparable elution volume (per c.c. of packing) to that of the first run. Since the product was still soluble in non-polar solvents, absence of denaturation could be inferred: products obtained by alternative procedures have sometimes rapidly become insoluble in non-polar solvents including chloroform as well as light petroleum, and precipitated. Decomposition of these complexes has been found to occur on silicic acid. Full details will be reported in a paper now being prepared on methods for the isolation and purification of "lipopurothionins", also including Sephadex LH-20* gel filtration, rubber dialysis and solvent precipitation, used individually and in combination.

Financial support of the U.S. Department of Agriculture is gratefully acknowledged.

Flour Milling and Baking Research Association,
Chorleywood, Rickmansworth, Herts. WD3 5SH (Great Britain)

N. FISHER

1 N. S. RADIN, F. B. LAVIN AND J. R. BROWN, *J. Biol. Chem.*, 217 (1955) 789.
2 C. L. TIPTON, J. W. PAULIS AND M. D. PIERSON, *J. Chromatog.*, 14 (1964) 486.
3 J. F. BERRY AND B. KAYE, *Lipids*, 3 (1968) 386.
4 J. B. DAVENPORT, *Lipids*, 4 (1969) 308.
5 A. K. BALLS, W. S. HALE AND T. H. HARRIS, *Cereal Chem.*, 19 (1942) 279.
6 N. FISHER, D. G. REDMAN AND G. A. H. ELTON, *Cereal Chem.*, 45 (1968) 48.
7 C. C. NIMMO, M. T. O'SULLIVAN AND J. E. BERNARDIN, *Cereal Chem.*, 45 (1968) 28.
8 D. G. REDMAN AND N. FISHER, *J. Sci. Food Agr.*, 19 (1968) 651.

Received December 15th, 1969

* Pharmacia Ltd.