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Note

Gel filtration of protected peptides on Enzacryl K2 in dimethylformamide and N-methyl-2-pyrrolidone

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We have recently shown[1] that large fully protected peptides can be purified by gel filtration on Sephadex G-50 using 5% water in hexamethylphosphoramide (HMPA). The routine application of this method is limited by the difficult removal of HMPA requiring subsequent gel filtration on Sephadex G-10–dimethylformamide (DMF) when precipitation methods of isolation are not feasible. Another more worrying feature, however, is the large-scale manipulation of HMPA, which has recently been exposed as a potential carcinogen[2,3]. During our continuing search for systems capable of coping with gel filtration of large-molecular-weight compounds our attention was drawn to the reported use[4] of *N*-methyl-2-pyrrolidone (NMP) as eluent in gel filtration with a polystyrene resin cross-linked with divinylbenzene. In addition the synthetic gel filtration matrix Enzacryl K2 has been developed by Epton *et al.*[5,6] and shown to be compatible with water, chloroform and tetrahydrofuran (THF) as eluents. The correlation of logarithm of molecular weight *versus* distribution coefficient, k_d, was close to ideal for polyethylene glycols and polysaccharides. Enzacryl K2 has been used[7] for thin-layer gel filtration of proteins using buffered aqueous eluents. It was therefore decided to examine the utility of Enzacryl K2 in gel filtration of protected peptides using DMF and NMP as eluents.

EXPERIMENTAL

Materials

Blue dextran 2000, Sephadex G series gels and 2.5 × 100 cm columns were purchased from Pharmacia (London, Great Britain). Enzacryl K2, bead size 40–70 μm (fine), was supplied by Koch-Light (Colnbrook, Great Britain)[8]. The columns (3.9 and 5.2 cm I.D.) were fitted with porous PTFE discs on machined PTFE end pieces. DMF and NMP were dried and distilled at 0.1 mmHg.

Procedures

The gels were swollen in either DMF or NMP at 50° for 16 h under degassed conditions. The columns were packed under maximum gravity flow and the samples loaded in various mixtures of DMF, NMP, dimethylacetamide (DMA) and HMPA followed by elution at flow-rates between 10 and 40 ml/h monitored by ultraviolet (UV) absorption (280 nm) and optical rotation (546 nm).

The void volume, V_0, was determined using modified Blue dextran 2000. Both the total bed volume, V_t, and elution volume, V_e, were determined as described previously and these parameters were used to determine the distribution coefficient, K_{av}, given by the expression:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}$$

The parameters for the columns used in gel filtration of the protected peptides are given in Table I.

TABLE I

COLUMN PARAMETERS

Column	*Diameter (cm)*	V_0 *(ml)*	V_t *(ml)*
Enzacryl K2–DMF	2.5	166	440
Enzacryl K2–DMF	5.2	785	2050
Enzacryl K2–NMP	2.5	122	450
Enzacryl K2–NMP	3.9	230	952
Sephadex G-75–NMP	2.5	136	464

RESULTS AND DISCUSSION

From Table II it can be seen that the swelling characteristics of Enzacryl K2 and the Sephadex G series gels were found to be superior in NMP compared with DMF. The complete range of Sephadex G series gels may be employed for gel filtration provided the solute is soluble in NMP, a problem arises, however, when the solute is dissolved in NMP–HMPA prior to application. It has been found that such mixtures cause shrinkage of the gel with ensuing reduction in flow-rate. For this reason we have favoured the Enzacryl K2–NMP system in which shrinkage problems are not serious when the sample is applied in mixed solvent systems containing HMPA. The Sephadex G-75–NMP system has been used, nevertheless, to purify the protected nonadecapeptide fragment of human big gastrin 10 (ref. 9). After chromatography the NMP may be removed *in vacuo* at 30°. Co-solvents such as HMPA are eluted close to the total bed volume.

TABLE II

SWELLING OF ENZACRYL K2 AND SEPHADEX G SERIES IN DMF AND NMP

Gel type	*Observed swelling (ml/g)*	
	DMF	*NMP*
Enzacryl K2	4.0	4.2
Sephadex G-10	2.5	1.8
Sephadex G-50	—	4.0
Sephadex G-75	—	5.8
Sephadex G-100	—	7.2
Sephadex G-200	—	18.0

It is important to our continuing programme of peptide synthesis to extend the use of gel filtration beyond the molecular weight limit of 3000 encountered when using Sephadex LH-20–DMF. A selection of protected peptide fragments, soluble in DMF, are illustrated in Table III. These were chromatographed on Enzacryl K2 and Fig. 1 shows the relationship of distribution coefficient, K_{av} against $\log_{10}$ M.W. Extrapolation of these results gives a molecular weight limit for this gel filtration system of approx. 10,000; at this molecular size, however, it would be expected that solubility in DMF would be a limiting factor. From Fig. 1 it can be seen that the K_{av} values for the carboxylic acids 5 and 9 are anomalous. We are currently investigating the remarkable retention of carboxylic acids on Enzacryl K2, compared with the corresponding esters, as a means of purifying hydrolysis products.

TABLE III

PROTECTED PEPTIDES PURIFIED BY GEL FILTRATION ON ENZACRYL K2 WITH DMF AS ELUENT

No.	*Compound*	*M.W.*	log_{10} *M.W.*	K_{av}
1	Bpoc-Phe-Asn-Thr(Bu^t)-Gln-Ala-Thr(Bu^t)-Asn-Orn(Adoc)-Asn-Thr(Bu^t)-Glu(OBu^t)-Gly-OPh	2027	3.307	0.29
2	Bpoc-Ser(Bu^t)-Thr(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-Gly-Leu-Leu-Gln-Ile-Asn-Ser(Bu^t)-Orn(Adoc)-Trp-Trp-Cys(Acm)-Ala-Asp(OBu^t)-Gly-Orn(Adoc)-Thr(Bu^t)-Pro-Gly-Ser(Bu^t)-Ala-Asn-Gly-OPh	3930	3.594	0.25
3	Bpoc-Phe-Asn-Thr(Bu^t)-Gln-Ala-Thr(Bu^t)-Asn-Orn(Adoc)-Asn-Thr(Bu^t)-Glu(OBu^t)-Gly-Ser(Bu^t)-Thr(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-Gly-Leu-Leu-Gln-Ile-Asn-Ser(Bu^t)-Orn(Adoc)-Trp-Trp-Cys(Acm)-Ala-Asp(OBu^t)-Gly-Orn(Adoc)-Thr(Bu^t)-Pro-Gly-Ser(Bu^t)-Ala-Asn-Gly-OPh	5625	3.750	0.15
4	Bpoc-Nle-Asn-Ala-Trp-Val-Ala-Trp-Orn(Adoc)-Asn-Arg(Adoc)$_2$-Cys(Acm)-Lys(Adoc)-Gly-Ser(Bu^t)-Asp(OBu^t)-Val-Ser(Bu^t)-Ala-Trp-Val-Orn(Adoc)-Gly-Cys(Acm)-Gly-Leu-OBu^t	4290	3.623	0.27
5	Bpoc-Cys(Acm)-Ala-Lys(Adoc)-Lys(Adoc)-Ile-Val-Ser(But^t)-Asp(OBu^t)-Gly-Asn-Gly-OH	1869	3.272	0.79
6	Bpoc-Cys(Acm)-Ala-Lys(Adoc)-Lys(Adoc)-Ile-Val-Ser(Bu^t)-Asp(OBu^t)-Gly-Asn-Gly-Nle-Asn-Ala-Trp-Val-Ala-Trp-Orn(Adoc)-Asn-Arg(Adoc)$_2$-Cys(Acm)-Lys(Adoc)-Gly-Ser(Bu^t)-Asp(OBu^t)-Val-Ser(Bu^t)-Ala-Trp-Val-Orn(Adoc)-Gly-Cys(Acm)-Gly-Leu-OBu^t	5902	3.772	0.15
7	Bpoc-Cys(Acm)-Asn-Ile-Pro-Cys(Acm)-Ala-Ala-Leu-Nva-Ser(Bu^t)-Gly-Asp(OBu^t)-Ile-Thr(Bu^t)-Ala-Ser(Bu^t)-Val-Gly-Cys(Acm)-Ala-Lys(Adoc)-Lys(Adoc)-Ile-Val-Ser(Bu^t)-Asp(OBu^t)-Gly-Asn-Gly-Nle-Asn-Ala-Trp-Val-Ala-Trp-Orn(Adoc)-Asn-Arg(Adoc)$_2$-Cys (Acm)-Lys(Adoc)-Gly-Ser(Bu^t)-Asp(OBu^t)-Val-Ser(Bu^t)-Ala-Trp-Val-Orn(Adoc)-Gly-Cys(Acm)Gly-Leu-OBu^t	7942	3.900	0.10
8	Glp-Leu-Gly-Leu-Gln-Gly-His-Pro-Leu-Leu-Val-Ala-Asp(OBu^t)-Pro-Ala-Lys(Adoc)-Lys(Adoc)-Gln-Gly-OPh	2441	3.388	0.25
9	Glp-Leu-Gly-Leu-Gln-Gly-His-Pro-Leu-Leu-Val-Ala-Asp(OBu^t)-Pro-Ala-Lys(Adoc)-Lys(Adoc)-Gln-Gly-OH	2365	3.374	0.71
10	Glp-Leu-Gly-Pro-Gln-Gly-His-Pro-Ser(Bu^t)-Leu-Val-Ala-Asp(OBu^t) Pro-Ser(Bu^t)-Lys(Adoc)-Lys(Adoc)-Gln-Gly-OPh	2527	3.403	0.25

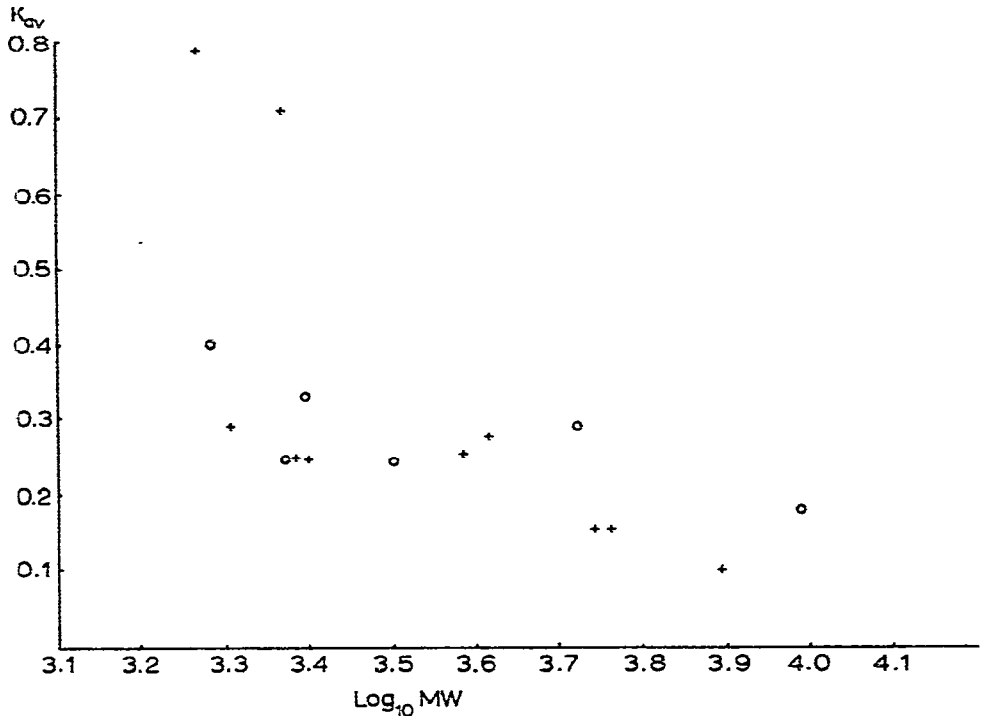

Fig. 1. Gel filtration on Enzacryl K2. + = DMF; ○ = NMP.

This problem of solubility can be obviated by utilising the Enzacryl K2–NMP system in which the solvating power of the mobile phase permits highly insoluble fragments to be chromatographed. In this system HMPA can be tolerated during sample application of large peptides. Table IV illustrates examples of fragments which have been satisfactorily purified by this method. The relationship of K_{av} against $\log_{10}$ M.W. for these protected peptides is shown in Fig. 1 which suggests a molecular weight limit of approximately 15,000. Fig. 2 shows typical chromatographic traces obtained during purification of fragments 13 and 14 and illustrates the separation to be expected from typical condensations of large protected peptide fragments.

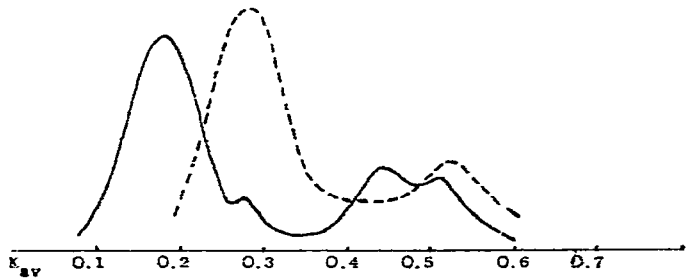

Fig. 2. Gel filtration on Enzacryl K2–NMP. Column, 2.5 cm I.D. monitored by UV (280 nm). ——— = Fragment 14; – – – = fragment 13.

TABLE IV

FRAGMENTS OF AN ANALOGUE OF LYSOZYME PURIFIED BY GEL FILTRATION ON ENZACRYL K2 WITH NMP AS ELUENT

No.	*Compound*	*M.W.*	log_{10} *M.W.*	K_{av}
11	Adoc-Lys(Adoc)-Val-Phe-Gly-Orn(Adoc)-Cys(Acm)-Glu(OBu^t)-Leu-Ala-Ala-Ala-Nle-Lys(Adoc)-Ala-Leu-Gly-OPh	2521	3.402	0.33
12	Bpoc-Leu-Ala-Gly-Tyr(Bu^t)-Orn(Adoc)-Gly-Tyr(Bu^t)-Ser(Bu^t)-Leu-Gly-Asn-Trp-Nva-Cys(Acm)-Ala-Ala-Lys(Adoc)-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh	3229	3.509	0.24
13	(Adoc)Lys(Adoc)-Val-Phe-Gly-Orn(Adoc)-Cys(Acm)-Glu(OBu^t)-Leu-Ala-Ala-Ala-Nle-Lys(Adoc)-Ala-Leu-Gly-Leu-Ala-Gly-Tyr(Bu^t)-Orn(Adoc)-Gly-Tyr(Bu^t)-Ser(Bu^t)-Leu-Gly-Asn-Trp-Nva-Cys(Acm)-Ala-Ala-Lys(Adoc)-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh	5418	3.734	0.29
14	(Adoc)Lys(Adoc)-Val-Phe-Gly-Orn(Adoc)-Cys(Acm)-Glu(OBu^t)-Leu-Ala-Ala-Ala-Nle-Lys(Adoc)-Ala-Leu-Gly-Leu-Ala-Gly-Tyr(Bu^t)-Orn(Adoc)-Gly-Tyr(Bu^t)-Ser(Bu^t)-Leu-Gly-Asn-Trp-Nva-Cys(Acm)-Ala-Ala-Lys(Adoc)-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-Phe-Asn-Thr(Bu^t)-Gln-Ala-Thr(Bu^t)-Asn-Orn(Adoc)-Asn-Thr(Bu^t)-Glu(OBu^t)-Gly-Ser(Bu^t)-Thr(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-Gly-Leu-Leu-Gln-Ile-Asn-Ser(Bu^t)-Orn(Adoc)-Trp-Trp-Cys(Acm)-Ala-Asp(OBu^t)-Gly-Orn(Adoc)-Thr(Bu^t)-Pro-Gly-Ser(Bu^t)-Ala-Asn-Gly-OPh	10711	4.031	0.18
15	Bpoc-Cys(Acm)-Asn-Ile-Pro-Cys(Acm)-Ala-Ala-Leu-Nva-Ser(Bu^t)-Gly-Asp(OBu^t)-Ile-Thr(Bu^t)-Ala-Ser(Bu^t)-Val-Gly-OPh	2373	3.375	0.25
16	Bpoc-Ser(Bu^t)-Asp(OBu^t)-Val-Ser(Bu^t)-Ala-Trp-Val-Orn(Adoc)-Gly-Cys(Acm)-Gly-Leu-OBu^t	1919	3.283	0.40

We have used Enzacryl K2–NMP or DMF for the isolation of pure compounds directly from reaction mixtures. The purity of the protected peptides isolated by these techniques was checked by the usual analytical techniques. From the experience gained so far, it is now feasible to purify protected fragments having molecular weight up to 15,000 by the gel filtration method and this may help to extend the range of the chemist engaged on the synthesis of large physiologically active peptides.

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REFERENCES

1 I. J. Galpin, G. W. Kenner, S. R. Ohlsen and R. Ramage, *J. Chromatogr.*, 106 (1975) 125.
2 Editorial, *Chem. Eng. News*, 53 (1975) 17.
3 Editorial, *Nature (London)*, 258 (1975) 94.

4 E. L. Smithwick, Jr., Eli Lilly and Co., *U.S. Pat.*, 3,857,829 (1974).
5 R. Epton, C. Holloway and J. V. McLaren, *J. Appl. Polym. Sci.*, 18 (1974) 179.
6 R. Epton, C. Holloway and J. V. McLaren, *J. Chromatogr.*, 90 (1974) 249.
7 R. Epton, S. R. Holding and J. V. McLaren, *J. Chromatogr.*, 110 (1975) 327.
8 *Enzacryl Gel*, Technical Data Sheet, Koch-Light Laboratories, Colnbrook.
9 R. A. Gregory, *J. Physiol.*, 241 (1974) 1.