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Note

Chromatographic separation and determination of tryptophan in foodstuffs after barytic hydrolysis using Fractogel TSK HW 40 S

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A knowledge of the total tryptophan content of food products is of major importance because tryptophan is an essential amino acid for man and monogastric animals. The degradation of the tryptophan molecule as a result of hydrolysis by hydrochloric acid requires the use of alkaline media[1], such as sodium hydroxide[2,3] or barium hydroxide[4], which implies a separation of tryptophan by a method other than ion-exchange chromatography. The destruction of tryptophan in the sodium hydroxide hydrolysates after acidification[3] led us to choose barytic hydrolysis and to investigate new supports for the adsorption chromatography of tryptophan.

The use of carbohydrate gels such as starch gel[2] or Sephadex G-25F[4] described in the earliest publications requires a considerable time to obtain a fair chromatographic separation. Sephadex G-10 has been used to study the complete composition of wheat grains as a function of nitrogen content[5]. However, such supports have a wide particle size distribution. Nowadays, soft supports are available, based on different chemical natures. We have deliberately excluded the study of expensive high-performance liquid chromatographic supports (*e.g.*, C_{18} silica gels) and focused on gels for low-pressure chromatography. In addition to dextran gels, modern supports such as Bio-Gel P-2 (ref. 6) and Trisacryl GF 05 (ref. 7) are known to adsorb tryptophan. Fractogel TSK HW 40 S has also been reported by the manufacturer[8] to be a potential support for the adsorption chromatography of tryptophan in pure water.

In this paper, we describe a comparison of the chromatographic parameters of tryptophan obtained using several gels: Fractogel TSK HW 40 S, Fractogel PGM 2000, Sephadex G-25 Superfine, Sephadex G-10, Sephacryl S-200 Superfine, Sephasorb HP Ultrafine, Trisacryl GF 05, Ultrogel AcA-202 and Bio-Gel P-2. Of these gels, the two Fractogels clearly exhibited the best resolution factor and the smallest peak width, giving a higher sensitivity and better accuracy in the determination of tryptophan. An *o*-phthaldialdehyde fluorimetric detection method adapted from Roth[9] was used. Under these conditions the recovery of protein tryptophan reaches 94 ± 1.7% in foodstuffs and 98 ± 1% in purified proteins.

EXPERIMENTAL

The samples used were either hen egg white lysozyme (Merck, 17 000 units

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mg^{-1}), with a purity of 90.4% determined by spectrophotometry[10], Kjeldahl nitrogen determination and amino acid analysis, or plant flours and ground foodstuffs, with lysozyme as an internal standard.

Barytic hydrolyses were performed according to Slump and Schreuder[4] using 100–300 mg of flour with 4.2 g of barium hydroxide octahydrate and 8 ml of Milli-Q water in 30-ml polymethylpentene plastic tubes. The samples were heated in an autoclave, in the absence of oxygen, for 16–18 h at 125°C (1.4 bar). The hydrolysates, chilled in an ice-bath, were acidified with 3 *N* hydrochloric acid to pH 3.5 using a Metrohm pHstat, diluted to 50 ml with water, then centrifuged at 14 000 *g*.

External calibrations were conducted with Calbiochem A-grade L-tryptophan, the purity of which was measured by spectrophotometry using a molar asbsorptivity of 5670 mol l^{-1} cm^{-1} at 279 nm and pH 3[11]. About 0.1 ml was injected on to the column using a Cheminert 20 PTFE sample loop.

A 300 × 9 mm I.D. Altex glass column was utilized, filled to 200 mm with one of the following gels: Fractogel TSK HW 40 S, Fractogel PGM 2000, 32–63 μm (Merck); Sephadex G-25 Superfine, Sephadex G-10, Sephacryl S-200 Superfine and Sephasorb HP Ultrafine (Pharmacia); Trisacryl GF 05 and Ultrogel AcA-202 (IBF); and Bio-Gel P-2, 200–4000 mesh (Bio-Rad Labs.).

Elutions were performed at a flow-rate of 30 ml h^{-1} (Milton-Roy Minipump) with pH 3.25 citrate buffer[12], in which phenol was replaced with caprylic acid, at room temperature. The eluate was mixed with the *o*-phthaldialdehyde (OPA) reagent (0.5 g of OPA dissolved in 5 ml of 95% ethanol, to which were added 2 ml of 2-mercaptoethanol and 2 ml of 30% Brij 35 solution, followed by dilution to 1 l with 1 *M* borate buffer, pH 10.6) delivered at the same flow-rate. After reaction for 90 s the effluent was monitored through a 2-mm cell with an Aminco fluoro-monitor, using a Corning 7-51 excitation filter and a Wratten 2A emission filter. The signal was recorded and integrated using a Spectra-Physics Autolab AA.

RESULTS AND DISCUSSION

In Fig. 1 are compared the chromatographic elution profiles obtained with Fractogel HW 40 S, Sephadex G-25, Sephadex G-10 and Bio-Gel P-2. These profiles exhibit two peaks: the first eluted corresponds to amino acids and primary amines reacting with OPA and the second to tryptophan. The profiles obtained with Fractogel PGM 2000, Sephasorb HP Ultrafine and Trisacryl GF 05 are comparable to the Fractogel HW 40 S, Sephadex G-10 and Bio-Gel P-2 profiles, respectively. It is clear that only the Fractogels and Sephadex G-10 allow a fair separation of tryptophan and that the peak obtained with Sephadex G-10 is far wider than that with Fractogel HW 40 S.

Table I illustrates the chromatographic parameters determined for all the supports. Two sets of gels can be identified when comparing the resolution coefficients: the gels with $R_s > 1.1$ give a good resolution (Fractogel HW 40 S, Fractogel PGM 2000, Sephasorb HP Ultrafine and Sephadex G-10), whereas the others are not able to separate tryptophan under these conditions. Nevertheless, the adsorption constants show that all the supports adsorb the tryptophan to various extents under pH and ionic strength conditions when no ion exchange is possible. Fractogel HW 40 S does not exhibit a greater K_{av} than the other gels giving a satisfactory resolution.

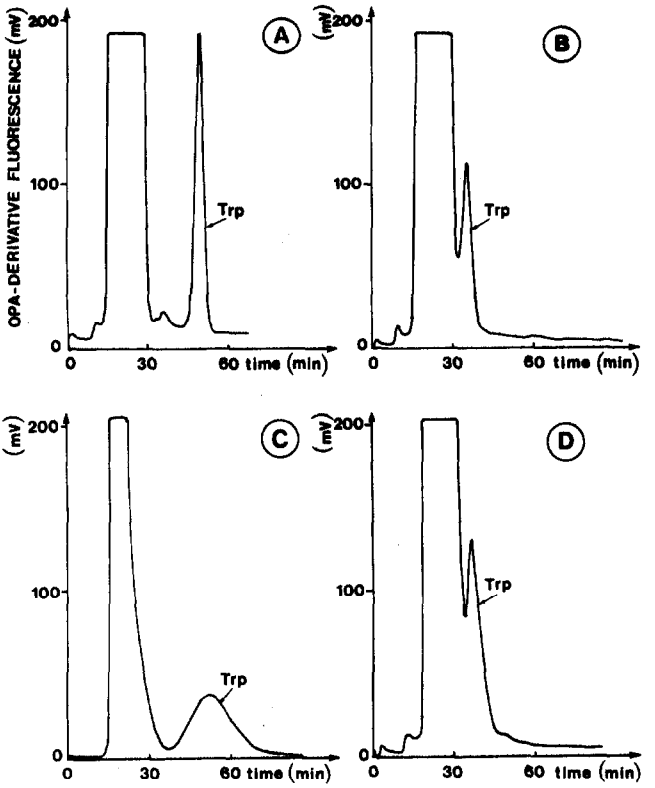

Fig. 1. Elution profiles of tryptophan after barytic hydrolysis of wheat flour. Separation on (A) Fractogel HW 40 S; (B) Bio-Gel P-2; (C) Sephadex G-10; (D) Sephadex G-25 Superfine. OPA fluorimetric detection (excitation wavelength 366 nm; emission wavelength >410 nm). The same flour sample was used with the four gels. Gel bed, 20 × 0.9 cm; flow-rate, 30 ml h^{-1}.

TABLE I

SEPARATION PARAMETERS OF TRYPTOPHAN USING DIFFERENT SOFT GELS

T = retention time; $W_{1/2}$ = peak width at half-height; K_{av} = adsorption constant; R_s = resolution coefficient; N = number of theoretical plates for a 20-cm column.

Gel	*T (min)*	*$W_{1/2}$ (min)*	*K_{av}*	*R_s*	*N*
Fractogel TSK HW 40 S	50	3.4	2.3	3.8	1200
Fractogel PGM 2000	46.5	4.3	2.1	3.0	660
Sephasorb HP Ultrafine	44	12	2.1	1.4	74
Sephadex G-10	52	16.3	2.3	1.2	56
Sephadex G-25 Superfine	35.5	5.5	1.4	1.0	230
Bio-Gel P-2 (200–400 mesh)	35.5	4.6	1.4	1.0	330
Trisacryl GF 05	36	5	2.3	0.9	290
Sephacryl S-200 Superfine	33	3.8	1.5	0.7	420
Ultrogel AcA-202	86	8.6	5	0.6	550

Owing to the peak width provided by this type of support, the efficiency of the column is far higher than with other gels, reaching 1200 theoretical plates per 20-cm column. All the gels that do not separate the tryptophan show a retention time less than 40 min, except for the peculiar case of Ultrogel AcA-202, which has a very low resolution coefficient. In contrast to the others, this gel does not absorb tryptophan specifically.

As regards the recovery of tryptophan from pure proteins and total wheat flour, we obtained 97.5 ± 1.1% (standard deviation for 14 assays) with a regression coefficient of 0.999 for lysozyme (using four different doses), and 94.1 ± 1.7% (standard deviation for 20 assays) with a regression coefficient of 0.997 for wheat flours to which were added the same lysozyme doses as the internal standard. Concerning the purified proteins, these results are in agreement with those from the literature[2,3] but are less scattered. As regards the determination of tryptophan in foodstuff protein, the recoveries we obtained with the internal protein standard are less scattered but comparable to results obtained with tryptophan adducts as internal standards[2,4].

Nevertheless, only the results obtained here by chromatographic means are reliable, in contrast to other methods[13] In addition, it is worth noting that under our hydrolytic conditions, tryptophan was stable for up to 2 months in the acidified hydrolysates.

CONCLUSION

The results show that Fractogel HW 40 S is a suitable support for the chromatographic determination of protein tryptophan after barytic hydrolysis. The advantage of Fractogel HW 40 S, like other good separating gels, is the moderate adsorption, but in addition it provides a very narrow tryptophan peak. This property may allow the ultraviolet detection of tryptophan instead of using fluorimetry of the OPA derivative. Nevertheless, it is important to emphasize that the ultraviolet detection of tryptophan is not specific, in contrast to the OPA reaction, which remains better for use in analyses of complex samples such as plant foodstuffs.

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