CHROM. 5846

Modification of the electrophoretic properties of certain jack bean proteins by chromatography on tanned gelatin

Chromium-formalin tanned gelatin is an easily prepared low-cost gel with mechanical properties ideally suited to preparative chromatography[1,2]. It is less suited to analytical work such as the estimation of molecular weights, however, as different proteins are not always eluted in the order of their molecular weights[2]. Evidence suggesting that modifications to the gel electrophoretic properties of certain proteins can be induced by chromatography on tanned gelatin is described here.

Experimental

An 8% chromium-formalin tanned gelatin gel was prepared as described previously[2]. This was packed into a jacketed glass column (4 × 150 cm), maintained at 6–8°, and equilibrated with buffer (see below). Before use in the separation described here this tanned gelatin column had been used several times previously to effect the same separation. Apart from a low recovery on the initial run, no subsequent change in the elution pattern was observed.

For comparative purposes, a separation was also carried out using a column (4 × 90 cm) of Sephadex G-100, prepared according to the recommended procedure[3]. As previous experience had shown that temperature had little effect on this particular separation, this column was packed and operated at room temperature.

The buffer used for equilibration, sample application and elution was in each case 0.01 *M* Tris, pH 7.6, containing 0.15 *M* NaCl and 0.2% NaN_3. The sample applied to each column consisted of 2 g of crude jack bean extract[4] dissolved in 20 ml of buffer and cleared by centrifugation before being applied to the column. The column effluent was monitored at 253.7 nm in a Uvicord absorptiometer and collected in approximately 20-ml fractions.

Samples (50 μl) for electrophoresis were taken directly from the fraction collector tubes with the aid of a micro-syringe (Hamilton Co., Whittier, Calif., U.S.A.) and applied to the sample applicator slots of a discontinuous slab electrophoresis apparatus developed in this laboratory. The gel and buffer systems of DAVIS[5] were used but the gels were cast as slabs 0.2 × 5.2 cm, six samples being run simultaneously on each slab. After electrophoresis, the gels were stained overnight with Amido Black (0.1% in 7% acetic acid), destained by transverse electrophoresis and scanned in a densitometric scanner (Zeiss Model 3) modified for this purpose.

Results

Elution profiles obtained by chromatography of a crude jack bean extract on chromium-formalin tanned gelatin and Sephadex G-100 together with electropherograms obtained from the eluted material are presented in Figs. 1 and 2, respectively. In the case of the tanned gelatin, samples for electrophoresis were taken from all of the fraction collector tubes containing UV-absorbing material, although for clarity only five representative electropherograms are presented in Fig. 1. These five pherograms nevertheless represent all of the bands obtained from the eluate from tanned gelatin. In the case of Sephadex G-100 samples were taken only from the fraction

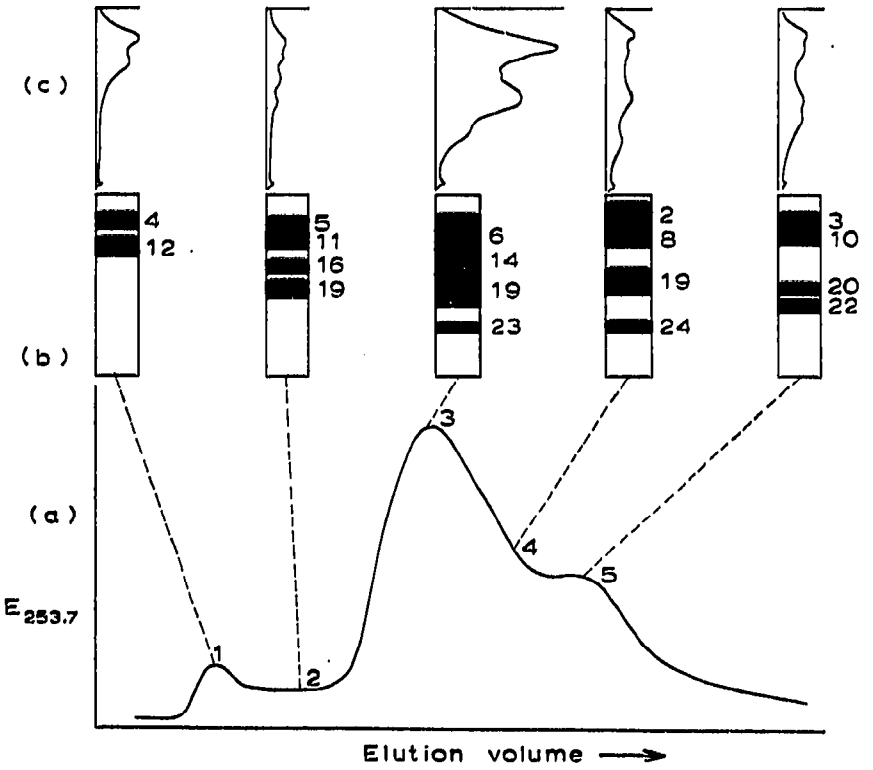

Fig. 1. (a) Elution profile obtained by chromatography of a crude jack bean extract on 8% chromium-formalin tanned gelatin. (b) Discontinuous slab gel electropherograms obtained from samples corresponding to the positions indicated on the elution profile. Band Nos. refer to Table I. (c) Densitometric scans of the discontinuous slab gel electropherograms.

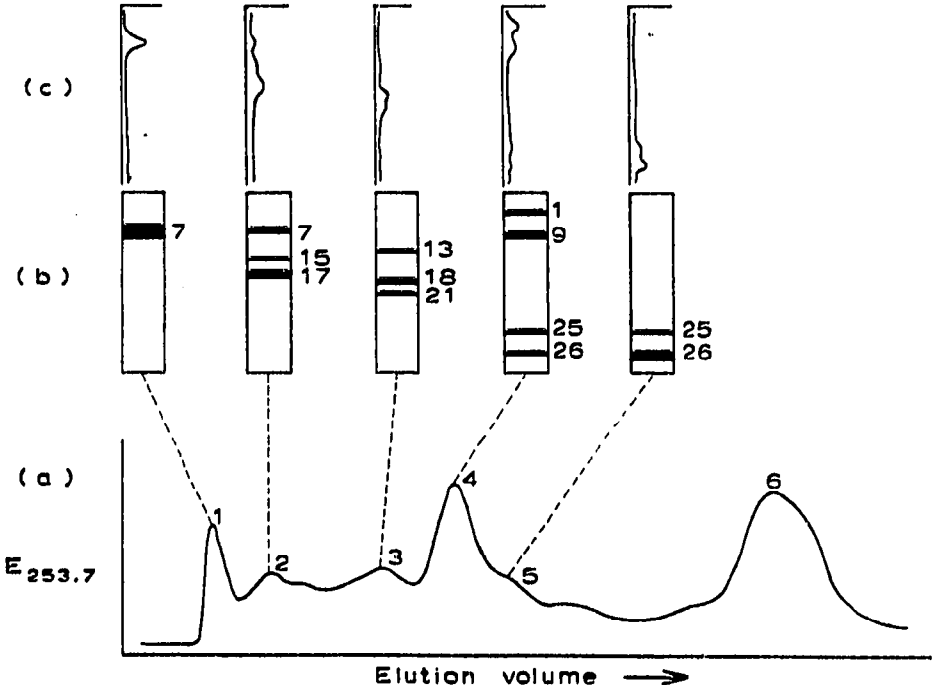

Fig. 2. (a) Elution profile obtained by chromatography of a crude jack bean extract on Sephadex G-100. (b) As in Fig. 1. (c) As in Fig. 1.

collector tubes corresponding to the apices of the six major peaks indicated in Fig. 2. Peak No. 6 yielded no bands upon electrophoresis and hence is not represented. A control electropherogram obtained from unfractionated crude jack bean extract is presented in Fig. 3.

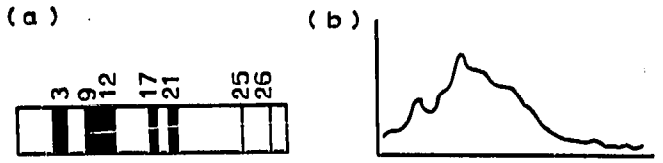

Fig. 3. (a) Discontinuous slab gel electropherogram obtained from unfractionated crude jack bean extract. Band Nos. refer to Table I. (b) Densitometric scan of the electropherogram in (a).

Comparison of the electropherograms obtained on the eluates from tanned gelatin and Sephadex reveals that the tanned gelatin eluate fractions yielded relatively broad, indistinct bands contrasting with the sharp, well-defined bands obtained from the Sephadex eluate fractions. This contrast in definition is also apparent in the overall elution profiles obtained from the two gel media.

Furthermore, comparison of the R_F values of the various bands (Table I) reveals that the Sephadex eluate yielded bands which corresponded to those of the

TABLE I

ELECTROPHORETIC R_F VALUES OF PROTEINS FROM A CRUDE JACK BEAN EXTRACT BEFORE AND AFTER CHROMATOGRAPHY ON SEPHADEX G-100 AND 8% TANNED GELATIN

Band No.	*Unfractionated*	*Chromatographed on Sephadex*	*Chromatographed on tanned gelatin*
1		0.118	
2			0.121
3	0.132		0.138
4			0.146
5			0.167
6			0.184
7		0.207	
8			0.223
9	0.239	0.235	
10			0.240
11			0.245
12	0.289		0.283
13		0.326	
14			0.357
15		0.363	
16			0.391
17	0.453	0.452	
18		0.488	
19			0.500
20			0.513
21	0.528	0.556	
22			0.592
23			0.719
24			0.730
25	0.767	0.779	
26	0.893	0.890	

unfractionated material but which were not yielded by the tanned gelatin eluate. This is particularly significant in the case of bands 25 and 26, which occur in a position in which possible masking by overlying bands is not a consideration. Although, conversely, bands from the tanned gelatin eluate were also not present in those Sephadex eluate fractions which were screened electrophoretically, this does not preclude the possibility that these might be present in fractions which were not screened.

The position of non-haemagglutinating toxic activity in the two eluates is also noteworthy. In the case of Sephadex, this was localized in peak 1, in agreement with the finding of DENNISON *et al.*[4], and gave rise to the single electrophoretic band 7. In the case of tanned gelatin, however, toxicity was found in the plateau region, giving rise to bands 5, 11, 16 and 19, none of which corresponds to band 7.

On the basis of this evidence it is tentatively concluded that the gel-electrophoretic properties of certain jack bean proteins are modified by chromatography on tanned gelatin.

Discussion

Legume proteins in general have proved to be complex oligomers, composed in certain instances of dissimilar subunits[6-9]. The nature of the forces involved in the maintenance of such quaternary structures is not clearly understood but that entropy effects (hydrophobic bonding) or hydrogen bonding plays a major role is demonstrated by the effect of high urea concentrations on the oligomeric structure[10,11].

It has previously been demonstrated that tanned gelatin interacts with certain simple proteins by a mechanism apparently sensitive to high concentrations of urea[2]. It would consequently seem possible that tanned gelatin could also interact with oligomeric proteins by the same mechanism, causing disaggregation of the subunits. With the subsequent re-association of subunits under the dynamic conditions pertaining during chromatography, this could result in the formation of new aggregates, either hybrids of different parent subunits or families of molecules comprised of different numbers of subunits from the same parent molecule. This could possibly explain the origin of new electrophoretic bands and the lack of definition of the bands obtained from the tanned gelatin effluent fractions.

STEAD[12] has previously observed the formation of different aggregates during successive re-chromatography runs of a toxic Natal round yellow bean (*Phaseolus vulgaris*) fraction on tanned gelatin, a phenomenon which he ascribed to the manipulations involved in concentrating and desalting the protein after each chromatography run. In the present study, however, samples were taken for assay directly from the fraction collector tubes without prior concentration and desalting so that these factors are not a consideration.

While the mechanism of formation of the new electrophoretic bands remains to be explained, it is nevertheless clear that care must be exercised in interpreting the results obtained by the chromatography of oligomeric proteins on tanned gelatin gels.

Department of Biochemistry,
Natal Agricultural Research Institute,
Pietermaritzburg, Natal (South Africa)

C. DENNISON

1 A. POLSON AND W. KATZ, *Biochem. J.*, 108 (1968) 641.
2 C. DENNISON, *J. Chromatogr.*, 53 (1970) 381.
3 *Sephadex-gel filtration in theory and practice*, Pharmacia Fine Chemicals, Uppsala, Sweden.
4 C. DENNISON, R. H. STEAD AND G. V. QUICKE, *Agroplantae*, 3 (1971) 27.
5 B. J. DAVIS, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
6 D. A. RIGAS, E. A. JOHNSON, R. T. JONES, J D. MCDERMED AND V. V. TISDALE, *Proc. Meet. "Journees Hellènes de Séparation Immédiate et de Chromatographie", Athens, 19–24 Sept., 1965*, Association of Greek Chemists, 1966, p. 151.
7 M. O. J. OLSON AND I. E. LIENER, *Biochemistry*, 6 (1967) 105.
8 N. CATSIMPOOLAS, C. EKENSTAM, D. A. RODGERS AND E. W. MEYER, *Biochim. Biophys. Acta*, 168 (1968) 122.
9 C. J. BAILEY AND D. BOULTER, *Biochem. J.*, 113 (1969) 669.
10 M. O. J. OLSON AND I. E. LIENER, *Biochemistry*, 6 (1967) 3801.
11 D. A. RIGAS AND C. HEAD, *Biochem. Biophys. Research Commun.*, 34 (1969) 633.
12 R. H. STEAD, unpublished results.

Received November 22nd, 1971