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Purification and Some Properties of a Group of Small Basic Proteins from Rapeseed

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The possibility to obtain high-quality protein for nutritional use from rapeseed, has evoked interest in the chemical and physico-chemical properties of the individual rapeseed proteins. Bhatty *et al.*[1] have isolated three main protein fractions soluble in salt solutions. One of these, containing 17 % of the total nitrogen in rapeseed, was shown to be basic and of low molecular weight. Using extraction with neutral buffer, followed by a single gel filtration step, a similar protein fraction has been isolated. This fraction consists of several basic proteins, which appear to be closely related.

Isolation of a low-molecular weight basic protein fraction. Rapeseed (*Brassica napus* L. var. *oleifera*), strain Panter, was a gift of Dr. Ragnar Olsson, Karlshamns Oljefabriker, Sweden. The seeds were put through a roll-mill and the flaky product was extracted 10 times on a Büchner funnel with an equal volume (w/v) of hexane (b.p. 66–69°). The hexane was removed by vacuum distillation in a Büchi Rotavapor at 50°, 2 h. 15 g dry rapeseed meal was extracted with 450 ml ice-cold 0.1 M sodium phosphate pH 7.0 in centrifuge bottles. The bottles were shaken vigorously for 5 min and the insoluble material was removed at 23 500 *g*, 10 min, 0°. The extract, containing 6520 mg protein, was immediately applied to a Sephadex G-50 column. The elution pattern is shown in Fig. 1. The basic protein fraction is collected with the second peak. The yield is 2760 mg.

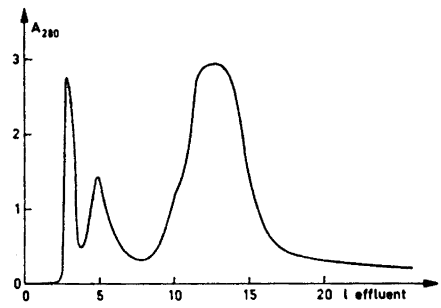

Fig. 1. Rapeseed meal extract on a Sephadex G-50 column. Column dimensions 95 × 12.0 cm. Sample volume 350 ml (6520 mg protein). Elution with 0.1 M sodium orthophosphate pH 7.0. Flow rate 0.84 l/h.

Extraction with neutral buffer solubilizes between 50 and 60 % of the total protein in rapeseed meal. The small basic proteins are completely solubilized. Thus the initial extraction in itself constitutes a purification step.

The short time and the low temperature used in the extraction were chosen in order to minimize the action of myrosinase, which is known to be present in rapeseed.[2] Myrosinase splits thioglucosides present in the extract and the products of this splitting may possibly form complexes with the proteins. Longer extraction times and higher temperatures only led to slight increases in yield.

Separation of individual basic proteins by ion exchange chromatography. The basic protein fraction from the gel filtration step was

precipitated by adding solid ammonium sulfate to 85 % saturation followed by centrifugation at 10 000 *g*, 30 min. The precipitate is dissolved in a minimum amount of 5 mM sodium pyrophosphate-HCl buffer pH 9.3 and dialyzed against a twentyfold volume of the same buffer overnight. The operations were carried out at 6°. The dialysate was applied to a Whatman CM-52 cellulose column and the column was eluted with a linear concentration gradient between 5 mM and 20 mM sodium pyrophosphate–HCl buffer pH 9.3. The elution pattern, in Fig. 2, shows

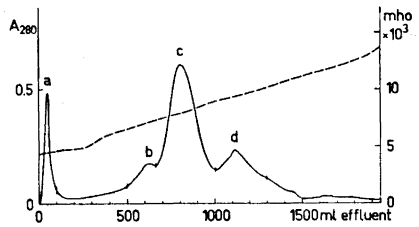

Fig. 2. CM-cellulose chromatography of low-molecular weight basic protein fraction. The column (4.7 × 3.2 cm) was equilibrated with 5 mM sodium pyrophosphate – HCl pH 9.3. 36 ml sample (580 mg protein) in the same buffer was applied, followed by elution with a linear gradient between 5 mM and 20 mM of the same buffer. Flow rate 77 ml/h.

4 distinct peaks. The effluent was divided into corresponding fractions, termed *a*–*d* as indicated in the figure. Typical yields, given as percent of the protein in the basic fraction from the gel filtration step, were: fraction *a* 5 %, *b* 9 %, *c* 42 % and *d* 16 %. Fraction *a* is a contaminant and not a basic protein. Further purification of the individual proteins was achieved by subjecting each fraction to a second ion-exchange run, using narrower gradients. The charge properties are, however, apparently similar to such an extent that even repeated fractionations were not able to separate completely the individual proteins from each other, as judged by gel electrophoresis performed according to Panyim and Chalkley.[3]

Properties of the basic proteins. Isoelectric focusing as described by Vesterberg and Svensson,[4] gave a single peak near the cathode end at a pH of about 11 for the group of basic proteins (fractions *b*, *c*, *d*). This pI is only an approximate one for the group. The individual proteins could not be resolved owing to the poor performance of the pH-gradient at high pH. Comparison with cytochrome *c*, with a pI of 10.8 (Ref. 5), established a more alkaline pI for the rapeseed proteins.

The molecular weight of the basic proteins, determined by gel filtration,[6] was 17 750 ± 1000. The minimum molecular weights of the fraction *c* and *d* proteins, calculated from the amino acid composition in Table 1, were 17 400 and 17 600, respectively, in good agreement with the gel filtration value. This value is significantly higher than the 13 800 ± 300 reported by Bhatty *et al.*[1] for a basic rapeseed protein fraction, using the approach to sedimentation equilibrium method.

The small basic proteins are soluble in pure water and extremely soluble in salt solutions between pH 3 and 11.

The amino acid compositions of fractions *c* and *d* are shown in Table 1. The proteins

Table 1. Amino acid composition of small basic proteins from rapeseed. Freeze-dried samples of protein fractions *c* and *d*, purified by repeated ion exchange chromatography and extensive dialysis, were hydrolyzed 24 h in 6 N HCl at 110°, *in vacuo*. The hydrolysate was analyzed essentially according to Spackman *et al.*[7] Results are expressed as the mean of duplicate analyses, followed by observed ranges.

Amino acid	Residues per tyrosine fraction *c*	fraction *d*
Lys	9.08 ± 0.42	8.85 ± 0.31
His	4.36 ± 0.30	3.93 ± 0.15
Arg	6.09 ± 0.15	6.63 ± 0.12
Asp	2.02 ± 0.10	1.88 ± 0.12
Thr	4.23 ± 0.24	4.11 ± 0.04
Ser	7.04 ± 0.27	6.06 ± 0.14
Glu	34.05 ± 0.6	34.7 ± 1.1
Pro	14.6 ± 0.6	14.0 ± 0.4
Gly	8.75 ± 0.35	9.91 ± 0.28
Ala	7.00 ± 0.23	6.73 ± 0.04
Cys–S–	7.07 ± 0.37	6.95 ± 0.24
Val	6.61 ± 0.12	6.19 ± 0.21
Met	2.69 ± 0.14	3.25 ± 0.03
Ileu	4.21 ± 0.07	4.59 ± 0.11
Leu	8.95 ± 0.26	8.35 ± 0.23
Tyr	1.00	1.00
Phe	2.94 ± 0.02	2.82 ± 0.10
(NH_3	29.7 ± 1.5	28.55 ± 0.8)

contain 7.6 % lysine, which should make them attractive from the nutritional point of view. A difference of one residue between the fraction *c* and *d* proteins may be detected for serine, glycine and, perhaps, arginine. The differences between the proteins in methionine and leucine content are of equal size but of different polarity. The same is true for the pair valine-isoleucine and suggests that these substitutions may have occurred.

Whether the rapeseed genome codes for a number of slightly different basic proteins or whether we are dealing with genetical heterogeneities within the rapeseed strain, is not known, nor is the possible biological function of these proteins. Further work characterizing the rapeseed proteins and exploring their use as human and animal food, is in progress at this laboratory.

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Circular Dichroism of Dihedral Rare Earth Carboxylates Chirally Stabilised in a Single-crystal

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This study is part of an investigation,[1] on one hand to study more generally the reliability of circular dichroism (CD) on pressed "randomised" crystalline powders and on the other to use this method to study compounds in a solid matrix, compounds which either do not exist in solution, or which are not stable towards racemisation.

The possibility to assign a certain observed CD effect to a defined environment of a chromophor will depend on a closer knowledge of the crystal structure. In a crystal the CD could originate from a *molecular configurational chirality* (*e.g.* due to a helical arrangement of ligands around the metal ion in a complex) but also from a *spiral crystal structure*. Examples of the latter case are circular dichroic crystals[2] of $[Ni(H_2O)_6]SO_4$,[3] SiO_2, and $NaClO_3$, *etc.*[2]

Previous CD [4] studies on single crystals were restricted by the demand of large specimens (crystals with an area of approx 100 mm^2 and a thickness of approx 1 mm). Furthermore, the measurements had to be made with the incident light propagated perfectly parallel to an optic axis.

We will in the following outline a method to study CD on a pressed "randomised" powder from a single crystal. The amount of material necessary corresponds to a single crystal of the size 0.5 – 1 mm^3. KCl was used as a matrix. By using a random orientation of the powdered dissymmetric single crystal, the CD tensors will be averaged and a CD similar to that of a random solution will be obtained. In this way it is also possible that a predominant crystal structure CD will be depressed or even averaged out; *e.g.* in a random sample of $[Ni(H_2O)_6]SO_4$ from a ground single crystal the CD was approximately zero.

The method outlined above can be used only if *a.* the incident light is not depolarized by the sample; *b.* if the CD/absorbance ratio is approximately constant with dif-