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FRACTIONATION OF PROTEIN COMPONENTS FROM BEER BY DENSITY GRADIENT CENTRIFUGATION

by

STEEN BECH SØRENSEN, TORBEN GRAVES PEDERSEN and MARTIN OTTESEN

Department of Chemistry, Carlsberg Laboratory,
Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby

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A protein-rich beer fraction obtained by alcohol precipitation has been fractionated by cesium chloride density gradient centrifugation into three fractions. One with buoyant density 1.27 $g \cdot ml^{-1}$ contained essentially pure protein, while a fraction with buoyant density 1.37 $g \cdot ml^{-1}$ contained protein and some carbohydrate, probably covalently bound to the protein. A large fraction with a buoyant density of 1.60 $g \cdot ml^{-1}$ consisted of carbohydrate.

Preparative isoelectric focusing of the protein-containing fractions revealed in both cases a protein peak with pI = 4.7, which for the 1.37 $g \cdot ml^{-1}$ fraction coincided with a carbohydrate peak.

1. INTRODUCTION

Beer proteins are a heterogeneous group of substances varying widely in isoelectric points and molecular weights, and also to some extent in firmly bound carbohydrates and polyphenols (1, 3, 6, 11, 13, 17). Hence, it is understandable that even the combined use of several protein fractionation methods has failed to produce beer protein fractions which meet the criteria for homogeneity (1, 13, 17).

Other categories of high molecular weight protein-carbohydrate complexes, e.g. animal mucins, present similar fractionation problems. However, purification of these substances has been greatly facilitated by the introduction of equilibrium density gradient centrifugation methods (5, 9, 12, 15, 16). In these methods the sample is mixed with a high density salt, like cesium chloride, and centrifuged at high speed until the cesium salt has formed a stable density gradient. High molecular weight components in the sample then form bands located at positions which correspond to the buoyant densities of the individual components. Proteins and carbohydrates have very different buoyant densities and for this reason they are widely separated. In addition, cesium salts are highly effective in promoting dissociation of non-covalently bound carbohydrate from protein. Consequently, any protein-

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carbohydrate material located in the density gradient between the protein region and the carbohydrate region would thus be expected to represent proteins covalently bound to carbohydrates.

The present work describes preliminary studies on the fractionation of beer proteins in density gradients.

2. MATERIALS AND METHODS

Beer proteins were isolated by fractional precipitation with ethanol (8). In a typical experiment one liter of Pilsner lager beer type "Hof" was lyophilized and the residue (34 g) redissolved in 375 ml water. Precipitation was performed at 0 °C by addition of ethanol until a final concentration of 20% (v/v). The pH was adjusted to 7.0 and after 3 hours the precipitate was isolated by centrifugation at 4,000 g for 15 min. The precipitate was redissolved in 3% EDTA, dialyzed extensively against distilled water and lyophilyzed. The yield was about 1 g of brownish coloured powder and this protein fraction corresponds to "fraction x" used in previous studies (8, 11). It contained 25% (w/w) of protein and 70% (w/w) of carbohydrate.

In preliminary experiments it was found that a density of 1.7 $g \cdot ml^{-1}$ was sufficient to prevent the most dense material in fraction x from precipitating during centrifugation. A gradient with this limiting density could be established by centrifuging 5 ml of a CsCl-solution of density 1.40 $g \cdot ml^{-1}$ for 44 hours at 250,000 g at 25 °C in a Beckman L5-65 ultracentrifuge equipped with a swinging-bucket rotor SW.50.1. When equilibrium was established this CsCl-gradient covered the range 1.2-1.7 $g \cdot ml^{-1}$ and it could be used for separation of approximately 100 mg of fraction x.

After termination of the centrifugation, the content of the gradient tube was divided in aliquots of 150 µl by suction from the surface using an Auto-Densi-Flow II C from Buchler Instruments (USA). The aliquots were used for determinations of density (from refractive index (10)), protein, carbohydrate and UV-absorption.

Aliquots from density gradient separations were pooled and further fractionated by isoelectric focusing after dialysis for 4 hours against distilled water in Ultra-Thimbles UH 100/10 Schleicher & Schüll, GmbH (W. Germany). Isoelectric focusing of the dialyzed fractions was performed in a sucrose gradient containing 1% Ampholines, pH 3.5-10 (4 °C) in an LKB 8100 electrofocusing column (110 ml). Equilibrium was reached after 45 hours and the content of the column was divided in 1 ml aliquots. These were used for measurement of pH and some selected aliquots were analyzed for protein and carbohydrate after extensive dialysis against distilled water.

Protein contents were determined from amino acid analyses after hydrolysis in 6 M-HCl for 24 hours at 110 °C and carbohydrate contents were determined colorimetrically (2).

3. RESULTS AND DISCUSSION

After density gradient centrifugation of fraction x a dark brown zone was observed in the narrow density range 1.48-1.52 $g \cdot ml^{-1}$ with lighter brown zones extending above and below the dark band, in the range from 1.32-1.62 $g \cdot ml^{-1}$ as indicated in Figure 1. This distribution of coloured material was also reflected in the UV-absorbance curve.

The carbohydrate material was concentrated in the lower part of the gradient with maximum about a density of 1.6 $g \cdot ml^{-1}$. This density corresponds to the density for carbohydrates calculated according to TRAUBE'S rules (18), and in other experiments it was verified that polymeric carbohydrates like pullulan and dextran after equilibrium centrifugation were located in this range. Since the beer protein fraction had been prepared by ethanol precipitation it is likely that this high density carbohydrate fraction partially consists of β-glucan. Amino acid analysis confirmed this fraction was essentially free from protein (Figure 1).

The protein-containing components accumulated in the upper parts of the gradient with two maxima at 1.27 $g \cdot ml^{-1}$ and 1.37 $g \cdot ml^{-1}$, respectively. The fraction with buoyant density 1.27 $g \cdot ml^{-1}$ is within the range reported in the literature for typical proteins, like bovine serum albumin (1.28 $g \cdot ml^{-1}$), ovalbumin (1.28 $g \cdot ml^{-1}$), subtilisin (1.32 $g \cdot ml^{-1}$) and lactate dehydrogenase (1.27 $g \cdot ml^{-1}$).

The amino acid composition of the 1.27 $g \cdot ml^{-1}$ protein fraction listed in Table I is seen to be similar to the albumin fraction from barley

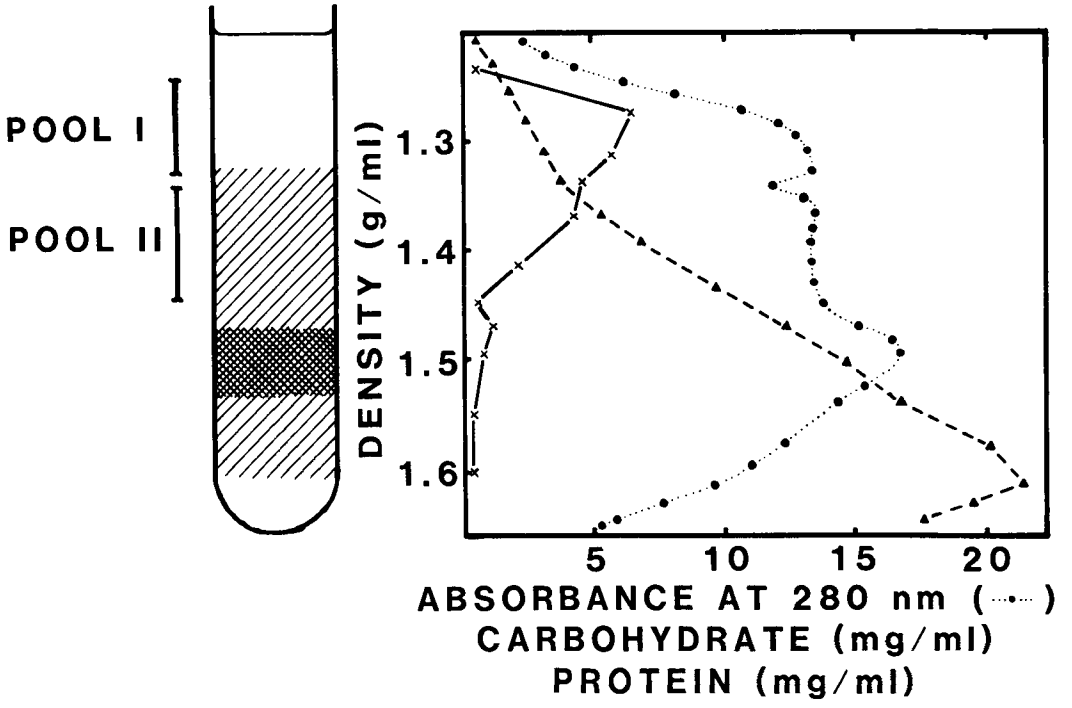

Figure 1. CsCl-density gradient centrifugation of fraction x from beer.

The carbohydrate content (- -▲- -) and the protein content (—x—) was determined by the phenol-sulfuric acid method (2) and by amino acid analysis, respectively.

(4). The protein in this density range (Figure 1, pool I) was collected, dialyzed and fractionated by isoelectric focusing. As seen in Figure 2 the protein accumulated in the acid range with maximum at pI = 4.7 and only small changes in the amino acid composition was observed (Table I).

The protein fraction located in the density range about 1.37 $g \cdot ml^{-1}$ has higher content of aspartic acid, proline, and glycine, and lower content of alanine, valine, leucine and phenylalanine than has the 1.27 $g \cdot ml^{-1}$ fraction. The formula derived by PEDERSEN and IFFT for estimation of the buoyant densities of proteins from their constituents (14) can be used to estimate how these variations in amino acid composition influence the buoyant density values. The calculation shows that the variations in amino acid composition can only account for an increase in buoyant density from 1.27 to 1.30, which is only one third of the observed density increase to 1.37 $g \cdot ml^{-1}$. Assuming the remaining two thirds of the density increase to originate from covalently bound carbohydrate of a density of 1.60 $g \cdot ml^{-1}$ a carbohydrate content of approximately 27% is estimated for this fraction.

This fraction was also collected (Figure 1, pool II) and subjected to isoelectric focusing. Again, the protein accumulated about pI = 4.7, but there was also an accumulation of carbohydrate in this pH-range, suggesting the protein to be covalently bound to carbohydrate (Figure 2). The amino acid composition was again essentially unchanged after isoelectric focusing, but differed significantly from the 1.27 $g \cdot ml^{-1}$ protein fraction (Table I).

The results of CsCl-density gradient centrifugation of fraction x indicate that the protein is separated in two groups. Two thirds of the protein is essentially pure protein while one third apparently contains approximately 27% carbohydrate. This indicates that only 2% of the carbohydrate in fraction x is bound to protein.

The dominating protein fraction resembles the previously described acid protein fraction with molecular weight about 44,000 $g \cdot mole^{-1}$ which crossreacted immunologically with a distinct bar-

Table I

Amino acid composition (mole-%) of fractions obtained after density gradient centrifugation and isoelectric focusing of fraction X.

	Density grad. centr.		IEF-fraction		Barley albumin
	$\rho = 1.27\ g \cdot ml^{-1}$	$\rho = 1.37\ g \cdot ml^{-1}$	Pool I pI = 4.7	Pool II pI = 4.7	(4)
Asx	8.6	11.4	8.8	10.5	10.4
Thr	5.2	5.4	4.8	5.1	4.6
Ser	8.3	8.1	8.4	8.3	5.4
Glx	15.3	16.8	14.2	15.4	14.5
Pro	4.8	8.7	6.1	7.5	7.3
Gly	8.6	10.8	8.1	9.8	8.9
Ala	8.5	6.7	8.8	7.7	10.6
Val	7.6	6.4	7.6	6.4	7.2
Met	1.6	1.5	1.6	1.6	1.9
Ile	4.3	4.0	4.1	4.2	4.1
Leu	10.1	6.6	10.7	8.6	7.9
Tyr	2.1	2.1	2.1	3.0	2.4
Phe	4.2	0.9	4.8	3.4	3.7
His	2.8	2.8	2.2	1.1	1.9
Lys	4.3	3.9	4.0	3.7	5.1
Arg	3.8	4.0	3.7	4.1	3.8

The analysis was performed with a Durrum D-500 analyzer after hydrolysis of the samples for 24 hours at 110°C with 6 M-HCl in evacuated tubes. No corrections were applied for hydrolysis losses.

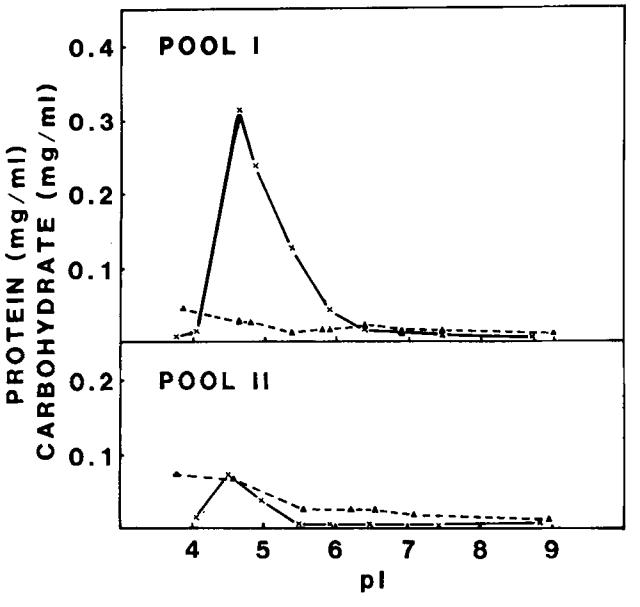

Figure 2. Isoelectric focusing of Pool I and II from density gradient centrifugation of fraction x.

Electrofocusing was performed in a sucrose gradient containing 1% Ampholine, pH 3.5-10. Protein content (—x—) and total carbohydrate content (- -▲- -) was determined on the fractions obtained.

ley protein (8, 17). Its composition is also reminiscent of the "foaming protein" fraction of molecular weight about 40,000 $g \cdot mole^{-1}$ isolated by ASANO and HASHIMOTO (1) and the barley protein Z recently isolated by HEJGAARD (7).

However, amino acid sequence analysis by the EDMAN procedure of the dominating protein fraction as isolated after isoelectric focusing still revealed inhomogeneity, although a dominating sequence could be observed.

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