Microheterogeneity at the C-Terminus of γ -Crystallin Fraction IVb

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The differentiation of the lens has been extensively reviewed (Clayton, 1970). In order to characterize events occurring during differentiation it is necessary to be able to distinguish between homologous proteins, which require different structural genes for their formation. This communication presents evidence for the existence of two polypeptide chains in γ -crystallin fraction IVb that differ at the C-terminus.

Materials and methods

γ -Crystallin fraction IVb was prepared from calf lenses by gel filtration on Sephadex G-75, followed by ion-exchange chromatography on sulphopropyl-Sephadex (Björk, 1961, 1964). Phospho-cellulose was obtained from Brown Co., Berlin, N.H., U.S.A. All other materials and methods were as described by Croft & Waley (1971) and Croft (1971). Amino acid analyses were performed on a Locarte amino acid analyser, with a single column for the determination of acidic, neutral and basic amino acids.

Results

Our preparation of γ -crystallin fraction IV, as isolated by ion-exchange chromatography on sulphopropyl-Sephadex, was further chromatographed on phospho-cellulose, when it was shown to consist of only fraction IVb. The amino acid composition was, in molar proportions: Lys (1.27), His (7.49), Arg (21.0), Cys (4.47), Asp (20.0), Thr (4.57), Ser (12.6), Glu (18.1), Pro (9.34), Gly (13.3), Ala (3.7), Val (5.64), Met (3.8), Ile (6.7), Leu (12.9), Tyr (15.6), Phe (7.98). This analysis is in agreement with that obtained by Björk (1964) for γ -crystallin fraction IVb. The protein was oxidized with performic acid, and the oxidized protein (100 mg) was digested with trypsin. The tryptic peptides were separated by ion-exchange chromatography (Fig. 1). The fractions from the main peaks were combined and dried down, and after being dissolved in water a small portion of each fraction was subjected to paper chromatography in solvent BAWP [butan-1-ol–acetic acid–water–pyridine (15:3:12:10, by vol.) (Waley & Watson, 1953)]. Peptides were detected with the ninhydrin reagent (Heilmann *et al.*, 1957), and arginine-containing peptides with the phenanthraquinone reagent (Itano & Yamada, 1966). By this method of staining,

the only peptides that are detected with ninhydrin yet fail to react with phenanthraquinone are the peptide containing the single lysine residue and the C-terminal peptide. The peptides from fractions 45–47, 49–50 and 79–82 were found to behave in this manner. The peptide from fractions 79–82 was the peptide containing the sole lysine residue. The peptide from fractions 45–47, peptide 1, had $m -0.32$ [m is the mobility on electrophoresis at pH 6.5, relative to aspartic acid ($m = -1.0$), the sign indicating the charge at this pH] and R_{Arg} 3.53 in solvent BAWP. Amino acid analysis indicated the composition: Asp (1.2), Ala (1.0), Ile (0.9), Leu (1.0), Tyr (0.9); sequence analysis by the dansyl–Edman method (Gray, 1967) indicated the sequence:

Ala-Ile-Asp-Leu-Tyr

Peptide 2, from fractions 49–50, had $m -0.34$ and R_{Arg} 3.33 in solvent BAWP. Amino acid analysis indicated the composition: Asp (1.2), Ala (0.9), Val (1.0), Tyr (1.0), Phe (0.9). Dansyl–Edman degradation indicated the sequence:

Ala-Val-Asp-Phe-Tyr

Evidence that these peptides were derived from the C-terminus of the protein was given by the reaction of carboxypeptidase A with the citraconylated oxidized protein. The amino acids released after digestion for 24 h were (mol/mol of protein): Tyr (1.01), Asp (0.83), Ala (0.69), Phe (0.58), Val (0.55), Leu (0.30) and Ile (0.24), on the basis of a molecular weight of 20000 (Björk, 1964). Thus it is evident that the two peptides were derived from the C-terminus of the protein and that the polypeptide chain terminating in peptide 2 was more abundant.

Discussion

Physicochemical studies (polyacrylamide-gel electrophoresis and isoelectric focusing) had suggested that γ -crystallin fraction IVb was homogeneous (Björk, 1964, 1970). It is significant that peptides 1 and 2 could not be resolved on paper chromatography or electrophoresis. It would therefore appear that γ -crystallin fraction IVb consists of two very similar polypeptide chains of different genetic origin, which vary at the C-terminus. Comparison of the sequences of peptides 1 and 2 with the sequence of

Fig. 1. Ion-exchange chromatography of peptides from digestion of performic acid-oxidized γ -crystallin fraction IVb with trypsin

Chromatography was carried out on a column (55 cm $\times$ 0.9 cm diam.) of Bio-Rad Aminex 50W (X2) at 40°C; elution was carried out with a gradient from 0.2 M-pyridine-acetate buffer, pH 3.1, to 2 M-pyridine-acetate buffer, pH 5, 200 ml of each buffer being used, with a flow rate of 6 ml/h. Peptides were detected with ninhydrin after alkaline hydrolysis.

the C-terminus of fraction II (Croft, 1971) indicates that the γ -crystallin proteins are homologous:

Fraction IVb: Ala-Ile-Val-Asp-Leu-Phe-Tyr

Fraction II: Val-Met-Asp-Phe-Tyr

In view of the organogenesis of the lens (Pirie & van Heyningen, 1956) it is noteworthy that γ -crystallin fraction IVb, which is heterogeneous, is more abundant in the nuclear region of the lens (Björk, 1961), and is comprised of the early foetal proteins, whereas fraction II, which is homogeneous (Croft, 1972), is more concentrated, relative to fraction IVb, in the cortex of the lens, which develops later.

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