ADDENDUM

Radioactive Peptide 'Maps' of the Major and Minor Components of Hen's Egg Ovotransferrin

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The amino acid sequence determinations described in the main paper were carried out on preparations of ovotransferrin that contained both the major and the minor electrophoretic forms of the protein. The object of the present experiments was to find whether these components have the same amino acid sequence. The major and minor forms were isolated by isoelectric fractionation and were then examined by peptide 'mapping' after treatment with radioactive iodine to label tyrosine and histidine residues. In some cases the iodination was carried out on the native iron-free proteins. In other cases the proteins were first denatured by reduction and alkylation.

METHODS

Isoelectric fractionation was carried out as described by Wenn & Williams (1968), with the following modifications. A 500 ml isoelectric column (SC.2), obtained from LKB Producter AB, Stockholm-Bromma 1, Sweden, was used to fractionate samples of fully iron-saturated native ovotransferrin (100–150 mg) in ampholyte-sucrose gradients, pH range 5–7. At equilibrium two broad pink bands of protein were observed, and these were syphoned from the column through narrow Teflon tubing inserted from the top of the column. The two protein fractions were dialysed, passed down a column of Sephadex G-25 in water and freeze-dried. Only protein from the middle region of each band was collected and each experiment yielded approx. 20 mg of minor component (pH_1 5.6) and 60 mg of major component (pH_1 5.8). The purity of the fractionated components was checked by starch-gel electrophoresis, with the buffer system of Poulik (1957) (Fig. 1).

Samples of the ovotransferrin components (10–25 mg) were dissolved in 1 ml of freshly prepared 8 M-urea–0.1 M-tris, adjusted to pH 8.5 with HCl. A 100-fold excess of β -mercaptoethanol over total half-cystine was added and reduction was carried out for 4 h at 20°C. A brown discolouration formed with some samples; this was considered to be the result of iron released from the protein. After the reduction, iodoacetic acid, in excess over the β -mercaptoethanol dissolved in 1.0 ml of 1 M-NaOH, was added slowly to the sample. The pH was maintained at 8.0–8.5 with NaOH. The reaction was

allowed to proceed in the dark for 30 min before dialysis of the product against distilled water. During dialysis the modified protein was precipitated; this precipitate was resuspended and finally freeze-dried.

Samples of fractionated native ovotransferrin were dissolved in 1.0 ml of 0.1 M-tris adjusted to pH 8.0 with 1 M-HCl. The same buffer made 4 M with respect to urea was used to dissolve the reduced carboxymethylated samples. A solution of ICl, containing 3.3 μ g-atoms of I/ml, was prepared as described by McFarlane (1963). A volume equivalent to 50 g-atom of I/mol of protein was taken and [131 I]iodide (The Radiochemical Centre, Amersham, Bucks., U.K.) was added to give a final radioactivity of 1 mCi/ml. All solutions were cooled on ice and the 131 ICl was then added to the protein samples. After mixing the reaction was allowed to continue for 3 min before being quenched by the addition of 0.1 ml of 1 M-ascorbic acid. The reaction mixture was dialysed against several changes of distilled water for 30 h and then freeze-dried.

Samples of iodinated protein were digested with various enzymes and combinations of enzymes under essentially the same conditions as described by Williams (1968). The digests were subjected to paper electrophoresis at pH 6.5. Radioautographs of this first dimension were prepared to

Fig. 1. Starch-gel electrophoresis of the major and minor forms of ovotransferrin obtained by isoelectric fractionation. (1) and (3) show the major component (pH_1 5.8). (2) shows the minor component (pH_1 5.6). The position of the origin is shown by O.

Fig. 2. Radioautographs of peptide 'maps' of pepsin-trypsin digests of reduced and alkylated ovotransferrin components. (1) shows the major component. (2) shows the minor component. O shows the position of the origin. The directions of electrophoresis at pH 6.5 and pH 3.5 are indicated.

Fig. 3. Radioautographs of peptide 'maps' of pepsin-trypsin digests of iron-free ovotransferrin components. (1) shows the major component. (2) shows the minor component. O shows the position of the origin. The directions of electrophoresis at pH 6.5 and pH 3.5 are indicated.

check the adequacy of loading and the separation achieved. The sample strip was then sewn to a fresh sheet of paper for electrophoresis at pH 3.5 in the second dimension and radioautographs were prepared.

RESULTS AND DISCUSSION

Fig. 2 shows the ^{131}I -labelled peptide 'maps' obtained from reduced carboxymethylated major

and minor ovotransferrins digested initially with pepsin and then with trypsin. The patterns are very similar, suggesting that the primary structures of the two components resemble one another. Such similarity between 'fingerprints' was also observed for pepsin, trypsin and trypsin-subtilisin digests of the reduced carboxymethylated proteins. Fig. 3 shows the peptide 'maps' obtained for the native ovotransferrin components, made iron-free (Warner & Weber, 1951) before iodination, and subsequently digested with pepsin and then with trypsin. Again these are essentially identical, as were peptide 'maps' of the native iron-saturated components.

The radioactive peptide 'mapping' technique employed is a sensitive test of identity, but it suffers the limitation that only those peptides possessing histidine or tyrosine are observed (Glazer & Sanger,

1964). Digestion of the iodinated protein in several different ways increased the numbers of labelled peptides produced and hence the reliability of the method, but the possibility remains that some small regions of the protein chain may not be represented.

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