Biosynthetic relationship between the major matrix proteins of adrenal chromaffin granules

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The matrix of the chromaffin granule contains a family of acidic proteins, collectively known as the chromogranins. It has been suggested that this family results from protease action on the major component, chromogranin A. Evidence for this has now been obtained from in vitro translation of adrenal medullary messenger RNA and immunoprecipitation of translation products using an antiserum directed against chromogranin A, but which also recognises other chromogranins.

Chromogranin *Chromaffin* *Messenger RNA* *Antibody*

1. INTRODUCTION

Chromaffin granules, the catecholamine storage organelles of the adrenal medulla, contain high concentrations of several low-M_r substances, including the catecholamines (noradrenaline and adrenaline), nucleotides (particularly ATP), calcium and magnesium ions, and ascorbate [1]. Protein components of the granule matrix include a soluble form of dopamine-β-hydroxylase, which is involved in catecholamine biosynthesis and occurs also as a granule membrane protein, enkephalins and their high-M_r precursors, and a family of acidic proteins, the chromogranins [1,2]. The major chromogranin, comprising 50% of matrix protein, has been named chromogranin A [3,4].

Chromogranin A has an M_r of about 70000 and NMR and ORD studies suggest that it exists mainly as a random coil interrupted by only a small segment of α-helix [5]. Amino acid analysis of chromogranin A and of the remaining chromogranins reveals a similar composition [6]. This in itself suggests a relationship between the chromogranins, and their isoelectric points are very similar [7]. This raises an important question about the biogenesis of the chromogranins: are they all encoded by a single gene which is transcribed into a single mRNA, giving rise to a precursor which generates a series of proteins by post-translational processing? Alternatively, is each member of the chromogranin family encoded by a distinct messenger RNA, transcribed from a family of genes and translated separately?

It has been suggested that the function of the chromogranins within the chromaffin granule is to stabilize a storage complex involving catecholamines and ATP. Recently, however, structural similarities between chromogranin A and secretory proteins of other hormone-storing granules have been detected by using immunological methods [8,9]. This raises the possibility that this type of protein plays a general role in storage or secretory processes and that similar proteins may be found in other secretory tissues.

2. MATERIALS AND METHODS

Chromogranin A was purified from a lysate of bovine chromaffin granules by chromatography on DEAE-cellulose [10], followed by passage through concanavalin A–Sepharose to remove dopamine-β-hydroxylase. The resulting mixture of chromogranins was resolved by electrophoresis on a 10% polyacrylamide slab gel in the presence of sodium dodecyl sulphate, briefly stained, and the

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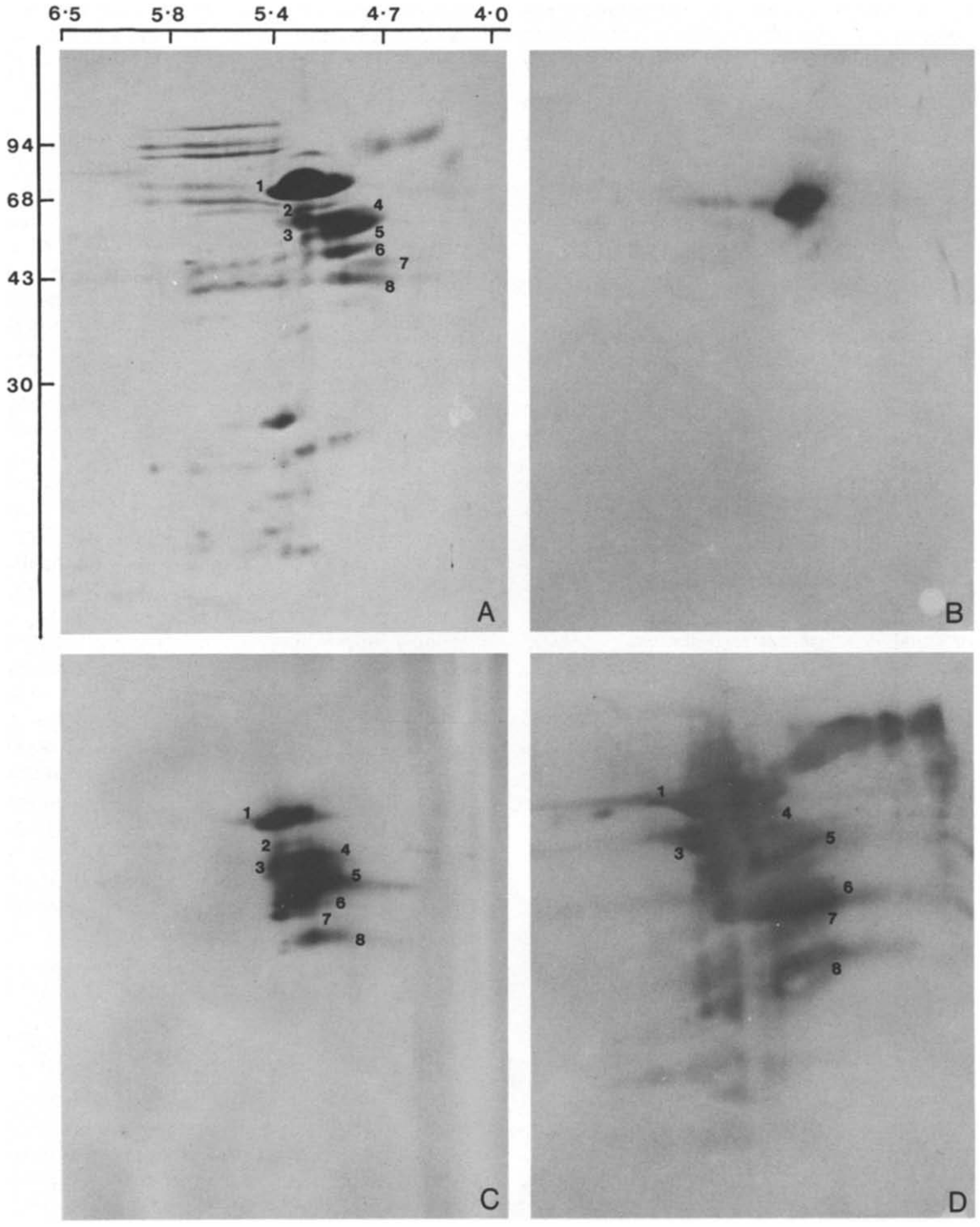
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A
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D

band of chromogranin A cut out. Pure chromogranin A was obtained by electroelution: this material was used to immunise New Zealand white female rabbits.

Radioiodinated chromogranins were prepared by iodination of total chromaffin granule lysate or of the mixed chromogranin peak from the DEAE-cellulose column, using the chloramine-T method.

Total cellular RNA was prepared from bovine adrenal medullae by guanidinium thiocyanate extraction [11]. Poly-adenylated RNA was subsequently purified by affinity chromatography on oligothymidilic acid cellulose [12] and translated in a message-dependent reticulocyte lysate [13] in the presence of [^{35}S]methionine (1.85×10^7 Bq/ml).

Immunoprecipitation of the translation product recognised by antiserum against chromogranin A was performed essentially as in [14].

Two-dimensional gel electrophoresis [15] was performed using Bio-Rad ampholines with a pH range of 4–6 in the first dimension and 8–15% SDS polyacrylamide gels in the second dimension.

Immune replicas [6] were made by electrophoretic transfer of proteins, separated by two-dimensional gel electrophoresis, onto nitrocellulose sheets. After thorough washing in a bovine serum albumin-containing buffer, the replica was decorated with antiserum to chromogranin A; after further washing, iodinated protein A (10^4 Bq/ml) was used to label the attached antibodies. The washed and dried nitrocellulose sheet was then autoradiographed.

3. RESULTS

3.1. *Cross-reaction of antiserum to chromogranin A with other chromogranins*

The result of two-dimensional gel electrophoretic separation of chromaffin granule lysate components, followed by staining with Coomassie blue, is shown in fig.1A. Chromogranin A is the dominant component with a p*I* in the range 4.5–4.9 and apparent M_r about 70000. The other chromogranins appear as spots of similar isoelectric point but decreasing M_r.

Two-dimensional electrophoresis of pure chromogranin A, followed by electroblotting and immune replication using anti-chromogranin A serum, demonstrates the purity of the chromogranin A used to raise the antiserum (fig.1B). However, an immune replica of total granule lysate, using the same serum, reveals that this antiserum can recognise other chromogranins present in the lysate in addition to chromogranin A (fig.1C). The number of cross-reacting lysate components revealed varies considerably with the amount of protein loaded. A light loading of protein (fig.1C) reveals a small, well-defined group of proteins with similar p*I* values, the largest of which is chromogranin A. These correspond to most of the major spots in fig.1A. On the other hand, a larger group of proteins with a greater range of isoelectric point and M_r are shown to be capable of reaction with anti-chromogranin A serum when a larger amount of protein is loaded (fig.1D).

Immunoprecipitation studies using ^{125}I-labelled granule lysate confirm that several chromogranins are recognised by antiserum to chromogranin A. Fig.2A shows an autoradiograph of a two-dimensional separation of the total ^{125}I-lysate components. The components of this unfractionated granule lysate which are immunoprecipitated by anti-chromogranin A serum are shown in fig.2B. In agreement with the results of fig.1C, this includes most of the chromogranin group (M_r in the range 70000–40000), but only traces of material of greater M_r than chromogranin A itself.

3.2. *Immunoprecipitation of in vitro translation products*

Anti-chromogranin A serum was added directly to the radiolabelled translation products from

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Fig.1. Two-dimensional electrophoresis of chromaffin-granule lysate proteins. (A) Total lysate, stained with Coomassie blue (gel loaded with 50 μg protein). The upper scale gives the pH calibration; M_r markers were phosphorylase (94000), bovine serum albumin (68000), ovalbumin (40000) and carbonic anhydrase (30000). Bands are numbered on the gel to aid identification in other figures; the numbers are not intended as a new classification of chromogranins. (B–D) Autoradiographs of immune replicas using anti-chromogranin A serum and ^{125}I-protein A: (B) purified chromogranin A (10 μg); (C) total lysate (2 μg); (D) total lysate (20 μg). The immune replica technique identifies material on the gel that cross-reacts with the serum; as used here, it does not give quantitative information about the amount of such material present.

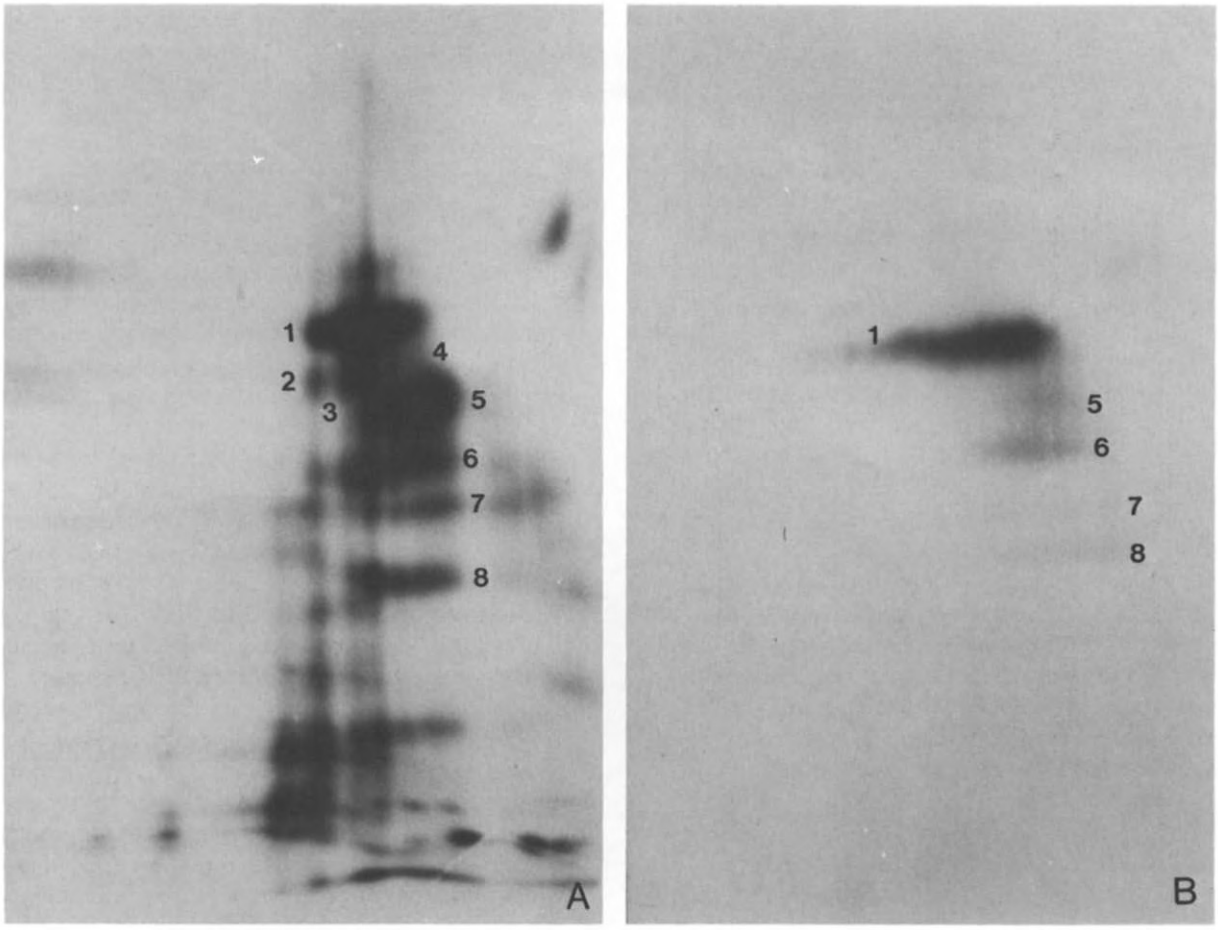

Fig.2. Two-dimensional electrophoretic separation of radioiodinated chromaffin-granule lysate proteins. (A) Autoradiograph of total lysate (compare with Coomassie blue-stained gel, fig.1A; the amount of ^{125}I incorporated into the polypeptides does not correlate closely with their staining intensity); (B) autoradiograph of proteins precipitated with anti-chromogranin A serum.

adrenal medullary mRNA, and immune complexes were precipitated by the addition of formalin-fixed *Staphylococcus aureus* cells. The immunoprecipitated translation product was eluted from the cells and 30 μg unlabelled lysate proteins added as carrier. A two-dimensional electrophoretogram of this mix was stained with Coomassie blue to reveal the position of the lysate proteins (fig.3A) and then autoradiographed to reveal the position of the ^{35}S-labelled translation product(s) recognised by the antiserum (fig.3B).

The two adjoining spots on the autoradiograph comigrate with the low-M_r end of the broad chromogranin A spot, with a pI slightly shifted towards the acidic side. No other translation products, either larger or smaller, were precipitated by the anti-chromogranin A serum.

It is interesting to note the effect of adding non-radioactive lysate proteins to the immunoprecipitated translation product on the pattern of radiolabelled spots obtained after autoradiography. A two-dimensional separation of the same immunoprecipitated translation products, but in the absence of lysate proteins as carrier, resulted in 4 distinct small spots in the same position as the two in fig.3B (see fig.3C). Presumably the higher loading of protein affected the resolution of the electrofocussing.

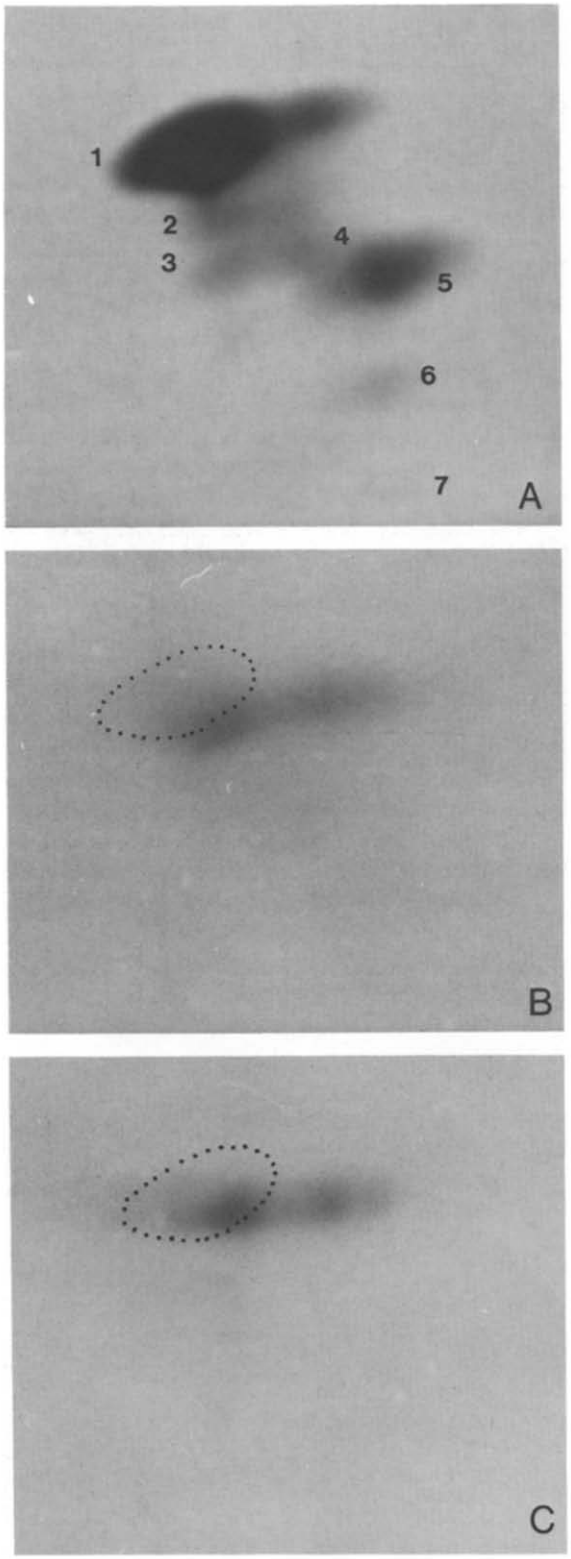

Fig.3. Two-dimensional electrophoretic separation of chromogranins synthesised by translation in vitro and precipitated with anti-chromogranin A serum. Only the region of the gel around the chromogranin A spot (fig.1B) is shown; the rest of each gel was blank. (A) Gel stained with Coomassie blue; lysate proteins were added as carrier to the immunoprecipitate; (B) autoradiograph of gel shown in (A), showing ^{35}S-labelled translation products; (C) autoradiograph of a similar gel run without carrier. In (B) and (C) the position of the major Coomassie blue-stained protein has been indicated.

4. DISCUSSION

The slight differences in M_r and isoelectric point between mature chromogranin A, and the product of in vitro translation, are presumably the result of post-translational processing. Chromogranin A is a glycoprotein containing about 4% carbohydrate with terminal sialic acid residues [4,17], so that the mature product would be expected to be of higher M_r and lower isoelectric point than the immediate product of translation. In fact, its isoelectric point is somewhat higher (fig.3B), suggesting that other modifications, such as proteolysis, may also occur during maturation in vivo.

As shown in fig.1A, the chromaffin granule lysate contains several minor components of higher M_r than chromogranin A itself, including one of somewhat lower isoelectric point. Using a heavy loading of the gels with protein, some of these components can be seen to cross-react with the antiserum against chromogranin A in an immune replica and are therefore structurally related to it; possibly they could arise through modification of the species of M_r 70000 which is produced by in vitro translation. It could be argued that this is itself the result of proteolysis, but we have detected no components of higher or lower M_r, suggesting that no proteolysis of translation products has occurred in vitro. While this work was in progress a report appeared in which a chromogranin precursor of M_r 100000 was identified after translation of adrenal mRNA in a cell-free system derived from wheat germ [18]. It is possible that this species is also produced by translation in the reticulocyte system, but is for some reason not precipitated by the antiserum. However we have shown (fig.1D) that our antiserum reacts with some components of apparent

M_r greater than 70000; the small amounts of these in the immune precipitate (fig.2B) may simply reflect their scarcity in the granule lysate. If they were produced by in vitro translation, particularly if they were the sole product, they would presumably be quantitatively precipitated. An alternative explanation is that they are translated from different mRNA species, which are either lost during isolation, or not efficiently translated in vitro. They might even arise through extensive post-translational modification of the major translated species, of apparent M_r 70000.

We therefore conclude that the acidic proteins of M_r between 40000 and 65000 (the chromogranins) that are released from the matrix of chromaffin granules are derived by proteolysis of one or two high-M_r precursors. We have isolated the primary translation product of the messenger RNA for chromogranin A, a polypeptide of M_r 70000: its structural relationship to mature chromogranin A is now under investigation.

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