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BIOCHEMICAL STUDIES OF THE CHROMAFFIN GRANULE

II. PROPERTIES OF MEMBRANE-BOUND AND WATER-SOLUBLE FORMS OF CHROMOGRANIN A AND DOPAMINE-β-HYDROXYLASE ACTIVITY

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SUMMARY

1. Chromogranin A has been isolated from the washed membrane fraction of highly purified chromaffin granules after solubilization in Triton X-100. A hydrated molecular weight of $2.9 \cdot 10^5$ has been obtained for chromogranin A_M which in its electrophoretic mobility and molecular volume appears very similar to chromogranin A_I of the water-soluble fraction.

2. Chromogranin A has been detected in a third form, A_{III}, in addition to the two forms A_I and A_{II} previously described. The former is predominant in phosphate-containing buffers, at high ionic strength or at very dilute protein concentrations.

3. Apparent molecular weights of $0.74 \cdot 10^5$–$0.78 \cdot 10^5$ and $0.33 \cdot 10^5$–$0.39 \cdot 10^5$ were obtained for A_{II} and A_{III}, respectively. A dissociation of chromogranin A_I into A_{II} and A_{III} was favoured by protein dilution, increase in ionic strength or by the presence of ATP or P_i. Electrostatic interactions are therefore assumed to be the forces involved in holding the subunits (A_{III}) together in dimeric (A_{II}) and hexameric (A_I) configurations.

4. Dopamine-β-hydroxylase activity has been associated with all chromogranins but with highest specific activity in A_M and A_I. This may indicate a dissociation of the membrane complex of dopamine-β-hydroxylase into less active water-soluble subunits parallel to the dissociation of one of its protein components, chromogranin A.

INTRODUCTION

Chromogranin A[1] and dopamine-β-hydroxylase[2] are both constituents of the specific subcellular organelle, the chromaffin granule, that is responsible for the storage of large quantities of catecholamines and ATP in the adrenal medulla.

Both protein constituents show a dual distribution in the chromaffin granule; they are present in the washed membrane fraction and in the water-soluble fraction of lysed granules[3–6]. Chromogranin A has been detected immunologically in the detergent-solubilized membranes in a molecular form which distinguishes itself from the main water-soluble chromogranin A with respect to electrophoretic mobility[7,8]. Dopamine-β-hydroxylase, on the other hand, has been detected in a highly active form associated with the membrane fraction[9] and in a less active form in the water-soluble protein fraction[7,10].

The aim of the present work has been to study the physical properties of the immunologically identical forms of chromogranin A and to determine their dopamine-β-hydroxylase activity.

METHODS

Chromaffin granules were prepared from bovine adrenals by sucrose density centrifugations as described elsewhere[11]. The highly purified chromaffin granules were lysed by hypo-osmotic shock and the membrane and water-soluble fractions were separated as described in the accompanying paper[7]. The washed membrane fraction (S4) was solubilized in 1 % Triton X-100 in 170 mM NaCl and centrifuged for $55 \cdot 10^5$ $g_{av} \cdot$min after 20 h in the cold. The supernatant thus obtained (SN4) and the first aqueous extract of the lysed granules (SN1) served as sources of membrane-bound and water-soluble chromogranin A in the present experiments.

Ethanol-precipitated chromogranin A was prepared as previously described[12] using SN1 as the starting material.

Protein was assayed by the method of LOWRY *et al.*[13].

Bound phosphate was determined in the dialysed protein fractions by the FISKE-SUBBAROW method[14] after digestion of the protein samples in 5 M H_2SO_4 and 30 % H_2O_2.

Lipids were extracted as described by GUSTAFSON[15]. The protein samples were dialysed against three changes of large volumes of 0.02 % (v/v) EDTA, pH 7.0 and dehydrated by freeze-drying. The dehydrated protein was extracted three times with a mixture of chloroform–methanol (2:1, v/v). The organic phases were pooled and washed with 0.1 M NaCl. The chloroform phases were assayed for bound P as described above. The phospholipids were qualitatively identified[16] by thin-layer chromatography of the organic phase and cholesterol was determined by the method of KLUNGSÖYR *et al.*[17].

Chromogranin A was assayed immunologically using the double diffusion technique previously described[4,18].

Dopamine-β-hydroxylase activity was assayed according to the method of GIBB *et al.*[19], using tyramine as the substrate. The unit of enzyme activity is expressed as $1 \cdot 10^{-5}$ mole product formed after 10 min of incubation at 37°.

Disc electrophoresis in polyacrylamide gels was carried out according to the method of DAVIS[20], with the modifications described in the accompanying paper.

Molecular sieve chromatography on Sephadex G-200 was performed in the cold room using a preparative LKB column (80 cm × 3.4 cm). The column was run in solvents containing 0.02 % (w/v) NaN_3 in order to prevent bacterial growth. The solvent was pumped through the column from the bottom with a flow rate of 11 ml/h. The protein samples were added to the column in 5-ml aliquotes and eluate fractions of 4.5 ml were automatically collected. The void volume (V_0) was determined using Blue Dextran 200 (Pharmacia) and the total volume (V_t) was taken as the elution volume of ATP and of catecholamines. The distribution coefficient (K_d) was calculated from the equation[21]

$$K_d = \frac{V_x - V_o}{V_t - V_o}$$

Unless otherwise stated 5 mM sodium succinate (pH 6.0) was used as the eluent.

Analytical ultracentrifugations were carried out in a Spinco Model E ultracentrifuge, running at +20°. The photographic plates were measured with the aid of a Leitz–Wentzlar microcomparator.

Sedimentation-equilibrium studies were made with the use of the YPHANTIS[22] centerpiece. Calculations were made from the interference patterns obtained photographically after 20 h of centrifugation at 24630 rev./min.

Density gradient centrifugation in CsCl was performed as described by DIRKX[23]. The protein samples were made 35 % (w/w) with respect to CsCl and the gradient pattern recorded with Schlieren optics after 18 h of centrifugation at 59780 rev./min. Calculations of buoyant densities, hydrated and anhydrous molecular weights were made from the photografic recordings.

Sedimentation-diffusion studies. The diffusion coefficients were obtained using the valve-type synthetic boundary cell with the cup emptying at 10000 rev./min. The first photo was taken immediately after the rotor had reached 10890 rev./min and 4 more pictures were taken at 8-min intervals. The speed was then increased to 59780 rev./min for the determination of the sedimentation coefficient in the standard manner. Calculations were made according to SCHACHMAN[24]. The protein concentration of the samples was calculated from measurements of the area under the peak of diffusing material.

Viscosity measurements were made using an Oswald viscometer at +20°.

Density measurements were made by weighing 0.5-ml aliquotes of the appropriate solutions in calibrated Carsberg micro-pipettes at +20°.

RESULTS

Isolation of chromogranin A from the membrane fraction (A_M)

The eluogram obtained for SN4 on Sephadex G-200 in 5 mM sodium succinate (pH 6.0) is given in Fig. 1A. One peak (I) of protein material was detected, with $K_d = 0.06$; well separated from the peak of Triton X-100. The protein fractions were rich in lipids, notably phospholipids, but appeared free of Triton X-100 as determined spectrophotometrically. Chromogranin A was detected in the protein peak, and in the concentrated, dialysed protein the immunological titre was 167 % of that obtained for the isolated water-soluble chromogranin A obtained after the first column of Sephadex G-200 (Fig. 1B, Peak II).

Chromogranin A_M thus isolated was found to contain an average of 1.5 μmoles bound P/mg protein of which 80 % were found to be of lipid nature. Extraction with organic solvents abolished the immunological reactivity, indicative of structural changes in the protein. The phospholipids detected in chromogranin A_M were lysolecithin, lecithin, phosphatidyl serine and cephaline, and a molar ratio of cholesterol/lipid P of 3:1 was obtained.

Chromogranin A_M appeared homogeneous by electrophoresis and in mobility identical to that of the source material (Fig. 2a, b) and very similar to A_I of SN1 (Fig. 2c).

Dopamine-β-hydroxylase activity was detected in SN4 (Fig. 1A) and in chromogranin A_M, and the results presented in Table I show that the ratio of dopamine-β-hydroxylase activity:chromogranin A in the isolated material was comparable to that of the source material and to that of A_I, but higher than that of A_{II} and A_{III}.

The method of density centrifugation in CsCl was employed for the determination of the molecular weight due to the low solubility of chromogranin A_M in aqueous buffers of low ionic strength. A hydrated molecular weight of $2.9 \cdot 10^5$ was obtained for the single peak detected in the Schlieren patterns of the developed gradients. The partial specific volume of the lipid-rich chromogranin A could not be determined due to scarcity of material and an anhydrous molecular weight could not be derived.

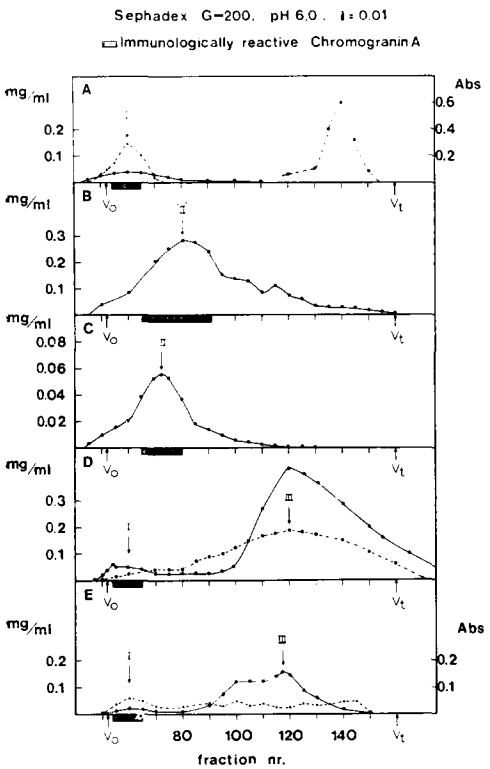

Fig. 1. Molecular sieve chromatography of granule fractions. Granule extracts were fractionated on Sephadex G-200 by elution with buffer at pH 6.0, $I = 0.01$. For chromogranin A_{EtOH} the buffer was sodium phosphate: in all other experiments sodium succinate was used. The protein samples were applied to the column in 5-ml volumes and the protein concentration in each sample is given in brackets. The samples were: (A) Membrane protein solubilized in Triton X-100 (4.3 mg/ml). (B) Water-soluble granule fraction SN1 (12 mg/ml). (C) Immunologically active chromogranin A in rechromatography of material in Peak II of Expt. B (1.2 mg/ml). (D) ———, water-soluble granule fraction SN1 (22 mg/ml); – – – –, ethanol-precipitated protein of SN1 (2.8 mg/ml) (-------). (E) Immunologically active chromogranin A in rechromatography of material in Peak I of Expt. D (6.0 mg/ml). The eluate fractions were assayed for protein (●—●), for dopamine-β-hydroxylase activity (▲—▲) and chromogranin A (shadowed bars). Triton X-100 was assayed in the fractions of Expt. A by its absorbance at 280 nm (■—■) and dopamine-β-hydroxylase activity in Expts. A and E by the absorbance at 330 nm of the product formed in the assay.

Isolation of chromogranin A from the water-soluble fraction (A_{WS})

Two electrophoretically distinct components, A_I and A_{II}, containing the chromogranin A antigen have previously been detected in SN1 (accompanying paper).

TABLE I

DOPAMINE-β-HYDROXYLASE ACTIVITY AND IMMUNOLOGICALLY ACTIVE CHROMOGRANIN A IN GRANULE FRACTIONS

The specific activity of dopamine-β-hydroxylase was 0.31 ± 0.12 (n = 7) and 2.01 ± 0.43 (n = 7) units/mg protein in SN1 and S_4 respectively[10]. For chromogranin A_{EtOH} a value of 1.66 units dopamine-β-hydroxylase activity/mg protein was obtained. Chromogranin A lost immunological activity after rechromatography. The concentrations of immunologically active chromogranin A in SN1 was 0.27 ± 0.11 (n = 7) and in SN_4 it was 0.21 ± 0.05 (n = 7) mg/mg protein[10]. The corresponding ratios of dopamine-β-hydroxylase activity/chromogranin A were 1.34 ± 0.69 (n = 7) and 10.60 ± 2.34 (n = 7) units/mg in SN1 and SN_4[10]. The concentration of immunologically active chromogranin A in the isolated peaks are given in parentheses as mg/mg protein.

Sephadex G-200 peak No.	*Units dopamine-β-hydroxylase/mg protein*			*Units dopamine-β-hydroxylase/mg immunologically active chromogranin A*		
	SN4	*SN1*		*SN4*	*SN1*	
	1st column	*1st column*	*2nd column*	*1st column*	*1st column*	*2nd column*
I	15.4 (1.00)	18.9 (1.00)	10.3 (1.00)	15.4	18.9	10.3
II	—	0.70 (0.66)	0.15 (0.25)	—	1.06	0.66
III	—	0.39 (0)	0.40 (0)	—	—	—

In the present experiments SN1 containing A_I as the conspicuous component (Fig. 2c) has been used as the starting material for the isolation of water-soluble chromogranin A (A_{WS}).

The eluogram obtained with SN1 on the first column of Sephadex G-200 in 5 mM sodium succinate (pH 6.0) is presented in Fig. 1B. It may be seen that the main protein peak (II) coincided with the peak of immunologically active chromogranin A. However, the peak was heterogenous in distribution, and the peak fractions were therefore rechromatographed. The eluogram obtained for the second column of Sephadex G-200 is given in Fig. 1C where it may be seen that the centre of the peak

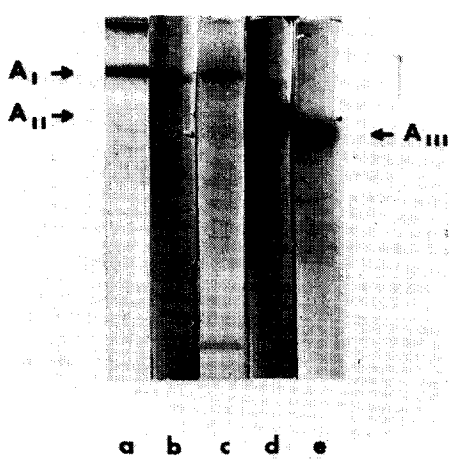

Fig. 2. Disc electrophoresis of chromogranins. The patterns were obtained by disc electrophoresis on polyacrylamide gels (7.5%) at pH 8.9 with 3 mA/gel for 1 h. The samples were: (a) Membrane protein solubilized in Triton X-100 (SN_4). (b) Chromogranin A_M (Fig. 1A, Peak I). (c) Water-soluble protein (SN1). (d) Chromogranin A_{WS} (Fig. 1C, Peak II). (e) Chromogranin A_{EtOH} obtained by ethanol-precipitation of the protein in SN1 (see also Fig. 1D, – – – –).

(II; K_d = 0.18) appeared symmetric but trailing material of higher and lower molecular dimensions were present. The peak fractions contained the immunologically active chromogranin A, and this material was used in the following as a source of purified water-soluble chromogranin A (A_{WS}). The immunological titre of A_{WS} was 36% of that applied to the column, indicative of a partial loss of the immunological response due to the purification procedure (Table I).

With high concentrations of protein the distribution pattern of SN1 differed from that described above. For the first column of Sephadex G-200 a main peak (Peak III, Fig. 1D) was obtained with K_d = 0.59 but no immunologically active chromogranin A was confined to this region. Chromogranin A was detected immunologically in Peak I, and the peak fractions were rechromatographed as before. The second column of Sephadex (Fig. 1E) revealed that the material of Peak I was recovered mainly in the form of Peak III. Again Peak I contained immunologically active chromogranin A while Peak III did not.

Ethanol-precipitated chromogranin A (A_{EtOH}) in 5 mM sodium phosphate (pH 6.0), was eluted as a broad peak with a K_d = 0.59 (Fig. 1D), similar to that of Peak III. The immunological activity in this material had been lost as a result of the ethanol-precipitation but the dopamine-β-hydroxylase activity was intact and similar to that obtained for SN1 prior to fractionation (Table I).

Dopamine-β-hydroxylase activity was found throughout the eluogram of SN1 (Fig. 1E). The specific activity of the various peaks are given in Table I, and it may be seen that no improvement was obtained in this parameter as a result of rechromatography.

No lipid phosphate could be extracted from A_{WS} although values of 0.2–0.02 μmole bound P/mg protein could be detected. The extraction with the organic solvents completely abolished the immunological response of A_{WS} similar to that observed for A_M and A_{EtOH}.

The electrophoretic mobility patterns of the starting material and the isolated peaks are given in Fig. 2c–e. The three peaks appear to correspond to three distinct components A_I, A_{II} and A_{III}, and in Peak II traces of A_I and A_{III} can still be seen.

An estimation of the molecular dimensions of A_M, A_{WS} and A_{EtOH} was obtained from a plot of K_d of the respective peaks on a reference diagram obtained with proteins of known molecular weights (Fig. 3). It may be seen that A_M and A_{WS} were

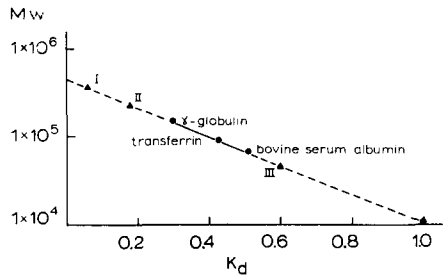

Fig. 3. Molecular weight estimation on Sephadex G-200. The Sephadex G-200 column was calibrated with reference proteins (human γ-globulin and transferrin, bovine serum albumin). Blue Dextran was used for the determination of the void volume (V_0) and ATP and adrenaline for the determination of the total volume (V_t). Chromogranin preparations were: I, chromogranin A_M (Fig. 1A, Peak I); II, chromogranin A_{WS} (Fig. 1C, Peak II); III, chromogranin A_{EtOH} (Fig. 1D, Peak III).

of apparently large sizes, equivalent to molecular weights of $4 \cdot 10^5$ and $2 \cdot 10^5$, respectively, while A_{EtOH} was of molecular weight $0.4 \cdot 10^5$ by this method.

Density centrifugation in CsCl revealed two molecular species in A_{WS}, with hydrated molecular weights of $0.96 \cdot 10^5$ and $0.46 \cdot 10^5$. These values correspond to anhydrous molecular weights of $0.79 \cdot 10^5$ and $0.39 \cdot 10^5$ for the heavy and the light component respectively, the latter predominant in the presence of 2 M CsCl.

The molecular weight of low concentrations of A_{WS} was determined by sedimentation–equilibrium centrifugation and a value of $0.33 \cdot 10^5$ was obtained from the slope of the plot of fringe displacement *vs.* rotor radius (Fig. 4). The straight line obtained indicates species homogeneity under these conditions.

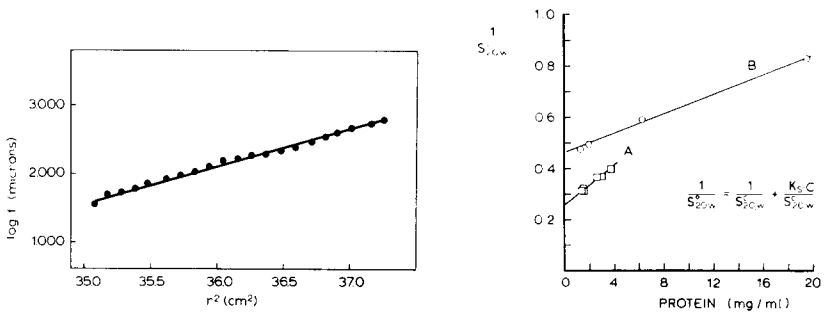

Fig. 4. Sedimentation–equilibrium centrifugation. Chromogranin A_{WS} was centrifuged for 21 h at 20° using the Yphantis centerpiece at 24690 rev./min in 5 mM sodium succinate (pH 6.0). The logarithm of the fringe displacement is plottet against the square of the distance from the center of rotation (r). From the slope of the straight line obtained the molecular weight could be calculated (33000).

Fig. 5. Sedimentation–velocity centrifugation. Chromogranin A_{WS} was studied in NaCl (A) and in ATP (B) of comparable ionic strength and pH (see Table II). The reciprocal of the sedimentation coefficient has been plotted as a function of protein concentration. The sedimentation coefficient at zero protein concentration was obtained from the inverse value of the intercept of the regression line with the ordinate. The slope of the line was used for the calculation of K_s according to the equation given.

Sedimentation–velocity centrifugations of A_{WS} have revealed that the sedimentation properties are markedly effected by the chemical composition of the solvent. Thus the sedimentation was strongly dependent on protein concentration (Fig. 5) and more so when ATP or P_i were the solvent ions. In the latter the aqueous solubility of A_{WS} appeared unlimited while not so in NaCl of comparable ionic strength. The molecular parameters calculated for A_{WS} in these solvents (Table II) suggest that in ATP chromogranin A is more condensed and less hydrated than in

TABLE II

MOLECULAR PARAMETERS OF CHROMOGRANIN A_{WS}

Solvent	*I*	*pH*	$s^\circ_{20,w}$	$1/s^\circ_{20,w}$	K_s (*ml/g*)	$[\eta]$ (*ml/g*)	$K_s/[\eta]$	$D^\circ_{20,w}$	*Mol. wt.*
NaCl	0.1	6.0	3.85	0.260	156	49	3.20	4.30	74 000
ATP	0.1	6.0	2.15	0.465	40	23	1.75	5.0	38 000

NaCl, but probably spherical in both solvents. The molecular weights were $0.74 \cdot 10^5$ and $0.39 \cdot 10^5$ in NaCl and ATP, respectively, indicative of a dissociative effect of ATP on the dimeric configuration.

DISCUSSION

The main functions of the chromaffin granule of the adrenal medulla are connected with their capacity for the synthesis and storage of catecholamines in presence of stoichiometric amounts of ATP[25] and the stable pool of catecholamine storage has been related to an complex with ATP and intragranular proteins[26]. The dual distribution of dopamine-β-hydroxylase[5,6] and chromogranin A[3,4], as well as the detection of an effect of ATP on the solubilization of both granule constituents from their membrane-bound forms[7], lead to the question of the possible function of the apparent dissociation of the large macromolecules in the intact granule.

The present results are in keeping with the previous reports on the physical properties of chromogranin A. A molecular weight of A_{WS} of $0.77 \cdot 10^5$ in Tris–sodium succinate has previously been reported[8,27] as well as a molecular weight of $0.40 \cdot 10^5$ for this protein in 6 M guanidinium·HCl in presence of mercaptoethanol[28]; a molecular weight similar to that obtained for the ethanol-precipitated chromogranin A using $\bar{V} = 0.71$ ml/g for the calculations[12]. The molecular weight of A_M closely resembles that reported for dopamine-β-hydroxylase purified from granule protein that had been solubilized in Triton X-100[29]. Thus the presently described species of chromogranin A (A_M, A_{WS} and A_{EtOH}) have molecular weights that compare well with those previously reported for the proteins specific to the chromaffin granule (Table III). However, the present findings strongly suggest that these molecular species have a common denominator (A) and thus represent different aggregates of the same polypeptide moiety. The smallest molecular weight so far detected for the subunit is $0.33 \cdot 10^5$, and the aggregated forms appear to dissociate into this subunit in the following manner:

$$\underset{(A)_6PL_x}{A_M} \longrightarrow \underset{(A)_6}{A_I} \longrightarrow \underset{3(A)_2}{A_{II}} \longrightarrow \underset{6(A)}{A_{III}}$$

TABLE III

MOLECULAR WEIGHTS OF CHROMOGRANIN A SPECIES

Molecular species	*Sephadex G-200*		*Apparent molecular weight* $\times 10^{-5}$*				
	Peak No.	K_d	*Mol. sieve*	*CsCl gradient*	*Sedimentation equilibrium*	*Sedimentation diffusion*	*Approach to equilibrium*
A_M	I	0.06	3.6	2.9**	—	—	—
A_{WS}	II	0.18	2.2	(a) 0.79	—	0.74_{NaCl}	—
				(b) 0.39	0.33	0.38_{ATP}	—
						0.32_{P_i}	—
A_{EtOH}	III	0.59	0.4	—	—	0.32_{P_i}***	0.34***

* For all the calculations the partial specific volume ($\bar{V}$) obtained for the anhydrous protein by calculation from its amino acid composition[8,12] has been used ($\bar{V}_{anhydr.} = 0.71$ g/ml).

** The hydrated molecular weight since $\bar{V}$ not known for the lipoprotein complex.

*** Values previously obtained[12] have been recalculated using $\bar{V}_{anhydr.}$.

The phospholipids associated with the membrane-form of chromogranin A have been symbolized by PL_x. The dissociating factors leading to a release of A_M from the intact membrane appears to be detergents, ATP or P_i. In the absence of lipids the hexamer (A_I) appears to be unstable and dissociates into A_{II} under the influence of protein dilution or an increase in ionic strength. The dimer (A_{II}) is more stable than A_I but still susceptible to a further dissociation in presence of high ionic strengths, at low protein concentrations or in the presence of small amounts of ATP or P_i.

Studies of the tryptic digests of chromogranin A have suggested that two polypeptides, identical or very similar in amino acid composition, is present in this protein[27] and recent observations on the effect of mercaptoethanol in 6 M guanidinium·HCl on the dissociation of the dimer have been taken as an indication of disulphide bridges involved in holding the two subunits together in the dimeric configuration[28]. However, the present data indicate that electrostatic interactions are the main forces responsible for the maintainance of the aggregated forms. The presence of lipids in A_M appears to stabilize the hexamer in the aqueous buffers but at the same time the lipids greatly reduces the aqueous solubility of this molecular form.

Dopamine-β-hydroxylase activity was associated with all species of chromogranin A studied, but the specific activity was highest in A_M and in A_I. In view of the similarity in molecular weight between that previously described for purified dopamine-β-hydroxylase ($2.9 \cdot 10^5$)[29] and that presently obtained for A_M it seems not unlikely that these two proteins may be identical. The water-soluble dopamine-β-hydroxylase was found to have its highest specific activity in A_I, very similar to A_M in electrophoretic properties. These findings suggest that also the enzyme dopamine-β-hydroxylase dissociates into subunits in a manner parallel to that obtained for chromogranin A. The complete immunological identity detected between A_M and A_{WS} furthermore strengthens the present assumption of a common subunit in these species. The gradual loss of immunological reactivity appears to be related to a reduction in size of the antigenic molecule, but this loss may also indicate structural rearrangements of the antigenic sites in the course of the dissociation of the large aggregates of antigen.

A recent report on the membrane proteins of the bovine chromaffin granule describes two main protein components, A and B, in the membrane fraction[30]. The electrophoretic mobility of protein A closely resembles that of A_M and A_I in electrophoretic mobility while a component of mobility comparable to protein B has not been detected in the present experiments with the membrane protein solubilized in Triton X-100. The amino acid composition of the granule membrane fraction indicated that in addition to a chromogranin A-like protein the membrane must also contain polypeptides which are rich in hydrophobic amino acids but low in glutamyl and prolyl residues[3,30]. It is therefore not unlikely that protein B belongs to the hydrophobic membrane fraction and that chromogranin A, *i.e.* protein A, is preferentially solubilized by the detergents and by ATP or P_i. In view of the finding that the whole water-soluble granule fraction has an amino acid composition very similar to that of the purified chromogranin A_{WS} (ref. 3) the possibility exists for the presence of yet other molecular species of chromogranin A than those described in this work.

The specific activity of electron transport enzymes in the Triton-extracts of the granule membrane fractions are lower than in the intact membranes[9], and a similar loss in the specific activity of dopamine-β-hydroxylase has been detected when Triton-

extracts are compared with membrane protein in potassium phosphate buffer[7] and when the water-soluble dopamine-β-hydroxylase activity is compared with that of the Triton-solubilized membrane protein[10]. These findings lend further support to the assumption that a dissociation of the highly active complex with dopamine-β-hydroxylase activity in the intact membranes leads to less active subunits of the enzyme. Furthermore, the similarity in dissociation patterns between chromogranin A and the dopamine-β-hydroxylase activity suggests that chromogranin A is one of the protein constituents of the intact membrane complex of high specific dopamine-β-hydroxylase activity.

A possible role for the dissociation of large aggregates of chromogranin A in the storage mechanism may be related to the tendency of this protein to become water-soluble in the presence of ATP[7] and to condense in volume with increase in ionic strength[8,12] or in the presence of ATP.

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REFERENCES

1 H. Blaschko, R. C. Comline, F. Schneider, M. Silver and A. D. Smith, *Nature*, 215 (1967) 58.
2 N. Kirshner, *J. Biol. Chem.*, 226 (1957) 821.
3 K. B. Helle and G. Serck-Hanssen, *Pharmacol. Res. Commun.*, 1 (1969) 25.
4 K. B. Helle, in H. J. Schümann and G. Kroneberg, *New Aspects of Storage and Release Mechanisms of Catecholamines, Bayer Symp. II*, Springer Verlag, Berlin, 1970, p. 45.
5 P. Laduron and F. Beliaire, *Biochem. Pharmacol.*, 17 (1968) 1127.
6 D. S. Duch, O. H. Viveros and N. Kirshner, *Biochem. Pharmacol.*, 17 (1968) 255.
7 K. B. Helle, *Biochim. Biophys. Acta*, 245 (1971) 80.
8 A. D. Smith and H. Winkler, *Biochem. J.*, 103 (1967) 483.
9 T. Flatmark, O. Terland and K. B. Helle, *Biochim. Biophys. Acta*, 226 (1971) 9.
10 K. B. Helle, H. Lagercrantz and L. Stjarne, *Acta Physiol. Scand.*, 81 (1971) 565.
11 K. B. Helle, T. Flatmark, G. Serck-Hanssen and S. Lönning, *Biochim. Biophys. Acta*, 226 (1971) 1.
12 K. B. Helle, *Mol. Pharmacol.*, 2 (1966) 298.
13 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
14 C. H. Fiske-SubbaRow, in W. W. Umbreit, R. H. Burris and J. F. Stauffer, *Manometric Techniques*, Burgess Publ. Comp., Minneapolis, Minn., 1957.
15 A. Gustafson, *J. Lipid Res.*, 6 (1965) 512.
16 U. Brockmann and G. Gercken, *Clin. Chim. Acta*, 23 (1969) 489.
17 L. Klungsöyr, E. Haukenes and K. Closs, *Clin. Chim. Acta*, 3 (1958) 514.
18 K. B. Helle, *Biochim. Biophys. Acta*, 117 (1966) 107.
19 J. W. Gibb, S. Spector and S. Udenfriend, *Mol. Pharmacol.*, 3 (1967) 473.
20 B. J. Davis, *Disc Electrophoresis*, Distillation Prod. Div., Eastman Kodak Comp., Rochester, N.Y., 1962.
21 P. Andrews, *Biochem. J.*, 96 (1965) 595.
22 D. Y. Yphantis, *Biochemistry*, 3 (1964) 297.

23 J. DIRKX, *Analytical Density Gradient Ultracentrifugation*, Beckman Instruments GMBH, 1964 p. 30.
24 H. K. SCHACHMANN, *Methods Enzymol.*, 4 (1957) 32.
25 H. BLASCHKO, G. V. R. BORN, A. D'ORIO AND N. EADE, *J. Physiol. London*, 133 (1956) 548
26 N.-Å. HILLARP, in G. E. W. WOLSTENHOLME AND M. O. O'CONNER, *Adrenergic Mechanism* Ciba Found. Symp., Churchill, London, 1960, p. 481.
27 W. J. SMITH AND N. KIRSHNER, *Mol. Pharmacol.*, 3 (1967) 52.
28 A. G. KIRSHNER AND N. KIRSHNER, *Biochim. Biophys. Acta*, 181 (1969) 208.
29 S. FRIEDMAN AND S. KAUFMAN, *J. Biol. Chem.*, 240 (1965) 4763.
30 H. WINKLER, H. HÖRTNAGL, H. HÖRTNAGL AND A. D. SMITH, *Biochem. J.*, 118 (1970) 303.