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SOME PROPERTIES OF SOLUBLE PROTEINS FROM CHROMAFFIN GRANULES OF DIFFERENT SPECIES

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Abstract—The soluble proteins of chromaffin granules from human, ox, horse and pig adrenals and from human phaeochromocytomata have been analysed by gel electrophoresis and their amino acid composition has been determined. The soluble proteins from human phaeochromocytoma chromaffin granules gave the same pattern on electrophoresis as did those from human adrenal, but this pattern differed from that given by proteins of bovine chromaffin granules. However, the amino acid compositions of the soluble proteins from human phaeochromocytoma chromaffin granules and from ox, pig and horse adrenal chromaffin granules were remarkably similar, being characterised by a high content of glutamic acid and a low content of cyste(i)ne. This typical amino acid composition of the total soluble proteins was very like that already reported for the purified main component of the soluble proteins from ox chromaffin granules.

A CHARACTERISTIC feature of adrenal chromaffin granules is their high content of water-soluble protein. Hillarp[1] isolated bovine chromaffin granules on a sucrose density gradient and found that 77 per cent of the total protein was soluble. The chromaffin granules isolated from the adrenals of pig and horse were found to contain 57 and 74 per cent, respectively, of soluble protein[2] and the granules isolated from a human phaeochromocytoma contained 64 per cent of soluble protein.[3] It has recently been proposed that the soluble protein fractions of chromaffin granules should be called 'chromogranins'.[4]

Hillarp[5] was the first to describe a procedure for the isolation of a soluble protein fraction free from amines and most of the adenine nucleotides; this was achieved by precipitation of the protein in 50% ethanol at pH 4. Blaschko and Helle[7] and Helle,[7] using this ethanol-precipitation method, found that the soluble lysate of bovine chromaffin granules contained several proteins; a major component was reported to have a molecular weight of 25,000. A different method of isolation was used by Kirshner *et al.*,[8] who found molecular weights of 39,000 and 21,000 for two main components. However, a higher molecular weight for the major component, i.e. about 75,000 was found by Blaschko *et al.*[9] and by Smith and Winkler,[10] and a very similar value for the molecular weight has since been reported by Smith and Kirshner[11] and by Helle.[12] This major component of the bovine chromogranins has been called chromogranin A.[13]

The granule proteins are of interest for two reasons: first it has been suggested that they may play a role in the storage of the catecholamines[14] and, second, it is now known that they are secreted from the adrenal gland.[4, 13, 15, 16] The present communication describes some of the properties of the soluble proteins from chromaffin granules of different species, since it was thought that properties which are of functional significance must be common to all, or to at least some, species.

METHODS

Highly purified chromaffin granules were obtained from homogenates of adrenal medullae by the simplified method of Smith and Winkler.[17] The same method was used for the isolation of granules from two human phaeochromocytomata; further details about these tumours (Nos. III and IV) will be described separately.[18] Soluble lysates of the granules were prepared as previously described.[10] Polyacrylamide-gel electrophoresis was carried out according to the simplified method of Clarke.[19] For amino acid analysis, 0·4 ml of the soluble lysate was dialysed for 16 hr at 2° against Tris Na-succinate buffer, pH 5·9, I 0·015. The dialysed soluble lysate was hydrolysed for 17 hr in 6 N HCl at 110° under anaerobic conditions, and the amino acids in the hydrolysate were determined with an automatic amino acid analyser (Evans Electroselenium Ltd.).

RESULTS AND DISCUSSION

The soluble proteins from ox, horse and pig chromaffin granules have been characterised by starch-gel electrophoresis,[2, 10] and similar results were obtained by polyacrylamide-gel electrophoresis. When the soluble proteins of chromaffin granules from ox adrenal, from human adrenal and from a tumour of the human adrenal medulla (phaeochromocytoma) were examined by polyacrylamide-gel electrophoresis, they could each be resolved into several components as shown in Fig. 1. The pattern given by the ox proteins is characterised by the presence of a slow-running main component, which is chromogranin A. A protein with the same mobility in gel electrophoresis has already been described in the granule proteins of horse and pig.[2] An immunochemical cross reaction was also reported between the soluble proteins of pig, horse and sheep chromaffin granules and an antibody prepared against ox chromogranin A.[20]

In contrast to the animal proteins, those from human adrenal and from human phaeochromocytoma granules did not contain a single main component with the same electrophoretic mobility as chromogranin A. The human proteins contained two main components which migrated faster than did bovine chromogranin A. However, it is noteworthy that the pattern given by the soluble proteins from the phaeochromocytoma chromaffin granules was identical to that given by the granule proteins of a human adrenal removed from a different patient. There is little or no correspondence between the electrophoretic mobilities of the minor components of the soluble proteins in each of the four species. It can be concluded from these and earlier[2] results that a similar major component of the soluble proteins from chromaffin granules is typical of some, but not all, species.

Ox chromogranin A has an unusual amino acid composition[7, 10, 11] and this is given (from the data of Smith and Winkler[10]) in Table 1 along with the amino acid composition of the total soluble proteins from ox chromaffin granules. Chromogranin

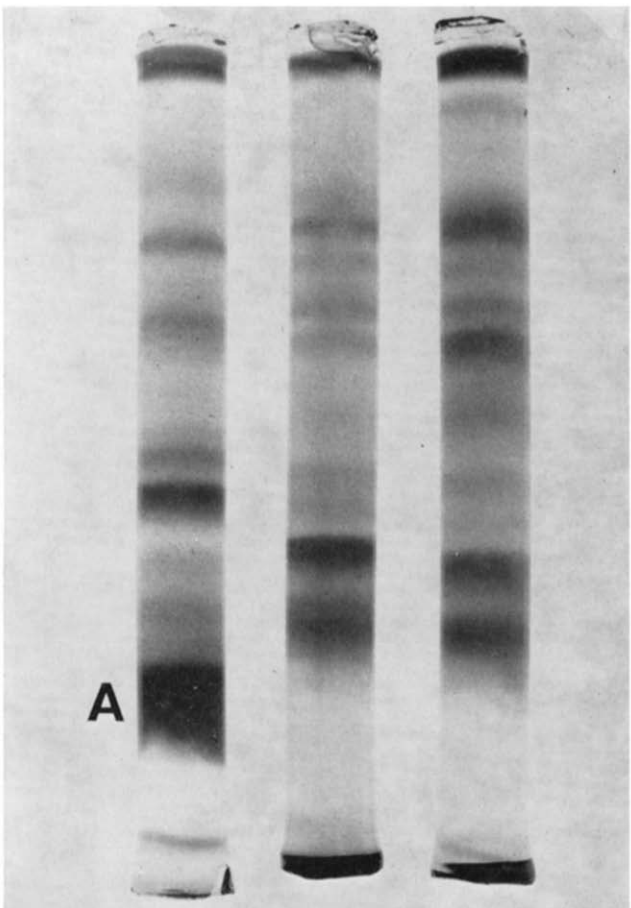

FIG. 1. Polyacrylamide-gel electrophoresis of soluble proteins from chromaffin granules. From the left to the right are shown photographs of the stained gels after electrophoresis of ox proteins, human adrenal proteins and proteins of human phaeochromocytoma respectively. The band corresponding to ox chromogranin A is indicated. The proteins migrated from the bottom to the top, which was the anode.

A is characterised by a very high content of glutamic acid, a relatively high content of proline and a low content of cyst(e)ine. It can be seen that the total soluble proteins from the ox granules have an amino acid composition remarkably similar to that of chromogranin A; the latter protein comprises about 40 per cent of the total soluble proteins.[10] Furthermore, this unusual amino acid composition, which determines the

TABLE 1. AMINO ACID COMPOSITION OF CHROMAFFIN GRANULE SOLUBLE PROTEINS FROM OX, HORSE AND PIG ADRENALS AND FROM TWO HUMAN PHAEOCHROMOCYTOMATA*

Amino acid	Ox chromogranin A	Total soluble proteins from chromaffin granules				
		Ox	Horse	Pig	Human (Case III)	Human (Case IV)
Glu	26·0	26·6	24·7	23·4	25·1	22·2
Lys	9·4	8·6	8·3	8·4	7·5	7·5
Asp	8·3	7·7	8·8	8·3	9·5	9·5
Pro	8·6	8·0	6·3	7·4	6·0	6·3
Leu	7·3	6·8	7·1	7·2	6·9	6·9
Arg	8·5	10·7	11·5	10·4	8·3	9·1
Ser	6·2	5·2	5·7	5·7	6·2	6·0
Ala	5·0	4·2	5·1	5·3	4·2	4·2
Gly	3·9	3·8	3·5	4·6	4·0	4·0
Val	3·2	2·7	3·0	2·5	3·2	3·1
His	2·3	3·6	3·7	3·0	4·2	5·1
Thr	2·4	2·3	2·9	2·9	3·2	3·2
Phe	2·1	2·3	2·2	2·7	2·9	3·1
Met	2·2	1·8	1·5	1·7	1·9	1·9
Tyr	1·7	2·2	2·3	2·7	3·5	3·6
Ile	1·1	1·0	1·2	1·4	1·5	1·5
Cys	0·4	0·6	0·5	0·5	0·4	0·8
NH_3	1·4	1·8	1·6	2·0	1·6	2·0

* The proteins were prepared for analysis as described in Methods. The results are expressed as grams of amino acid per 100 g protein and refer to a single analysis. The hydrolysates from each species also contained small amounts of glucosamine and glactosamine. The amino acid composition of purified chromogranin A is the mean of 3 determinations and is taken from Smith and Winkler.[10]

properties of the proteins, is not confined to the proteins from the ox. Included in Table 1 are the amino acid compositions of the total soluble proteins from pig and horse adrenal chromaffin granules and from two human phaeochromocytomata. The amino acid compositions of the proteins from these different origins are very similar, and have the same characteristic features as those of bovine chromogranin A described above. The analytical variation in the amino acid analysis of ox chromogranin A, under the same experimental conditions used in the present work, was up to $\pm$ 10 per cent for individual amino acids.[10] It can be seen that for most of the amino acids the differences between their proportions in the total soluble proteins of different species could have been the result of experimental variation.

The high glutamic acid content of these proteins is noteworthy, since only two other animal proteins are known that contain more. These are poly-α-glutamic acid, which has been isolated from fowl oviduct,[21] and tropomyosin, a protein that contains 29 per cent (w/w) of glutamic acid.[22] It has been proposed by Smith and Winkler[10] that the high content of polar amino acids and of proline, together with the low cystine content, is the reason why bovine chromogranin A has a conformation approaching that of a random coil polypeptide. Since the total soluble proteins from the chromaffin

granules of several species also have this characteristic amino acid composition, it is suggested that these proteins will tend to behave as random coils in solution.

What is the significance of these properties of the soluble proteins? It has been established that all the soluble proteins of chromaffin granules are secreted from the bovine adrenal gland[13] and if these proteins have any extra-adrenal function, then the similarity in their amino acid composition in different species may be significant. The other possible role of the soluble proteins is in the storage of the catecholamines, and it is suggested that the similar amino acid composition and conformation of these proteins may be relevant to this function. Random coil polymers form gels (e.g. gelatin) and the diffusion of a low molecular weight compound is restricted in a gel.[23] It has recently been found that it is possible to convert a solution of bovine chromogranin A or a solution of the total ox soluble proteins into a gel at low temperature (H. Winkler and A. D. Smith, unpublished observations) and further work is in progress to see whether the properties of the soluble proteins described above are related to the binding of catecholamines in the chromaffin granules.

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