Elemental composition of secretory granules in pancreatic islets of Langerhans

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ABSTRACT We have characterized, by electron probe microanalysis, rapidly frozen cultured rat islets at the level of individual secretory granules. Elemental analysis of thin, dried cryosections showed that beta granules could be distinguished by high Zn, Ca, and S, whereas non-beta (mainly alpha) granules contained elevated P and Mg. Although a single granule type predominated in a particular cell, some rebel granules were found in A cells that had the compositional fingerprint of B cell granules. Zn, which was found in millimolar concentrations in B cell granules, was considered a marker for the insulin storage complex. The data indicate that non-B islet cells in the adult pancreas may produce insulin-containing organelles and that, when glucagon and insulin are coexpressed, these hormones are packaged in separate granules.

INTRODUCTION

Secretory granules, intracellular membrane-bound organelles containing hormones destined for regulated secretion, are generally characterized by high concentrations of hormones, adenosine triphosphate (ATP), Ca, acid pH, and a positive membrane potential (Greider et al., 1969; Hutton, 1982, 1984, 1989; Winkler and Carmichael, 1982; Mellman et al., 1986; Rojas et al., 1986). Since the granules are dynamic (Orci et al., 1986; Schwartz, 1990), turning over at significant rates, processing prohormones, changing internal pH as they traverse the cell from their origin at the Golgi apparatus to the plasma membrane, fusing with the plasma membrane, and releasing their contents, the granules are affected by the environment of the cell, secretions of neighboring cells, and gap junctions between cells (Meda et al., 1984, 1986). Indeed, granules in different secretory cells have distinctive elemental compositions. This has been demonstrated by previous electron probe studies of secretory granules in chromaffin cells, labial gland, mast cells, atrial cells, mucous acinar cells, parotid acinar cells, pancreatic acinar cells, and beta cells of ob/ob mouse (Izutsu et al., 1985, 1986; Mueller et al., 1985; Roomans and Wei, 1985; Kendall and Warley, 1986; Norlund et al., 1987; Ornberg et al., 1988; Somlyo et al., 1988). Few studies have been done, however, on the composition of secretory granules in neighboring cells in a heterogeneous tissue. In such a tissue, large differences in granule composition may occur within a short distance. The aim of the present study was to measure the extent of compositional heterogeneity among granules within individual cells and between cells to gain information about relationships between cellular environment and granular structure and function.

We have used electron probe microanalysis to measure elemental concentrations of secretory granules in situ in pancreatic islets of Langerhans. Pancreatic islets of Langerhans, small organelles composed mainly of insulin-secreting beta or B cells, also contain glucagon-secreting alpha or A cells, as well as somatostatin-secreting D cells and pancreatic polypeptide-secreting cells (Baetens et al., 1979; Bonner-Weir, 1989; Weir and Bonner-Weir, 1990). They afforded us an opportunity to study granules in neighboring secretory cells that originated in embryo from common progenitor cells capable of expressing more than one hormone (Alpert et al., 1988) and that in the adult function cooperatively. Zn is a distinctive marker for the insulin-containing secretory granule in B cells, for a high concentration of Zn is characteristic of the insulin complex but is not expected in millimolar concentrations in other cellular organelles (Greider et al., 1969; Blundell et al., 1972; Sudmeier et al., 1981; Gold and Grodsky, 1984; Norlund et al., 1987; Hutton, 1989).

Quantitative electron probe microanalysis of rapidly frozen, cryosectioned, and freeze-dried tissue can yield concentrations of elements—Na, Mg, P, S, Cl, K, Ca, and Zn—in subcellular organelles in situ (Shuman et al., 1976; Somlyo et al., 1985, 1989; Ornberg et al., 1988; LeFurgey et al., 1991). Distributions of diffusible elements are preserved by specialized cryopreparation techniques. The previous electron microprobe study of secretory granules in ob/ob mouse pancreatic islets (Norlund et al., 1987) reported measurements only on beta granules, since B cells constitute 99% of the mass of these islets. Although B cells constitute ~80% of the normal rat islet mass (Baetens et al., 1979; Bonner-Weir, 1989; Weir and Bonner-Weir, 1990), cryosections cut from near the surface of frozen islets, where freezing quality is best, would be expected to have comparable numbers of B and non-B cells (Fig. 1).

From electron probe microanalysis of fast-frozen, cryosectioned rat pancreatic islets, we found that secretory granules in B cells were characterized by high Zn, Ca, and S and those in non-B, presumably A cells, by

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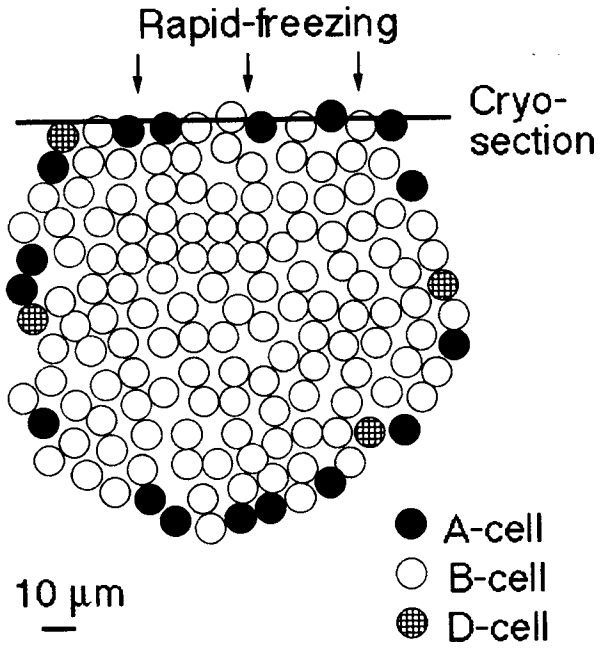

FIGURE 1 Schematic distribution of cell types in rat pancreatic islet. Plane of cryosection to be analyzed is indicated. Beta or B cells, which secrete insulin, make up ~80% of the islet mass. Alpha or A cells, which secrete glucagon, are localized mainly at the periphery. Two other less common types of cell are found in vertebrate islets, the somatostatin-secreting D cell, which is distributed sparsely at the periphery of the cluster, and the pancreatic polypeptide-secreting or PP cell, which replaces A cells in ~25% of the islets (Baetens et al., 1979; Bonner-Weir, 1989; Weir and Bonner-Weir, 1990). Since cryosections were taken near the periphery of the islet, they sampled both B and non-B cells.

high P and high Mg. Average concentrations of other elements were also different in the two granule types. Although most granules in a cell were of the same type, ~10–15% of the granules in A cells had the characteristic elemental fingerprint of granules in B cells. We concluded that A cells in the adult may be capable of expressing insulin and that when insulin and glucagon are coexpressed, they are packaged in separate granules.

MATERIALS AND METHODS

Islets were isolated from collagenase-digested pancreases of adult rats (National Institutes of Health stock). To assess islet function, some islets from a batch were incubated for 2 h in 5.6 mM glucose or in 16.8 mM glucose; in high glucose, insulin secretion was doubled from 1.1 to 2.2 ng/ml/h. The islets intended for x-ray microanalysis were incubated with 5% CO_2 for 2–3 h in culture medium (CMRL 1066; Biofluids, Inc., Rockville, MD) at 37°C in the presence of 5.6 mmol/l glucose, transferred to a gold freeze-fracture "hat" (Bal-Tec Products, Inc., Middlebury, CT), and frozen on the liquid helium-cooled block of a Medvac Cryopress freezing machine (Medvac, St. Louis, MO).

Cryosections, 100 nm thick, were cut from within the first 20 μm of the frozen islet surface, placed on a carbon-coated formvar film supported by a folding copper grid, inserted under liquid nitrogen into a specimen cryoholder (model 626; Gatan Inc., Pleasanton, CA), transferred to the electron microscope (model H700H; Hitachi Scientific Instruments, Mountain View, CA), and freeze-dried in situ. Samples were warmed to 30°C to complete dehydration and then cooled to −60°C for collection of x-ray spectra.

x-ray spectra were recorded from individual granules and cell nuclei with a energy dispersive x-ray detector (Tracor Northern Microtrace; Noran Instruments, Inc., Middleton, WI). The samples were probed with an electron beam (100 keV, 0.5 or 1.0 nA) in a raster scan for 100-s acquisition time. Concentrations (mmol/kg dry weight) were determined from the x-ray spectra by standard procedures (Shuman et al., 1976; Ornberg et al., 1988; Somlyo et al., 1989). Spectra were digitally filtered and analyzed using a multiple least-squares fitting routine (Tracor Northern BIOQ program). Elemental concentrations per unit dry mass were determined from ratios of counts in characteristic peaks to counts in a region of the continuum from 1.37 to 1.61 keV (method of Hall, 1971). Spectra were analyzed for Na, Mg, P, S, Cl, K, Ca, and Zn. Corrections were made in Ca quantitation according to the method of Kitazawa et al. (1983) to eliminate errors from the overlap of calcium K-α and the potassium K-β peaks. Counts in the Zn L peak, which overlaps the Na K peak, were subtracted from spectra on the basis of fits to the Zn K-α and the measured ratio of counts in Zn L to those in Zn K-α. It was assumed that the percent mass loss induced by the electron beam was the same for the sample and the standards. Standards were frozen solutions of polyvinylpyrrolidone containing known concentrations of salt supported between a carbon film sandwich (Ornberg et al., 1988).

Spectra were obtained from three islets, each from a different animal. Different numbers of spectra were recorded from each islet: (*a*) 50 cells, 308 granules; (*b*) 17 cells, 110 granules; and (*c*) 8 cells, 51 granules. A nested analysis of variance analysis was performed on data from all three islets, with Scheffe's procedure used as a post-hoc test to compare pairs of means. From multivariate analysis of variance, the Wilke's lambda statistic was obtained. The probability P is reported that estimated means could differ by as much or more than that measured and still be drawn from the same population.

RESULTS

In cryosections prepared with quick freezing, beta granules did not show the condensation observed after preparation by normal fixation and embedding, and they could not be distinguished from non-beta granules by their morphology (Fig. 2). However, differences between islet cells were evident in the x-ray spectra recorded from the secretory granules, as illustrated in Fig. 3. Granules in one cell exhibited an easily visible Zn peak, whereas those in another cell showed no detectable Zn. Although there were systematic differences in other elemental concentrations, Zn was used to classify granules as "a" or "b," as described below.

The two broad peaks in the histogram of Zn concentrations (Fig. 4), obtained from 469 granules, suggested the existence of two distinct types of granules in our samples. A least-squares fit to the sum of two Gaussians gave one population with a Zn concentration of 1.1 ± 0.2 mmol/kg and a width of 3.0 ± 0.2 mmol/kg and another population at 20.4 ± 1.2 mmol/kg and a width of 10.1 ± 1.2 mmol/kg. A Zn concentration of 8 mmol/kg dry wt, which was at the minimum between the two peaks in the histogram, was selected as the cutoff between a and b granules. Granules were classified as b or beta if they had >8 mmol Zn/kg dry weight and a or alpha otherwise.

The percentage of granules misclassified by this cutoff was estimated from the fit to two Gaussians. If the population near zero represented alpha granules, then ~1%

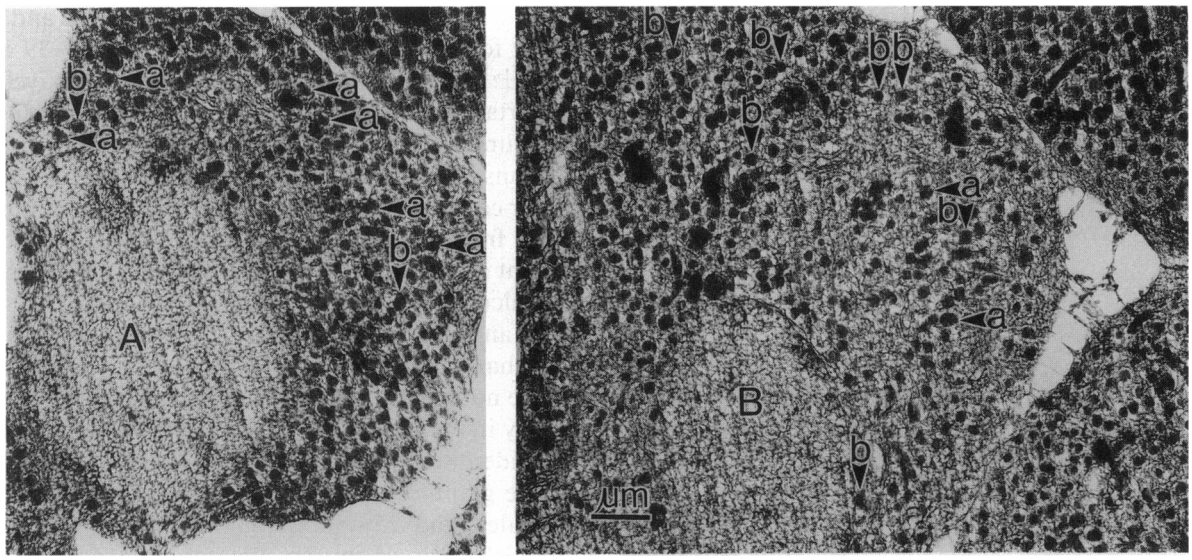

FIGURE 2 Transmission electron micrographs of freeze-dried cryosection of rat pancreatic islet: non-B cell (probably A cell), with few Zn-containing granules and B cell with most granules containing Zn. Granules were indistinguishable from their morphology. Arrows point to secretory granules that were analyzed by electron probe x-ray microanalysis. Those labeled a contained little or no Zn (<8 mmol/kg dry weight), those labeled b contained significant Zn (>8 mmol/kg dry weight). Cells were classified as A (non-B) or B according to whether the majority of granules measured in that cell were a or b, respectively.

of the alpha granules would have Zn concentrations > 8 mmol/kg dry weight. If the population near 20 mmol/kg dry weight represented beta granules, then ~10% of the beta granules would have Zn concentrations < 8 mmol/kg dry weight.

Cells were classified as B or non-B according to whether the majority of granules measured in that cell were beta or non-beta. Non-B cells were considered to be A cells, which are known to predominate near the surface of the islet where the cryosections were taken. Spectra summed from granules measured in A and B cells demonstrate the distinctive composition of granules in the two cell types (Fig. 3). A multivariate analysis comparing the average concentrations of Na, Mg, P, S, Cl, K, Ca, and Zn of all granules in A and B cells showed that these cells are significantly different ($P < 0.001$).

Although the majority of granules in an individual cell were found to be of the same type, in some cells a few granules of the other type were also found (Fig. 2). The number of "rebel" granules in a cell was small enough to have a negligible effect on the average concentration. The average granule spectrum for the A cell shown in Fig. 3, for example, included rebel granules. The observation that granule concentrations averaged for a single cell had distinctive a or b fingerprints supported our classification of cell type. No correlation was found between concentration of Zn and proximity to the nucleus or to the plasma membrane. Neighboring granules were sometimes found to have strikingly different concentrations of Zn.

Nuclei of A and B cells had concentrations of elements that were not significantly different ($P > 0.05$ for all elements measured) (Table 1). High K, low Na, and low Ca concentrations in the nuclei and mitochondria were consistent with measurements on other cells (Somlyo et al., 1985; Norlund et al., 1987; Ornberg et al., 1988) and indicated physiological viability at the time of freezing.

The a and b granules were selected according to their Zn content, as described above, and average concentrations of S, P, Mg, Ca, Na, Cl, and K were calculated for a and b granules separately. Those granules that were in the majority in each cell (conformist granules) were examined first.

Elemental concentrations for conformist a and b granules were significantly different ($P < 0.001$). Although there were small differences among islets, the same conclusions could be drawn from each islet. The b granules with high Zn also had high S, and the a granules with low Zn had high P and high Mg (Table 1 and Fig. 5). Calcium was higher on the average and more variable in beta granules than in alpha granules (Table 1 and Fig. 5). Linear regressions of granule concentrations showed positive correlations between S and Zn (slope: 5–7 S mol to 1 Zn mol) and between Mg and P (slope: 1 Mg mol to 13 P mol). No correlation was observed between Ca and Zn concentrations. The average Na concentration was higher and the K concentration lower in b granules than in a granules, and the average Cl concentration was higher in b granules than in a granules (Table 1).

The average number of continuum counts in the spectra, taken to be a measure of relative dry mass, was the same for a and b granules, within experimental uncertainty, and the ratio of the continuum for granules to that for nuclei was 2.14 ± 0.15. These measurements indicate that the a and b granules had the same water

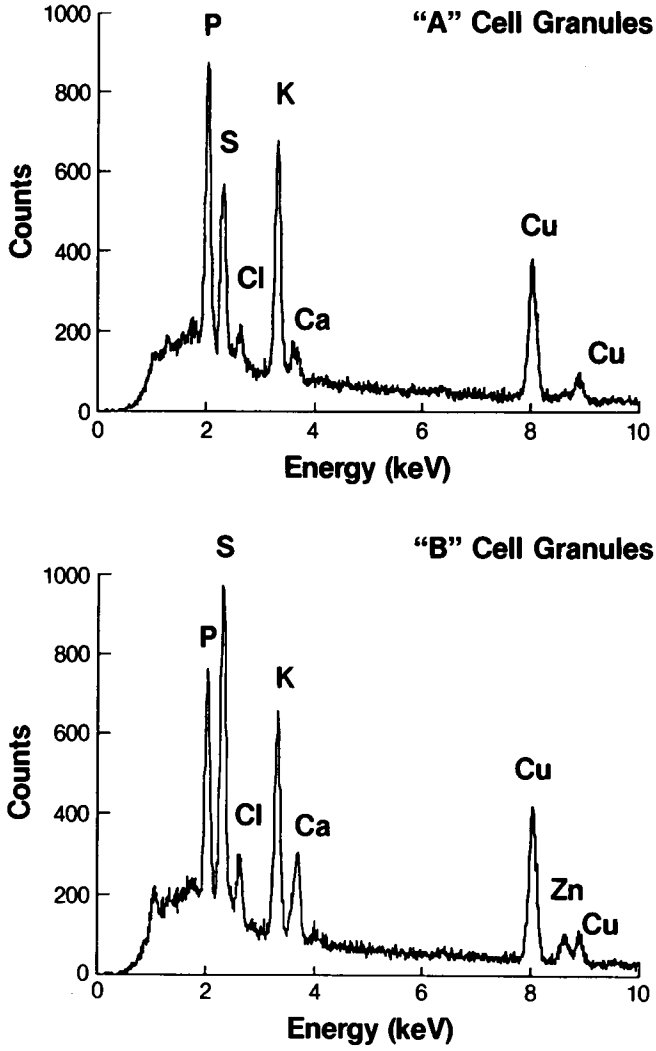

FIGURE 3 x-ray spectra from secretory granules in two pancreatic islet cells. Spectra are shown summed from 10 granules in each cell: non-B cell with predominantly non-Zn-containing granules and B cell with predominantly Zn-containing granules. Characteristic x-ray peaks are labeled. Notice that Zn is visible only in the B cell granules. Cu Kα and Kβ peaks are produced by extraneous x-rays from the support grid.

content, since the relative continuum was considered to be equal to the relative dry mass of the organelles. If it is further assumed that the weight percent water of the hydrated nuclei was 81% (LeFurgey, et al., 1991), then an estimate of 59% may be obtained for the weight percent water of granules.

Elemental concentrations in mmol/kg H_2O were then calculated for granules and nuclei with these estimates for water content. The sum of Na + K was 362 ± 32 and 312 ± 28 mmol/kg H_2O in a and b granules, respectively, and 206 ± 8 and 216 ± 9 mmol/kg H_2O in nuclei of A and B cells. Average Cl concentrations of 45 ± 4 and 69 ± 6 mmol/kg H_2O were calculated for a and b granules, respectively, and of 29 ± 2 and 33 ± 2 mmol/kg H_2O for A and B cell nuclei. Chloride Nernst potentials, calculated with respect to the culture medium that contained 125 mmol Cl^-/kg H_2O, were −27 ± 2 and −16 ± 1 mV for a and b granules, respectively, and −39 ± 3 and −35 ± 2 mV for A and B cell nuclei, respectively. The uncertainties indicated above reflect uncertainties in measurements of continuum and of dry weight concentrations and not the uncertainty in the value assumed for water content of nuclei. If the nuclear water content differed from that assumed, then different values for wet weight concentrations and for chloride potentials would be calculated. Relative elemental concentrations, however, and differences in chloride Nernst potentials would not change.

We next examined those granules that were in the minority in each cell (nonconformist or rebel granules). In cells identified as non-B cells (probably A cells), 10–15% of the granules had significant Zn, typical of beta-type granules, and 25% of the granules in B cells had <8 mmol/kg dry weight Zn. Beta granules in non-B cells were especially distinctive because such high Zn concentrations are rarely found in other cells and organelles and also because only 1% of the alpha granules measured would be expected to have Zn concentration larger than the cutoff value of 8 mmol/kg dry weight selected for a and b granules. Beta granules were found in 4 out of 10 non-B cells in which 6 granules were measured and 6 out of 10 non-B cells in which 10 granules were measured. We found no detectable Zn in other organelles of the islet cells.

What is so striking is that the rebel granules in A cells had concentrations of S, P, Ca, Mg, Na, K, Cl, and Zn that were typical of granules in B cells ($P > 0.05$) and atypical of other A cell granules ($P < 0.001$) (Fig. 5 and Table 1). This suggests that non-B cells may be capable of producing insulin-containing granules.

DISCUSSION

We found that the composition of secretory granules in situ in normal rat pancreatic islets of Langerhans, as measured by electron probe analysis, fell into two

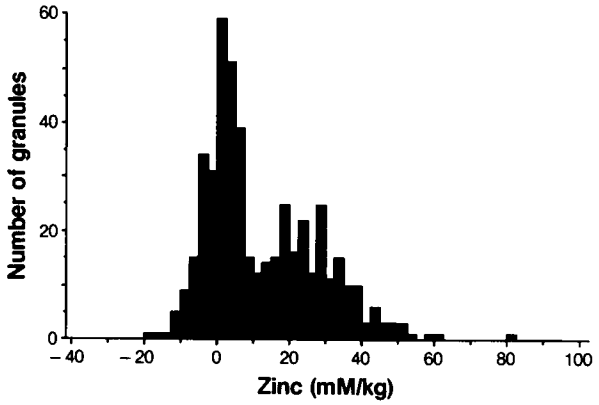

FIGURE 4 Histogram of Zn concentrations of secretory granules in pancreatic islet cells, showing a bimodal distribution. Data were obtained from 469 granules in three islets.

TABLE 1 **Average concentrations of elements in nuclei and granules of A and B pancreatic islet cells**

Element	Nucleus		Conformist granules		"Rebel" granules
	A Cell (n = 40)	B Cell (n = 42)	A Cell (n = 192)	B Cell (n = 179)	A Cell (n = 36)
Na	199 ± 26	194 ± 21	145 ± 6	187 ± 9	197 ± 16
Mg	67 ± 7	75 ± 4	46 ± 1	26 ± 1	24 ± 3
P	740 ± 27	796 ± 31	581 ± 8	432 ± 11	376 ± 3
S	136 ± 9	132 ± 7	145 ± 6	318 ± 11	331 ± 30
Cl	124 ± 8	143 ± 10	65 ± 2	100 ± 3	97 ± 8
K	683 ± 23	728 ± 32	383 ± 6	269 ± 6	255 ± 14
Ca	0.3 ± 1.6	2.1 ± 1.8	11 ± 1	58 ± 7	29 ± 5
Zn	0.2 ± 1.3	−3.7 ± 1.8	0.2 ± 0.3	27 ± 1	21 ± 1

Conformist granules are those that comprise the majority of granules in a cell. "Rebel" granules are those granules that are in the minority in a cell. Note the differences between conformist granules in A and B cells and the similarities between rebel granules in A cells and conformist granules in B cells. Data are summed from three islets, each from a different animal. Means ± SEM are indicated; average concentrations are in mmol/kg dry weight.

classes: one characterized by high Zn and S (called b) and the other by no measurable Zn and higher P and Mg (called a). Average concentrations of all elements measured were found to be significantly different in the two classes. Islet cells were classified as A or B cells, according to whether the majority of granules in that cell were a or b. For those cells containing both granule classes, multipotential stem cells cannot be ruled out, although it seems unlikely that a relatively high percentage of islet cells would be stem cells.

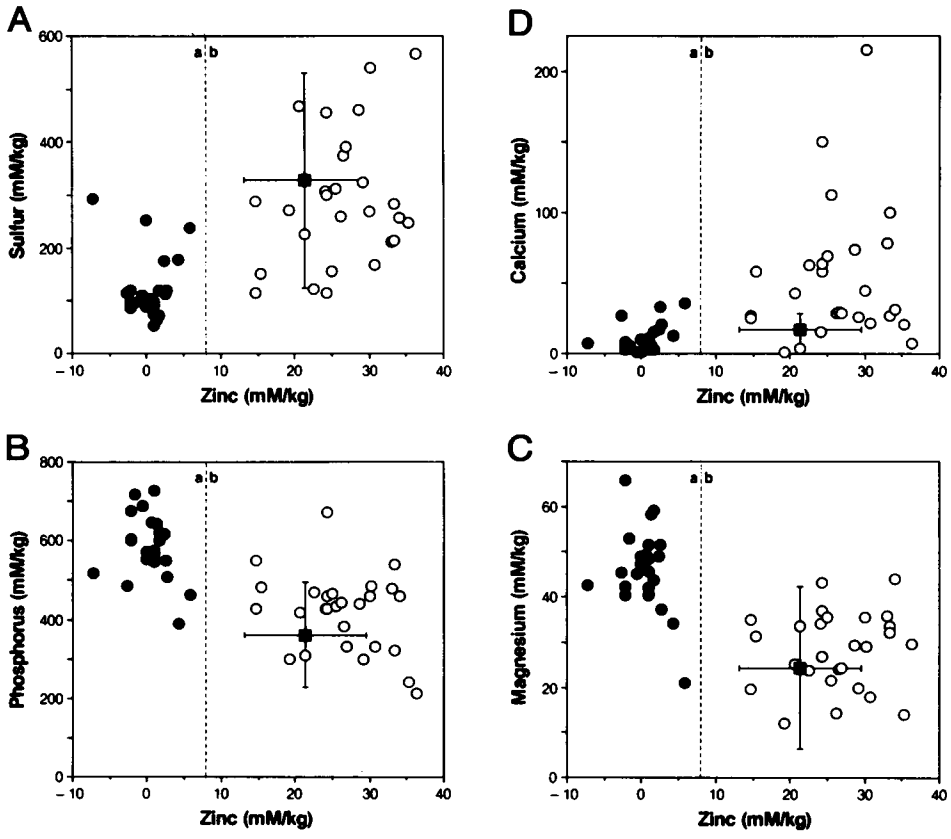

FIGURE 5 Scattergrams of average granule concentrations in 50 cells from one islet. (*A*) Sulfur versus zinc. (*B*) Phosphorus versus zinc. (*C*) Magnesium versus zinc. (*D*) Calcium versus zinc. Each circle represents the measured average granule composition for all "conformist" granules analyzed in a given cell: A cells (*solid circles*); B cells (*open circles*). Conformist granules were the predominant type of granule in a particular cell. The solid square represents the average "rebel" granule composition in all A cells analyzed in the islet. (Error bars indicate standard deviation). The rebel granules in A cells had the characteristic elemental composition of beta granules. In this islet, rebel granules were found in 7 out of 23 A cells.

Since granule elemental composition reflects, for example, protein content, nucleotide content, and transport properties of the granules, inferences concerning granular structure and function may be derived from the elemental distributions. In addition, the spatial distribution of the granules obtained by in situ measurements may have implications for granule expression, packaging, processing, and secretion.

In our study, Zn was taken to be a marker for the insulin molecule and Zn-containing granules were called b granules, since Zn is commonly associated with the insulin molecule and is not found in millimolar concentrations in other cellular organelles (Greider et al., 1969; Blundell et al., 1972; Sudmeier et al., 1981; Gold and Grodsky, 1984; Norlund et al., 1987; Hutton, 1989). Support for the use of Zn as a marker comes also from a previous microprobe study of secretory granules in ob/ob mouse pancreatic islets, in which insulin secreting B cells account for 99% of the mass of the islet. That study reported only one class of granule composition, which was characterized by high Zn (Norlund et al., 1987).

The average insulin concentration in a single beta granule could be estimated from the S concentration, since insulin has a high S content and accounts for ~80% of granule protein (Hutton, 1989). There are six S per insulin molecule (Ryle et al., 1955), so that 318 mmol/kg dry weight S in B cell beta granules represents an upper limit for insulin of 53 mmol/kg dry weight. Insulin concentration in granules has been estimated at 10 and 42 mM (Hutton et al., 1983; Hutton, 1984). Zn and S concentrations were correlated, and Zn was present somewhat in excess of that which would be stoichiometrically bound to crystalline insulin. With two specific binding sites for Zn in a crystalline insulin hexamer (Blundell et al., 1972), the average b granule Zn of 27 mmol/kg dry weight is about a factor of 1.5 greater than the maximum of 18 mmol/kg dry weight Zn that could be stoichiometrically bound to 53 mmol/kg dry weight insulin. Previous measurements of the granular Zn:insulin ratio have shown that Zn concentration is consistent with or in excess of that required for the two zinc sites in the insulin hexamer (Hutton et al., 1983; Grodsky and Schmid-Formby, 1985; Norlund, et al., 1987). The absence of a correlation between Ca and Zn or S content indicated that Ca was contained in beta granules in excess of that specifically bound to insulin (Sudmeier et al., 1981).

The broad distribution found for Zn and S concentrations indicated a variable insulin content from granule to granule. The measurement uncertainty for Zn can be no larger than the Gaussian width of the narrower population taken to represent a granules. This means that the width of the population of b granules at high Zn, which was more than three times that for a granules, represented a real variation of Zn content and not an uncertainty in the measurement. A variation of hormone content of pancreatic zymogen granules has been reported previously (Mroz and Lechene, 1986).

Granules with little or no Zn were assumed to be non-beta granules. Alternate interpretations such as immature beta granules do not seem likely, since (*a*) the insulin prohormone also binds Zn; (*b*) beta granules would then have to acquire Zn as they matured, whereas Zn exchanges very slowly across the granule membrane (Figlewicz et al., 1980); (*c*) all granules measured in many of the cells would be immature; and (*d*) the granules without Zn were not clustered around nuclei, as would be expected for immature granules (Orci et al., 1986). Because A cells are the most common non-B cell in pancreatic islets, the non-beta granules were assumed to be glucagon-containing alpha granules, although some could be somatostatin or polypeptide-containing granules. The S content measured for alpha granules was lower than that measured beta granules, as expected, since there is only one S per glucagon molecule (Bromer et al., 1971).

The phosphorus concentrations measured in alpha and beta granules are consistent with high ATP, as has been found, for example, in the catecholamine-containing granules of bovine adrenal chromaffin cells (Rojas et al., 1986; Winkler and Carmichael, 1982) and in isolated granules from insulinoma (Hutton et al., 1983). The P content reported for chromaffin granules (Ornberg et al., 1988) is comparable with that of alpha granules. If ATP concentrations are proportional to P concentrations, our results suggest that the ATP content of the alpha-granule storage complex may be higher than that of the beta-granule complex. The Mg content is higher in both a and b granules than that reported for chromaffin granules (Ornberg et al., 1988). The correlation of Mg with P concentrations suggests that Mg in granules from pancreatic islets may be associated with ATP and other nucleotides.

Although simplifying assumptions were made, the water content of 59% obtained for islet granules is within the range of that previously reported for granular water content. Hutton (1982) quotes 66% water content found for isolated beta granules. For chromaffin granules, Winkler and Carmichael (1982) quote ~60% water content (range 52–68.5%) and Ornberg et al. (1988) use 66% for the water content of chromaffin granules. Somlyo et al. (1988) report 53% water content for atrial granules.

The Na + K concentrations calculated for a and b granules, 362 ± 32 and 312 ± 28 mmol/kg H_2O, respectively, are considerably higher than the concentration of $Na^+ + K^+$ in the medium (149 mmol/kg H_2O). Maintenance of osmotic equilibrium would imply that the cations were not free in the water compartment but rather complexed with negative sites in the granule. Na was higher and K lower in b granules than in a granules. This inverse relationship between Na and K in a and b granules suggests that these granules have different transport properties.

The higher Cl content of b granules also suggests differences in structure or metabolism for a and b granules. Higher Cl content could reflect fewer fixed negative charges. It could also reflect differences in Cl-proton pump rates, pH, or intragranular membrane potential. In isolated beta granules, the pH and intragranular membrane potential have been shown to depend on the cytosolic pH, the presence of ATP, and the concentration of permeant anions and bases (Hutton, 1982).

The distinctive compositions of alpha and beta granules suggest that glucagon and insulin were packaged in different granules. A mixture of hormones in single granules would result in a continuum of compositions, whereas the average composition measured for beta granules in A cells was similar to that measured for beta granules in B cells.

When hormones are coexpressed but separately packaged, the question is raised of how hormones are sorted into separate secretory granules. One possible mechanism for separate packaging could involve specific "sorting domains" or three-dimensional epitopes on the insulin and glucagon prohormones. A general sorting epitope apparently separates regulated hormones destined for secretory granules and hormones destined for vesicles of the constitutive pathway (Moore and Kelly, 1986; Burgess and Kelly, 1987; Chung et al., 1989; Schwartz, 1990).

Although coexpression in the same cell of insulin and glucagon, insulin and somatostatin, and glucagon and pancreatic polypeptide have been reported (Kaung, 1985; Alpert et al., 1988), we believe this is the first report of beta granules in non-B cells of adult vertebrates. It would be of interest to confirm these results by immunolabeling techniques at the level of individual granules. Some pancreatic islet tumors coexpress and cosecrete more than one hormone (Schein et al., 1973; Gazdar et al., 1980; Madsen et al., 1986; Kloppel and Heitz, 1988; Wynick et al., 1988), but it is not known if the different hormones are secreted from the same cells and, if so, whether they are packaged in separate granules.

It remains to determine if these rebel b granules in A cells are secreted and, if so, what stimulus is required. A high blood glucose level stimulates the B cells to release insulin, whereas a low blood glucose level stimulates A cells to release glucagon (Atwater et al., 1989; Weir and Bonner-Weir, 1990). Secretion of both hormones requires intracellular ATP and extracellular Ca. With separate granules for glucagon and insulin, the possibility exists that secretion of these two hormones could be separately controlled, even in the same cell. Differential modulation of secretion from three different granule populations in human neutrophils has been observed (Lew et al., 1986).

It has been believed that insulin biosynthesis in adult vertebrates is strictly confined to pancreatic B cells, perhaps because of the complexity of the regulatory region of the insulin gene (Maniatis et al., 1987; Walker, 1990). Since B cells are selectively destroyed in some types of diabetes (Colman et al., 1989), it would be of clinical interest to control induction, as well as secretion, of insulin in non-B cells.

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