# SUBCELLULAR DISTRIBUTION OF ADENYLATE CYCLASE IN RAT-LIVER TISSUE

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## 1. Introduction

It is generally assumed that adenylate cyclase is located in the plasma membrane. In rat liver however multiple subcellular localizations of this enzyme have been described. According to [1] more adenylate cyclase could be associated with the endoplasmic reticulum than with the plasma membrane, whereas in [2] the highest specific activity of the enzyme was found in Golgi membrane preparations. The possible multiplicity of the subcellular localization of this enzyme should be considered with respect to its function in the cell and its utilization as a specific membrane marker. We have re-investigated the intracellular localization of adenylate cyclase in rat liver by making use of the analytical approach of centrifugation. Such an approach allows the distribution pattern of an enzyme in different centrifugation systems to be established and when compared with that of the reference enzymes in the same systems, to propose the most plausible subcellular localization of this enzyme. More the centrifugation systems are numerous more the information is precise. Here, the distribution of adenylate cyclase was established after differential and gradient centrifugations of homogenates and particulate fractions. Adenylate cyclase distribution has been compared with the distribution of markers for the plasma membrane (5′-nucleotidase), Golgi membranes (galactosyl transferase) and endoplasmic reticulum (glucose 6-phosphatase). We show that when rat-liver adenylate cyclase is stimulated by fluoride, its behaviour in different centrifugation systems is similar to that of an enzyme quasi-exclusively associated with the plasma membrane.

## 2. Experimental

### 2.1. *Tissue fractionation*

Experiments were performed with male Wistar rats (200–250 g). The animals were decapitated after fasting for ~20 h. The liver was removed, chilled in ice-cold 0.25 M sucrose and homogenized in the same medium by means of a smooth glass tube fitted with a Teflon pestle rotating at 3000 rev./min. The suspension was brought to ~40 ml and spun for 10 min at 1700 rev./min (600 × $g_{av}$) in an International PR2 refrigerated centrifuge. The sediment was washed twice at 1500 rev./min (470 × $g_{av}$). Supernatants from the centrifugation were pooled to make the cytoplasmic extract. This extract was fractionated following [3]. A nuclear fraction (N), a heavy mitochondrial fraction (M) a light mitochondrial fraction (L), a microsomal fraction (P) and a soluble fraction (S) were isolated using the fractionation scheme in [3].

Density gradient experiments were done as in [4] with a Spinco model L2-65B ultracentrifuge; for details see text.

### 2.2. *Enzyme assays*

5′-AMPase was measured as in [5], glucose 6-phosphatase as in [3] and galactosyl transferase as in [6]. Adenylate cyclase was assayed in 60 μl containing [α-$^{32}$P]ATP ($10^6$ cpm), 0.33 mM ATP, 5 mM $MgCl_2$, 25 mM Tris-buffer (pH 7.6), 5 mM theophylline, 20 mM NaF, 20 mM phosphocreatine and 0.25 mg creatine phosphokinase/ml. The reaction was initiated by the addition of the enzyme; incubation was at 30°C for 10 min maximum. Measurements were usually done with 4 enzyme concentrations. The reaction was terminated and cyclic AMP measured as in [7]. The proteins were measured following [8].

## 3. Results and discussion

### 3.1 *Differential centrifugation*

Fig.1 shows the distribution patterns of enzymes after differential centrifugation according to the scheme in [3]. The distribution of adenylate cyclase is similar to that of 5′-AMPase, a plasma membrane marker. Both enzymes are mainly recovered and exhibit the highest relative specific activity in nuclear (N) and microsomal (P) fractions. Such a distribution pattern is different from that of galactosyltransferase (Golgi membranes) and of glucose 6-phosphatase (endoplasmic reticulum) which is typically microsomal.

### 3.2. *Gradient centrifugation*

The enzyme distribution was established after centrifugation in a sucrose gradient in different conditions.

In fig.2A, the distributions observed after the centrifugation of a whole homogenate are represented, the time integral of the square angular velocity ($\omega$) was 480 rad$^2$/ns. The homogenate was homogeneously distributed throughout the whole gradient before centrifugation. Although adenylate cyclase distribution is relatively less flattened, it resembles

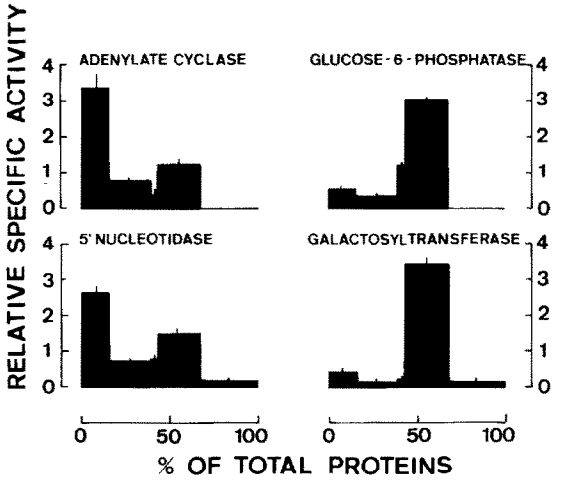


Fig.1. Distribution patterns of enzymes after differential centrifugation. Ordinate: mean relative specific activity of fractions (% of total recovered activity/% of total recovered proteins). Abscissa: relative protein content of fractions (cumulatively from left to right). Mean recoveries (6 fractionations) were 102% for adenylate cyclase, 103% for 5′-AMPase, 93% for galactosyltransferase and 102% for glucose 6-phosphatase.

that of 5′-AMPase and is clearly distinct from that of glucose 6-phosphatase and galactosyltransferase. Fig.2B illustrates the results obtained after centrifugation of a whole particulate fraction (NMLP) with $\omega$ = 144 rad$^2$/ns. In this experiment the granules were deposited at the bottom of the gradient before centrifugation. As shown in [5] in these conditions, the difference between the distribution pattern of 5′-AMPase and of glucose 6-phosphatase is well marked. Adenylate cyclase behaves like 5′-AMPase, its distribution pattern is different from those of endoplasmic reticulum and Golgi markers. Fig.2C,D show the enzyme distribution curves established after centrifugation of a microsomal P fraction treated (or not) with digitonin, as in [9].Granules were layered at the top of the gradient before centrifugation and $\omega$ = 480 rad$^2$/ns. The resemblance of adenylate cyclase and 5′-AMPase is apparent when centrifugation has been performed on untreated granules. This similarity is strikingly confirmed after the digitonin treatment. The shift of the distribution curve towards higher density regions is remarkably the same for 5′-AMPase and adenylate cyclase. Digitonin does not affect the distribution of glucose 6-phosphatase [9]; it causes a shift of the galactosyltransferase distribution, but even in this case, the Golgi enzymes are mainly found in the zones of lower densities than those where adenylate cyclase and 5′-AMPase are found. The fact that the behaviour of adenylate cyclase parallels that of 5′-AMPase in our gradient experiments may also be made conspicuous by comparing the median densities calculated from the distribution curves (table 1). The median densities of both enzymes are very close.

Our experiments show that the distribution of rat-liver adenylate cyclase is similar to that of 5′-nucleotidase and different from that of glucose 6-phosphatase and galactosyltransferase. Therefore, as far as the distributions of marker enzymes corre-

Table 1
Median densities (g/ml) of particle-bound enzymes

| Enzyme | A | B | C | D |
|---|---|---|---|---|
| Adenylate cyclase | 1.183 | 1.195 | 1.150 | 1.185 |
| 5′-AMPase | 1.179 | 1.192 | 1.150 | 1.188 |
| Glucose 6-phosphatase | 1.183 | 1.223 | 1.172 | 1.180 |
| Galactosyltransferase | 1.138 | 1.161 | 1.119 | 1.148 |

Median densities were calculated from the distributions in fig.2

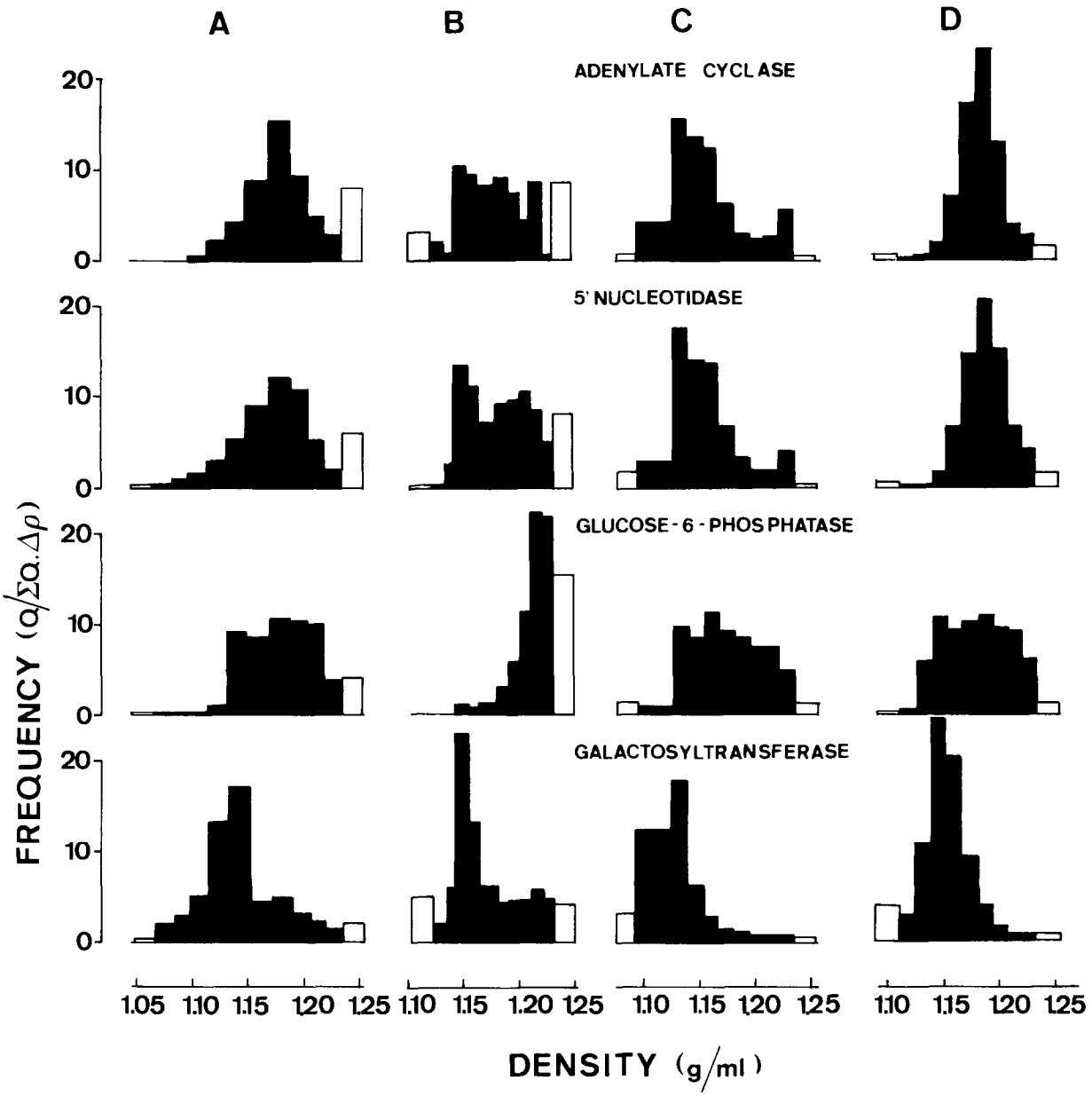


Fig.2. Distribution of enzymes after centrifugation in a sucrose gradient. (A) Centrifugation was performed at 65 000 rev./min ($\int_0^t \omega^2 dt$ = 480 rad²/ns) in the Spinco SW 65. A whole homogenate was initially homogeneously distributed throughout the whole gradient before centrifugation; gradient, $\rho$ = 1.09–1.26 g/ml. (B) A granule preparation (N + M + L + P) was prepared by centrifuging the homogenate at 40 000 rev./min for 45 min in the Spinco rotor 40. This preparation was diluted in sucrose ($\rho$ = 1.25 g/ml) and layered at the bottom of the tube, under a $\rho$ = 1.09–1.24 g/ml gradient. Centrifugation was performed at 39 000 rev./min ($\int_0^t \omega^2 dt$ = 144 rad²/ns) in the Spinco rotor SW 65. (C) A microsomal fraction P was layered at the top of $\rho$ = 1.09–1.26 g/ml gradient and centrifugation was done at 65 000 rev./min ($\int_0^t \omega^2 dt$ = 480 rad²/ns) in the Spinco rotor SW 65. (D) A microsomal fraction P was treated with digitonin as in [9] and centrifuged as in (C). Ordinate: average frequency of the components for each fraction $Q/\epsilon Q \cdot \Delta\rho$ where $Q$ represents the activity found in the fraction, $\epsilon Q$ the total recovered activity and $\Delta\rho$ the increments of density from top to bottom of the fraction. Recovery values for all the experiments were 97–112%.

spond to the distributions of a well-defined membrane system, one has to conclude that the major part or even the totality of rat liver adenylate cyclase is associated with the plasma membrane. If some adenylate cyclase is located in the endoplasmic reticulum and in the Golgi membranes, it must represent only a minor proportion of the enzyme. Our results do not agree with those in [1] showing that a high proportion of adenylate cyclase is located in the endoplasmic reticulum. The reason for such a discrepancy is not apparent although in [1] an analytical approach was not used in the centrifugation experiments. The adenylate cyclase intracellular localisation proposed in [1] is founded on measurements mainly performed on purified fractions and the results do not permit a clear balance sheet of the enzyme distributions. With respect to the presence of adenylate cyclase in Golgi membranes [2], our observations do not exclude the possibility that a minor proportion of the enzyme could be associated with these membranes and therefore do not necessarily disagree with the results in [2].

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