Transverse-plane Topography of Long-chain Acyl-CoA Synthetase in the Mitochondrial Outer Membrane*

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Transverse-plane topography of mitochondrial outer-membrane long-chain acyl-CoA synthetase was investigated using proteases as probes for exposure of crucial domains, i.e. domains containing the active site or otherwise required for enzymatic activity. Incubation of intact mitochondria with the nonspecific proteases proteinase K and subtilisin resulted in a time-dependent loss of 90% or more of the long-chain acyl-CoA synthetase activity compared to control incubations. The integrity of the outer membrane before and during this treatment was shown by cytochrome c oxidase latency as well as the stability of adenylate kinase activity in the presence of protease. After a 15-min incubation in these conditions, site-specific proteases such as trypsin and chymotrypsin had only a limited inhibitory effect (29 and 58%, respectively) but, treatment of hypotonically disrupted mitochondria with these proteases resulted in increased (71 and 77%, respectively) loss of activity. Exposure of trypsin-sensitive crucial domains on the inner surface of the membrane was directly demonstrated by incubation of trypsin-loaded outer-membrane vesicles. Together, these results suggest that mitochondrial long-chain acyl-CoA synthetase is a transmembrane enzyme, possessing crucial domains on both sides of the outer membrane. However, the cytosolic exposure of the enzyme does not appear to be affected by a change in the medium ionic strength as seen previously for other outer-membrane enzymes. In an experiment investigating the topography of the active site of the enzyme, an immobilized substrate analog, desulfo-CoA-agarose, was preincubated with intact mitochondria. This resulted in up to a 42% loss of the activity of long-chain acyl-CoA synthetase, consistent with a cytosolic exposure for at least the CoA-binding domain of the active site.

Recent studies have shown the mitochondrial outer membrane to have several important functions within the cell. As the external boundary of the mitochondrion, the outer membrane interacts with other organelles, the cytoskeleton, as well as the cytosol (1, 2). The vast majority of mitochondrial proteins are nuclear-coded, are synthesized extramitochondrially, and must be imported into the mitochondrion during its biogenesis in a process controlled by the outer membrane (3, 4). Also, the outer membrane, through the selective permeability of a pore protein or “porin” (5), is capable of maintaining a composition in the intermembrane space of the mitochondrion that is distinct from that of the cytosol.

The outer membrane is also active metabolically primarily as a site of lipid biosynthesis. Indeed, it is at the level of the membrane that the metabolic fate of cellular fatty acids is largely determined. Long-chain fatty acids are activated to acyl-CoAs by the activity of outer-membrane long-chain acyl-CoA synthetase (EC 6.2.1.3) (6). Fatty acyl-CoAs are then utilized for the sequential acylation of glycerol 3-phosphate by outer-membrane glycerol-3-phosphate acyltransferase (EC 2.3.1.15) (6, 7) and acyl-CoA:1-acyl-sn-glycerol-3-phosphate acyltransferase (8) to form phosphatidic acid. The phosphatidic acid synthesized in the outer membrane is thought to serve as a precursor for cellular glycerolipid (9-11) or cardiolipin biosynthesis in the mitochondrial inner membrane (12). Alternatively, long-chain acyl-CoAs are utilized catabolically during β-oxidation in the mitochondrial matrix after translocation across the inner membrane by the carnitine shuttle.

Since compositionally distinct compartments can be maintained on either side of the outer membrane, the positioning of the fatty acid-activating and -utilizing enzymes within the transverse plane of this membrane determines the milieu of these reactions as well as the accessibility of the products of these reactions to their ultimate subcellular destination. Hence, it is necessary to determine the transverse-plane topography of these enzymes in the outer membrane to more completely understand outer-membrane lipid metabolism. In a previous study we have determined that one of these enzymes, glycerophosphate acyltransferase, spans the transverse plane of the outer membrane and the exposure of the cytosolic domain of the enzyme is modulated by the ionic strength of the medium (13). In this report we extend our investigation of the topography of the outer membrane to include long-chain acyl-CoA synthetase. The results of these experiments indicate that acyl-CoA synthetase possesses one or more protease-sensitive essential (“crucial”) domains on the cytosolic surface of the membrane, in addition to having trypsin-sensitive sites on the outer surface of the membrane. Also, the use of an immobilized substrate analog for acyl-CoA synthetase suggests that at least a portion of the active site of the enzyme is accessible on the cytosolic surface of the

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1 The abbreviation used is: glycerophosphate acyltransferase, acyl-CoA:1-acyl-sn-glycerol-3-phosphate acyltransferase.
outer membrane. Thus, similar to glycerophosphate acyl-transferase, mitochondrial long-chain acyl-mitochondria synthetase appears to be a transmembrane enzyme. However, unlike glycerophosphate acyl-transferase, the results show the exposure of the cytosolically exposed domain(s) to be not significantly affected by change in the ionic strength of the medium.

**EXPERIMENTAL PROCEDURES**

**Materials**—[9,10-^3^H]Palmitic acid (23.5 Ci/nmol) was obtained from Du Pont-New England Nuclear. Coenzyme A (lithium salt, reference standard grade) and palmitic acid (free acid) were purchased from Sigma. Dithiothreitol and soybean trypsin inhibitor were obtained from Sigma Chemical Co. 

**Methods**—Long-chain acyl-CoA synthetase was assayed by quantitating the conversion of[^H]palmitic acid into a non-ether-extractable product,[^H]palmityl-CoA. The method used was a modification of that described by Suzue and Marcel (15). The 0.2-ml reaction mixture contained a final concentration of 350 mM Tris-HCl (pH 7.4), 8 mM MgCl₂, 0.5 mM CoA, 10 mM ATP, 1 mg/ml Triton WR-1339, and 30 μM[^H]palmitic acid (specific radioactivity = 6 × 10⁶ cpm/nmol). The assay mixture was contained in 15 × 100-mm Teflon-lined screw-capped culture tubes. The reaction was initiated by the addition of the mitochondrial fraction (3.5 μg of protein) which had been diluted with 100 mM Tris-HCl (pH 7.4), 1 mg/ml dithiothreitol. The assay mixture was incubated for 4 min at 37°C and stopped by the addition of 0.8 ml of 1% perchloric acid. Excess[^H]palmitic acid was removed by extracting the aqueous assay mixture four times with 5 ml of water-saturated diethyl ether. Aliquots of 0.2 ml of the washed aqueous phase were counted with 2.8 ml of Biofluor liquid-scintillation mixture in a Packard Instrument Co. Tri-Carb 3325 liquid-scintillation spectrometer. Residual[^H]palmitic acid in the aqueous phase was determined using control reactions in which the reactants (ATP and CoA) were omitted. The incorporation of[^H]palmitic acid into aqueous-soluble product was linear with time and protein concentration. Specific activities presented are expressed as nanomoles of[^H]palmitic acid incorporated into aqueous-soluble product per min/mg mitochondrial protein and are the mean of duplicate assays. In Fig. 4, freshly prepared mitochondrial fraction (3.5 μg of protein) was preincubated in the acyl-CoA synthetase assay medium minus the CoA, [^H]palmitic acid, and Triton WR-1339 for 30 min at 37°C with the indicated volumes of cross-linked 4% agarose (Sepharose CL-4B) was obtained from Pharmacia LKB. 

**RESULTS**

Fig. 1 documents the results of time-course experiments in which the non-specific proteases, proteinase K (Fig. 1a) or subtilisin (Fig. 1b), were used to probe for cytosolically exposed crucial domains of several outer-membrane enzymes. During digestion of preparations of intact rat liver mitochondria with either protease, long-chain acyl-CoA synthetase was the most rapidly inactivated outer-membrane enzyme studied in these experiments. Under these conditions, maximal inactivation (95%) of acyl-CoA synthetase occurred within 5 min of incubation. Proteolysis by either proteinase K or subtilisin caused a moderately rapid and extensive degree of inactivation of mitochondrial glycerophosphate acyltransferase, in keeping with results presented previously for 15- and 46-53; 0, glycerophosphate acyltransferase, 3.3-4.5; 0, rotenone-insensitive NADH-cytochrome c reductase, a marker enzyme for the outer surface of the outer membrane (21), was inactivated by these proteases at an initial rate exceeding that of glycerophosphate acyltransferase. In either experiment, both of these enzymes were not maximally inactivated until 20 min of incubation in hypotonic medium (0.025 M sucrose, 5 mM potassium phosphate buffer (pH 7.4)). In Fig. 3, the ionic strength of aliquots of mitochondria in low ionic isotonic medium was adjusted by the addition of 4 M KCl as previously described (13). Aliquots of the mitochondrial fraction were preincubated for 5 min at 30°C, and then proteolytic enzyme was added and incubation continued for 15 min unless otherwise indicated. The proteinase K, subtilisin, and chymotrypsin reactions were stopped by the addition of phenylmethylsulfonyl fluoride at 1 ml/ml. Trypsin reactions were stopped by adding soybean trypsin inhibitor to 0.12 mg/ml. The samples were immediately chilled and then stored at -70°C until they were used for enzyme activity analyses within 1 week.

**Preparation and Incubation of Trypsin-loaded Outer-membrane Vesicles**—Trypsin-loaded outer-membrane vesicles were prepared from the mitochondrial fraction by a modification of the method of Sottocasa et al. (20) as previously described (13) in which trypsin is added (0.5 mg/ml) after sonication or, to control aliquots, added after sonication or not at all. Following sonication, soybean trypsin inhibitor was added (1 mg/ml) to inhibit externally accessible trypsin. Aliquots of the sonicated mitochondria were incubated at 30°C for the indicated time and then the reaction was stopped by the addition of phenylmethylsulfonyl fluoride.

Fig. 1. Time course of effect of non-specific proteases on activity of mitochondrial outer-membrane enzymes and adenylate kinase. Aliquots of mitochondria, suspended in low ionic isotonic incubation medium at a protein concentration of 4 mg/ml, were treated with either proteinase K (a) or subtilisin (b) at 40 μg/ml as described under "Experimental Procedures" for the indicated time periods. Resulting specific activities are expressed as percentage of that of similarly treated control (no protease) incubations. The enzymes assayed and the range of specific activities (nanomoles/min/ mg mitochondrial protein) as previously described (a) long-chain acyl-CoA synthetase, 46-53; (b) glycophosphate acyltransferase, 3.3-4.5; (c) rotenone-insensitive NADH-cytochrome c reductase, 272-293; (d) monoamine oxidase, 11.0-12.5; (e) adenylate kinase, 392-418.
of incubation. At all time points the integrity of the outer membrane was not significantly affected, as shown by the stability of the activity of adenylate kinase in the protease-treated mitochondria. Under conditions in which the outer membrane is lysed, allowing protease access to the inner surface of the outer membrane and the intermembrane compartment, this intermembrane enzyme is readily inactivated (Refs. 21 and 22, and Table I). Also, the stability of monoamine oxidase activity in the experiments presented in Fig. 1 indicates that proteolysis did not lead to exposure of enzymes which are deep buried within the bilayer and are therefore not protease-accessible (23, 24).

Since maximal inactivation of acyl-CoA synthetase had already occurred by the earliest time point of incubation examined in Fig. 1, the effect on this enzyme of treatment of mitochondria with reduced concentrations of nonspecific protease was examined. At a protease concentration one-fifth that used in Fig. 1, an incubation time-dependent loss of acyl-CoA synthetase activity in either proteinase K or subtilisin treated mitochondria was obtained (Fig. 2). Stability of adenylate kinase activity in the treated samples again demonstrated the maintenance of intact outer membranes during the course of the digestions. Thus, the inactivating effect of these proteases on acyl-CoA synthetase is shown to be due to proteolysis during the course of the incubation, similar to the inactivation of mitochondrial glycerophosphate acyltransferase and rotenone-insensitive NADH-cytochrome c reductase (Fig. 1). However, much less active proteolytic conditions are necessary to produce comparable rates of inactivation for acyl-CoA synthetase.

Further experiments examined the dependence of proteolytic inactivation of mitochondrial long-chain acyl-CoA synthetase on the ionic strength of the incubation medium. The degree of inactivation observed following a 15-min incubation with nonspecific protease was completely unaffected (40 pg/ml, proteinase K at 8 pg/ml for the indicated time periods. Protease-treated and control (no protease) samples were assayed for acyl-CoA synthetase and adenylate kinase (C, D) and the data expressed as percentage of the control incubations. Specific activities (nanomoles/min/mg) in the control samples ranged from 46 to 54 and 384 to 397, respectively.

![Fig. 2. Time course of inactivation of mitochondrial long-chain acyl-CoA synthetase by nonspecific protease.](http://www.jbc.org)

![TABLE I](http://www.jbc.org)

**TABLE I**

**Effect of treatment of mitochondria with various proteases on mitochondrial enzymes and outer-membrane intactness**

| Proteolytic enzyme | ACS* | GAT* | NCR | MAO | AK | Outer-membrane lysis |
|--------------------|------|------|-----|-----|----|---------------------|
| A. Isotonic medium |      |      |     |     |    |                     |
| Trypsin            | 71   | 91   | 66  | 104 | 97 | 7                   |
| Chymotrypsin       | 42   | 92   | 93  | 108 | 83 | 5                   |
| Proteinase K       | 9    | 5    | 9   | 98  | 87 | 15                  |
| Subtilisin         | 8    | 27   | 7   | 102 | 89 | 10                  |
| B. Hypotonic medium|      |      |     |     |    |                     |
| Trypsin            | 29   | 58   | 45  | 99  | 8  |                     |
| Chymotrypsin       | 23   | 73   | 85  | 103 | 3  |                     |
| Proteinase K       | 8    | 13   | 17  | 105 | 4  |                     |
| Subtilisin         | 6    | 5    | 14  | 106 | 12 |                     |

* Long-chain acyl-CoA synthetase.
* Glycophosphate acyltransferase.
* Rotenone-insensitive NADH-cytochrome c reductase.
* Monoamine oxidase.
* Adenylate kinase.

![Fig. 3. Effect of different ionic media on the inactivation of mitochondrial long-chain acyl-CoA synthetase by nonspecific protease.](http://www.jbc.org)

medium was increased to physiological levels by the addition of KCl (Fig. 3). Addition of other salts such as MgCl₂ or (NH₄)₂SO₄ also failed to significantly alter the proteolytic inactivation rate of acyl-CoA synthetase. The percentages of control activities remaining following subtilisin digestion at 8 µg/ml in medium containing 0.15 units of additional ionic strength MgCl₂ or (NH₄)₂SO₄ under the conditions described in Fig. 3 increased only 9 and 1%, respectively, over those in unadjusted medium. These results for acyl-CoA synthetase contrast with results of previous experiments conducted under similar conditions showing a marked decrease (52–75%) in the inactivation of mitochondrial glycerophosphate acyltransferase by these proteases when the ionic strength of the medium was increased by 0.10 or 0.15 unit (12). Also, previous studies have shown that the inactivation of mitochondrial outer-membrane rotenone-insensitive NADH-cytochrome c reductase (21) and GDP-mannose: dolichyl-monophosphate mannosyltransferase (25) by protease could be substantially reduced or eliminated by increasing the ionic strength of the
proteolyis medium to this range.

The above results demonstrate the presence of one or more nonspecific protease-sensitive domains of acyl-CoA synthetase on the outer surface of the mitochondrial outer membrane. To probe the inner surface of the membrane, the effect of limited-specificity proteases (trypsin and chymotrypsin) on both intact and osmotically disrupted mitochondria was studied. Table I presents the effect on the activities of acyl-CoA synthetase as well as other mitochondrial enzymes assayed following proteolytic digestion of mitochondria during suspension in either isotonic or hypotonic medium. As expected, the nonspecific proteases extensively inactivate all three cytosically exposed enzymes in either medium. Similar to glycerophosphate acyltransferase, acyl-CoA synthetase activity was slightly (chymotrypsin) or markedly (trypsin) more susceptible to site-specific protease digestion following osmotic lysis of the outer membrane in the hypotonic medium, suggesting the possibility that additional crucial domains of acyl-CoA synthetase exist on the inner surface of the membrane. However, unlike glycerophosphate acyltransferase, a significant amount of inactivation of acyl-CoA synthetase by these proteases occurred in isotonie medium. This inactivation exceeded by 22% (trypsin) or 53% (chymotrypsin) the percentage of outer membrane lysis as estimated by cytochrome c oxidase latency. The results of this method of estimating the percentage of the mitochondrial population with disrupted outer membranes showed a close correlation with the percentage of inactivation of adenylate kinase by the various proteolytic enzymes (Table I). Thus, it appears that limited trypsin- and chymotrypsin-sensitive sites of acyl-CoA synthetase exist on the cytosolic surface of the membrane. Therefore, the increased inactivation of the enzyme by these proteases in hypotonic medium may be due to exposure of additional sensitive sites on the inner surface of the membrane, or may instead simply reflect increased exposure of the cytosolic domain.

The experimental approach taken to resolve this question was to measure the activity of acyl-CoA synthetase after direct exposure of only the inner surface of the outer membrane to protease activity. Right-side-out outer-membrane vesicles containing trypsin trapped internally by sonication were prepared as previously described (13) by a modification of the method of Sottocasa et al. (20). Previous studies of permeability as well as protease- or immunochemical-treated vesicles have shown that virtually all of the outer-membrane vesicles prepared by this method have the same "outside-out" surface orientation as the intact mitochondrion (26) and are sealed (26, 27). Also, proteins that are deeply imbedded within the bilayer do not become accessible to protease activity due to this treatment (26). This protocol was previously used for preparation of protease- or lactoperoxidase-loaded outer-membrane vesicles in other topography studies (24, 28). Trypsin-loaded and control outer-membrane vesicles were incubated in the presence of soybean trypsin inhibitor to inactivate any external protease which would have access to the cytosolic membrane surface. The impermeability of these vesicles to molecules greater than 13,000 daltons (27) precludes inactivation of internally trapped trypsin. The activities in the incubated control vesicles which were prepared either without trypsin or with trypsin added after sonication remained stable for the entire incubation period (Table II). In contrast, incubation of the trypsin-loaded vesicles caused a gradual loss of up to 36% of the initial control acyl-CoA synthetase activity after 1 h (Table II). Loss of monoamine oxidase activity following incubation of control or trypsin-loaded vesicles was less than 10% (data not shown), indicating that proteins buried within the bilayer were not exposed by the sonication procedure. Thus, this approach demonstrates the presence of protease-accessible crucial domains of this enzyme on the inner surface of the membrane as well.

In further experiments, using nondisrupted mitochondria, we investigated the topography of the CoA-binding portion of the active site of outer-membrane long-chain acyl-CoA synthetase using an irreversibly inhibitory immobilized substrate analog for this enzyme. Intact mitochondria were preincubated for 30 min in the presence of cross-linked 4% bead agarose containing up to 4 nmol of desulfo-CoA attached covalently with a Ca spacer. Control incubations contained an equal volume of agarose without desulfo-CoA. Following this preincubation, an assay reaction to measure remaining acyl-CoA synthetase activity in the mitochondrial samples incubated with the desulfo-CoA-agarose. The inhibitory effect was specific for the desulfo-CoA moiety since, at any dose, the activities in the agarose-alone incubations did not differ significantly from the no-agarose control (Fig. 4). These results indicate a cytosolic exposure of the CoA-binding domain of the enzyme on the outer membrane, since the accessibility of the inhibitor during the preincubation was limited to this surface. The pretreatment with the agarose under these conditions did not lead to disruption of the outer membrane as detected by release of adenylate kinase into the medium.

**TABLE II**

| Treatment                | Specific activity at 0 min | Incubation time | Specific activity | nmol/min/mg |
|--------------------------|---------------------------|-----------------|------------------|-------------|
| Control (no trypsin)     | 48.1                      | 45.6            | 47.3             | 53.2        |
|                          | (100)                     | (95)            | (98)             | (111)       |
| Trypsin added            |                           |                 |                  |             |
| Postsonication           | 52.6                      | 55.3            | 50.0             | 46.4        |
|                          | (109)                     | (115)           | (104)            | (96)        |
| Presonication            | 42.8                      | 41.0            | 37.6             | 30.7        |
|                          | (50)                      | (85)            | (78)             | (64)        |

**DISCUSSION**

In the present study we have investigated the transverse-plane topography of long-chain acyl-CoA synthetase within the rat liver mitochondrial outer membrane using, as one approach, proteases as probes for exposed crucial domains of the enzyme. In this study we define crucial domains to include the catalytic domain(s) of the enzyme as well as any regulatory site domains or structural portions of the protein involved in maintaining an active conformation of the enzyme. Crucial
brane vesicles containing trapped trypsin (Table II) provides the active site of the enzyme. Proteases, the use of a variety of proteolytic enzymes, preferentially inactivating kinase and cytochrome c oxidase latency as controls for outer-membrane integrity, digestion of mitochondria using the nonspecific proteases subtilisin and proteinase K (Table I) indicate that all possess a cytosolic exposure. The present results show mitochondrial outer membrane long-chain acyl-CoA synthetase to be similar to both the rat liver microsomal (36) and the peroxisomal (37) forms of the enzyme in possessing exposed crucial domains on the cytosolic side of their respective membranes. In all three subcellular fractions these essential domains are accessible for hydrolysis by nonspecific proteases such as proteinase K or subtilisin (Figs. 1 and 2) or Pronase (36, 37) in the absence of disrupted membranes.

Previous studies of outer-membrane topography have defined two categories of integral membrane proteins. One group consists of proteins such as monoamine oxidase (21, 23) and porin (26, 38) which are deeply buried within the membrane and thus completely protected from protease activity. The other group is those enzymes such as glycerophosphate acyltransferase (13), rotenone-insensitive NADH-cytochrome c reductase (21), or GDP-mannose:dolichyl-monophosphate mannosyltransferase (25) whose accessibility to proteases is dependent upon the ionic strength of the membrane's environment. Mitochondrial long-chain acyl-CoA synthetase is shown here (Fig. 3) to be an example of a third group of mitochondrial outer-membrane proteins, which possess exposed crucial domains whose protease accessibility is not significantly affected by a shift in the medium ionic strength. This may reflect a more prominent exposure on the membrane such that changes in the microenvironment of the proteins, perhaps due to increases in the membrane fluidity with increasing ionic strength (39), do not lead to masking of crucial domains as for those enzymes whose accessibility changes. The much greater susceptibility of acyl-CoA synthetase to protease activity than glycerophosphate acyltransferase or rotenone-insensitive NADH-cytochrome c reductase (Fig. 1) is also consistent with a greater degree of exposure of acyl-CoA synthetase on the membrane's cytosolic surface.

The use of immobilized substrates or substrate analogs as active site probes is another investigational approach to the study of membrane enzyme topography. Previous experiments in our laboratory demonstrating the ability of mitochondria with intact outer membranes to use palmitoyl-CoA-agarose as an acyl donor have provided evidence for a cytosolic exposure of the active sites of both glycerophosphate acyltransferase and acyl-CoA synthetase.
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and acyl-CoA-1-acyl-sn-glycerol-3-phosphate acyltransferase (40). In this study we have taken a similar approach using an immobilized substrate analog for long-chain acyl-CoA synthetase, desulfo-CoA-agarose. This reagent is specifically inhibitory to the enzyme following preincubation with mitochondria with intact outer membranes (Fig. 4). Although further investigation of the topography of the active site is necessary, this result is consistent with a cytosolic exposure for at least the CoA-binding domain of the active site. Thus, it appears that the active sites for long-chain acyl-CoA-generating and -utilizing enzymes in the outer membrane may have a similar cytosolic topographical orientation; an orientation which would allow them to draw directly on cytosolic substrate pools and to be regulated by cytosolic factors.

REFERENCES
1. Yaffe, M., and Schatz, G. (1984) Trends Biochem. Sci. 9, 179-181
2. Hirokawa, N. (1982) J. Cell Biol. 94, 129-142
3. Ernster, L., and Schatz, G. (1981) J. Cell Biol. 91, (suppl.) 227-255
4. Schatz, G., and Rutzow, R. A. (1983) Cell 32, 316-318
5. Roos, N., Benz, R., and Brdiczka, D. (1982) Biochim. Biophys. Acta 686, 204-214
6. Daae, L. N. W., and Bremer, J. (1970) Biochim. Biophys. Acta 210, 92-104
7. Monroy, G., Rolla, F. H., and Pullman, M. E. (1972) J. Biol. Chem. 247, 6884-6884
8. Haldar, D., Kelker, H. C., and Pullman, M. E. (1983) Trans. N. Y. Acad. Sci. 41, 173-182
9. Stern, W., and Pullman, M. E. (1978) J. Biol. Chem. 253, 8047-8055
10. Haldar, D., Tso, W.-W., and Pullman, M. E. (1979) J. Biol. Chem. 254, 4502-4509
11. Rutzow, B., Schlieme, M., Rabe, H., Reichmann, G., and Kunze, D. (1988) Biochim. Biophys. Acta 1002, 961-963
12. Davidson, J. B., and Stanacev, N. Z. (1971) Can. J. Biochem. 49, 1117-1124
13. Heeler, C. B., Carroll, M. A., and Haldar, D. (1986) J. Biol. Chem. 260, 7452-7456
14. Carroll, M. A., Morris, P. E., Grosjean, C. D., Anzalone, T., and Haldar, D. (1982) Arch. Biochem. Biophys. 214, 17-25
15. Suzue, G., and Marcel, Y. L. (1972) Biochemistry 11, 1704-1708
16. Wojtczak, L., Zaluska, H., Wroniszewska, A., and Wojtczak, A. B. (1972) Acta Biochim. Pol. 19, 227-234
17. Schnaitman, C., and Greenswaal, J. W. (1968) J. Cell Biol. 38, 158-175
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
19. Stoller, C. L., Kuylenstierna, B., Ernster, L., and Bergetrand, A. (1967) J. Cell Biol. 32, 415-438
20. Kuylenstierna, B., Nicholls, D. G., Hovmoller, S., and Ernster, L. (1970) Eur. J. Biochem. 12, 419-426
21. Mayer, R. J., and Hubscher, G. (1971) Biochem. J. 124, 491-500
22. McCauley, R. (1978) Arch. Biochem. Biophys. 189, 8-13
23. Weiss, S., and McCauley, R. (1979) in 4th International Catecholamine Symposium (Usdin, E., Konin, I., and Barchas, J. eds) pp. 198-200, Pergamon Press, New York
24. Gasnier, F., Louilot, P., and Gateau-Roesch, O. (1969) Biochim. Biophys. Acta 980, 339-347
25. Reizman, H., Hay, R., Gasser, S., Daum, G., Schneider, G., Witte, C., and Schatz, G. (1983) EMBO J. 2, 1105-1111
26. Wojtczak, L., and Sottocasa, G. L. (1972) J. Membr. Biol. 7, 313-324
27. Smith, D., and McCauley, R. (1980) in Monoamine Oxidase: Structure, Function and Altered Functions (Singer, T. J., ed) pp. 273-278, Academic Press, New York
28. Coleman, R. A., and Bell, R. M. (1960) Biochim. Biophys. Acta 595, 184-188
29. Wiemken, V., and Bachofen, R. (1982) Biochim. Biophys. Acta 681, 72-76
30. Nimmo, H. (1979) FEBS Lett. 101, 362-364
31. Gasnier, F., Louilot, P., and Gateau-Roesch, O. (1989) Biochim. Biophys. Acta 1019, 227-234
32. Coleman, R., and Bell, R. M. (1978) J. Cell Biol. 76, 245-253
33. Delong, J. W., and Hulsmann, W. C. (1970) Biochim. Biophys. Acta 197, 127-136
34. Tanaka, T., Hosaka, K., Hoshimaru, M., and Numa, S. (1979) Eur. J. Biochem. 96, 165-172
35. Philipp, D. P., and Parsons, F. (1979) J. Biol. Chem. 254, 10776-10784
36. Coleman, R., and Bell, R. M. (1978) J. Cell Biol. 76, 245-253
37. Manner, G. P., VanVeldhoven, P., VanBroekhoven, A., Vandebroek, G., and Debeer, L. J. (1982) Biochem. J. 204, 17-23
38. Freitag, H., James, M., and Neupert, W. (1982) Eur. J. Biochem. 126, 197-202
39. Ohyashiki, T., Taka, M., and Mohri, T. (1985) J. Biol. Chem. 260, 6857-6861
40. Haldar, D., and Sheehan, D. J. (1988) J. Cell Biol. 107, 348 (abstr.)
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