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Iodination of Liver Plasma Membranes Using Lactoperoxidase: Effects on Adenylate Cyclase Activity (39389)

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In recent years, studies of receptors for many polypeptide hormones have focused on adenylate cyclase activity and hormone binding sites in isolated cells and purified plasma membrane preparations. These studies have provided major insights regarding the physiology of hormone action. However, relatively little has been learned of the molecular properties of receptors or of the chemical mechanisms involved in hormone receptor interactions. The major obstacle to obtaining a more basic understanding of receptors is their membrane location. While some information has been obtained with solubilized hormone-sensitive adenylate cyclase preparations (1–3), receptors must ultimately be studied in their natural membranous environment.

The lactoperoxidase-catalyzed iodination of exposed tyrosines has been used to label protein components of plasma membranes of several types of cells (4–6). The surface specificity of this enzyme suggested that it could provide useful information regarding the spatial arrangement of receptor components within an intact membrane. This report demonstrates the effects of iodination of highly purified plasma membranes from rat liver on adenylate cyclase activity in the membranes.

Materials and Methods. Lactoperoxidase, B grade, estimated purity 60%, was obtained from Calbiochem. Carrier-free ^{125}I was from Amersham/Searle. Acrylamide, *N,N'*-methylenebisacrylamide, and *N,N,N,N*-tetramethylethylenediamine were obtained from Eastman.

Plasma membranes were prepared by a modification (7) of the procedure of Neville (8). Adenylate cyclase was assayed as described previously (7) except that [^{32}P]cAMP was separated from [^{32}P]ATP by the aluminum oxide method devised by Ramachandran (9). Protein was measured by the procedure of Lowry *et al.* (10).

For amino acid analysis, liver membranes, 2 mg of protein, were hydrolyzed for 24 h at 100° in 6 *N* HCl. Amino acids were then estimated using a Spinco 120C amino-acid analyzer.

Results. Iodination of liver plasma membranes with lactoperoxidase has not been reported previously. As shown in Fig. 1 this procedure results in incorporation of iodine into several membrane proteins ranging from mol wt 20,000 to 100,000. The resolution of peaks of radioactivity is not adequate to determine the exact number or to compare extent of iodination with the quantity of protein in a given band.

The effects of iodination on membrane adenylate cyclase activity are illustrated in Fig. 2. The maximal glucagon and fluoride stimulated activities are reduced by 63 and 46%, respectively. The extent of incorporation of iodine is also shown in Fig. 2. Although full saturation was not obtained in this experiment, the incorporation of iodine clearly approaches a maximum under these conditions. Maximal effects on adenylate cyclase activity are seen with less than 10% of the total incorporation of iodine which could be obtained indicating that the affected component(s) of adenylate cyclase is particularly susceptible to iodination. The inhibition of adenylate cyclase is partial and no further inhibition is seen despite increased incorporation of iodine at higher KI concentrations. The effect on glucagon-stimulated activities is seen with less incorporation of iodine than is the effect on fluoride-stimulated activity.

Table I shows the effects of omitting each of the components of the iodination mixture and demonstrates that inhibition of adenylate cyclase activity is enzymatic and depend-

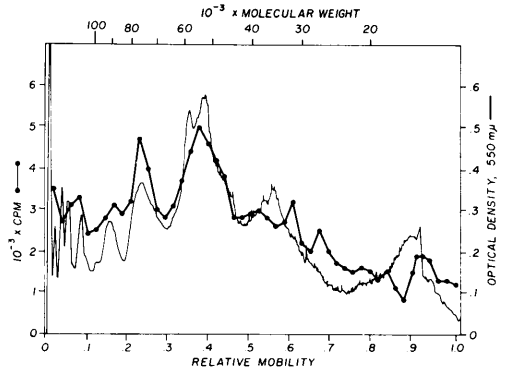

FIG. 1. Incorporation of ^{125}I into plasma membranes and resolution of labeled proteins by polyacrylamide gel electrophoresis. Plasma membranes, 1 mg of membrane protein, were suspended in 1 ml of 0.1 *M* Tris-HCl, pH 7.6, 10^{-6} *M* KI, 1 μCi $Na^{125}I$, and 10^{-6} *M* lactoperoxidase. The reaction was performed at ambient temperature and was initiated by addition of 5 μl H_2O_2 to give a final concentration of 0.3 μ/ml. Additional 5-μl aliquots of dilute H_2O_2 were added every 15 sec for a total of 2 min and the reaction mixture was placed on ice. Membranes were dissolved, dialyzed, and subjected to electrophoresis in sodium dodecyl sulfate polyacrylamide gels as described by Neville (11) using a pH-10 lower buffer. The gels were stained with Coomassie blue by the method of Fairbanks *et al.* (12). The stained gels were scanned at 530 nm in a spectrophotometer fitted with a linear transport device (Gilford Instruments). Parallel gels containing proteins of known molecular weight were used to calibrate the gels. The gels were fractionated with a Maizel gel extruder (Savant). The fractions were counted in a gamma scintillation counter.

ent on the presence of H_2O_2. The data show further that basal adenylate cyclase activity is inhibited by iodination and that the effect on glucagon-stimulated activity is on the maximal activity (10^{-6} *M* glucagon; (7)) rather than on the apparent affinity for glucagon estimated from the activity in the presence of 4×10^{-9} *M* glucagon.

Amino acid analysis revealed that the membrane used in this study contained 18 nmole tyrosine and 15 nmole histidine per mg of membrane protein. In addition to tyrosine, lactoperoxidase catalyzes the iodination of monoidotyrosine and histidine as free amino acids (13). However, it has not been established whether these amino acids are substrates in membranes. Consequently the number of iodine acceptors could be as low as 18 nmole or as high as 51 nmole per mg of protein. Since the maximum iodination achieved in the experiment described in Fig. 2 is 3 natoms per mg protein, this would correspond to 6 to 17% of the total potential iodine acceptors. The number required to produce maximal effects on adenylate cyclase activity is 10-fold less.

Discussion. The lactoperoxidase catalyzed iodination of membrane proteins has generally been used as a labeling procedure to study the disposition of proteins within a membrane. In this study lactoperoxidase is used as a probe of a specific membrane function, the glucagon receptor-adenylate cyclase system. The inhibition of adenylate cyclase produced by iodination indicates that a component of the receptor-adenylate cyclase system containing a tyrosine, or possibly a histidine, is susceptible to attack by lactoperoxidase.

A current hypothesis regarding the molecular organization of the glucagon receptor system is that it is composed of a catalytic subunit, adenylate cyclase, on or near the inner surface of the plasma membrane and a separate regulatory subunit on or near

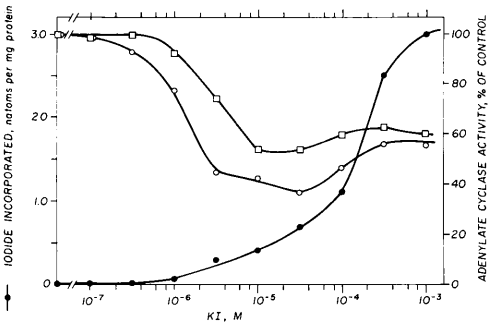

FIG. 2. Effects of iodination on membrane adenylate cyclase activity. Membranes were iodinated as described in Fig. 1 in the presence of varying concentrations of KI and in the presence and absence of a tracer quantity of $Na^{125}I$. Incorporation of iodine into the membranes was estimated by layering 0.1-ml aliquots of the reaction mixtures containing ^{125}I over 0.3 ml of 1% bovine serum albumin in a microfuge tube. The membranes were sedimented for 5 min in the microfuge (Beckman). The supernatant was aspirated and the tube was rinsed once with water. The tip containing the membrane pellet was cut off and counted in a gamma counter. Adenylate cyclase activity in the membranes treated without ^{125}I was measured as described under Methods. Glucagon (—○—) was 10^{-6} *M*. Fluoride (—□—) was 15 mm. Values are expressed as percentage of no I^- control and are the means of triplicate determinations.

TABLE I. ADENYLATE CYCLASE ACTIVITY.[a]

Lacto-peroxi-dase	H_2O_2	KI	Basal	Glucagon (4×10^{-9} M)	Glucagon (10^{-6} M)	NaF 15 mM
			(nmoles per 10 min per mg protein)			
−	−	0	0.89 ± 0.02	2.69 ± 0.26	6.77 ± 0.12	2.02 ± 0.07
+	−	0	0.93 ± 0.01	2.57 ± 0.24	7.14 ± 0.14	2.27 ± 0.01
+	−	$10^{-6}M$	0.84 ± 0.12	2.90 ± 0.54	6.96 ± 0.09	2.10 ± 0.03
+	−	$10^{-5}M$	0.88 ± 0.01	2.35 ± 0.22	7.22 ± 0.08	2.11 ± 0.08
+	−	$10^{-4}M$	0.86 ± 0.09	2.17 ± 0.32	6.71 ± 0.03	2.13 ± 0.07
+	+	0	0.90 ± 0.01	1.88 ± 0.04	6.52 ± 0.10	2.05 ± 0.06
+	+	$10^{-6}M$	0.55 ± 0.04	1.21 ± 0.14	4.06 ± 0.15	1.42 ± 0.01
+	+	$10^{-5}M$	0.21 ± 0.05	0.36 ± 0.04	0.80 ± 0.05	0.48 ± 0.01
+	+	$10^{-4}M$	0.32 ± 0.01	0.32 ± 0.01	1.78 ± 0.04	0.75 ± 0.01
−	+	$10^{-5}M$	0.82 ± 0.02	2.61 ± 0.54	6.51 ± 0.13	1.82 ± 0.02

[a] Liver plasma membranes were incubated in the presence of absence of 10^{-6} M lactoperoxidase and varying concentrations of KI for 2 min at room temperature as described in Fig. 1. Water was added in place of H_2O_2 to the minus H_2O_2 controls. At the end of the iodination, the reaction mixtures were placed on ice and adenylate cyclase activity was measured in the presence of no addition, 4×10^{-9} M glucagon, 10^{-6} glucagon, or 15 mM NaF was measured as described under Methods. Values are the mean ± SE of triplicate determinations.

the outer surface to which glucagon binds. The inhibition of basal and fluroride-stimulated activities by iodination indicates that the catalytic subunit is affected. The incompleteness of the inhibition of adenylate cyclase by iodination may be due to the heterogeneity of membrane preparation. The liver membrane preparation is composed of a mixture of open sheets and closed vesicles (7). Adenylate cyclase on the inner surface of the vesicles may be protected from attack by lactoperoxidase.

The amino acid analysis indicates that no more than about 20% of the total iodinatable substrates (tyrosine, monoiodotyrosine, histidine) in the membrane are iodinated under the conditions employed here. The remainder are probably buried within the membrane and less accessible for iodination. The observation that maximal effects on adenylate cyclase occur with only about 10% of the total possible incorporation of iodine suggests that the affected component is very superficially situated in the membrane and is readily accessible for attack by lactoperoxidase. Neer has shown that solubilized renal adenylate cyclase has very little associated lipid or detergent and therefore is probably located on the surface of the membrane (3). The data presented here support this hypothesis. If this interpretation is correct, then the regulatory component(s) of the system must traverse the hydrophobic core of the membrane. The importance of membrane phospholipids in hormone sensitivity of adenylate cyclase has been amply demonstrated (1, 14).

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