Biochemical Pharmacology, Vol. 32, No. 7, pp. 1251–1257, 1983.
Printed in Great Britain.

0006-2952/83/071251-07 $03.00/0
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PHOTOREACTIVITY OF LYSERGIC ACID DIETHYLAMIDE AND ITS POSSIBLE UTILITY AS A PHOTOAFFINITY LABELING REAGENT*

EDWARD B. WALKER, STEVEN J. MCNALL and TAG E. MANSOUR†
Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305, U.S.A.

(*Received* 1 *June* 1982; *accepted* 28 *September* 1982)

Abstract—Aqueous solutions of lysergic acid diethylamide (LSD) are extremely sensitive to light in the near-ultraviolet region of the spectrum. This rather efficient photoreaction yields a variety of products which have very low affinity for LSD-binding sites on plasma membranes from *Fasciola hepatica*. Since this photoreaction may be elicited by normal white fluorescent lighting in the laboratory, it represents a potential source of error in determining the binding affinity of LSD. Utilizing this photoreactivity advantageously, [^{3}H]LSD was used to photolabel membrane proteins. Covalent binding of [^{3}H]LSD was shown to be a function of the duration of illumination and was inhibited by 5-hydroxytryptamine and nonradioactive LSD. Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis of [^{3}H]LSD labeled membranes from *F. hepatica* showed two proteins which were selectively labeled by the photoreactive [^{3}H]LSD. This method of direct photolabeling with non-derivatized [^{3}H]LSD may allow identification of LSD-binding proteins in a variety of systems.

Affinity labeling of proteins with photoreactive ligands has attracted much attention in recent years as a powerful tool in probing biological targets [1, 2]. In particular, photoaffinity labeling of membrane proteins [3, 4] has provided methods for identifying, purifying, and characterizing receptor proteins. Photolabeling of hormone receptors with specifically designed photoreactive ligands has been utilized to identify steroid [5–7], glucagon [8], and insulin [9] receptors. The acetylcholine receptor has been the subject of a number of photolabeling studies [10–13]. Guillory [14] has reviewed the use of photoaffinity reagents in studying active sites of energy transduction.

Ideally, perturbation of the binding affinity of a photoreactive ligand by its reactive group should be minimized to preserve specificity in photolabeling. In the case where the non-derivatized ligand itself is photoreactive, such perturbation is avoided. A few examples of non-derivatized photoreactive ligands have been reported, most of which are nucleic acids. Ferguson and co-workers have used both cAMP and cGMP to label protein extracts from testis and adrenal cortex [15, 16] and phosphofructokinase [17, 18].

Lysergic acid diethylamide (LSD) and other ergot alkaloids have been reported to undergo photodecomposition in $CHCl_3$ to yield a variety of products [19]. Lysergic acid derivatives in acidic solutions or in alcohol, when irradiated with intense u.v. light, form enantiomers of a so-called lumi derivative, in which the 9,10-double bond has been hydrated [20, 21]. These reports indicate the lability of these agents under non-physiological conditions.

Previous work in our laboratory showed that both 5-hydroxytryptamine (5-HT) and LSD stimulate the rhythmical movement of the liver fluke *Fasciola hepatica* [22] and activate adenylate cyclase [23, 24]. Kinetic data showed that LSD is a partial agonist to serotonin, having an efficacy of about 25% that of serotonin. The serotonin receptors have a high affinity to LSD with a half-maximal activation of 46 nM. In addition to the kinetic data, recent binding studies using [^{3}H]LSD [25] revealed that LSD has greater affinity than serotonin to membrane particles.

The high affinity of LSD for 5-HT binding proteins indicates that it may be useful in the detection and isolation of these proteins. The photoreactivity of LSD suggests some interesting biological applications. In the present work we have investigated the possible use of [^{3}H]LSD as a photoaffinity agent which could covalently bind to proteins to which it is bound. It could then serve as a marker for the identification of these proteins. The present work characterizes the photoreactions of LSD in aqueous solution at pH 7.5 and shows some preliminary data using LSD as a photoaffinity agent in the liver fluke.

MATERIALS AND METHODS

Crystalline LSD was obtained from the National Institute of Mental Health as a tartrate salt. Purity of both [^{3}H]LSD and LSD was established by high-pressure liquid chromatography and thin-layer chromatography in a variety of solvents, and by absorption and fluorescence spectroscopy. [^{3}H]LSD was purchased from the New England Nuclear Corp., Boston, MA (40–45 Ci/mmole). Sucrose and $MgCl_2 \cdot 6H_2O$ were from Fisher, Pittsburgh, PA, chromatographic grade hexane was from Baker, Phillipsburg, NJ, while all other chemicals were obtained from the Sigma Chemical Co., St. Louis, MO, unless otherwise specified.

* This research was supported by Grants AI 16501 and MH 23464 from the National Institutes of Health.
† To whom reprint requests should be addressed.

HPLC analysis was performed with a Waters HPLC system, including a variable-wavelength absorption monitor. Separation of LSD from its photoreaction products was achieved using a Varian CN-10 Micropak No. 491 column at a flow rate of 3.0 ml/min of (10/90) 2% NH_4OH in ethanol/hexane. HPLC solvents were of the highest chromatographic grade and were both filtered and degassed before use. Thin-layer chromatography on pre-coated Kodak silica-gel plates was routinely performed. Silica-gel plates with fluorescent indicator were used in identifying photoproducts; however, gel without indicator was used when the recovery of LSD for spectral analysis or for [^{3}H]LSD scintillation analysis was desired. TLC solvents most commonly used were $CHCl_3$–methanol (4:1), $CHCl_3$–methanol–NH_3 (90:5:1), $CHCl_3$–ethanol–acetic acid (18:10:1), and $CHCl_3$–benzene–ethanol (4:2:1).

The photoreaction of LSD was accomplished by irradiating the solution with a u.v. Mineralight UVSL-25 (Ultra-Violet Products, Inc.) from a distance of 3.0 cm. The intensity of longwave irradiation was 330 microwatts per square centimeter at a distance of 15 cm. Spectral output of the u.v. Mineralight was maximum at 365 nm with a broad 80 nm bandpath (320–400 nm). When monitoring the photoreaction in the absence of protein, irradiation was carried out in a 1 cm cuvette at 20°, with light entering from the side of the cell.

Photolabeling of membranes with [^{3}H]LSD was also at a distance of 3 cm; however, the solution was spread out in a thin layer (~ 1 mm depth) on a petri dish and irradiated from above, while the dish was slowly rotated to ensure even distribution of the light. All photolabeling of membranes was carried out at 4°.

Absorption spectra were determined on a Cary 118 spectrophotometer. Corrected fluorescence spectra were measured on a Perkin–Elmer scanning spectrofluorometer.

Gel filtration chromatography was performed at 4°. The G-50 superfine column (1.5 × 20 cm) was equilibrated with 10 mM glycylglycine buffer, pH 7.5, for preparative separation of [^{3}H]LSD components.

Initial experiments in this investigation were carried out on membrane particles of the liver fluke *F. hepatica*. Cell-free particles were prepared as described previously [24], with only slight modifications. Fresh intact liver flukes were frozen in Wollenberger clamps chilled in dry ice and stored at −80° until needed. A chilled mortar and pestle (−80° with dry ice) was used to pulverize frozen flukes, before suspending them in 6 vol. (w/v) of homogenization buffer (0.33 M sucrose, 5 mM dithiothreitol, and 1 mM EDTA). The suspension (in 200 ml) was subjected to four 30-sec pulses with a Tekmar tissumizer at 4°. After centrifuging for 20 min at 2000 *g*, the pellet was resuspended to the original volume in 0.25 M sucrose, 5 mM dithiothreitol, and 1 mM EDTA. After another centrifugation at 2000 *g* for 20 min, the particles were resuspended in the second (0.25 M sucrose) solution and frozen at −80° until needed.

To enhance the specific binding of the crude homogenates, an enriched membrane preparation was used for binding studies. Fresh liver flukes were homogenized in 3 vol. (w/v) of homogenization buffer in a motor-driven Teflon homogenizer. The homogenate was spun at 10,000 *g* for 15 min, and the pellet was resuspended in resuspension buffer [0.25 M sucrose, 25 mM glycylglycine (pH 7.5), 5 mM dithiothreitol, 5 mM $MgCl_2$, 2 mM EDTA]. This suspension was brought up to 53% sucrose by the addition of a solution of 69% sucrose + 5 mM dithiothreitol. A 42.3% sucrose + 5 mM dithiothreitol solution was layered on top, and the mixture was spun at 25,000 rpm for 2 hr in a Beckman SW 27 rotor. Material at the interface (enriched membrane fraction) was collected, diluted 3-fold with homogenization buffer and spun at 10,000 *g* for 15 min, and the pellet was resuspended in homogenization buffer. This suspension was frozen and stored in liquid nitrogen until needed.

Protein concentrations were determined by the method of Bradford [26], using the Bio-Rad protein assay reagent with bovine serum albumin as a standard.

Photolabeling of membrane-bound proteins with [^{3}H]LSD was accomplished by thawing either particles or membranes and, after centrifuging at 10,000 *g* for 20 min, resuspending the pellet in binding buffer to a final concentration of 50 mM glycylglycine, 0.25 M sucrose, 5 mM dithiothreitol, 1 mM EDTA, 2 mM $MgCl_2$, and 0.2% ascorbate at pH 7.5. After 30 min of incubation with [^{3}H]LSD at 4°, the suspension was poured into a petri dish (depth ~1 mm) and irradiated with longwave u.v. light for 30 min as described above. After irradiation, the particle suspension was washed three times with 8–10 vol. of binding buffer before either solubilizing the membranes with detergent or assaying for covalent binding.

Freshly thawed enriched fluke membrane fractions were used to measure [^{3}H]LSD binding. The final incubation mixture consisted of membrane protein (1–2 mg/ml), 0.25 M sucrose, 50 mM glycylglycine (pH 7.5), 5 mM dithiothreitol, 1 mM EDTA, 2 mM $MgCl_2$, and [^{3}H]LSD and other ligands as indicated in a final volume of 0.5 ml. Samples were incubated at 37° for the indicated times. After incubation, three identical aliquots were removed, added to 4 ml of cold wash buffer (10 mM glycylglycine, pH 7.5), and passed through Whatman GF-C glass fiber filters under vacuum. The filters were washed with wash buffer and counted for ^{3}H-activity. Specific [^{3}H]LSD binding was determined by subtracting from the total amount of [^{3}H]LSD binding the amount of [^{3}H]LSD binding of an identical sample containing an excess of unlabeled LSD (10^{-4} M). Covalent binding was determined by first washing the irradiated membranes three times with 8 vol. of binding buffer, centrifuging each cycle at 9000 *g* for 10 min. The final pellet was resuspended in 0.5 ml of binding buffer, and binding was determined as above.

For analysis of labeled proteins following detergent disruption, labeled and washed membranes were solubilized with 2% sodium dodecylsulfate (SDS), 62.5 mM Tris buffer (pH 6.8) and 5% β-mercaptoethanol in a boiling water bath for 5 min. The resulting suspension was centrifuged for 40 min at 200,000 *g*, and the supernatant fluid was dialyzed

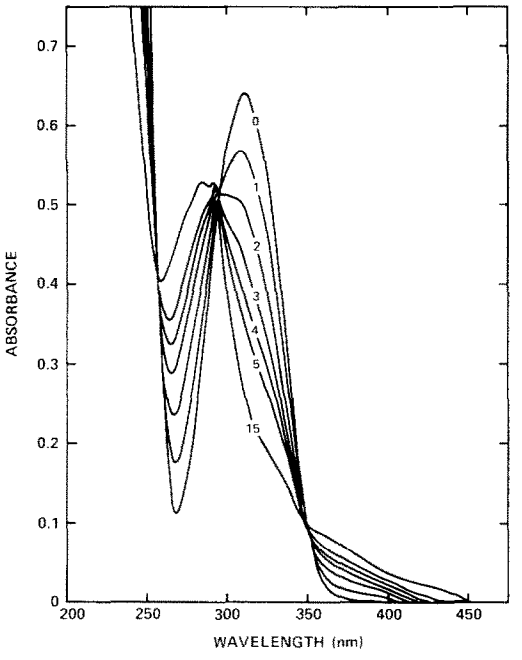

Fig. 1. Absorption spectra of LSD after various periods of illumination of longwave u.v. light (320–400 nm). An aqueous solution of LSD (6.4×10^{-5} M, $\varepsilon = 1 \times 10^4\ M^{-1}$) was irradiated with longwave u.v. light from a distance of 3 cm in an absorption cuvette. At the times indicated, irradiation was interrupted and the absorption spectrum was scanned. Numerical values on the curves indicate minutes of irradiation.

for 36 hr against four changes of 1000-fold excesses of SDS dialysis buffer (0.1% SDS, 0.05 M Tris buffer, 0.5% β-mercaptoethanol, pH 6.8). Prior to electrophoresis, solubilized proteins were dialyzed against sample buffer (2% SDS, 0.0625 M Tris, 10% glycerol, 5% β-mercaptoethanol, and 0.001% bromophenol blue, pH 6.8) for 6 hr before boiling for 2 min and applying to a 15% acrylamide gel according to the procedure of Laemmli [27]. Preparative slab gels (1 mm thick) were sliced latitudinally across an 8 cm center section of the gel, dried, dissolved in 0.7 ml of 30% H_2O_2 at 50° overnight in capped vials, and counted in Insta-Gel. A representative lane with molecular weight standards was cut longitudinally and stained for protein with Coomassie blue for comparison.

Adenylate cyclase activity was assayed by the method of Salomon *et al.* [28] as described previously for liver fluke particles [23]. Basal rates of enzymatic activity were assayed as well as serotonin (5-HT/GTP) and fluoride-stimulated activity. Concentrations of the various components were 0.1 mM ATP, 0.1 mM 5-HT, 0.1 mM GTP, and, where indicated, 10 mM NaF.

RESULTS

Photoreactivity of LSD. Aqueous solutions of LSD at room temperature are extremely sensitive to light. Exposure to indirect sunlight and to fluorescent lighting for a brief period of time in our laboratory significantly altered the characteristics of the ligand. However, when precautions were taken to eliminate sunlight and the cool-white fluorescent lights were exchanged for red fluorescent ones (GE F96T12-R), no reaction was observed in aqueous solutions of LSD at room temperature over a period of several hours. When LSD samples were wrapped in foil and stored at 4°, no changes were observed over a period of 10 days. Longwave ultraviolet light also initiated the same reaction. As shown in Fig. 1, loss in absorption at 310 nm was accompanied by an increase at both 375 nm and 265 nm. Isosbestic points were seen at 288 and 334 nm.

Fluorescence offers an excellent method to follow the kinetics of the photoreaction of LSD. Figure 2 shows that, upon u.v. irradiation, there was a loss of LSD fluorescence ($\lambda_{ex} = 288$ nm) and the appearance of a new peak at 355 nm, with an isoemissive wavelength of 360 nm. The excitation wavelength of 288 nm was chosen to avoid interference due to changes of the absorption spectrum. The non-amidated lysergic acid underwent an identical reaction, yielding the same spectral changes and associated kinetics, indicating that the photoreaction occurred in the conjugated pi electron system. The absence of oxygen slightly accelerated the rate of the LSD photoreaction ($k_{N_2}/k_{O_2} = 1.3$), indicating that oxygen was not directly involved in the reaction. When D_2O was used as the solvent, the rate decreased perceptibly ($k_H/k_D = 1.7$). The photoreaction in ethanol or methanol was similar to that in water with respect to absorption, fluorescence and HPLC analysis of the products. More than one product was formed by irradiating aqueous solutions of LSD at pH 7.5. HPLC analysis revealed at least four products, varying in polarity and absorption maxima from the original LSD. All of the detected products appeared at the same relative rates, indicating that the kinetics of product formation were identical and corresponded to the decrease of LSD. No changes in product or reactant concentrations were observed when irradiated solutions were stored for up to 48 hr in the dark at 4°.

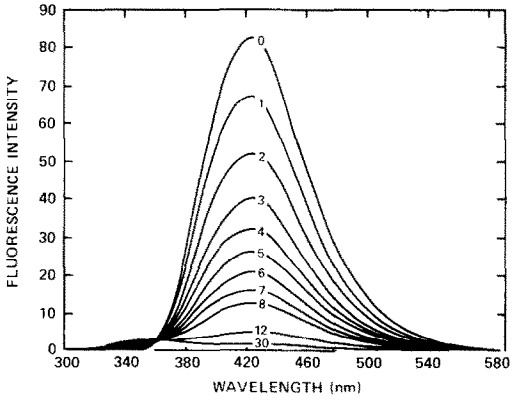

Fig. 2. Fluorescence spectra of LSD after various periods of illumination of longwave u.v. irradiation. After irradiation as described in Fig. 1, emission spectra were scanned. Numerical values indicate the length of irradiation in minutes at an excitation wavelength of 288 nm with a 10 nm bandpass and an emission bandpass of 3 nm. Initial LSD concentration was 6.4×10^{-5} M.

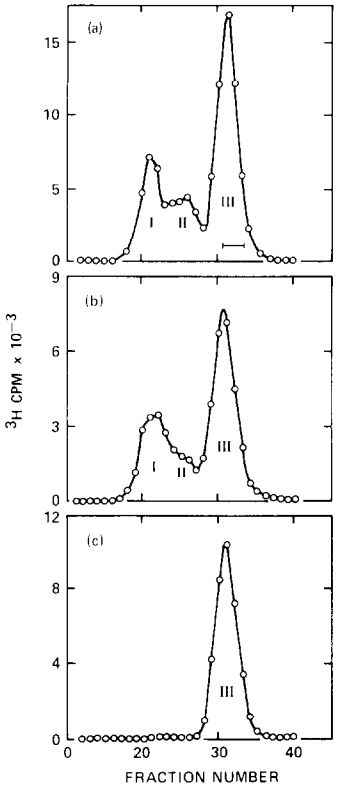

Fig. 3. Elution profiles from G-50 Superfine gel filtration chromatography (1.5 × 20 cm) of [^{3}H]LSD, utilizing 10 mM glycylglycine buffer (pH 8.2) as elution buffer. Fractions (1.0 ml) were collected, and aliquots of each fraction were counted. The column was wrapped in foil and eluted at 4° in the dark. Elution peaks are labeled as I, II and III in order of their elution. An amount of 0.75 ml of 10^{-8} M LSD (aged 1 month after receiving, stored at 4° in original container with only brief exposures to light) was applied, and 200 μl aliquots of each fraction were counted (a). Fractions 31–34 (III) were pooled as shown by bar and subjected to further analysis. A 0.75-ml amount of III was allowed to stand at room temperature under normal lighting in laboratory for 4 hr, and was then reapplied to the same G-50 Superfine column. The resulting elution profile is seen in (b). Another portion of III was incubated at 37° under red light for 4 hr before reapplying to the column. The resulting elution profile is shown as (c).

Characterization of LSD photoreaction products. Photoproducts that are distinguishable from LSD by their different chromatographic properties were formed as a result of irradiating aqueous solutions of [^{3}H]LSD with either white or u.v. light. When photodecomposed [^{3}H]LSD was chromatographed on G-50 superfine gel (10 mM glycylglycine elution buffer, pH 7.5) and aliquots of the resulting fractions were counted, an elution profile such as Fig. 3a was obtained. The elution profile 3a shows that at least three fractions were present in the commercial preparation of [^{3}H]LSD after only brief exposure to white light. These are labeled I, II, and III in order of their elution volumes. When fractions 31–34 (III) were pooled and stored in the dark at 4° for a period of 10 days to 2 weeks, no decomposition was seen. Fraction III was tested by thin-layer chromatography, and it was found to comigrate with LSD in the solvent system used, as described under Materials and Methods. Brief irradiation with u.v. light or exposure of fraction III to 4 hr of white light at room temperature resulted in extensive decomposition, as is shown in Fig. 3b. However, when a portion of fraction III was incubated at room temperature or 37° under red light for a period of 4 hr, no decomposition was observed (Fig. 3c). Frozen aqueous solutions of [^{3}H]LSD at −80° were still subject to photodecomposition but, instead of fraction I being the major product, the majority of the photoreacted ligand appeared in fraction II, with only small amounts of fraction I present. Reapplication of fraction I to the column after 24 hr in the dark at 4° yielded only fraction I, with 100% of the counts eluting in the initial band.

None of these products were retained by a dialysis membrane that allowed molecules of <10,000 daltons through, suggesting that fractions I and II contained molecular weights between 3,000 and 10,000. Although polymerization reactions are common with indole systems, it was unexpected that such diffusion-controlled reactions would occur at concentrations of 1×10^{-8} M during brief exposure to light.

The most retarded fraction (III, Fig. 3), which was the unaltered LSD, exhibited a much higher affinity for fluke membranes than the early fractions (I and II, Fig. 3). This is demonstrated in Fig. 4, where binding of the two major fractions from the G-50 elution profile is shown. It may be clearly seen that products of the LSD photoreaction in fraction I possessed a much lower affinity for LSD-binding sites on the fluke membranes. In support of these findings, it was found that the removal of the nonspecific ^{3}H-labeled decomposition products from [^{3}H]LSD by gel filtration resulted in a decrease in the apparent K_d of binding to the membrane particles from 80 to 50 nM. None of the photoreaction products were retained on the glass fiber filters in the absence of protein. It should be noted that this decrease in the K_d was after only a brief exposure

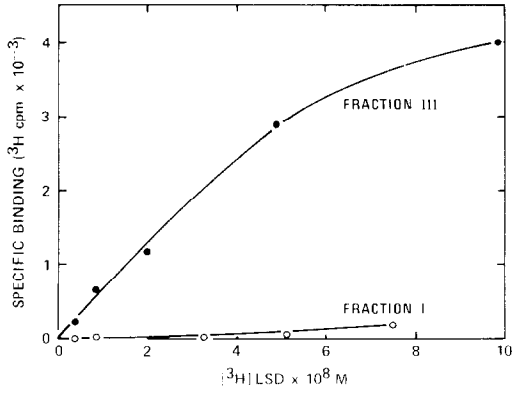

Fig. 4. Specific binding of [^{3}H]LSD and its photodecomposition products. The binding of the two major components from the G-50 Superfine solution (I and III from Fig. 3) to membranes of *F. hepatica* is shown. Binding was assayed by filtration, as described in Materials and Methods, after incubation with membranes for 5 min at 37°. All procedures were conducted in the dark or under red light.

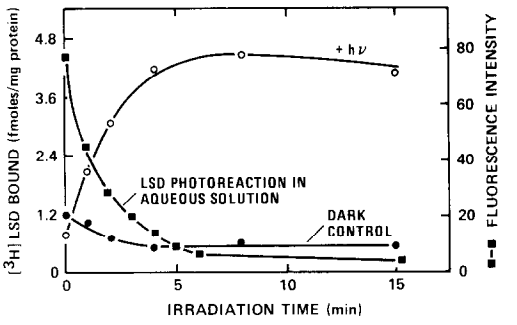

Fig. 5. Time–course of photolabeling of fluke membranes with [^{3}H]LSD. After 30 min of incubation of 10^{-8} M [^{3}H]LSD with fluke membranes (1.9 mg/ml) at 4° in the dark, samples were irradiated with longwave u.v. light. At the indicated times, 150 μl aliquots were taken and washed three times with buffer before filtering, as described in Materials and Methods. Specific covalent binding for each point was the difference between [^{3}H]LSD binding and [^{3}H]LSD binding in the presence of a 100-fold excess of LSD. Units on the left ordinate are the number of femtomoles incorporated per mg protein. The progress of the photoreaction of LSD in binding buffer under identical conditions is also plotted for comparison purposes. Fluorescence intensity was used to monitor the reaction of LSD.

to light. Longer exposure to light without the precautions taken would be expected to cause a more drastic change.

Photolabeling of LSD binding proteins with [^{3}H]LSD. In an attempt to use the efficient photoreactivity of LSD to label the serotonin receptor in its native environment, [^{3}H]LSD was first allowed to bind to membrane suspensions at 4° and subsequently irradiated directly with longwave u.v. light. As would be expected, non-covalent specific binding decreased rapidly upon exposure to light, which corresponded well with the photodestruction of the ligand. However, a small amount of [^{3}H]LSD became covalently bound to the membranes and was not removed with three or more successive washes of binding buffer or overnight dialysis. Figure 5 shows the time–course of photolabeling membranes with [^{3}H]LSD and u.v. light. Specificity of the covalent binding was ascertained by photoirradiation of [^{3}H]LSD after it achieved equilibrium with a 100-fold excess of unlabeled LSD. The specific covalent binding to the membranes increased with time of irradiation and paralleled the loss of LSD in aqueous solution under similar conditions. Figure 5 shows that the amount of total binding was very low, in the femtomoles/mg protein range. Scatchard binding analysis showed that only 5×10^{12} sites/mg protein existed in cytoplasmic membrane preparations. Of these, only 0.05 to 0.1% were labeled at an initial concentration of [^{3}H]LSD of 5×10^{-8} M. The low concentrations of labeled receptors may explain the fact that no significant change occurred in the responsiveness of adenylate cyclase to serotonin following irradiation (see below).

Tritium-labeled LSD ([^{3}H]LSD) was also used to investigate the photoreaction. We found that the affinity of the membrane receptors for [^{3}H]LSD decreased gradually, when the aqueous stock solutions of [^{3}H]LSD were exposed to white fluorescent light at room temperature. Similarly, when aqueous solutions of [^{3}H]LSD were irradiated with longwave u.v. light, subsequent binding studies showed that the specific binding of [^{3}H]LSD to membrane receptors decreased rapidly. This was naturally assumed to have been due to photoreaction of the LSD.

Both LSD and 5-HT have been shown to displace [^{3}H]LSD binding in the cytoplasmic membranes of *F. hepatica* [25]. This was also the case in photolabeling of the membranes with u.v. light and [^{3}H]LSD. LSD and 5-HT decreased both total and specific covalent binding of the [^{3}H]LSD upon longwave u.v. irradiation. At 10^{-8} M concentrations of [^{3}H]LSD, no specific covalent binding of the ligand was observed in the presence of either 10^{-4} M LSD or 10^{-3} M 5-HT. These competition experiments were done under normal reversible binding conditions. Ultraviolet irradiation was carried out only after equilibrium was reached. The ability of unlabeled LSD at different concentrations (10^{-7} to 10^{-4} M) to inhibit both noncovalent and covalent binding was examined. The results showed that inhibition of covalent binding paralleled the decrease in noncovalent binding, indicating that the photocovalent labeling occurred at the high affinity site(s).

To ensure that the properties of the adenylate cyclase system were not changed as a result of u.v. irradiation, fluke membrane fractions were exposed to u.v. light in the absence of any exogenous ligands. Both 5-HT-stimulated adenylate cyclase activity and specific [^{3}H]LSD binding showed no decrease in membranes irradiated up to 30 min with u.v. light compared to untreated control membranes.

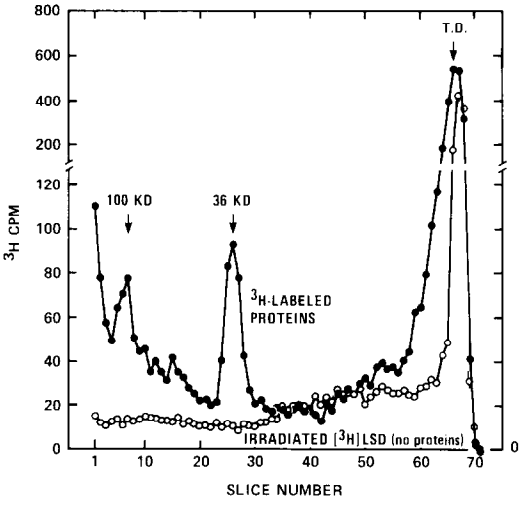

Fig. 6. Denaturing SDS-gel electrophoresis of [^{3}H]LSD photolabeled proteins from membranes of *F. hepatica*. As described in Materials and Methods, the labeled membranes were solubilized in 2% SDS and electrophoresed on a slab gel. Preparative slices were cut across the gel and counted (●—●), representing 450–500 μg of total applied protein. One lane was used for calibration proteins and another for irradiated [^{3}H]LSD in the absence of protein (○—○). KD denotes kilodaltons; T.D. represents the migration of the tracking dye. The apparent molecular weights of the two prominent bands were calculated by comparison with molecular weight standards in the same gel.

Although the photo-incorporated ligand could not be removed by successive washings or dialysis against buffer, detergent treatment solubilized the covalently bound [^{3}H]LSD adduct. Greater than 80% of the label in the membranes was released into a 200,000 g supernatant fraction upon treatment with 20 mM cholate detergent, and more than 95% of the label was solubilized in the presence of 2% SDS. Analysis of labeled proteins by SDS-denaturing gel electrophoresis is seen in Fig. 6, in which at least two prominent bands are seen (100,000 and 36,000 daltons). The incorporation of the label into two major bands indicates its specificity.

DISCUSSION

The photoreactivity of LSD can be both helpful and detrimental to the investigator using it as a probe of receptor phenomenology. As is seen from the decreased binding of the photodegradation products of [^{3}H]LSD, significant errors may be introduced in the determination of affinities of receptor binding if the ligand has been exposed to light before or during binding experiments (Figs. 1–4). However, the photoreactivity may be beneficially utilized to photolabel and identify LSD binding proteins. All binding studies in which [^{3}H]LSD is utilized as a ligand should be carried out under red light or wavelengths of light not absorbed by LSD.

At this time little is known concerning the exact nature or mechanism of the LSD photoreaction. The photoreaction in the polar protic solvents, such as water (Figs. 1 and 2) or in methanol or ethanol [29], yielded at least one product containing an indole ring system similar to tryptophan. Saturation of the 9,10-double bond would result in such a product. In their studies on the derivatives of LSD, Stoll and Hofmann [29] reported that, when acidic solutions of LSD were exposed to intense u.v. light, addition of 1 molecule of water at the 9,10-double bond occurred, forming two isomers of lumi-LSD. The catalytic hydrogenation of the 9,10-double bond has also been described [20, 29], in which the resulting dihydrolysergic acid exhibited an absorption spectrum [29] matching the photoreacted products in Fig. 1. It appears that the major absorbing and fluorescing product of the photoreaction in neutral aqueous solution (pH 7.5) is spectrally similar to the lumi-LSD formed in acidic solutions. Hydration of the 9,10-double bond as a result of the u.v.-irradiation offers an explanation for the slower rate of reaction in D_2O as compared to H_2O ($k_H/k_D = 1.7$), and for the fact that the products of the aqueous photoreaction were significantly different from those formed in DMSO or $CHCl_3$. The slightly retarded rate of the aqueous photoreaction in the presence of O_2 may have been due to quenching of an excited state of the LSD.

Separation of three components via G-50 gel filtration demonstrates that at least some of the products of the [^{3}H]LSD photoreaction had substantially different chromatographic properties. These products of the photoreaction exhibited binding affinity that is different from that of the monomeric forms of LSD to proteins in particulate fractions of *F. hepatica*. This may be a source of substantial errors in binding studies unless precautions are taken.

Initially, our goal in studying the photoreactivity of LSD and its derivatives was to utilize it as a labeling reagent for proteins that bind LSD in the liver fluke membranes. Since LSD is extremely susceptible to racemization, isomerization, and decomposition under conditions used to form photoreactive intermediates [30], we examined the possibility of using the inherent photoreactivity of the nonderivatized [^{3}H]LSD to label membrane proteins that bind LSD.

Figure 5 shows that, when [^{3}H]LSD was allowed to bind to fluke membranes and exposed to longwave u.v. light, a small amount of the ligand was bound very tightly to specific binding site(s) and could not be removed. Some of the LSD binding was released following detergent disruption of the membrane. This SDS-solubilized fraction containing the labeled proteins routinely represented 30–40% of the total photobinding. Another important observation was the fact that 10^{-3} M concentrations of 5-HT blocked the specific photobinding of [^{3}H]LSD. Due to the high affinity of LSD for the serotonin receptor in a variety of systems, including fluke membranes, this photolabeling technique might provide a method to visualize the solubilized receptors for the purpose of its isolation and characterization.

One problem encountered with this technique is the rather low quantum yield of protein labeling. This is not an unusual problem when utilizing ligands for photolabeling which do not contain specifically designed photoreactive groups. One such case is seen in the comparison between labeling purified phosphofructokinase with [8-^{3}H]cAMP [17] as compared to labeling the same protein with O^2-(ethyl-2-diazomalonyl)cAMP [31]. The diazoderivative of cyclic AMP labels phosphofructokinase with a quantum yield of 0.3 to 0.5, while the [^{3}H]cAMP quantum yield was roughly 3000 times less [17]. Ferguson [18] reported that the photoincorporation of the [^{3}H]cAMP can be enhanced by irradiating the ligand–protein complex at low temperatures in a frozen state, yet the photolabeling efficiency remains substantially less than the diazoderivative. No enhancement of photolabeling was observed by irradiation of our system at −80° in the solid state. One complication associated with visualizing the LSD-binding proteins from *F. hepatica* was the fact that the number of LSD-binding sites is low.

Despite the rather small amounts of label incorporated, the selectivity of the photolabeling process was demonstrated. This is shown by the fact that a large percentage of the incorporated label appeared in distinct bands at 36,000 daltons and 100,000 daltons of the electrophoretic gel.

The question arises as to the functional significance of the photolabeled proteins. The possibility of any of these proteins being the receptors that are coupled to adenylate cyclase cannot be determined until these proteins can be bioassayed in a reconstitution system showing the biological function of these receptors, namely serotonin/LSD mediated cyclase activation. Such a system is as yet not available. Nevertheless, the selectivity of the photoreactive [^{3}H]LSD in labeling membrane proteins affords a powerful biochemical probe for visualizing these proteins in the solubilized state. Hopefully this technique will be

useful in identifying LSD-binding proteins in a variety of systems.

Acknowledgement—We would like to thank Mr C. Crawford Mechem for his excellent technical assistance.

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