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STABILIZATION OF THE PERIPHERAL-TYPE BENZODIAZEPINE ACCEPTOR BY SPECIFIC PHOSPHOLIPIDS

PAUL N. MOYNAGH and D. CLIVE WILLIAMS*
Department of Biochemistry, Trinity College, University of Dublin, Dublin 2, Ireland

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Abstract—The peripheral-type benzodiazepine acceptor from rat adrenal gland was solubilized with Triton X-100. The soluble acceptor exhibited isoquinoline carboxamide and benzodiazepine binding activity only when supplemented with lipid. Phosphatidylserine and phosphatidylinositol were especially efficacious. Such selectivity may reflect a specific structural requirement for acceptor activity. The requirement for lipid for acceptor activity in fractions subsequent to solubilization was demonstrated. This stabilization of acceptor activity by the phospholipids promises to facilitate purification of the functional acceptor.

Benzodiazepines bind to two pharmacologically distinct types of high affinity sites [1]. The first type is the "central-type" benzodiazepine receptor which is associated with the neuronal γ-aminobutyrate (GABA)-gated chloride channel [2, 3]. The clinical effects of benzodiazepines are mediated through this receptor [4]. The second type is the "peripheral-type" benzodiazepine acceptor which is present in several peripheral organs [1] and is localized primarily to the mitochondrial outer membrane [5–7]. Peripheral-type benzodiazepines (e.g. Ro 5-4864) which bind selectively to the acceptor have been reported to cause numerous diverse effects including inhibition of cell proliferation [8], induction of differentiation of cells [9], stimulation of phospholipid methylation [10], inhibition of mitochondrial respiratory control [11] and stimulation of steroidogenesis [12, 13]. However, it is by no means certain that all of these effects are mediated through the peripheral acceptor.

The peripheral-type benzodiazepine acceptor also binds isoquinoline carboxamides (chemically unrelated to the benzodiazepines), including PK 11195 [14]. The binding of the latter precludes benzodiazepine binding. The binding sites for the two classes of compounds are not identical based on their differential sensitivity to reagents such as diethylpyrocarbonate [15], unsaturated fatty acids [16] and detergents [17]. Such sites may reside on different portions of the same molecule or on different, closely interacting proteins. An 18-kDa polypeptide which possesses the isoquinoline carboxamide binding site has been purified from rat adrenal gland [18, 19] exploiting PK 14105, the nitrophenyl derivative of PK 11195, as a selective photoaffinity label for the site [20]. The question of whether this polypeptide also contains the benzodiazepine binding site remains to be answered. In order to resolve this unequivocally, it is necessary to purify the peripheral benzodiazepine acceptor in an intact form which retains its binding activities and analyse its constituent subunits. This may further our understanding of the physiological role of the acceptor.

Purification of the intact acceptor has proved a challenging task due to the lability of the functional acceptor upon detergent solubilization. Martini *et al.* [21] described the solubilization of rat kidney [^{3}H]Ro 5-4864 binding sites using Triton X-100. However, the detergent potently inhibited the binding assay and thus necessitated prior removal of detergent. Gavish and Fares [22] reported successful solubilization of [^{3}H]Ro 5-4864 binding sites from rat kidney using digitonin whilst CHAPS, deoxycholate, Triton X-100 and Tween 20 proved ineffective. Similarly, Benavides *et al.* [23] described solubilization of [^{3}H]PK 11195 binding sites from rat adrenal gland using digitonin whereas CHAPS and Triton X-100 were unsuitable. However, a subsequent study [24] reported the denaturation of these sites when subjected to digitonin solubilization. The sensitivity of the peripheral benzodiazepine acceptor to removal from its *in situ* membrane environment was suggestive of an essential interaction with a membrane component(s), possibly lipid.

This paper describes Triton X-100 solubilization of the peripheral-type benzodiazepine acceptor from rat adrenal mitochondria and stabilization of the soluble acceptor by lipid, especially phosphatidylserine and phosphatidylinositol. The importance of such lipid species in purification of the functional acceptor is discussed.

MATERIALS AND METHODS

Materials. [^{3}H]PK 11195 (90 Ci/mmol) was from N.E.N. (Dreieich, Germany). Ro 5-4864 was from Fluka Chemicals (Glossop, U.K.). PK 11195 was a gift from Dr Gerard Le Fur (Pharmuka Laboratories, Gennevilliers, France). Triton X-100 and lipids were from the Sigma Chemical Co. (Poole, U.K.). The PL-SAX column was from Polymer Labs (Church Stretton, U.K.).

* Corresponding author. Tel. (353) 1-7021802; FAX (353) 1-772400.

Table 1. Stabilization of [^{3}H]PK 11195 binding site in Triton X-100 solubilizate from rat adrenal mitochondria by soybean lipid

[Triton X-100] (%) (w/v)	Protein recovery in supernatant (%)	Recovery of [^{3}H]PK 11195 binding activity in supernatant (%) −lipid	+lipid
0.05	26 ± 2	2.3 ± 0.5	21 ± 1
0.1	48 ± 4	0	26 ± 2
0.25	44 ± 4	0	3.7 ± 0.8
0.5	46 ± 2	0	0.5 ± 0.3

Rat adrenal mitochondrial membranes (1 mg protein/mL) were solubilized with Triton X-100 (range of concentrations) as described in Materials and Methods. The soluble extracts were each divided into two portions, one of which was supplemented with soybean lipid (final concentration, 0.02% (w/v)). Membranes and supernatants were assayed for specific binding of 1.7 nM [^{3}H]PK 11195 (90 Ci/mmol). Recoveries of protein and binding activity are expressed relative to the membrane preparations.

Values represent the means ± ranges of two separate experiments.

Triton X-100 solubilization of mitochondrial membranes from rat adrenal gland. Mitochondrial membranes were prepared from rat adrenal gland as described previously [19]. The membranes were incubated at a protein concentration of 1–3 mg/mL, in 50 mM Tris–HCl buffer, pH 7.4, containing the stated concentration of Triton X-100. The mixtures were agitated for 1 hr at 4° and subsequently ultracentrifuged at 100,000 *g* for 1 hr at the same temperature. The resultant supernatants were used as soluble preparations. Protein content was determined by the method of Markwell *et al.* [25].

Emulsions of lipids which were subsequently added to the soluble extracts were prepared by sonication. This consisted of four bursts (10 sec each) using a Dawe soniprobe (Type 7532A) at a setting of 80 W. The samples were kept on ice between sonications to minimize heating.

[^{3}H]*Ligand binding assays.* Mitochondrial membranes or Triton X-100 soluble extracts (10–20 μg protein) were incubated with 0.2–100 nM [^{3}H]PK 11195 (8 or 90 Ci/mmol) in 50 mM Tris–HCl buffer, pH 7.4, in a total volume of 0.5 mL at 4°. Assays for soluble extracts contained the appropriate concentration of Triton X-100 as used in solubilization and the stated concentration of lipid. All samples were incubated in triplicate for 60 min. After incubation, 50 μL of γ-globulin (15 mg/mL) and 200 μL of 30% (w/v) polyethylene glycol were added to each sample, vortexed and filtered through Whatman glass fibre filters (GF/B, 2.5-cm dia.), presoaked in 0.1% (w/v) polyethyleneimine. The test tubes were rinsed twice with 1 mL of 8% (w/v) polyethylene glycol and the filters subsequently washed twice with 5 mL of 8% (w/v) polyethylene glycol (total time for filtration and washing, 15 sec). When dried, the filters were counted for radioactivity by scintillation counting.

Total and non-specific/non-saturable binding in each case was determined in the absence and presence of 10 μM unlabelled ligand, respectively. Specific/saturable binding was calculated from the difference between total and non-saturable binding.

Inhibition of [^{3}H]*PK 11195 binding to Triton X-100 soluble extract.* Triton X-100 soluble extracts (14 μg protein) were incubated with 5 nM [^{3}H]PK 11195 (90 Ci/mmol) in the absence and presence of various concentrations of Ro 5-4864 for 60 min at 4° in a final volume of 0.5 mL. The remainder of the assay was performed as above.

RESULTS

Rat adrenal mitochondrial membranes were solubilized with a range of Triton X-100 concentrations. The specific binding of [^{3}H]PK 11195 to the soluble supernatants was assessed and this demonstrated the quantitative loss of binding activity upon Triton X-100 solubilization (Table 1). However, in the presence of 0.02% (w/v) soybean lipid, 20–30% of the initial activity could be recovered (Table 1). Subsequent solubilization of mitochondrial membranes (1 mg protein/mL) was performed using 0.05% (w/v) Triton X-100. This concentration of detergent produced a soluble extract which, in the presence of exogenous lipid, possessed the highest number of [^{3}H]PK 11195 binding sites relative to protein and advocates its use in further purification studies. Increasing the Triton X-100 concentration to 0.1% (w/v) led to slightly improved recovery of [^{3}H]PK 11195 binding activity in the soluble extracts but this was coupled with an even higher recovery of solubilized protein which negated the former effect. Higher levels of Triton X-100 resulted in a dramatic decrease in the recovery of [^{3}H]PK 11195 binding activity in the soluble extracts despite lipid supplementation (0.02% (w/v)) (Table 1).

The effects of various concentrations of soybean lipid on the specific binding of [^{3}H]PK 11195 to 0.05% Triton X-100 (w/v)-solubilized membranes was assessed (Fig. 1). Half-maximal stabilization of [^{3}H]PK 11195 binding sites occurred at 0.004% (w/v) soybean lipid. Specific binding reached a plateau at 0.02% (w/v) soybean lipid and this level of lipid was used in further studies because it represented the minimum concentration of lipid required to unveil the maximum number of [^{3}H]PK 11195 binding sites. Soybean lipid itself exhibited no [^{3}H]-PK 11195 binding activity over the same range of concentrations (data not shown).

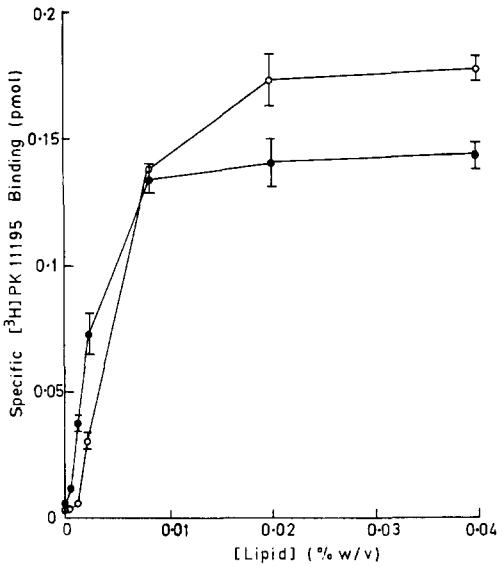

Fig. 1. Effect of various concentrations of soybean lipid and phosphatidylserine on specific [^{3}H]PK 11195 binding to Triton solubilizate from rat adrenal mitochondrial membranes. Rat adrenal mitochondrial membranes (1 mg protein/mL) were solubilized with 0.05% (w/v) Triton X-100 in 50 mM Tris–HCl buffer, pH 7.4. Aliquots (50 μL) of the supernatant were assayed for specific binding of 1.7 nM [^{3}H]PK 11195 (90 Ci/mmol) in the absence and presence of various concentrations of soybean lipid (○) or phosphatidylserine (●), as described in Materials and Methods. Each point is the mean of triplicates and the error bars represent the SEM. Absence of error bars indicates that the error was smaller than the size of the symbol.

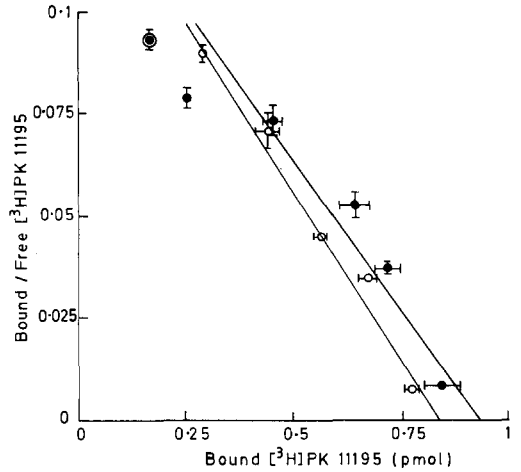

Fig. 2. Scatchard plots of [^{3}H]PK 11195 binding to Triton X-100-solubilized rat adrenal mitochondrial membranes in presence of lipid. Mitochondrial membranes (1 mg/mL) were solubilized with 0.05% (w/v) Triton X-100 in 50 mM Tris–HCl buffer, pH 7.4. Aliquots (50 μL) of the solubilized supernatant, supplemented with 0.02% (w/v) soybean lipid (○) or 0.02% (w/v) phosphatidylserine (●), were incubated with 0–100 nM [^{3}H]PK 11195 (8 Ci/mmol) for 60 min at 4° in the presence (non-specific binding) and absence (total binding) of unlabelled PK 11195 (10 μM). Incubation buffer consisted of 50 mM Tris–HCl buffer, pH 7.4, containing 0.05% (w/v) Triton X-100 and 0.02% (w/v) soybean lipid or phosphatidylserine. Equilibrium dissociation constants (K_d) and the maximal binding capacities (B_{max}) were obtained by fitting the data to a rectangular hyperbola describing the binding of ligand to a single class of binding sites [30]. K_d and B_{max} values of 6.1 ± 0.6 nM and 81 ± 3 pmol/mg protein, respectively, were calculated for the soybean lipid-supplemented preparation. K_d and B_{max} values of 7 ± 1 nM and 91 ± 5 pmol/mg protein, respectively, were calculated for the phosphatidylserine-supplemented preparation. The data are shown in the form of Scatchard plots for clarity. The lines were constructed using the appropriate K_d and B_{max} values from above. K_d and B_{max} values of 2.4 ± 0.3 nM and 77 ± 3 pmol/mg protein were determined for [^{3}H]PK 11195 binding to mitochondrial membranes.

Specific binding of peripheral-type benzodiazepine ligands to 0.05% Triton X-100 (w/v)-solubilized membranes was studied in the presence of 0.02% soybean lipid (w/v). Specific binding of [^{3}H]PK 11195 displayed linearity with respect to quantity of protein (at least in the range 0–30 μg). The association and dissociation rates for [^{3}H]PK 11195 binding were determined to be $1.64 \times 10^7\ M^{-1}\ min^{-1}$ and $0.0153\ min^{-1}$, respectively. The ratio (k_{diss}/k_{assoc}) of these two constants is 0.93×10^{-9} M which is sensibly consistent with the K_d values quoted below. The association constant is also consistent with binding equilibrium being reached before 1 hr for the concentrations of ligand and binding sites used in these studies. The lack of deviation of the Scatchard plots at low ligand concentrations again is diagnostic of complete equilibration of binding. Specific binding of [^{3}H]PK 11195 was found to be saturable over the range 0–100 nM (Fig. 2). B_{max} and K_d values obtained from two different preparations were 72 ± 9 pmol/mg protein and 5 ± 1 nM, respectively, where each value represents the mean and its associated range. A concentration-dependent inhibition of [^{3}H]PK 11195 binding by Ro 5-4864 was observed (Fig. 3) being described quantitatively by a K_i value of 160 ± 20 nM (mean ± range of two separate experiments).

The crude nature of soybean lipid prompted an investigation into the stabilization of [^{3}H]PK 11195 binding sites by various individual lipids (Table 2). Phosphatidylserine was the most efficient and indeed was comparable in effect to soybean lipid. Phosphatidylinositol was next in order of potency. The remaining phospholipids and the representatives of other lipid classes were relatively impotent. Phosphatidylserine produced its half-maximum effect at 0.002% (w/v) and a minimum concentration of 0.008% (w/v) was required to obtain maximum [^{3}H]PK 11195 binding (Fig. 1). Phosphatidylserine, itself, exhibited no specific [^{3}H]PK 11195 binding activity (data not shown). Triton X-100-solubilized membranes, in the presence of phosphatidylserine, displayed binding of [^{3}H]PK 11195 with association and dissociation rates of $1.3 \times 10^7\ M^{-1}\ min^{-1}$ and $0.0174\ min^{-1}$, respectively. Subsequent equilibrium binding analysis demonstrated the saturable high

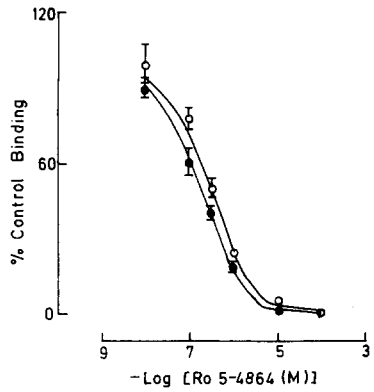

Fig. 3. Characterization of Ro 5-4864 binding to Triton X-100-solubilized rat adrenal mitochondrial membranes in presence of lipid. Mitochondrial membranes (1 mg/mL) were solubilized with 0.05% (w/v) Triton X-100. Aliquots (50 μL) of the solubilized supernatant, supplemented with 0.02% (w/v) soybean lipid (○) or 0.02% (w/v) phosphatidylserine (●) were assayed for specific binding of 5 nM [^{3}H]PK 11195 (90 Ci/mmol) in the absence and presence of various concentrations of unlabelled Ro 5-4864. Control binding is defined as specific binding in the absence of Ro 5-4864. The inhibition curves were fitted by eye to single-site displacement curves. Inhibition constants (K_i values) of 180 nM and 120 nM were calculated for the soybean lipid- and phosphatidylserine-supplemented samples, respectively. Each point represents the mean ± SEM of triplicates.

Table 2. Effects of different lipids on [^{3}H]PK 11195 binding site in Triton solubilizate from rat adrenal mitochondria

Supplementary lipid	Recovery of [^{3}H]PK 11195 binding activity (%)
Soybean lipid	100
None	3.7 ± 0.9
Phosphatidylcholine	14 ± 0.6
Phosphatidylethanolamine	3.1 ± 0.5
Phosphatidylinositol	67 ± 6
Phosphatidylserine	88 ± 4
Phosphatidic acid (free acid)	1.2 ± 0.4
Phosphatidic acid (sodium salt)	2.3 ± 0.6
Lysophosphatidylserine	28 ± 3
Lysophosphatidylcholine	11 ± 0.4
Cholesterol	2.7 ± 0.7
Tristearin	1.6 ± 0.2

Rat adrenal mitochondrial membranes (1 mg protein/mL) were solubilized with 0.05% (w/v) Triton X-100. Aliquots (50 μL) of the solubilized supernatants, supplemented with various lipid species (all 0.02% (w/v)), were assayed for specific binding of 2 nM [^{3}H]PK 11195 (90 Ci/mmol). Assay buffer contained the appropriate lipid at a concentration of 0.02% (w/v). Recoveries of binding activity are expressed relative to the soybean supplemented samples.

The values shown are means ± ranges of two separate experiments.

The recovery of [^{3}H]PK 11195 binding activity in supernatants supplemented with serine (0.02 (w/v)) was 5.2 ± 0.8%.

affinity nature of the [^{3}H]PK 11195 binding (Fig. 2). B_{max} and K_d values (± ranges) obtained from two different preparations were 87 ± 4 pmol/mg and 8 ± 1 nM, respectively. Ro 5-4864 inhibited [^{3}H]PK 11195 binding to solubilized membranes in a concentration-dependent manner (Fig. 3). Potency of inhibition was quantitated by a K_i value of 140 ± 20 nM (mean ± range of two separate experiments).

Dilution of Triton X-100-soluble extracts from rat adrenal mitochondrial membranes in the absence of exogenous lipid led to quite significant decreases in the recovery of [^{3}H]PK 11195 binding activity (Table 3) despite the subsequent addition of soybean lipid to the diluted soluble extracts. However, addition of lipid to the soluble extracts, prior to dilution, protected against the deleterious effects of dilution on [^{3}H]PK 11195 binding activity. Similarly, dialysis of the extracts in the absence of exogenous lipids resulted in low recovery of binding activity whereas prior addition of lipid and dialysis in its presence produced dialysates with [^{3}H]PK 11195 binding activity comparable to untreated soluble extracts, supplemented with soybean lipid (Table 3). Dialysis of lipid-treated samples in buffer lacking lipid resulted in slightly lower recovery of binding activities.

Triton X-100 soluble extracts of rat adrenal mitochondrial membranes were subjected to Sephacryl S-200 gel filtration in the presence of soybean lipid. Eluted fractions from the column were assayed for [^{3}H]PK 11195 binding sites. Binding activity eluted in the void volume of the column (Fig. 4a). Active material from the Sephacryl S-200 gel filtration column was further fractionated by anion exchange chromatography in the presence of soybean lipid (Fig. 4b). Two major peaks of A_{280} eluted during the applied salt gradient. The eluate was monitored for the presence of [^{3}H]PK 11195 binding sites and two peaks of binding activity were apparent. SDS–PAGE analysis of the material constituting the latter peaks demonstrated heterogenous mixtures of proteins. However, an 18-kDa polypeptide was a major component in both cases. Sephacryl-200 gel filtration or anion exchange chromatography in the absence of lipid resulted in total loss of [^{3}H]PK 11195 binding activity despite subsequent addition of lipid to fractionated samples (data not shown).

DISCUSSION

The adrenal gland was selected as a source for the peripheral benzodiazepine acceptor because it contains one of the highest acceptor densities among all tissues examined [26]. Triton X-100 was the detergent of choice because it is both inexpensive and non-ionic: desirable features for detergents used in purification of membrane proteins.

Triton X-100 solubilization of rat adrenal mitochondrial membranes resulted in the loss of [^{3}H]PK 11195 binding activity, consistent with a previous report [24]. However, a significant proportion of activity was recovered by subsequent addition of lipid. Soybean lipid was used initially because although it consists primarily of phosphatidylcholine (20%, w/w) it also contains various other lipids. The

Table 3. Effect of dilution and dialysis on [^{3}H]PK 11195 binding to Triton X-100-solubilized rat adrenal mitochondrial membranes

Lipid addition	Recovery of [^{3}H]PK 11195 binding activity (%) Dilution factor 2	4	Dialysis buffer ± soybean lipid +	−
Before	95 ± 5	100 ± 5	88 ± 3	67 ± 5
After	35 ± 3	18 ± 2	ND	15 ± 1

Rat adrenal mitochondrial membranes (1 mg protein/mL) were solubilized with 0.05% (w/v) Triton X-100 in 50 mM Tris–HCl buffer, pH 7.4. (a) Aliquots (50 μL) of the solubilized supernatant were (i) supplemented with 0.02% (w/v) soybean lipid, (ii) diluted 1:1 (by vol.) or (iii) 1:3 (by vol.) with solubilization buffer before/after being supplemented with soybean lipid. Recoveries of binding activity in the diluted samples are expressed relative to the undiluted samples, supplemented with lipid ((i) above). (b) Aliquots (0.5 mL) of the solubilized supernatant were dialysed overnight against 1 L solubilization buffer of 1 L solubilization buffer containing 0.02% (w/v) soybean lipid. The dialysates were supplemented with soybean lipid to a final concentration of 0.02% (w/v) either before or after dialysis. Recoveries of binding activity in the dialysed samples are expressed relative to undialysed samples of the solubilized supernatant which were supplemented with lipid.

Aliquots (50 μL) of samples were assayed for specific binding of 2 nM [^{3}H]PK 11195 (90 Ci/mmol) in solubilization buffer which also contained 0.02% (w/v) soybean lipid.

Values represent the means ± ranges of two separate experiments.

ND, not determined.

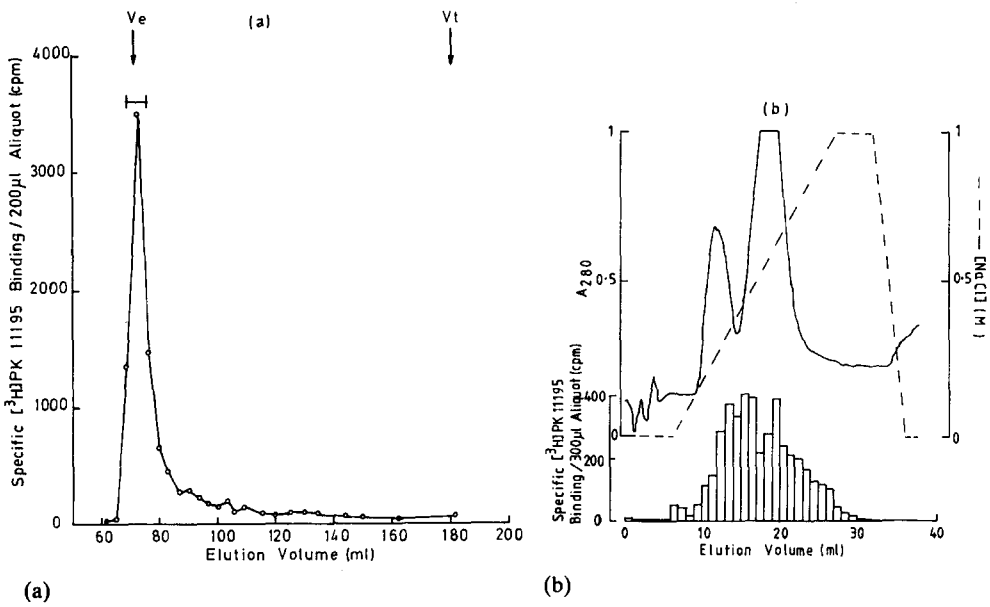

Fig. 4. Fractionation of Triton X-100 soluble extracts of rat adrenal mitochondria. (a) A Triton X-100 soluble extract (3.5 mL, 1 mg protein) of rat adrenal mitochondria was applied at a flow rate of 14 mL/hr to a Sephacryl S-200 column (76 × 1.8 cm), equilibrated in 50 mM Tris–HCl buffer, pH 7.4, containing 100 mM NaCl, 0.05% (w/v) Triton X-100 and 0.02% (w/v) soybean lipid. Aliquots (200 μL) of fractions (2.5 mL) were assayed for specific binding of 1.5 nM [^{3}H]PK 11195. Results are plotted as specifically bound radioactivity (cpm) per aliquot against the corresponding elution volume. The two arrows represent the void (V_e) and total (V_t) volumes as determined by blue dextran and pyridoxal phosphate, respectively. (b) An aliquot (1 mL) of an eluted fraction from the Sephacryl S-200 column (indicated by the bar in (a)), containing [^{3}H]PK 11195 binding sites, was applied to a PL-SAX column (50 × 4.6 mm). The gradient profile (dashed line) was as illustrated. Fractions (1 mL) were collected at a flow rate of 1 mL/min and aliquots (300 μL) of each assayed for specific binding of 1.5 nM [^{3}H]-PK 11195. Results are plotted as specifically bound radioactivity per aliquot (histogram) against the corresponding elution volume. The absorbance at 280 nm was measured continuously. Buffer A consisted of 30 mM Tris–HCl buffer, pH 8, containing 0.05% (w/v) Triton X-100 and 0.02% (w/v) soybean lipid. Buffer B consisted of buffer A with 1 M NaCl.

binding sites which were recovered exhibited, approximately, the same affinity and density as in the membranes, at least with respect to [^{3}H]PK 11195. In contrast, the affinity for Ro 5-4864 was significantly reduced demonstrating the non-identical properties of the binding sites for these ligands.

The mechanism of restoration of binding activity by supplementary lipid is not understood but may be due to stabilization of binding site conformation. Such a phenomenon has been observed for other membrane proteins including the nicotinic acetylcholine receptor [27], the GABA transporter [28] and the $GABA_A$ receptor [29]. Indeed, the latter contains the "central-type" benzodiazepine receptor which in the light of the present work suggests a general lipid requirement for benzodiazepine binding to its different sites. In the present study, phosphatidylserine and phosphatidylinositol appear to constitute the major components responsible for stabilization of acceptor activity. Such selectivity in terms of lipid species may reflect the provision of a specific environment appropriate to a functional protein conformation. The single net negative charge common to both these phospholipids may be an important feature in their efficacy.

The deleterious effect of dilution and dialysis on [^{3}H]PK 11195 binding activity might be the result of the increase in detergent:lipid ratio. It is envisaged that this would lead to "stripping" of endogenous lipid associated with the binding sites which might lead to irreversible denaturation. Exogenous lipid could then no longer exert its stabilizing effect. However, prior addition of lipid could possibly prevent the stripping action of the detergent. This is an important feature to consider before embarking on the purification of the peripheral benzodiazepine acceptor. It was thus decided to perform chromatography of Triton X-100 soluble extracts in the presence of exogenous lipid.

Elution of [^{3}H]PK 11195 binding sites in the void volume upon Sephacryl S-200 gel filtration is indicative of the sites being part of a large molecular mass complex. This might reflect incomplete solubilization of binding sites, despite the fact that they failed to sediment by centrifugation at 100,000 *g* for 1 hr. Alternatively, the high molecular mass may be due to a large number of Triton X-100 and lipid molecules which associate with the solubilized binding sites. The fractionation of [^{3}H]PK 11195 binding sites into two peaks of activity upon anion-exchange chromatography is consistent with a previous report [31]. This may be due to the partitioning of binding sites into two classes of protein–lipid–detergent micelles. The fractionation studies, while not achieving purification of the polypeptide(s) constituting the binding sites, have demonstrated the continual requirement of exogenous lipid for preservation of binding activity during fractionation. Such a stabilizing effect of lipid promises to facilitate purification of the peripheral-type benzodiazepine acceptor in a form which retains its functional integrity, possibly leading to elucidation of its biological role(s).

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