Drug-binding Macromolecular Lipids from L1210 Leukemia Tumors

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ABSTRACT

The isolation and purification of at least two distinct macromolecular lipids from murine L1210 tumors either sensitive or resistant to the terephthalanilide NSC 38280 are described. The lipids are extracted from the tumors with chloroform:methanol and purified utilizing a series of gel permeation and affinity chromatography columns with chloroform:methanol elution. Preliminary characterization of the macromolecular lipids indicates that they have low molecular weights (>10,000 but <25,000) and are comprised of lipid, carbohydrate, and polyamino components. Significant differences were found between the component composition, molecular size, and column elution properties of the two lipids. One of the macromolecular lipids is extractable from both sensitive and resistant tumors and binds terephthalanilides, Cain quinoliniums, a carbanilide, bis(substituted aminoalkyl)antraquinones, and Adriamycin. The other macromolecular lipid appears to occur only in resistant tumors and does not bind the terephthalanilides, Cain quinoliniums, or the carbanilide. Further, the binding studies may predict a correlation between drug binding to the lipids in vitro and drug activity against L1210 leukemia and other tumors in vivo. The polyamine, spermine, also binds to the macromolecular lipids and can displace drugs bound to the lipids. Such spermine displacement of drug binding is either competitive or noncompetitive in nature depending on the cell line origin of the lipid. The isolated macromolecular lipids may represent components of binding sites for cancer-chemotherapeutic drugs in vivo.

INTRODUCTION

The terephthalanilides are a class of substituted phthalanilides which were first synthesized in 1962 (11) and subsequently found to be highly active against animal leukemias (16, 22, 24). Studies on the molecular action of the drugs indicated that their polycationic nature enabled their complexing with biological molecules including nucleic acids, proteins, and known anionic lipids in vitro (25, 34). Extraction of terephthalanilide complexes from animal tissues, however, has shown that the drugs complex primarily with unidentified lipids in vitro (32, 34, 36) and that such lipid complexing occurs chiefly in the nuclear and mitochondrial fractions of treated cells (24, 34). In addition, the degree of terephthalanilide:lipid complexing in leukemia cells appears to correlate directly with chemotherapeutic response (32, 35) and in normal tissues with toxicity (33). Terephthalanilide-binding lipids have also been extracted from dog, rat, mouse, and monkey tissues (31, 36); from Escherichia coli (32); and from P388 and L1210 leukemia tumors either sensitive or resistant to the terephthalanilide NSC 38280 (24, 35, 36). Drugs structurally similar to the terephthalanilides have also been found to complex with lipids from normal and tumor-bearing tissues. For example, the Cain quinolinium NSC 113089, which has been found active against murine L1210 leukemia (1), was found to complex with lipid extracts from rat liver, kidney, heart, and skeletal muscle (31). Preliminary studies in this laboratory (37) have found that lipids extracted from L1210 leukemic tumors complex with aminoantraquinones such as NSC 287513. These latter compounds represent a new series of cancer-chemotherapeutic drugs which are very active against a number of tumor systems in vivo (15, 38).

It thus appears that specific lipids occur in both normal and neoplastic tissues and complex with structurally similar cancer-chemotherapeutic drugs. This report describes efforts to first purify and then characterize, both chemically and pharmacologically, the cancer-chemotherapeutic drug-binding lipids from L1210 leukemia tumors.

MATERIALS AND METHODS

Drugs and Chemicals. The Cain quinoliniums NSC 113089 and 114347; the terephthalanilides NSC 38280 and 35843; the carbanilide NSC 109555; Adriamycin NSC 123127; and the aminoantraquinones NSC 287513, 281248, 279836, 281249, 279847, and 279837 were all obtained from Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. All the drugs used exhibit in vivo antitumor activity against L1210 leukemia implanted in mice except the 3 aminoantraquinoines NSC 281249, 279847, and 279837 (1, 2, 7, 15, 18, 22). Sephadex LH-20 and Sepharose CL-4B were from Pharmacia Fine Chemicals, Piscataway, N. J. Spermine tetrahydrochloride, spermidine trihydrochloride, and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, Mo. All solvents were analytical grade or better.

The purity of all the drugs was assayed prior to use in binding studies. The Cain quinoliniums, terephthalanilides, carbanilide, and Adriamycin were analyzed spectrophotometrically and found to be >95% pure according to their reported extinction coefficients. The aminoantraquinoine compounds were also assayed spectrophotometrically as well by high-performance liquid chromatography (28) and found to be >95% pure.

Affinity Supports. Synthesis of the affinity supports utilized standard methods modified for application to organic solvent-resistant gel media (27). Two supports were made (Chart 1) by linking either the Cain quinolinium NSC 113089 or the polyamine spermidine to Sephadex LH-20 through succinylaminoaikyl side chains. The degree of alkylation, succinylation, and ligand coupling to each support was monitored by withdrawing 2-ml aliquots of the beads after each reaction step and analyzing the bound amino group content by a standard method utilizing trinitrobenzene sulfonate (9). In addition, the amount of NSC 113089 bound to the support could be quantitated by its extinction coefficient in the reaction solution ($\varepsilon_{330} = 30,580$ in 40% aqueous dimethylformamide) before and after coupling. Such analysis showed...
that 0.54 ± 0.11 µmol of NSC 113089 or 0.69 ± 0.12 µmol of NGC 38280 (32) were used in these studies. A 10° cell inoculum of either tumor was grown i.p. in 18- to 22-g BALB/c x DBA/2 F2 columns. All other columns in the purification sequences were 1.5 (inside diameter) x 75 cm in size.

Chemical Assays and Analysis. To determine the amount of a lipid, aliquots from the TLE and of the purified drug-binding lipids were taken to dryness under a stream of nitrogen and dried in vacuo to a constant weight (±0.1 mg) in a desiccator over KOH. Protein and hexose content in extracts and pooled column fractions were determined by the standard method of Lowry et al. (17) and orcinol methods as modified for protein-lysides (10). Bovine serum albumin and galactose, respectively, were used as standards. Phosphorus was determined after perchloric acid digestion according to the ascorbic acid:myoglobin- date method of Chen et al. (4) as modified for microdeterminations (20) utilizing KH2PO4 as the standard. Nitrogen (Method 1) and total sugars were determined according to standard methods (8, 14) utilizing ammonium sulfate and galactose, respectively, as standards. Elemental analysis for carbon, hydrogen, and nitrogen was carried out by Spang Microanalytical Laboratory, Eagle Harbor, Mich. Fatty acids and glyceroles analyses were carried out utilizing standard methodologies (19, 36). UV-visible spectroscopic analysis of the purified macromolecular lipids and of the drugs used in the partition assay was carried out on a Varian Cary 118C UV-visible spectrophotometer. Fluorescence spectra were determined on a Perkin-Elmer Hitachi MPF-44 fluorescence spectrophotometer.

Polyacrylamide Electrophoreses. PAGE, both in the presence and absence of sodium, dodecyl sulfate, was utilized to assess the homogeneity and to determine the approximate molecular weights of the purified macromolecular lipids. All equipment and chemicals were purchased from Bio-Rad Laboratories, Inc., Richmond, Calif. Lipid samples containing from 2 to 10 µg of phosphorus were dried under a stream of nitrogen and solubilized according to the standard method of Weber and Osborn (30) but with the substitution of dithiothreitol for mercaptoethanol. The samples were then applied to the PAGE system described by Davis (5) but with the substitution of precast Bio-Phore gels (Bio-Rad Laboratories, Inc.) containing 7.5% monomer. Molecular weight estimates of the macromolecular lipids were made by analysis of standard protein markers under identical electrophoretic conditions. The separated lipids and protein markers were located on the gels by scanning at 280 nm using a Gilford Model 2520 gel scanner (Gilford Instruments, Oberlin, Ohio) either before or after staining with Coomassie Blue G-250 with or without formaldehyde fixation (26, 30). The use of the latter stains for detection of the lipids on the gels resulted, however, in only faintly visible bands at best, due to the low nitrogenous content of the lipids and their highly hydrophobic nature.

High-Performance Liquid Chromatography. HP-GPC of the macromolecular lipids utilized a Micromeritics Model 701 liquid chromatograph (Micromeritics, Norcross, Ga.) equipped with a variable UV-visible detector and an FS 970 fluorometer (Schoeffel Instruments Corp., Westwood, N. J.). Five-a Styragel columns (Waters Associates, Milford, Mass.) were linked in the following order of pore size (inlet to outlet): 10,000 to 1,000 to 1,000 to 500 to 500 Å. This arrangement was found to result in excellent retention and resolution of macromolecular standards with molecular weights of <100,000 as determined with narrow range polystyrene molecular weight standards (Waters Associates). Column elution was at 2.0 ml/min (±1500 psi) using either tetrahydrofuran or chloroform:methanol (4:1, v/v) as solvent. Lipid samples were dissolved in either of the latter 2 solvents prior to analysis. The column effluent was monitored at either 254 (polystyrenes) or 280 (lipids) nm and then routed to a fraction collector set to collect 2-ml fractions. When required, the effluent was also monitored for Adriamycin fluorescence with excitation at 480 nm and an emission filter with a 550-nm cutoff. Drug binding and component assays were carried out on each fraction to locate the lipids. The retention volumes of the lipids were then compared to the polystyrene standards for estimation of lipid molecular weights.

**Macromolecular Lipids from L1210 Leukemia Tumors**

**Chart 1.** Postulated structures of the Sephadex LH-20:NSC 113089 and Sephadex LH-20:spermidine affinity supports. Spermidine or NSC 113089 was coupled to the free hydroxyl groups on the Sephadex LH-20 beads through a succinyl-aminoalkyl spacer arm.
Drug-binding Studies. All drug-binding experiments were carried out with a modified version of a standard partition method (31, 32) which utilizes the hydrophobicity of the macromolecular lipids and the hydrophilicity of the drugs in a biphasic mixture. Initially, a mixture of chloroform:methanol:water (2:1:1, v/v/v) is equilibrated in a separatory funnel to yield a lower chloroform phase and an upper aqueous phase. For an assay, the drug is dissolved in 3.0 ml of upper phase and added to 3.0 ml of lower phase containing a known amount of lipid (from 0.01 to 0.3 µg of phosphorus). The 2 phases are mixed for 20 sec and then allowed to separate at 4°C (12 to 16 hr). The drug in each phase is then quantitated by its extinction coefficient in that phase or, in the case of [14C]spermine, by the amount of radioactivity in each phase. The amount of lipid bound drug in the lower phase of any reaction mixture was calculated after necessary correction for normal drug partitioning between the phases as determined from nonlipid-containing control assays. Unless otherwise indicated, drug concentrations in the upper phase of an appropriate assay mixture were 16.7 µM (NSC 113089, 38280, and 287513) or 3.33 µM (spermine).

In the case of saturation experiments, increasing amounts of drug were present in the upper phases of 2 parallel reaction series. The lower phases of the first series contained no lipid and acted as controls to indicate the amount of drug normally partitioning into the lower phase at each drug concentration. The lower phases of the second series contained a fixed amount of the lipid to be saturated. Typically, each saturation series utilized 11 mixtures with varying drug or spermine concentrations of 1.7 to 670 µM in the upper phases.

Each drug saturation series was then reassayed in the presence of spermine to determine the effects of spermine on such saturation. After quantitating the amount of drug in the 2 phases of a mixture, the phases were recombined, and 0.1 ml of a spermine tetrahydrochloride stock solution was added to both the test and control mixtures to result in a final spermine concentration of 16.7 µM. After mixing and overnight reequilibration, the amount of drug bound by the lipid was requantitated.

RESULTS

Lipid Purification. Extensive studies were carried out on the separation of L1210 lipid extracts by gel permeation chromatography and affinity chromatography in organic solvents. Initial separation of the extracts into a number of peaks was accomplished using stepwise elution of Sephadex LH-20 and Sepharose CI-4B columns with increasing amounts of methanol in chloroform. Each peak was then analyzed for drug binding, lipid component content, and UV-visible absorbance characteristics in order to locate and quantify the drug-binding lipids. Even though the bulk of the classical lipids and some nonlipid material were removed in this step, the drug-binding peak containing the macromolecular lipid was not homogeneous. Thus, the affinity supports were used to further purify the macromolecular lipids initially fractionated on the first Sephadex LH-20 column. The choice of binding ligands for the affinity columns was based upon preliminary studies which characterized the binding of drugs to the TLE and the displacement of such binding by naturally occurring polyamines. These studies and other studies (31, 32, 34–36) further led us to search for more than one drug-binding macromolecular lipid. As a result, we found one macromolecular lipid in sensitive L1210 tumors (Ba) and 2 in resistant L1210 tumors (Ar and Br).

Lipid Ar eluted in the void volume while Lipids Br and Bs were eluted with chloroform:methanol (1:1) from Sephadex LH-20 columns. Lipid Ar was further purified by chromatography and Sepharose CI-4B and eluted with chloroform:methanol (1:1). The partially purified lipids were then chromatographed on either the Sephadex LH-20:spermidine (Lipid Ar) or the Sephadex LH-20:NSC 113089 (Lipids Br and Bs) affinity support. Attempts to isolate a lipid similar to Ar in sensitive cells indicated that such a lipid does not occur in the sensitive cells in any significant amount.

Approximately 300- and 50-fold purification of Lipids A and B was achieved, respectively, utilizing affinity chromatography purification (Table 1). Similar purification was achieved utilizing HP-GPC instead of affinity chromatography. All 3 lipids resulted in a single band on PAGE and one elution peak upon HP-GPC analysis (Chart 2).

Preliminary Characterization Studies. The macromolecular lipids comprise 5% of the total lipid extracted from the L1210 cells and 0.1% of the total cell weight (Table 2). While homogenous by PAGE and HP-GPC analysis, the lipids exhibit the solubility and multicomponent characteristics of macromolecules. The lipids are soluble in chloroform containing 5 to 50% methanol but are insoluble in solutions containing >50% methanol, or in aqueous solutions. Attempts to transfer the lipids to methanol, ethanol, or tetrahydrofuran led to precipitation. Lipids retained their full drug-binding capacity and specificity for at least 6 months when stored in 10% methanolic chloroform under nitrogen at ~4°C. Molecular weight determinations on the lipids (Table 2) found that Lipid Ar is larger than Lipids Br and Bs. The HP-GPC estimates of the lipid molecular weights were constantly lower than those found with PAGE. This is presumably due to the inconsistencies in comparing a natural macromolecule to rigid, linear polystyrene molecular weight standards in HP-GPC analysis.

Elemental analysis of the lipids for carbon, hydrogen, nitrogen, and phosphorus accounted for 65% of the lipid weight. The remainder of the lipid weight is presumably oxygen and possibly inorganic salts. Component analysis of the lipids confirmed the presence of diverse subunits including fatty acids, glycerol, sugars, and nitrogenous material giving a positive reaction by the method of Lowry. In general, the compositions of Lipids Br and Bs were similar but significantly different from that of Lipid Ar. UV-visible spectra of the lipids showed one major absorbance peak for each lipid, indicative of aromatic amino acid residues. The fluorescence spectra of all 3 lipids suggest the presence of a component with the excitation and

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Drug bounda</th>
<th>Fold purifi-</th>
<th>Drug bounda</th>
<th>Fold purifi-</th>
<th>Drug bounda</th>
<th>Fold purifi-</th>
</tr>
</thead>
<tbody>
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<tr>
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<td>21</td>
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<td></td>
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<tr>
<td>Purified lipid</td>
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<td>44</td>
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<td>43</td>
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<td>47</td>
<td>2.29</td>
<td>51</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a NSC 287513 (mol/mol lipid).

NU, Sepharose CI-4B not used in Lipid B purification.
Macromolecular Lipids from L1210 Leukemia Tumors

Binding Studies. The binding of drugs to the macromolecular lipids could be quantitated directly from the amount of drug migrating into the lower phase of a partition mixture in excess of any drug found in this phase in the absence of drug-binding lipid. Previous studies (31, 32, 34–37) have established that, during drug:lipid binding in this assay, no migration of drug:lipid complex into the upper phase occurs. In addition, drug:lipid binding does not alter the physical characteristics of the lipids. This is illustrated in Chart 3 which compares the HP-GPC elution characteristics of Lipid Bs both before and after reaction with Adriamycin. The same results were found before and after Adriamycin reaction with Lipids Ar and Br.

Saturation of the macromolecular lipids was possible with most of the drugs tested. Comparisons of the saturation characteristics of the 3 lipids studied, however, showed significant differences. As reported in Table 3, the most active drugs in vivo saturate Lipids Br and Bs at the lowest drug concentrations and have the highest affinities for the lipids as indicated by their $K_m$ values. Lipids Br and Bs differ, however, in that Lipid Bs has 2 saturable drug-binding sites as determined by double reciprocal and Scatchard (21) plots of the saturation data. For example, Chart 3 illustrates the plots obtained from saturation of Lipids Br and Bs with the aminoanthraquinone NSC 287513. The plots indicate that 2 distinct and saturable binding sites are present on Lipid Bs, with differing $K_m$ values and each requiring different amounts of drug for saturation. Similar analyses of Lipid Bs saturation with the other drugs tested indicated that all but the in vivo inactive aminoanthraquinones interact with 2 sites on the lipid. Site 1 of Lipid Bs has saturation characteristics similar to the single drug-binding site observed on Lipid Br, although the lower $K_m$ values found for drug binding to Lipid Br indicate that the drugs have a higher affinity for Lipid Br than for Site 1 of Lipid Bs. Site 2 on Lipid Bs required from 15 to 20 times more drug for saturation and has $K_m$ values for the drugs tested from 5 to 30 times greater than Site 1.

Lipid Ar from terephthalanilide-resistant L1210 tumors does not bind the Cain quinoliniums, terephthalanilides, or the carbamidine. Saturation of the lipid with Adriamycin and the in vivo active aminoanthraquinones showed that from 2 to 10 times more drug is required to saturate Lipid Ar than for Site 1 of Lipid Bs. Site 2 on Lipid Bs required from 15 to 20 times more drug for saturation and has $K_m$ values for the drugs tested from 5 to 30 times greater than Site 1.

Another important feature of drug binding to Lipid Ar is that such displacement is antagonistic at the drug-binding sites. For example, the double reciprocal plots of Lipid Br and Bs saturation by NSC 287513 (Chart 3) show that, in the presence of spermine, the drug $K_m$ value is increased while the ordinate intercept remains the same. This indicates that spermine acts as a competitive inhibitor at the drug-binding site(s) on Lipids Br and Bs. This type of inhibition by spermine was observed in all cases of saturable drug binding to Lipid Br and to both sites on Lipid Bs with 2 exceptions. In the case of Adriamycin, no drug was displaced from Site 2 of Lipid Bs at the spermine concentration used. In the case of NSC 281249, double reciprocal plots of the saturation data resulted in identical $K_m$ values but different ordinate intercepts, indicating that spermine inhibition of NSC 281249 binding to Lipids Br and Bs is noncompetitive in nature. Such noncompetitive inhibition to drug binding by spermine was found with all those drugs which saturate Lipid Ar (Table 3).

DISCUSSION

The present studies describe the isolation from L1210 leukemia tumor cells of at least 2 macromolecular lipids which bind spermine and cancer-chemotherapeutic drugs. Purification of the lipids has been accomplished using gel permeation and affinity chromatography in chloroform:methanol. The affin-
repeated gel permeation chromatography and PAGE analysis. Molecular weight determinations are the mean of at least 4 different analyses by each method with no deviation from the mean by more than ±10.6%. Component analyses data are the means of at least 3 determinations for each lipid with a maximum deviation for any single value deviating from the mean by more than ±7.5%. Lipid yield values are the mean of at least 2 separate extractions from each cell line with no single value deviating from the mean by more than ±11.1%.

Aminoanthraquinones (inactive)  
NSC 6  
Table 2  
Preliminary characterization of the macromolecular lipids  
All analyses were carried out on macromolecular lipid samples purified through affinity chromatography or HP-GPC and shown to be a single macromolecule by repeated gel permeation chromatography and PAGE analysis. Molecular weight determinations are the mean of at least 4 different analyses by each method with no deviation from the mean by more than ±10.6%. Component analyses data are the means of at least 3 determinations for each lipid with a maximum deviation for any single value deviating from the mean by more than ±7.5%. Lipid yield values are the mean of at least 2 separate extractions from each cell line with no single value deviating from the mean by more than ±11.1%.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Molecular wt</th>
<th>Elemental composition (wt %)</th>
<th>Component composition (wt %)</th>
<th>Spectroscopic analysis*</th>
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<tbody>
<tr>
<td>Ar</td>
<td>20,633</td>
<td>PAGE 14,700</td>
<td>54.9</td>
<td>13.7</td>
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<tr>
<td>Br</td>
<td>16,100</td>
<td>Method 1 10,200</td>
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<td>13.7</td>
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<tr>
<td>Bs</td>
<td>15,800</td>
<td>Method 2 9,300</td>
<td>56.1</td>
<td>13.7</td>
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Table 3  
Drug saturation of the macromolecular lipids in the absence and presence of spermine  
Drug saturation of each lipid was studied with a series of 11 biphasic assay mixtures containing increasing amounts of drug (1.7 to 670 μM) in the upper phase and a constant amount of lipid in the lower phase. After reaction, the amount of drug bound by the lipid at any drug concentration was determined by quantitating the amount of drug in the lower phase spectrophotometrically and correcting for normal drug partitioning into the lower phase from a control mixture which contained the drug at that concentration but no lipid. The amount of drug bound by the lipid at saturation and the Km values were determined from double reciprocal and Scatchard plots. The PAGEdetermined molecular weight (Table 2) of each lipid was used in the calculation of mol of drug bound per mol of lipid. Drug binding was also determined in the presence of spermine tetrahydrochloride at a final concentration of 16.7 μM in the upper phase. The amount of drug bound in each reaction mixture was again determined, and the resulting data were used to construct double reciprocal plots for drug binding in the presence of spermine.

<table>
<thead>
<tr>
<th>Lipid Ar</th>
<th>Lipid Br</th>
<th>Lipid Bs</th>
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<tr>
<td>Km (μM)</td>
<td>Kd (μM)</td>
<td>Kd (μM)</td>
</tr>
<tr>
<td>Drug bound* - Spermine + Spermine</td>
<td>Drug bound* - Spermine + Spermine</td>
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<tr>
<td>Cross-resistant to NSC 38280</td>
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<td>NSC 382843</td>
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<td>Anthracine glycoside</td>
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</tr>
<tr>
<td>NSC 278387</td>
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</tr>
</tbody>
</table>

a In 10% methanolic chloroform.  
b Based on the PAGE molecular weights.

d Drug bound (mol/mol lipid) at saturation.

e Activity against murine L1210 leukemia tumors (15).  
f NS, not saturable with drug at the concentrations used.

Table 4  
Lipid properties of the macromolecular lipids  
Lipid properties of the macromolecular lipids are notable since, to date, there are only 2 other reported instances of successful synthesis of affinity supports for use in organic solvents (6, 27). Observations that each lipid had the same binding characteristics for polyamine and the Cain quinolium NSC 113089, regardless of whether the latter compounds were bound to the Sephadex LH-20 support or not, show that drug:lipid-binding interactions are not inhibited by immobilization of a drug on the support. This premise is further supported by the observation that the lipids can be released from the affinity supports by compounds which they bind in vitro.

Preliminary characterization of the macromolecular lipids has shown them to be multicomponent. Standard assay procedures as well as UV-visible absorbance spectra, thin-layer chromatography, and PAGE indicate that phospholipid-, carbohydrate-, and amino acid-containing materials are present in the lipids and that the ratio of these components differs significantly between Lipids A and B. The component ratios must, however,
Macromolecular Lipids from L1210 Leukemia Tumors

Charts. Scatchard plots for the saturation of macromolecular Lipids Br (Plate 1) and Bs (Plate 3) with the aminoanthraquinone NSC 287513 illustrate the differences in binding site affinities for the drug by the 2 lipids and the presence of a second saturable binding site on Lipid Bs. Double reciprocal plots of NSC 287513 saturation of Lipids Br and Bs in the absence and presence of spermine are shown in Plates 2 and 4, respectively. Spermine acts as a competitive inhibitor at the drug-binding site(s) on both lipids.

be viewed with caution since they do not represent absolute values and, especially in the case of protein determinations, are prone to error from interference in the assay by the other components present (10, 27). The assays and resulting ratios do, however, provide proof for the presence of the components and a relative comparison method for the lipids. For example, Lipid A appears to contain either less phospholipid than does Lipid B and/or increased nitrogen-containing component. Since Lipid A has a larger molecular weight than does Lipid B, it is thus possible that Lipid A contains increased amounts of nonphosphorus-containing lipids and/or increased amounts of hydrophobic nitrogenous material (Table 2). Other studies on the drug-binding lipids from P388 leukemia cells either sensitive or resistant to the terephthalanilides have shown that the lipids from resistant tumors have increased hydrophobicity and molecular size (32, 34), indicating that the lipids from such cells also contain more lipid or hydrophobic material by weight. The PAGE and HP-GPC results confirm that the multicomponent macromolecular lipids are homogenous. Our studies thus do not allow us at this time to conclude which component(s) of

NOVEMBER 1981
the lipids contain the drug-binding site(s).

Drug binding by a macromolecular lipid does not, in itself, correlate with in vivo drug activity. For example, at the concentrations tested, the in vivo inactive aminoaanthraquinones bind to all 3 lipids at levels comparable to the in vivo active aminoaanthraquinones (Table 3). The lipid saturation characteristics of the drugs may, however, predict correlation with in vivo activity. Thus, the inactive aminoaanthraquinones are required in the highest concentration for saturation of Lipid Br and Site 1 of Lipid Bs and have the highest \( K_m \) values (i.e., the lowest affinity) of all the drugs with these lipids. Further, with the exception of NSC 281249, saturation of Lipid Ar and Site 2 of Lipid Bs is not possible with the inactive drugs. The binding of the active in vivo drugs of Lipids Br and Bs is also inhibited in a competitive manner by spermine. This is not the case in the binding of the inactive aminoaanthraquinones to Site 2 or Lipids Bs or any of the drugs to Lipid Ar. Rather, in the latter case, spermine displacement of the drug is noncompetitive in nature. These results may indicate that drug binding to the lipids must satisfy specific site concentration and orientation criteria in order to achieve in vivo activity.

The differences observed in drug binding by the macromolecular lipids from sensitive and resistant tumors also appear to correlate with in vivo drug activity. Previous studies on the uptake and retention of terephthalanilides in P388 leukemia tumors either sensitive or resistant to NSC 60339 (the free base of NSC 38280) have shown that sensitive cells retain higher intracellular drug concentrations and that such retention correlates with the chemotherapeutic activity of the drug (32, 35). Similar results have been reported in the case of Adriamycin activity on Adriamycin-sensitive and -resistant P388 tumors (13). Other studies have found that the drug-binding lipids from normal animal tissues have properties more similar to Lipid Bs than to Lipids Br or Ar. For example, lipid extracts from rat kidney, liver, heart, and skeletal muscle are all saturable with the Cain quinolinium NSC 113089 at levels of 30 nmol of drug per \( \mu \)g of phosphorus and with associated \( K_m \) values of 1.5 \( \mu \)M for each tissue (31). It is thus possible that Lipids Ar and Br are unique to the L1210 tumor which has acquired resistance to the terephthalanilides and related drug classes. This is supported by the reported data which show that Lipid Ar does not bind the bisquaternary ammonium heterocycles studied, all of which are inactive against the resistant tumor in vivo (25, 32). On the other hand, Adriamycin and the aminoaanthraquinones NSC 298513 and 279836 can saturate Lipid Ar and are effective in vivo against the resistant tumor.

The effects of spermine on drug binding to the macromolecular lipids raise questions concerning possible in vivo interactions of polyamino compounds with the macromolecular lipids. For example, the competitive inhibition by spermine of drug binding to Lipids Br and Bs indicates that the polyamine binds to these lipids at site(s) common to drug binding. Previous studies have shown that spermine can also displace drugs from macromolecular lipids isolated from normal tissues (31). Thus, further studies need to be directed at definition of the interactions between polyamino compounds and the macromolecular lipids. It is possible that the increased polyamine levels observed in cancer could relate to increased synthesis of the macromolecular lipids and their eventual derepression of DNA transcription (33). Our present studies indicate that the macromolecular lipids may represent a primary drug-binding site necessary for in vivo activity of antineoplastic agents. This is supported by the changes in drug-binding specificity between the macromolecular lipids in drug-sensitive and -resistant cell lines and by previous studies which have established that the lipids occur in the nucleoplasm and mitochondria of leukemia cells (23, 29, 34). In addition, while compounds such as the terephthalanilides bind to nucleic acids, proteins, and lipids in vitro (23, 33), they are extracted from normal and neoplastic tissues primarily as drug:lipid complexes (25, 32, 35). Further studies are now necessary to explain the function of these unique macromolecular lipids in both normal and neoplastic tissues.

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Drug-binding Macromolecular Lipids from L1210 Leukemia Tumors

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