INTERACTION OF LIVER PLASMA MEMBRANES AND GTP WITH GTP HYDROLYSIS

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SUMMARY: [^{14}C]GTP or a metabolic product of GTP binds to liver membranes. Less label was associated with membranes when membranes were incubated with increasing concentrations of carrier GTP; ATP did not displace the label. Chromatography of extracted incubation mixtures of [^{14}C]GTP and membranes revealed that over 96% of the nucleotide was hydrolyzed to 5'GMP and guanosine, Exposure of liver membranes to GTP prevented the separation of characteristic membrane bands that could be obtained when centrifugation was carried out without GTP. These studies indicate that GTP-effected alteration of liver plasma membranes is concomitant with GTP hydrolysis. These effects may be in addition to direct effects of GTP on enzymes and membrane proteins.

Guanosine triphosphate modifies the adenyl cyclase system of liver plasma (1-3), platelet (4) and thyroid (5) membranes. In the liver GTP enhances basal adenyl cyclase activity (1-3) as well as glucagon-(1,2) and epinephrine-(2) stimulated activities. Adenyl cyclase of platelet membranes requires GTP for activation by prostaglandin E_1 (4). It has been proposed that the nucleotide binds to sites in the membranes (1) and has allosteric effects (4) on catalytic subunits of the enzymes.

The present study was carried out to determine the nature of the GTP effects on liver membranes. To that end we have observed that GTP or a product of GTP hydrolysis binds to membranes. In the presence of GTP liver membranes aggregate and exhibit altered sedimentation properties.

Methods: Liver plasma membranes were prepared by the method of Neville (6) from livers of hypophysectomized rats, weighing 140-160 g, obtained from Charles River Laboratories. Membrane purification was carried out to Step 11 (6) from which membranes were obtained, that both in our hands (7) and as described elsewhere (1,2), exhibit sensitivity to GTP and glucagon. 5'-nucleotidase, used as a marker enzyme for plasma membranes, was assayed by the method of Song and Bodansky (8). Protein was determined by the method of Lowry et al (9).

GTP metabolism was evaluated by incubating guanosine[U-^{14}C]-triphosphate

(99.3% pure) obtained from Schwartz, with isolated membranes in 1 mM $NaHCO_3$. After incubation at 37° for 10 min in 25 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.5, 10% CCl_3COOH was added. GTP and its metabolites, GDP, 5'GMP, cyclic GMP and guanosine were extracted (10) into the acid which was removed by washing the extract six times with 5 vols each of water saturated diethylether. These were separated by thin layer chromatography in the LiCl system of Randerath and Randerath (11) on PEI-cellulose sheets, obtained from the Brinkmann Instrument Co. Strips of the plates corresponding to chromatographed lanes were cut out and the radioactivity thereon detected by liquid scintillation counting of 1 cm segments. Areas of radioactivity were compared to standards of labeled GTP and its metabolites run in parallel with the samples.

Results: Rat liver plasma membranes obtained by the Neville procedure bound radioactivity when incubated with [^{14}C]GTP (Fig. 1). The membranes exhibited similar binding properties if prepared in the presence of Ca^{2+} added to the 1 mM $NaHCO_3$ buffer during homogenization to increase the apparent yield of membranes (12). When radioactivity was diluted with unlabeled GTP less radioactivity from [^{14}C]GTP was associated with the membranes. As calculated from the specific activity of GTP, in the presence of the labeled, carrier-free nucleotide, 6.6 pmoles of GTP or a GTP metabolite bound per mg of membrane protein to membranes prepared without Ca^{2+}. Membranes prepared with Ca^{2+} bound 4.2 pmoles of GTP or a GTP metabolite per mg protein.

Partially purified membranes (Step 11, Ref. 6) were further fractionated to determine whether certain components present in the partially purified membranes bound radioactivity more specifically after incubation with [^{14}C]GTP. Instead of the 37%-3% sucrose gradient (6), sucrose layers were utilized. We had observed that in the sucrose gradient as described (6) two distinct membranes layers appeared at the 50%-37% sucrose interphase and one more in the 10% sucrose region. As seen in Fig. 2, by interposing a 10 ml layer of 37% sucrose over the 50% cushion, the two closely adjacent peaks were resolved and three fractions

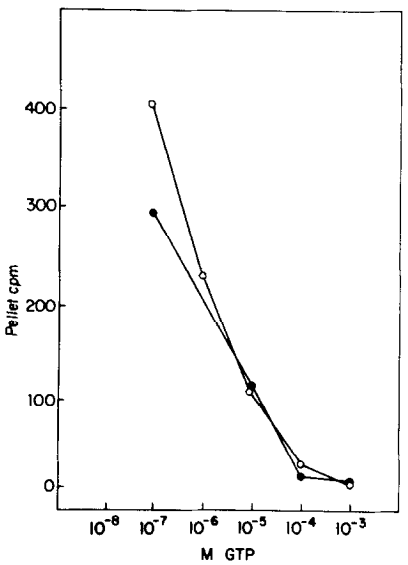

Fig. 1

Binding of radioactivity after incubating [^{14}C]GTP with partially purified rat liver plasma membranes. Membranes were prepared without Ca^{2+} as described (6) and with the addition of 0.5 mM Ca^{2+} (12). Membranes were incubated at 30° for 10 min with 0.2 μCi carrier-free [U-^{14}C]GTP (S.A. 355 mCi/mmole) and varying concentrations of GTP in 25 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.4 buffer. After incubation the samples were chilled, centrifuged at 12,000 x g for 15 min and the supernate was removed. The pellets were resuspended in 0.5 ml buffer (without GTP), centrifuged, and washed two times more. Radioactivity present in the entire pellet was measured by liquid scintillation counting. Each sample of the Ca^{2+}-free liver membrane contained 95 μg protein. Samples of membrane with Ca^{2+} contained 219 μg protein. ●----●, Ca^{2+}-free membranes; o----o, Ca^{2+} membranes.

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were obtained, one at each of the sucrose interphases 50%:37%; 37%:10%; 10%:buffer. 5'-nucleotidase which was used as a membrane enzyme marker (8) was present in the two denser membrane peaks at greater specific activities than the peak at the top. Cytochrome oxidase was present with the greatest specific activity in the top peak. The bulk of the membrane protein was present in the middle layer. When partially purified membranes were incubated with [^{14}C]GTP and centrifuged on sucrose layers, radioactivity was associated with membranes at 5.3 moles of GTP or a GTP metabolite per mg membrane protein (Fig. 3A). However,

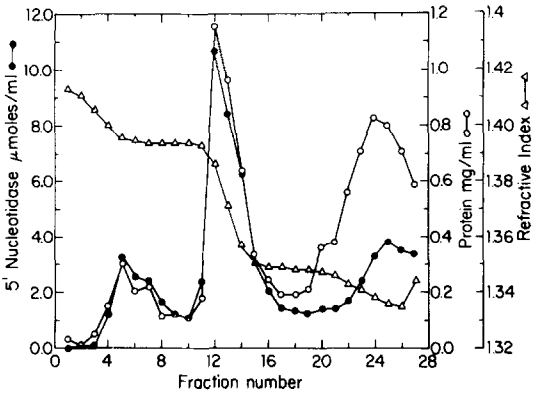

Fig. 2

Fractionation of partially purified rat liver plasma membranes. Partially purified membranes (Step. 11, ref. 6) were washed in 25 ml 1 mM $NaHCO_3$ and centrifuged at 25,000 x g for 10 min. The pellet containing 28.5 mg of protein was resuspended in 3 ml of 1 mM $NaHCO_3$ and applied to sucrose layers made up in SW-25.1 (Beckman Instrument Co.) tubes as follows: 50% sucrose, 4.1 ml; 37%, 10 ml; 10%, 10 ml. The membranes were centrifuged at 2000 x g for 1 hr in a Model L-2 Beckman ultracentrifuge. Fractions of 1 ml were collected from the bottom and assayed for protein and 5'-nucleotidase activity.

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instead of the three bands of membrane obtained upon centrifugation (Fig. 2) addition of GTP prevented the membranes from separating. The membranes sedimented as a major band to the 37%-10% sucrose interphase (Fig. 3A). The radioactivity from [^{14}C]GTP was not displaced and was not prevented from binding by ATP added to the incubation mixture (Fig. 3B).

[^{14}C]GTP was incubated with membranes to determine whether it was associated with the membranes as the nucleoside triphosphate or modified by its association with the membranes into metabolites of GTP. As seen in Fig. 4, over 90% of added GTP was converted to 5'GMP and guanosine. After a 10 min incubation 48.3% of the [^{14}C]GTP was hydrolyzed to 5'GMP, 43.2% to guanosine and 6% to GDP; 4% of the GTP remained unaltered.

The membrane fractions that were obtained by sucrose gradient centrifugation were incubated with each of the probable products of GTP hydrolysis: GDP, 5'GMP, cyclic GMP and guanosine. The partially purified membranes

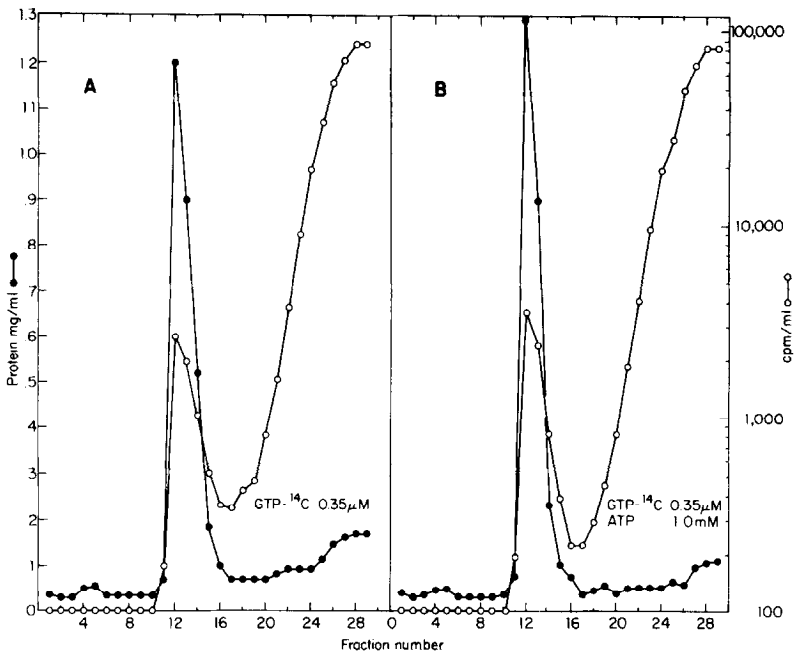

Fig. 3

Binding of radioactivity to membrane fractions after incubation with [^{14}C]GTP. Membranes, 28.5 mg of protein, were washed in 25 ml 1 mM $NaHCO_3$ and centrifuged at 25,000 x g for 10 min. The membrane pellet was resuspended in 4 ml 25 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.4, and divided into 2 samples, A and B. These were incubated at 37° for 10 min with 0.25 μCi (0.7 x 10^{-9} moles) GTP. To one sample only (B) were added 2 μmoles ATP. After incubation 1 ml of 1 mM $NaHCO_3$ was added to both samples which were centrifuged on sucrose as described in legend to Fig. 2. Fractions of 1 ml were collected and protein and radioactivity determined.

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and the fractions derived therefrom, converted GTP, GDP, and 5'GMP to guanosine. GTP and GDP were hydrolyzed to 5'GMP. Cyclic GMP was not readily metabolized by these membranes as 85% of the initial material was recovered.

Discussion: The present findings demonstrate that [^{14}C]GTP, or label originating therefrom, was bound to liver plasma membranes. The labeled nucleotide was displaced by added carrier nucleotide. Extraction and chromatography of GTP and its derivatives, obtained after a short incubation of membranes and the labeled nucleotide indicate that 96% of added GTP was rapidly converted to GDP, 5'GMP, and guanosine, either during binding or as a consequence of

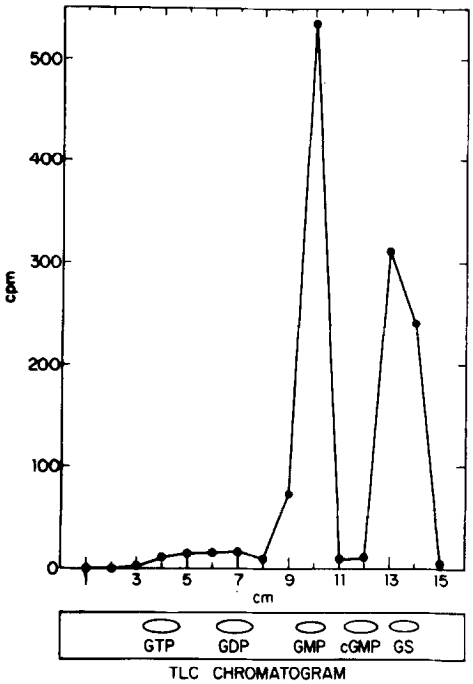

Fig. 4

Phosphorolysis of GTP by liver plasma membranes. Membranes, 1.3 mg protein, were incubated at 37° for 10 min with 0.275 μCi [^{14}C]GTP in 1 ml 25 mM Tris-HCl, pH 7.4, and 1 mM $MgCl_2$. After incubation 1 ml of 10% CCl_3COOH was added and the mixture kept at 4° for 1 hr. The sample was centrifuged and the CCl_3COOH was removed from the supernate by extraction six times with 5 vols each, of water-saturated diethylether. An aliquot of the membrane extract was applied to PEI-cellulose plates and developed in LiCl as described (11). The lanes were cut out into 1 cm segments and radioactivity thereon detected by liquid scintillation counting.

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binding. It is possible that GTP is metabolized, at least, by an ATPase that is present in liver plasma membranes that has specificity towards nucleotide-triphosphates (13).

We have demonstrated (3) that GTP, in addition to activating adenyl cyclase, modified liver plasma membrane ATPase and NADase activity, while effecting conformational changes in liver plasma membrane proteins (3,7). In related studies from this laboratory utilizing ε-ATP, a fluorescent analog of ATP, we observed changes in fluorescence in liver plasma membranes by added GTP (14). Thus,

it appears that GTP modifies overall structure of liver plasma membrane proteins and specific proteins which leads to altered catalytic properties.

Acknowledgements

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