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Sulfhydryl groups in glycolipid transfer protein: formation of an intramolecular disulfide bond and oligomers by Cu^{2+}-catalyzed oxidation

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Glycolipid transfer protein (GLTP) purified from pig brain facilitates the transfer of various glycolipids between lipid bilayers. Purified GLTP migrates as two bands of different mobility in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions. The slower component and the faster component constituted about 80% and about 15%, respectively, of purified GLTP. Treatment of GLTP with 45 μM $CuSO_4$ resulted in a decrease in the slower component, an increase in the faster component, and the formation of oligomeric components. The faster and oligomeric components were quantitatively converted to the slower component by reduction with 2% 2-mercaptoethanol in the presence of 1% SDS. The formation of oligomeric components was enhanced by increasing the concentration of $CuSO_4$ to 450 μM and 4.5 mM. Oxidation of GLTP catalyzed by $CuSO_4$ resulted in a decrease in the transfer activity and an increase in the apparent binding affinity of GLTP to 1-*O*-(β-D-galactopyranosyl)-*N*-[10-(1-pyrenyl)decanoyl]-D-*erythro*-sphingosine (PyrGalCer). The oligomeric components and the monomeric components were isolated by chromatography on a Sephadex G-75 column. It was found that GLTP in fractions enriched with the monomeric components had very high transfer activity and is responsible for most of the transfer activity in the oxidized GLTP. Treatment of GLTP with 1.27 mM $HgCl_2$ resulted in a formation of components unresolvable on SDS-PAGE and also resulted in a reduction of the transfer activity to one-third. However, no obvious change in the binding affinity of GLTP to PyrGalCer was observed by $HgCl_2$ treatment. Treatment with 2-mercaptoethanol restored the activity of GLTP inactivated by $HgCl_2$, whereas the activity inactivated by $CuSO_4$ was not restored by treatment with 2-mercaptoethanol. These results suggest that the transfer activity depends on the turnover rate of the GLTP-PyrGalCer complex which is affected by modification of sulfhydryl groups of GLTP. The sulfhydryl group content of GLTP was estimated by the use of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). A value of 2.2 mol sulfhydryl groups per mol of GLTP was found in the presence of 0.5% SDS and one sulfhydryl group in a GLTP molecule was very rapidly oxidized in the native state, from which it is assumed that the slower component contains three sulfhydryl groups per GLTP molecule and the faster component contains one sulfhydryl group and one disulfide bond per GLTP molecule.

Introduction

Glycolipid transfer protein (GLTP) facilitates the transfer of glycolipids between lipid bilayers [1–4]. The protein has been found in various animal tissues. GLTP in some tissues was partially purified and the properties were studied [1,2,4,5]. We purified GLTP to apparent homogeneity from pig brain [6]. The transfer activity of GLTP purified from pig brain was inhibited by treatment with *N*-ethylmaleimide (NEM) and $HgCl_2$ [7]. Metz and Radin also reported the same results on GLTP partially purified from bovine spleen [1,8]. It is possible that the transfer activity of GLTP is regulated by oxidation and reduction of sulfhydryl groups present in GLTP.

In this paper, we studied the formation of disulfide bond in GLTP by oxidation in the presence of $CuSO_4$

Abbreviations: GLTP, glycolipid transfer protein; PyrGalCer, 1-*O*-(β-D-galactopyranosyl)-*N*-[10-(1-pyrenyl)decanoyl]-D-*erythro*-sphingosine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); *E*/*M* ratio, eximer/excited monomer ratio.

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and the effect of oxidation on the transfer activity of GLTP. Cu^{2+}, a transition metal ion, catalyzes oxidation of sulfhydryl groups in protein by O_2 [9]. The oxidation catalyzed by Cu^{2+} is mild and accelerates the formation of intra- and/or inter-molecular disulfide bonds between suitably placed sulfhydryl groups.

Materials and Methods

Materials. PyrGalCer was purchased from Sigma Chemical Co. (St. Louis). PyrGalCer was kept in chloroform/methanol (2:1, v/v) at −20°C in the dark. The concentration of PyrGalCer was determined by measuring the absorbance at 343 nm in ethanol. A molar absorption coefficient of 50000 $M^{-1} \cdot cm^{-1}$ was used. Phosphatidylcholine from rat liver was prepared by the method described previously [10].

Preparation of lipid vesicles. Small unilamellar vesicles were prepared by ethanol injection technique [11]. An ethanolic solution of lipids was injected into buffer A (0.15 M NaCl, 20 mM Tris-HCl (pH 7.4), 1 mM EDTA). The vesicles thus prepared were dialyzed against the same buffer at room temperature in order to remove ethanol. In the assay of transfer activity, donor vesicles were prepared as follows; a lipid mixture (170 nmol) of PyrGalCer and rat liver phosphatidylcholine at a molar ratio of 9:91 was dissolved in 35 μl ethanol and the ethanolic solution was injected into 3 ml of buffer A. Acceptor vesicles were prepared by injecting 1643 nmol of rat liver phosphatidylcholine in 70 μl of ethanol into 3 ml of buffer A.

Fluorescence measurement. The measurement was carried out essentially as described previously with a Hitachi 650-10S spectrofluorometer equipped with thermostatically controlled cuvett holder [7]. In the assay of transfer reaction, 100 μl each of the PyrGalCer-containing donor vesicles and the acceptor vesicles were mixed with 2 ml of buffer A. This mixture gives a vesicle suspension with 27 nmol of lipids/ml and an acceptor-to-donor vesicle ratio of 9.66 in lipid molar ratio. Transfer reaction was started by the addition of GLTP. Emission spectra of PyrGalCer were recorded at suitable time intervals by scanning from 360 to 600 nm. The excitation wavelength was fixed at 347 nm. The slit at excitation was 2 nm and that at emission was 10 nm. Eximer emission intensity (E) at 475 nm and excited monomer emission intensity (M) at 398 nm were determined from peak height. The transfer of PyrGalCer from donor to acceptor vesicles was measured by a decrease in the E/M ratio [7]. The transfer rate was determined by the equations developed by Roseman and Thompson [12] and Correa-Freire et al. [13].

The formation of GLTP-pyrene labeled glycolipid complex was shown by Abe et al. [7] and Brown et al. [14]. They observed that the decrease of the E/M ratio brought about by the addition of GLTP was dependent on the amount of GLTP [7,14]. The assay of binding reaction was carried out as described above but in the absence of acceptor vesicles.

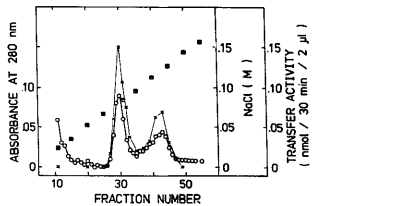


Fig. 1. Elution pattern of GLTP from a phosphocellulose column. Protein (3.6 mg) obtained from Phenyl-Sepharose chromatography step [6] was applied to a phosphocellulose column (1.4×8 cm) equilibrated with 10 mM sodium phosphate (pH 6.0), 1 mM EDTA. GLTP was eluted from the column with 240 ml of linear gradient of NaCl from 0 to 200 mM in 10 mM sodium phosphate (pH 6) and 1 mM EDTA. Fractions of 3.5 ml were collected and 2-μl aliquots were assayed for PyrGalCer transfer activity (×). The assay was carried out at 20°C for 30 min. Absorbance at 280 nm (○) and NaCl concentration (■) were shown.

Purification of GLTP. GLTP was purified from pig brain by the method described previously [6]. Fig. 1 shows the elution profile of GLTP from a phosphocellulose column, which was used in the final step of purification. Elution of the column with sodium chloride gradient separated applied protein into two protein peaks (fractions 1 and 2), both of which showed the transfer activity of PyrGalCer. Both of these peaks contained the same major protein with estimated molecular mass of 22 kDa when analyzed by SDS-PAGE [15]. The protein(s) present in these peaks was found capable of binding PyrGalCer because an immediate drop in E/M ratio was observed on mixing each of the two protein fractions with the donor vesicles containing PyrGalCer. In the present studies, fraction 1 (fractions 28–32) in Fig. 1 was used as purified GLTP. Protein was determined by the method of Bensadoun and Weinstein [16].

Oxidation of GLTP. Purified GLTP was dialyzed against 0.15 M NaCl, 20 mM Tris-HCl (pH 7.4). A portion (250 μl) of the dialyzed GLTP (143 μg/ml) was incubated in the presence of various concentrations of $CuSO_4$ in a polyallomer tube (9 × 60 mm). The incubation was done at 20°C with vigorous shaking in a reciprocal shaker.

Isolation of the monomeric component and oligomeric components of GLTP. Purified GLTP (530 μg) was incubated with 450 μM $CuSO_4$ for 4 h at 20°C. The reaction mixture (1 ml) was applied to a Sephadex G-75 column (1.2 × 45 cm) and chromatographed in a cold room. The chromatography was carried out in 0.15 M

NaCl, 20 mM Tris-HCl (pH 7.4) at a flow rate of 10 ml/h. Fractions of 1 ml was collected. The GLTP activity in the collected fraction was determined by the fluorimetric assay.

Determination of the free sulfhydryl content in GLTP with DTNB. Before DTNB assay, the purified GLTP was chromatographed on a PD-10 (Pharmacia) column pre-equilibrated with 50 mM Tris-HCl, pH 8, 1 mM EDTA. The chromatography was carried out in the same buffer at room temperature. Fractions of 1 ml were collected. The content of free sulfhydryl group in GLTP was determined according to the method of Fernandez Diez et al. [17]. In the determination of total sulfhydryl groups, the reaction mixture contained 760 μl of GLTP (90.7 μg) and 40 μl of 10% SDS. Reference solution contained all the reagents except protein. Reaction was started by the addition of 6.67 μl of DTNB solution (40 mg of DTNB in 10 ml of 0.1 M sodium phosphate, pH 7) to the reaction mixture. The absorbance at 412 nm was followed as a function of time. To calculate the content of sulfhydryl groups in GLTP, a molar absorption coefficient of 13600 at 412 nm was used.

Results and Discussion

Effect of copper ion on GLTP

Purified GLTP consisted of a major band of the slower component and a minor band of the faster component (Fig. 2a). The major band accounted for 75–85% of GLTP and the minor band about 15%. Boiling of GLTP in a SDS-PAGE sample buffer under nonreducing conditions resulted in an increase in the faster component (data not shown). Boiling of GLTP in an SDS-PAGE buffer containing 2% 2-mercaptoethanol converted all faster component to the slower component (Fig. 2a). We interpreted these results in the following way: the formation of an intramolecular disulfide bond in the slower component of GLTP resulted in the formation of a compact form of GLTP, the faster component. This was proved by experiments described below. No significant change was observed in protein banding on SDS-PAGE after incubation of GLTP at 20 °C for 4 h in the absence of 2-mercaptoethanol (Fig. 2a).

The activity of GLTP is inactivated partially by NEM and completely by $HgCl_2$ [7]. It has been shown that metal cations, particularly Cu^{2+}, catalyze the oxidation of sulfhydryl groups in protein by O_2, which results in the formation of intra- and inter-molecular disulfide bonds [9]. GLTP was incubated with various concentrations of $CuSO_4$ at 20 °C for 32 min, 105 min, 174 min, and 244 min, after which EDTA was added and GLTP was analyzed by SDS-PAGE under non-reducing conditions. Incubation of GLTP with 45 μM $CuSO_4$ resulted in a decrease in the slower component, an increase in the faster component and the formation of oligomeric components (Fig. 2c). These changes became progressively evident in the course of incubation up to 4 h. The main component of oligomers was dimers, which were found as doublet bands in SDS-PAGE (Fig. 2c). Reduction of the oxidized GLTP with 2-mercaptoethanol in SDS-PAGE sample buffer almost completely converted the faster component and oligomers to the slower component (Fig. 2c). When the concentration of $CuSO_4$ in incubation mixture was lowered to 4.5 μM, no change in GLTP mobility on SDS-PAGE was observed even after incubation for 4 h at 20 °C (Fig. 2b).

When GLTP was incubated with 450 μM $CuSO_4$, the

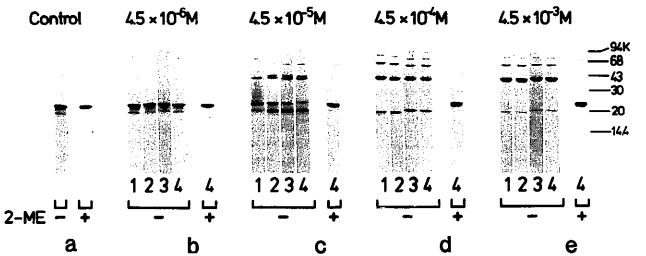


Fig. 2. Formation of disulfide bridges by the addition of $CuSO_4$ to GLTP. GLTP was incubated in the presence of various concentrations of $CuSO_4$ as described under Materials and Methods and analyzed by SDS-PAGE according to the method of Laemmli and Favre [15]. The upper figures indicate concentrations of $CuSO_4$. GLTPs applied to lanes 1–4 were incubated for different times: lane 1, 31.5 min; lane 2, 105 min; lane 3, 174 min; lane 4, 244 min. The samples for SDS-PAGE contained 3.6 μg of protein per one lane, 1% SDS, 0.005% Bromophenol blue, and 2.5% 2-mercaptoethanol (2-ME) where indicated. The samples were not boiled.

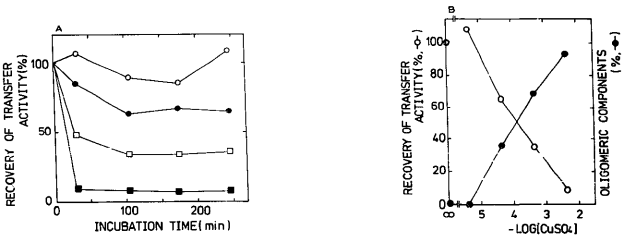


Fig. 3. Effect of copper ion concentrations on the transfer activity and oligomer formation of GLTP. Fig. 3A shows time course of the transfer activity of GLTP in the presence of various concentrations of copper ion. The activity was measured at 25 °C in 0.15 M NaCl, 20 mM Tris-HCl (pH 7.4). Concentrations of $CuSO_4$ were as followed: ○, 4.5 μM; ●, 45 μM; □, 450 μM; ■, 4.5 mM. Fig. 3B shows recovery of the transfer activity of GLTP (○) and the amounts of oligomeric components of GLTP (●) after oxidation for 244 min. The fluorimetric assay was carried out as described under Materials and Methods.

slower component disappeared completely, and the formation of oligomers (dimers, trimers etc.) was markedly enhanced (Fig. 2d). Change in protein banding as revealed by SDS-PAGE was complete by 30 min incubation. When GLTP was incubated with 4.5 mM $CuSO_4$, the slower component disappeared completely with concomitant formation of dimer (Fig. 2e). The faster component was formed in only a small amount and the formation of trimer was also small. All components formed by incubation with 450 μM and 4.5 mM $CuSO_4$ were converted to the slower component by reduction with 2% 2-mercaptoethanol in SDS-PAGE sample buffer (Fig. 2d, e). Fig. 3B summarizes the extent of oligomers formation after 4 h of oxidation in the presence of various cupric ion concentrations. Incubation of GLTP with 450 μM of Zn^{2+}, Mn^{2+}, or Ca^{2+} did not accelerate the formation of intra- or inter-molecular disulfide bond (data not shown).

These results suggest that the faster component was produced from the slower component not by a proteolytic degradation but by the formation of an intramolecular disulfide bond because all faster components were converted to the slower component by reduction. It is also clear that oligomeric components are produced from monomeric components by the formation of intermolecular disulfide bond(s).

Effect of copper ion on GLTP activity

We studied the effect of oxidation of GLTP in the presence of copper ion on the transfer and binding activities of GLTP. The transfer activity of purified GLTP was found to be stable at least for 8 h at 20 °C. When GLTP was incubated with 4.5 μM $CuSO_4$, there was no significant change in the transfer activity of GLTP even after incubation for 4 h (Fig. 3A). Incubation of GLTP with higher concentration of copper ion resulted in a decrease in the transfer activity (Fig. 3). The transfer activity of GLTP was reduced to 60% of the control value when incubated with 45 μM $CuSO_4$, to 40% when incubated with 450 μM $CuSO_4$, and to 10% when incubated with 4.5 mM $CuSO_4$. It is obvious that there is a correlation between a decrease in transfer activity and the formation of oligomers.

In a previous study, we showed complex formation between GLTP and [^{3}H]GalCer and the transfer of [^{3}H]GalCer from the complex to liposomes [6]. Thus, GLTP-GalCer complex functions as an intermediate in the transfer reaction. The rate of glycolipid transfer reaction is therefore dependent on the dissociation constant of the intermediate and the turnover rate of the intermediate. We isolated GLTP-PyrGalCer complex by a Sephadex G-75 chromatography and showed the transfer of PyrGalCer from the complex to liposomes (data not shown). The spectrum of GLTP-PyrGalCer complex lacked the eximer fluorescence of PyrGalCer (data not shown). Therefore, the complex is most likely one-to-one complex of GLTP and PyrGalCer. A marked decrease of E/M ratio was immediately observed after the addition of GLTP to the vesicles containing PyrGalCer [7]. This decrease was taken to indicate the formation of GLTP-PyrGalCer complex, and change in E/M ratio was taken as a measure of the amount of the GLTP-PyrGalCer complex. Thus, any change in the binding affinity of GLTP to PyrGalCer can be monitored by measuring the E/M ratio [7].

The decrease in E/M ratio following the addition of GLTP treated with high concentration of $CuSO_4$ to the vesicles containing PyrGalCer was larger than that of the control GLTP (data not shown). This result suggests that a complex between oligomeric GLTP and PyrGalCer is more stable than the complex formed by monomeric GLTP.

Isolation of monomeric and oligomeric GLTP by chromatography on a Sephadex G – 75 column

The results described above suggest that monomeric components (faster and slower) and oligomeric components are different in the transfer and binding of PyrGalCer. The faster component and oligomeric component were isolated by subjecting oxidized GLTP to a Sephadex G-75 chromatography (Fig. 4). The oxidized GLTP was prepared by incubating 530 μg of GLTP with 450 μM $CuSO_4$ at 20 °C for 4 h. Most oligomeric components were eluted in the fractions from 21 to 30, which contained low transfer activity (Fig. 4). Most of the transfer activity was found in the fractions 34–36, which corresponds to the fractions most enriched in the faster component (Fig. 4). Absorption at 280 nm by the fractions 53–67 is due to the elution of $CuSO_4$ in these fractions (Fig. 4). Fraction 28, in which most of the GLTP was found to be oligomeric, was assayed for binding of PyrGalCer. It was found that there was a marked difference in the PyrGalCer binding between GLTP in fraction 28 and the control GLTP when the binding was determined by the decrease in E/M ratio upon GLTP addition (data not shown). GLTP in fraction 28 had a high binding affinity to PyrGalCer and a very low transfer activity (Fig. 4). Thus the transformation of GLTP from monomers to oligomers by the formation of intermolecular disulfide bond(s) results in a decrease in the turnover rate of the GLTP-PyrGalCer complex and in a decrease in the transfer rate.

We have confirmed in primary experiments that reduction of the oxidized GLTP (enriched in oligomer) by 2-mercaptoethanol in the absence of denaturing reagent does not lead to the complete conversion of oligomeric components to the slower component: we have observed that the GLTP after reduction consists of about 30% of oligomeric components and about 70% of monomeric components (data not shown). The monomeric components seem to be the species containing an intramolecular disulfide bond on a native PAGE according to the method of Reisfeld et al. [18] (data not shown). It is useful to propose two structures of GLTP (Fig. 5) postulated from the results shown in Fig. 7. We think that the transfer activity of GLTP containing an unnatural disulfide bond must be considerably lower than that of GLTP containing a natural disulfide bond (form B in Fig. 5). The results shown in Fig. 3B and those shown in Fig. 4 indicate that the faster component produced by Cu^{2+}-catalyzed oxidation may consist to a large extent of a form containing an unnatural intramolecular disulfide bond.

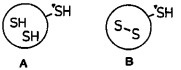
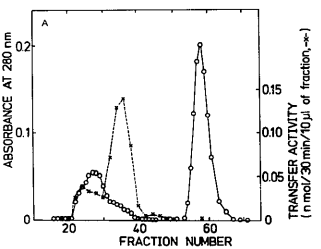


Fig. 5. The postulated structures of GLTP. Purified GLTP from pig brain is constituted from about 80% of form A and about 15% of form B. Form A and form B correspond to the slower component and the faster component, respectively, on SDS-PAGE under nonreducing conditions. SH is a sulfhydryl group reactive with DTNB under nondenaturing conditions.

Comparison of the effect of copper in and mercury ion on GLTP

Purified GLTP was incubated with 1.27 mM of $CuSO_4$ or $HgCl_2$ for 4 h at 20 °C. The oxidation was terminated by the addition of EDTA and GLTP was subjected to SDS-PAGE under nonreducing conditions.



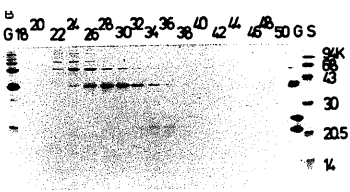


Fig. 4. (A) Sephadex G-75 chromatography of the oxidized GLTP. GLTP was oxidized in the presence of $CuSO_4$. Oxidation was carried out in 0.15 M NaCl, 20 mM Tris-HCl (pH 7.4) in the presence of 450 μM $CuSO_4$ under vigorous shaking for 4 h at 20 °C. Oxidized GLTP (0.53 mg in 1 ml) was applied to a Sephadex G-75 column (1.2×45 cm) and eluted with 0.15 M NaCl, 20 mM Tris-HCl (pH 7.4). 1-ml fractions were collected. Absorbance at 280 nm (○) and transfer activity (×) were shown. (B) SDS-PAGE [15] analysis of each fraction: each sample (35 μl) applied to the lanes numbered from 18 to 50 contained a 23.3-μl portion of the numbered fraction obtained in the chromatography, 1% SDS, and 0.005% Bromophenol blue. The SDS-PAGE samples were not boiled. GLTP pretreated with $CuSO_4$ was applied to lane G (left). Lane G (right) appears to be partially reduced by 2-mercaptoethanol leaked out from standard sample (lane S) during electrophoresis.

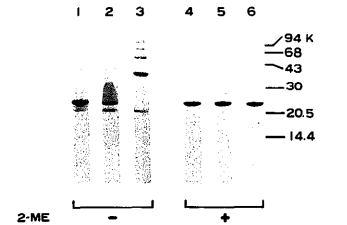


Fig. 6. Effect of mercuric ion on GLTP. GLTP was incubated with either 1.27 mM $HgCl_2$ or $CuSO_4$ for 4 h at 20 °C. After addition of 9.1 mM EDTA, the treated GLTP (3.6 μg) was analyzed by SDS-PAGE under the same conditions as shown in Fig. 2. The samples on the lanes 1–3 contained no 2-mercaptoethanol whereas the samples on the lanes 4–6 contained 2.5% 2-mercaptoethanol. Lanes 1 and 4, control GLTP; lanes 2 and 5, $HgCl_2$-treated GLTP; lanes 3 and 6, $CuSO_4$-treated GLTP.

On incubation with $CuSO_4$, the slower component disappeared completely and large amounts of oligomers were formed as described above (Fig. 6). On the other hand, incubation with $HgCl_2$ produced a diffuse component above 22 kDa in addition to slower and faster components, all of which were completely converted to the slower component by treatment with 2% 2-mercaptoethanol in SDS-PAGE sample buffer (Fig. 6).

About 15% and 25% of the original transfer activity was found after incubation of GLTP with $CuSO_4$ and $HgCl_2$, respectively (data not shown). Reduction with 2-mercaptoethanol restored the inactivation caused by

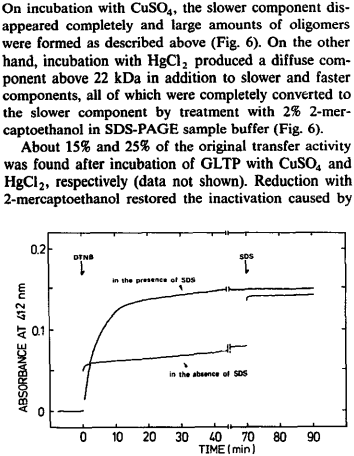


Fig. 7. Spectrophotometric determination of sulfhydryl groups of GLTP. DTNB assay was carried out as described under Materials and Methods. The reaction was started by the addition of 6.67 μl DTNB solution (40 mg DTNB in 10 ml of 0.1 M sodium phosphate (pH 7)) to 800 μl GLTP (90.7 μg) in 50 mM Tris-HCl (pH 8.0), 1 mM EDTA in the presence or absence of 0.5% SDS. Absorbance at 412 nm was followed as a function of time. In the time course of the reaction in the absence of SDS, 40 μl 10% SDS was added to the reaction mixture at the point indicated by an arrow.

$HgCl_2$. However, the inactivation caused by $CuSO_4$ was not restored by reduction with 2-mercaptoethanol (data not shown). Addition of 8.55 μg of GLTP treated with $HgCl_2$ to the vesicles containing 0.46 nmol PyrGalCer resulted in a decrease in E/M ratio of PyrGalCer, which was slightly larger than the change in E/M ratio found by the use of untreated GLTP under the same conditions (data not shown).

These results suggest that the treatment of GLTP with $HgCl_2$ produces mercaptides or S-Hg-S bond(s) between sulfhydryl groups and Hg. This results in a decrease in the turnover rate of the GLTP-PyrGalCer complex and a strong inhibition of the transfer activity of GLTP.

Quantitative analysis of sulfhydryl groups in GLTP

Free sulfhydryl groups present in purified GLTP were determined by the method of Fernandez Diez et al. [17]. Fig. 7 shows that 2.2 mol sulfhydryl groups per mol GLTP were titrated in the presence of 0.5% SDS. It was found that one mol sulfhydryl group out of 2.2 mol reacted immediately with DTNB under nondenaturing conditions (Fig. 7). Pretreatment of GLTP with NEM under nondenaturing conditions prevent oligomer formation by subsequent oxidation catalyzed by $CuSO_4$. Therefore, the sulfhydryl group reactive with DTNB and NEM under nondenaturing conditions seems to participate in the formation of intermolecular disulfide bond.

In the presence of 0.5% SDS, the DTNB method gave a value of 3.7 mol sulfhydryl groups per mol ovalbumin. This value corresponds to about 90% of total sulfhydryl groups in an ovalbumin. The measured value of sulfhydryl group in GLTP, 2.2 residues per molecules, seems to be explained by the heterogeneity of GLTP, the slower (form A in Fig. 5) and faster (form B in Fig. 5) components. Densitometry indicates the ratio of form A and form B to be about 80 : 15. Therefore, it is assumed that form A contains three sulfhydryl groups per GLTP molecule and form B contains one sulfhydryl group and one disulfide bond per GLTP molecule (Fig. 5). The functional and structural implications of intramolecular disulfide bond in GLTP are the subject of the following paper.

Acknowledgments

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References

1 Metz, R.J. and Radin, N.S. (1980) J. Biol. Chem. 255, 4463–4467.
2 Yamada, K. and Sasaki, T. (1982) Biochim. Biophys. Acta 687, 195–203.

3 Abe, A., Yamada, K. and Sasaki, T. (1982) Biochem. Biophys. Res. Commun. 104, 1386–1393.
4 Wong, M., Brown, R.E., Barenholz, Y. and Thompson, T.E. (1984) Biochemistry 23, 6498–6505.
5 Gammon, C.M., Vaswani, K.K. and Ledeen, R.W. (1987) Biochemistry 26, 6239–6243.
6 Abe, A. and Sasaki, T. (1985) J. Biol. Chem. 260, 11231–11239.
7 Abe, A., Yamada, K., Sakagami, T. and Sasaki, T. (1984) Biochim. Biophys. Acta 778, 239–244.
8 Metz, R.J. and Radin, N.S. (1982) J. Biol. Chem. 257, 12901–12907.
9 Liu, T.-Y. (1977) in The Protein (Neurath, H., Hill, R.L. and Boeder, C.-L., eds.), Vol. 3, pp. 240–402, Academic Press, New York.
10 Sasaki, T. and Sakagami, T. (1978) Biochim. Biophys. Acta 512, 461–471.
11 Batzri, S. and Korn, E.D. (1973) Biochim. Biophys. Acta 298, 1015–1019.
12 Rosenman, M.A. and Thompson, T.E. (1980) Biochemistry 19, 439–444.
13 Correa-Freire, M.C., Barenholz, Y. and Thompson, T.E. (1982) Biochemistry 21, 1244–1248.
14 Brown, R.E., Stephenson, F.A., Markello, T., Barenholz, Y. and Thompson, T.E. (1985) Chem. Phys. Lipids 38, 79–93.
15 Laemmli, U.K. and Favre, M. (1973) J. Mol. Biol. 80, 575–599.
16 Bensadoun, A. and Weinstein, D. (1976) Anal. Biochem. 70, 241–250.
17 Fernandez Diez, M.J., Osuga, D.T. and Feeney, R.E. (1964) Arch. Biochem. Biophys. 107, 449–458.
18 Reisfeld, R.A., Lewis, U.J. and Williams, D.E. (1962) Nature (Lond.) 195, 281–283.