Preparation, Purification, and Characterization of a Reversibly Lipidized Desmopressin with Potentiated Anti-Diuretic Activity

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Purpose. To prepare and characterize a reversibly lipidized dipalmitoyl desmopressin (DPP), and to compare its anti-diuretic efficacy and biodistribution with that of unmodified desmopressin (DDAVP).

Methods. Dithiothreitol (DTT) was used to reduce the intramolecular disulfide bond in DDAVP, and the reduced DDAVP was treated with a thiopyridine-containing disulfide lipidization reagent, Pal-CPD. The product, DPP, was purified by acid precipitation and, subsequently, by size-exclusion chromatography. Reversed-phase HPLC was used to analyze the purity and to evaluate the hydrophobicity of the product. Mass spectrometry was employed to characterize its molecular structure. The biological activity of DPP was demonstrated by the anti-diuretic effects in vasopressin-deficient Brattleboro rats. Preliminary pharmacokinetic and biodistribution studies of intravenously injected DDAVP and DPP were carried out in CF-1 mice.

Results. DDAVP was readily reduced by a 2-fold molar excess of DTT at 37°C for 0.5 hr. DPP was formed by the reaction of reduced DDAVP with Pal-CPD. Each DPP molecule contains two palmitic acid moieties, which link to the peptide *via* two disulfide bonds. After acid precipitation and size-exclusion chromatography, the purity was found to be approximately 95%, and the overall yield was 57%. When DPP was administered subcutaneously to Brattleboro rats, the potency of the anti-diuretic activity of DDAVP was enhanced to more than 250-fold. The plasma concentration of intravenously injected DDAVP in mice decreased rapidly during the first 20 min and followed by a slow elimination rate. However, in DPP administered mice, the plasma concentration actually increased in the first 20 min, followed by a slow elimination with a rate similar to that in DDAVP-injected mice. The regeneration of DDAVP was detected in the plasma of mice treated with DPP. Studies of the organ distribution in mice indicated that the liver retention of DPP was longer than that of DDAVP. On the other hand, the intestinal excretion of DPP was significantly less than that of DDAVP.

Conclusions. The 250-fold increase of the anti-diuretic potency in DPP is most likely due to a slow elimination and prolonged tissue retention, together with the regeneration of active DDAVP, in the animals. Our results indicate that reversible lipidization is a simple and effective approach for improving the efficacy of many peptide drugs.

KEY WORDS: desmopressin; palmitic acid; conjugate; anti-diuretic activity.

INTRODUCTION

An increasing number of new peptides have emerged from the biotech industry as drug candidates for the treatment of various human diseases, posing a delivery challenge to pharmaceutical scientists (1,2). Problems associated with using peptides as drugs include their enzymatic and/or chemical degradation, and their rapid elimination by the kidneys. These problems often result in short half-lives and, consequently, short duration of action. In addition, because of their large molecular size, instability, and hydrophilicity, peptides are difficult to encapsulate in certain types of drug carrier systems such as liposomes and microspheres (3).

One simple approach to overcome these problems is to increase the lipophilicity of the peptide by conjugating with one or several lipid moieties. This conjugation, known as lipidization, is usually accomplished by forming an amide bond between a carboxyl group of a lipid molecule and an amino group of a peptide. Fatty acids and phospholipids have been used previously as lipid moieties to modify peptides such as thyrotropin-releasing hormone (4), tetragastrin (5), insulin (6), and inhibitors of the HIV protease (7). It has been reported that lipidization can increase gastrointestinal absorption (5), enhance transport across blood-brain barrier (8,9), decrease proteolytic degradation (8,10), and prolong plasma half-life (7,11) of peptide drugs. However, due to the irreversibility of the linkage and hydrophobicity of the lipid moiety, products of lipidization often have limited water solubility and are generally less potent when compared with the native peptide (12).

Recently, we have reported a novel reversible lipidization method for the modification of Bowman-Birk protease inhibitor (BBI), an 8 kDa polypeptide isolated from soybean and a potential cancer chemopreventive agent (13). The lipidization reagent, *N*-palmitoyl cysteinyl 2-pyridyl disulfide (Pal-CPD), is structurally designed to be water soluble and thiol-reactive. The palmitic acid-peptide conjugate is formed by a reversible thiol-disulfide exchange reaction between the reagent and thiol-modified BBI (13). As expected, the product was water soluble and the anti-carcinogenic activity was preserved (13). When injected intravenously to mice, the lipidized BBI showed a great improvement on plasma half-life and stability (11), and consequently, a more than 10-fold increase in the area under the curve (AUC) when compared with the original polypeptide (11). These findings prompted us to extend our studies to other peptide drugs for improving therapeutic efficacies. In this report, we present our findings on the lipidization of desmopressin (DDAVP).

DDAVP is a therapeutic peptide widely used for treating diabetes insipidus, primary nocturnal enuresis, hemophilia and Type I Von Willebrand's disease (14). It also has the ability to improve human memory functions (15). Structurally, it is an analog of naturally occurring arginine vasopressin (AVP), in which the terminal amino group is removed and the amino acid residue Arg8 is replaced by *D*-Arg (Fig. 1) (16,17). Even though the stability of DDAVP is remarkably increased due to the elimination of the α-amino group and the introduction of *D*-Arginine, the half-lives of DDAVP in patients are still short (7.8 and 75.5 min for the fast and slow phases, respectively) and daily administration are required to obtain optimal effects (14). An analog with longer duration of action would minimize the number of injections, reduce the medical costs, and improve patient compliance. The existence of a disulfide bond in the molecule of DDAVP makes it a suitable candidate for reversible

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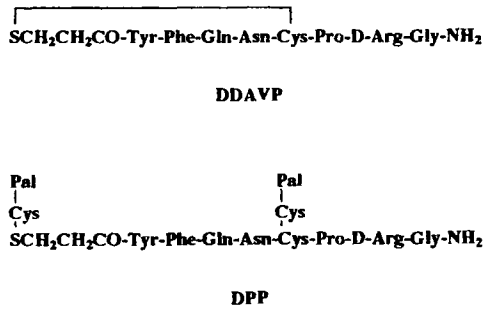

Fig. 1. Structure of desmopressin (DDAVP) and DPP. Pal-Cys: *N*-palmitoyl-cysteine which is linked to the peptide backbone *via* a disulfide bond.

lipidization using Pal-CPD. In this report, we describe the synthesis of palmitic acid conjugate of DDAVP. The lipidized DDAVP was used as an anti-diuretic drug in Brattleboro rats which express an inhereditary hypothalamic diabetes insipidus due to the deficiency in vasopressin genes and are widely used as an *in vivo* animal model for the human disease of diabetes insipidus (18).

MATERIALS AND METHODS

Materials

DDAVP was purchased from Penta Biotech (Foster City, CA). Cysteine hydrochloric acid, dithiothreitol, dithiopyridine, palmitic acid and Sephadex® G-15 and G-25 gels were obtained from Sigma (St. Louis, MO). Silica gel 60 F254 TLC plates (EM Science) and all solvents were provided by Fisher Scientific (Ivine, CA). All chemicals were used without further purification. Brattleboro (*di/di*) rats and CF-1 mice were purchased from Harlan Sprague Dawley, Inc (Indianapolis, IN). Animal experiments were compliant with the "Principles of Laboratory Animal Care" (NIH Publication #85-23, revised 1985) and approved by the IACUC at USC.

Reversible Lipidization of DDAVP

DDAVP (4 mg) was dissolved in 2 ml of PBS (pH 7.4) and treated with 74.8 μl of DTT (0.1 M) at 37°C. The reaction was monitored by using thin-layer chromatography (TLC). A small aliquot of the reaction mixture was spotted on a TLC plate and was developed twice using the organic layer of a mixture of n-butanol:water:acetic acid (4:5:1). The reduction of the disulfide bond in DDAVP was completed within 30 min, as indicated by the conversion of DDAVP (Rf = 0.15) to a single UV-absorbing spot (Rf = 0.20). The DTT-reduced DDAVP (dithiodesmopressin) was used without further purification for the subsequent conjugation. The reduced DDAVP solution was mixed with 2.24 ml of a Pal-CPD solution (10 mM, pH 7.6) for 30 min at 25°C and, subsequently, acidified to pH 3 using HCl (1N). The precipitate formed in the acidified reaction mixture, which consisted of the lipidized DDAVP, i.e., DPP, and the excess reagent, was isolated by using centrifugation (11,220 × g/20 min) and re-dissolved in 1 ml of dimethylformamide (DMF). DPP was subsequently purified by using a Sephadex® G-15 column (40 ml) eluted with DMF. DPP-containing fractions at the void volume of the column were identified by TLC analysis (DPP: Rf = 0.24) and pooled. After the removal of the solvent under vacuum, 3.8 mg of purified DPP was obtained (yield 57%).

Characterization of DPP

Reversed-Phase HPLC Analysis

The purity and hydrophobicity of DPP and DDAVP were analyzed using a reversed-phase Shimadzu HPLC system, which consisted of a LC-6A dual pump, a SCL-6A controller, a SPD-6A UV detector with the wavelength set at 214 nm, and a C-R3A recorder. Ultrasphere Protein C-4 column (Vydac, Hesperia, CA) was used with a flow rate of 1 ml/min. The mobile phases used were: (A) 0.1% trifluoroacetic acid in distilled water, (B) 0.0925% trifluoroacetic acid in isopropanol: acetonitrile (1:1). A linear gradient was programmed starting with 3% of solvent B to 97% of solvent B in 30 min and the column was eluted with 97% of solvent B for an additional 20 min.

Mass Spectrometric Analysis

The definite molecular identification of DPP was conducted on a Krotos Kompact III MALDI-TOF Mass Spectrometer. Nitrogen 370 nm was the source of the laser beam and dihydroxybenzoic acid was used as the matrix. The reflectron high power was set at 95.

Chemical Reversibility

In vitro conversion of DPP to DDAVP was demonstrated in the presence of DTT as the reducing reagent. Twenty μl of DPP (1 mg/ml, PBS) was treated with 10 μl of DTT (0.1 M) at 37°C. Samples (1.5 μl each) at time 0, 15, 30, 60 and 90 min were taken to be analyzed by TLC under conditions described previously.

Anti-Diuretic Activity of DDAVP and DPP

Brattleboro rats, which carry the hereditary disease of hypothalamic diabetes insipidus, were used to compare the effects of DDAVP and DPP for alleviating the disease symptoms, i.e., polyuria and polydipsia. A group of three Brattleboro rats were kept separately in three metabolic cages. Their body weight, water intake and urine output were measured daily. DDAVP and DPP were formulated in 10% Liposyn® II (Abbott, Abbott Park, IL) and injected subcutaneously to each rat at a dose of 5 μg/kg. An average washout period of 2 days was used following the completion of each experiment.

The dose-response relationship of DPP was studied in the same group of Brattleboro rats using doses ranging from 0.02 to 50 μg/kg.

Preliminary Pharmacokinetic Studies of Intravenously Administered DDAVP and DPP in Mice

The preliminary pharmacokinetic studies of DDAVP and DPP were performed in female CF-1 mice. Both DDAVP and DPP were iodinated with ^{125}I using the Chloramine T method (19). Two groups of twelve mice, 6 weeks old and weighting between 20 and 25 g each, were injected intravenously *via* the

tail vein with ^{125}I-DDAVP or ^{125}I-DPP, respectively, at 1×10^6 cpm per mouse which corresponds to a dose of 6.7 μg/kg. Three animals from each group were sacrificed at 5, 15, 60 and 240 min post-injection and blood (0.8–1.0 ml) was collected by heart puncture. Organs of each animal, including kidneys, liver, spleen, small intestine, and large intestine, were also collected. The radioactivity in each organ or in 0.2 ml of the blood was measured by counting in a gamma counter. Area under the curve (AUC) was calculated from the blood levels of radioactivity from 0 to 240 min using the trapezoidal method.

Analysis of Blood Radioactivity Composition

A previously reported procedure (11) was used to analyze the composition of total blood radioactivity. To each 0.2 ml of the 1-hr and 4-hr blood samples collected from mice treated with DPP, 0.8 ml of distilled water was added. After vigorous mixing, the mixture was incubated in a 37°C water bath for 10 min to lyse the red blood cells. The contents were pooled by combining a 0.33 ml aliquot from the three mice of the same time point. The pooled blood was centrifuged at $200 \times g$ for 10 min to remove cell ghosts. A 0.8 ml aliquot of the supernatant was applied to a Sephadex G-25 column (20 ml) and eluted with PBS, pH 7. Two column volumes of eluent (40 ml) were collected in 1-ml fractions, and the radioactivity in each fraction was determined by counting in a gamma counter.

RESULTS

Characterization of DPP

DPP was synthesized by a 2-step procedure without the isolation of the intermediate, dithiodesmopressin. The reactions were performed under mild conditions and no major side-product formation was noticed. After the hydrochloric acid precipitation, centrifugation, and Sephadex G-15 column purification, DPP was obtained with an overall yield of 57%. HPLC and MS analyses were conducted to characterize the purity and the molecular identity of the product. Under the HPLC conditions described in Materials and Methods, DDAVP and DPP were eluted from a reversed phase C-4 column with retention times of 10.6 and 25.0 min, respectively. DPP was found to be more than 95% pure. Using a time-of-flight mass spectrometer, the molecular weights of DDAVP and DPP were found to be 1069.80 and 1784.00, respectively. The mass difference between DPP and DDAVP, 714.20, corresponds to the mass of the two lipid moieties from the reagent, Pal-CPD.

The reversibility of DPP to DDAVP was demonstrated *in vitro* using DTT as a reducing agent. TLC was used to monitor the reactions. The gradual disappearance of DPP (Rf = 0.24) and a concomitant regeneration of DDAVP (Rf = 0.15) were observed under the conditions described. After 1 hr, the conversion of DPP to DDAVP was apparently completed.

Anti-Diuretic Activity of DPP

Figure 2 shows a typical response of Brattleboro rats to the DDAVP treatment at a dose of 5 μg/kg which maintained the rats symptom-free (daily urine output <50 ml) for less than 1 day. On the other hand, DPP at an identical dose could maintain the rats symptom-free for more than 4 days, as indicated by the reduction of both water intake and urine output without significant change of the body weight.

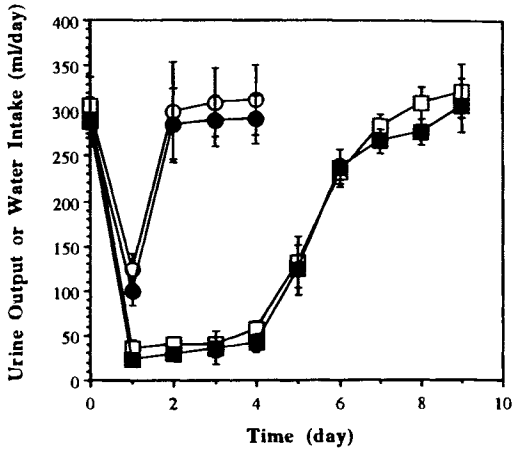

Fig. 2. Effects of DDAVP and DPP on the water intake and urine output of Brattleboro rats. Three Brattleboro rats were subcutaneously injected with 5 μg/kg of either DDAVP or DPP. The animals were kept in separate metabolic cages. The daily urine output (DDAVP, ●; DPP, ■) as well as the water intake (DDAVP, ○; DPP, □) of each animal was measured and presented as the average ml per day. (Bars indicate standard deviations, n = 3).

Dose-Response Relationship

In order to evaluate the dose-dependent responses to DDAVP and DPP in Battleboro rats, we define the effectiveness of anti-diuretic activity as the length of time that a more than 50% reduction of the urine volume (T50) can be maintained in the treated animals. A substantial enhancement of the anti-diuretic effect of DDAVP was observed in DPP. As shown in Fig. 3, a similar effect was obtained when animals were treated

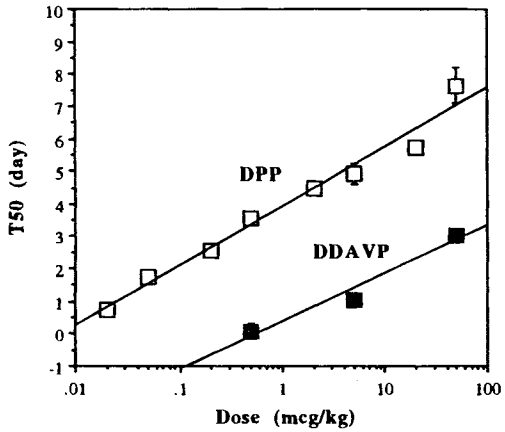

Fig. 3. The dose-response relationship of DPP for the treatment of diabetes insipidus in Brattleboro rats. The effect of DPP, T50, is the number of days that a 50% reduction of the urine volume can be maintained. Three animals were injected subcutaneously with 0.02, 0.05, 0.2, 0.5, 2.0, 5.0, 20 and 50 μg/kg of DPP (□). In addition, injections with 0.5, 5.0 and 50 μg/kg of DDAVP (■) were used as controls for the comparison of the potency between DPP and DDAVP. (Bars indicate standard deviations, n = 3).

with 0.02 μg/kg of DPP or 5 μg/ml of DDAVP. Therefore, it was concluded that DPP was about 250-fold more potent than DDAVP when administered subcutaneously for the treatment of diabetes insipidus in Brattleboro rats. Without exception, longer duration of anti-diuretic effect was also observed when the dose was increased gradually from 0.02 to 50 μg/kg. When T50s are plotted against doses on logarithmic scale, regression analysis reveals the following linear relationship:

$$T50 = 1.8280 \log(dose) + 3.9113$$

with a high correlation coefficient of 0.99 (Fig. 3).

Preliminary Pharmacokinetics

To understand the mechanism behind the prolonged anti-diuretic activity of DPP, preliminary pharmacokinetic study was conducted in CF-1 mice. Although complete kinetic analysis was not intended, several interesting observations were made from this study. As previously reported by others (20), DDAVP was bi-phasically eliminated from the circulation in rats with a fast distribution phase and a slow elimination phase (Fig. 4). Interestingly, in the case of DPP, there was an absorption phase proceeding a monoexponential elimination phase. This phenomenon suggests that the intravenously injected DPP, but not DDAVP, tends to stay at local injection site, i.e., the tail vein, before gradually entering the central circulation. However, as judged by the slopes of the two curves, the terminal half-life of DPP is not very different from that of DDAVP. Therefore, the apparent 7-fold increase of AUC of DPP is largely due to a slow release of DPP into the blood stream and the lack of a fast distribution phase, rather than the change of the half-life of the terminal elimination phase.

Biodistribution

A comparison of biodistribution of DDAVP and DPP in kidneys, liver, spleen, small intestine, and large intestine at various time points is shown in Table 1. The major excretion pathways for DDAVP were the small intestine and the kidneys (20). The time-dependent distribution patterns of DPP in kidneys and small intestine were consistent with the pharmacokinetic data as shown in Fig. 4, i.e., the clearance of DPP from the blood was slower than that of DDAVP. Table 1 also shows that DPP accumulated in the liver longer than DDAVP. Another important observation as shown in Table 1 was the high localization of DDAVP and, to a less extent, DPP in the spleen. If the weight of each organ was considered, i.e., 1.2, 0.1, 0.3, 1.1, and 0.7 grams for liver, spleen, kidneys, small intestine, and large intestine, respectively, the spleen would be the organ with the highest amount of DDAVP or DPP per tissue weight at 5 min, followed by liver, kidneys, small intestine, and large intestine.

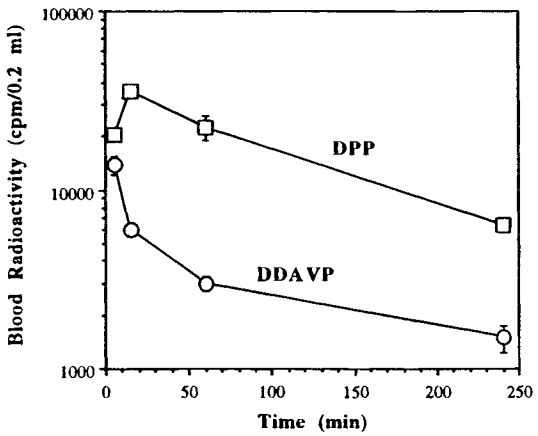

Fig. 4. Total radioactivity in blood following iv injections of DDAVP or DPP in CF-1 mice. CF-1 mice were injected intravenously with ^{125}I-DDAVP (○) or ^{125}I-DPP (□) at 1×10^6 cpm/animal. At each time point, i.e., 5, 15, 60 and 240 min, three mice were sacrificed, blood samples were collected by heart puncture, and various organs were then removed. A 0.2 ml aliquot of blood from each blood sample was counted in a gamma counter, and the data were presented as cpm/0.2 ml blood. (Bars indicate standard deviations, n = 3).

Blood Analysis

It was assumed that lipidized peptide *via* disulfide linkage would regenerate the original peptide *in vivo*. To test this hypothesis, blood samples from 1 hr and 4 hr following intravenous injection of ^{125}I-DPP were analyzed on a Sephadex® G-25 size exclusion column. As shown in Fig. 5, 66% of the radioactivity in the 1-hr sample was associated with plasma proteins, which eluted at the void volume (Fractions 7–9). This represented the sum of plasma protein-bound DPP. Eight percent of the radioactivity was co-eluted with DDAVP in Fractions 16–19, representing DDAVP regenerated from DPP. The rest of the radioactivity eluted after fraction 20 and appeared to be degradation products. The elution profile of the 4-hr sample was essentially the same, except that the radioactivity in the fraction of regenerated DDAVP was increased to about 20% of the plasma DPP. Since the plasma half-life of DDAVP is relatively short (Fig. 4), the increase of percentage of free DDAVP to total DPP in the 4-hr plasma sample indicates that DDAVP was regenerated continuously in the animals after DPP administration.

DISCUSSION

We show in this paper that by using a reversible lipidization method, the anti-diuretic activity of DDAVP was enhanced 250-fold when administrated subcutaneously in vasopressin-deficient Brattleboro rats. Our findings indicate that the reversibly lipidized peptide can possess a prolonged retention in the tissue, and that the active peptide can be regenerated slowly either in the tissue or in the blood to exert its pharmacological activity.

Several lines of evidence support the notion that the original DDAVP can be regenerated from DPP both *in vitro* and *in vivo*. First, by using an *in vitro* reducing system, DDAVP was detected by TLC analysis. Second, the disulfide ring structure is essential for the anti-diuretic activity of DDAVP (21). Since subcuteneously injected DPP, a derivative of DDAVP without a disulfide ring, displayed a potency which is higher than that of DDAVP, a regeneration of the disulfide ring structure must occur after the administration to the animal. Third, the most direct evidence came from the analysis of blood radioactivity in DPP-administered animals; DDAVP was clearly detectable in 1-hr and, even at a higher percentage, in 4-hr samples (Fig. 5).

The dose-response relationship of DPP displays a linear correlation between the pharmacological response (T50) and

Table 1. Biodistribution of Intravenously Injected ^{125}I-DDAVP or ^{125}I-DPP in CF-1 Mice

Time (min)		5	15	60	240
Liver	DDAVP	15.51 ± 0.70	8.13 ± 0.90	3.32 ± 0.57	1.72 ± 0.23
	DPP	14.45 ± 1.47	10.5 ± 0.99	8.17 ± 1.70	4.12 ± 0.34
Spleen	DDAVP	4.11 ± 0.48	4.23 ± 0.32	3.16 ± 0.78	2.00 ± 0.50
	DPP	2.16 ± 0.16	2.07 ± 0.22	2.38 ± 0.42	2.02 ± 0.30
Kidneys	DDAVP	0.95 ± 0.16	0.66 ± 0.08	0.21 ± 0.02	0.11 ± 0.02
	DPP	1.05 ± 0.12	1.25 ± 0.06	0.84 ± 0.11	0.31 ± 0.01
Small Intestine	DDAVP	4.95 ± 2.43	20.7 ± 0.75	20.6 ± 2.74	3.25 ± 0.47
	DPP	2.57 ± 0.12	4.17 ± 0.78	8.10 ± 0.94	2.66 ± 0.67
Large Intestine	DDAVP	1.07 ± 0.13	0.65 ± 0.15	0.52 ± 0.39	17.6 ± 7.83
	DPP	0.52 ± 0.05	0.62 ± 0.08	0.71 ± 0.50	6.25 ± 0.35

Note: (Percentage of total administered dose, ± standard deviation, n = 3)

log(dose) (Fig. 3). This observation, together with the preliminary pharmacokinetic profile (Fig. 4 and Table 1), suggests that the prolonged anti-diuretic activity of DPP is most likely due to the depot and sustained releasing effect, rather than the slow elimination from the blood. We have reported previously that reversible lipidization can increase significantly the cellular uptake of a polypeptide, BBI, in cultured Caco-2 cell monolayers, even in the presence of serum-containing culture medium (13,22). An increase of DPP absorption on the surface of endothelial cells may conceivably cause a slow releasing of this lipidized peptide from the tail vein endothelium to the blood circulation. Such a "depot" effect was observed as the increase of DPP in the plasma during the first 20 min after the intravenous administration (Fig. 4). It seems likely that the anti-diuretic activity is controlled by the rate of DDAVP release from tissue-associated DPP, which is apparently a first order kinetics. The biodistribution of DDAVP in mice (Table 1) is consistent with the report from the study of DDAVP in rats (20), i.e., this peptide analog is eliminated via both the kidney and intestinal excretion. However, Table 1 indicates that the metabolic pathway of DPP may be very different from that of DDAVP. The most apparent difference is that the intestinal localization of DPP is very low. A prolonged accumulation and elimination of DPP in kidneys, which is different from that of DDAVP, is also observed. Furthermore, data in Table 1 suggest a transport and accumulation of DPP from blood to liver, an observation that is consistent with the report on the study of intravenously injected lipidized BBI in mice (22). It is noteworthy that the releasing of BBI from the intravenously injected lipidized BBI in mice has been suggested to occur in the liver rather than in the blood (22). If the stability of the disulfide linkage in DPP is comparable to that of lipidized BBI, the releasing of active DDAVP from DPP most likely also occurs in the tissue such as the liver rather than in the blood. However, for subcutaneously administered DPP, it is not yet determined whether DDAVP is regenerated locally at the injection site before entering into the blood, or is regenerated after DPP is released from the injection site into the circulation and, subsequently, absorbed by the target tissue.

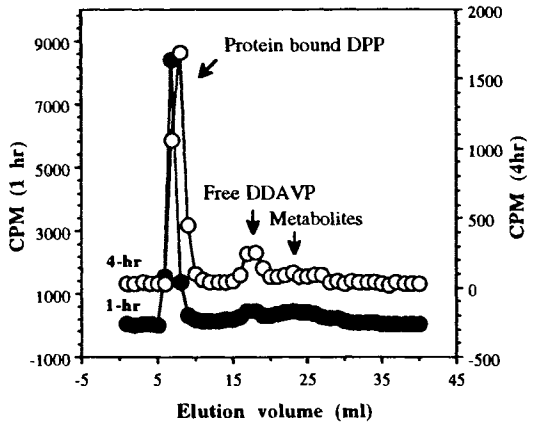

Fig. 5. The analysis of blood samples with Sephadex G-25 gel filtration. Plasma samples, 1-hr (●) and 4-hr (○) after intravenous injection in mice, were separated in a Sephadex G-25 column (20 ml).

As described in this and other reports (11,13,22) from our laboratory, reversible lipidization for increasing lipophilicity of peptide drugs offers several advantages over conventional lipidization methods. The lipidized products have good water solubility and are suitable for various formulations. Reversibly lipidized peptides are fully bioactive because they are capable of regenerating the native peptides in a living system. Furthermore, significantly prolonged pharmacological activity, as in the case of DPP, can be achieved due to the unique drug biodistribution and release kinetics of lipidized peptides. Consequently, it is possible to reduce the numbers of injections and lower the dose to achieve the desirable clinical response in patients. In addition, the break-down products of lipidized peptides are palmitic acid and cysteine, which are endogenous components of the living system. These by-products are unlikely to cause any serious toxicity, especially in the dose ranges which are insignificant when compared with other drug carriers.

In summary, we demonstrate here that, DPP, a reversibly lipidized DDAVP, can be prepared under aqueous conditions by reducing DDAVP with DTT and, subsequently, reacting with a disulfide lipidizing agent, Pal-CPD. DDAVP can be slowly regenerated from DPP *in vivo*, resulting in a potentiated and prolonged anti-diuretic effect. The anti-diuretic effect of the subcutaneously injected DPP in Brattleboro rats indicates a 250-fold increase of the therapeutic potency of DDAVP. These results, together with our previous findings from the studies of BBI (11,13,22), suggest that reversible lipidization is a simple but effective approach for increasing the therapeutic efficacy of peptide and protein drugs.

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