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THE TRANSPORT MECHANISM OF THIAMINE PROPYL DISULFIDE INTO HUMAN BLOOD CELL MEMBRANE

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SUMMARY

Thiamine propyl disulfide (TPD), a lipotropic derivative related to thiamine, has a specific ability to cross the biological membrane. In the present paper the mechanism of reduction and uptake of TPD in blood is described.

TPD is converted to thiamine by a heat stable factor(s) in blood cells and to a slight extent by plasma. This reducing ability of blood cells is increased with the aid of plasma. From the fact that this conversion is inhibited by *p*-chloromercuribenzoic acid, it is postulated that SH groups may play a major role in this reduction process.

A possible explanation on the mechanism for accumulation of TPD in blood cells is presented.

INTRODUCTION

Thiamine propyl disulfide (TPD) is referred to as a structure closely related to thiamine (Fig. 1). There is considerable evidence that this compound has the specific ability to cross biological membranes more readily than thiamine. Because of this property this compound may be more effective than ordinary thiamine for thiamine deficiency or some neurological diseases therapeutically[1–4].

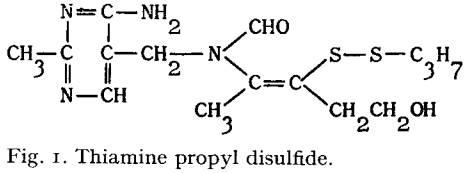

Fig. 1. Thiamine propyl disulfide.

Evidences of the property may be summarized as follows:

(a) TPD is readily absorbed from the intestinal tract when it is given orally and the absorption increases almost indefinitely in proportion to the amount of intake. In contrast, the absorption of ordinary thiamine is limited in extent and no further increase is found even if the amount of intake is increased over 5 to 10 mg[5–8].

(b) A more marked elevation of thiamine concentration in the blood cell and organs occurs when TPD is given orally or parenterally than when ordinary thiamine is administered[9–14].

Abbreviations: TPD, thiamine propyl disulfide; PCMB, *p*-chloromercuribenzoic acid.

(c) The high turnover rate of thiamine in the organs and each subcellular fraction is confirmed by experiments with labeled TPD[12, 15, 16].

However, the mechanism of the cell membrane transport and reduction of this compound have not been completely clarified yet. In an attempt to elucidate these mechanisms the following *in vitro* experiments were carried out.

MATERIALS

Red blood cell suspensions. Blood was obtained from a normal healthy human subject (to 10 ml blood 0.1 ml of 1 % heparin solution has been added to prevent coagulation). The red blood cells were separated from the plasma by centrifugation and washed twice with an about equal volume of isotonic saline. The washed red blood cells were suspended in an equal volume of isotonic saline.

Isotonic saline solution. The isotonic saline solution was composed of 85 parts of 0.9 % NaCl and 15 parts of 0.1 M Na_2HPO_4–HCl buffer solution (pH 7.4).

TPD solution. 25 mg ($7 \cdot 10^{-5}$ moles) TPD was dissolved in isotonic saline and made up to a final volume of 10 ml.

TPD had been donated by the Takeda Pharmaceutical Company, Japan.

p-Chloromercuribenzoic acid (PCMB) solution. $7 \cdot 10^{-5}$ moles of PCMB were dissolved in a few drops of 1 M NaOH solution and to this solution 2 ml of 0.5 M Na_2HPO_4–HCl buffer (pH 6.0) solution was added and the mixture made to a total volume of 10 ml with distilled water (pH 7.4).

METHODS

Incubation method. A given volume of either blood cell suspension or plasma was transferred into a 20 ml glass-stoppered centrifuge tube. An adequate volume of TPD solution was added and the mixture was made a total volume of 7.0 ml with isotonic saline. The mixture was allowed to react in an incubator at 37° for a given length of time (see Tables).

Determination of thiamine. Free thiamine was assayed by the thiochrome method of FUJIWARA AND MATSUI[17].

RESULTS

The parameters of the incubation system for TPD reduction mechanism were studied using red blood cells and plasma.

Table I illustrates the relationship between volumes of red blood cells or plasma and the amounts of free thiamine which were formed from TPD. The reduction ability of TPD was higher in red blood cells than in plasma and increased linearily in proportion to the volume of blood cells or plasma.

The limit of the reduction ability of blood cells is shown in Fig. 2. The maximum amount of free thiamine formed from TPD with 0.5 ml of blood cells was 600 μg, most of which was in blood cell. However, amounts in excess of 600 μg remained as TPD.

In contrast, incorporation of ordinary thiamine into blood cells was very small even when a large amount (3 mg) of thiamine was added to the reaction mixture (Table II).

Blood cell membrane ghosts and cell contents from 0.5 ml of blood cells were prepared by centrifugation at 3000 × *g*·25 min after hemolysis by water. The membrane fraction was washed 3 times with 5 ml of isotonic saline and the washings were added to the hemolysate. To the membrane ghosts and hemolysate, TPD solution and isotonic saline were added, the mixture incubated 37° for 60 min and free thiamine determined in each fraction. As shown in Table III, most of the reduction

TABLE I

RELATIONSHIP BETWEEN VOLUMES OF BLOOD CELLS OR PLASMA AND REDUCTION ABILITY OF TPD

Tubes containing isotonic saline medium, 1.0 ml of TPD solution and various volumes of blood cell or plasma were incubated at 37° for 30 min. Blood cells and supernatant were separated by centrifugation at 3000 × *g* for 15 min. Free thiamine content in blood cell, supernatant or plasma were determined.

	Volume of blood cells (*ml*)	*Free thiamine in blood cells* (*nmoles*)	*Free thiamine in supernatant* (*nmoles*)	*Total* (*nmoles*)
Blood cells	0.25	436	267	703
	0.50	899	445	1344
	1.00	1840	855	2694
		Volumes of plasma (*ml*)	*Free thiamine* (*nmoles*)	
Plasma		0.25	119	
		0.50	261	
		1.00	484	

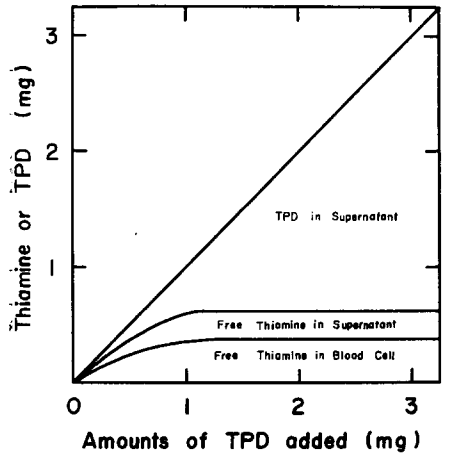

Fig. 2. Reduction and incorporation of TPD in blood cell. To tubes containing 0.5 ml blood cells, various amounts of TPD were added and with isotonic saline made to 7.0 ml (pH 7.4). Samples were incubated at 37° for 30 min. After incubation blood cell and supernatant were separated, free thiamine in both fractions were determined.

ability was in the hemolysate and the amounts reduced by cell membranes were relatively small.

In the above experiments reduction of TPD was tested in red blood cells and in plasma separately and indicated that most of the ability to reduce TPD exists in the red blood cells. However, in the animal body both red blood cells and plasma react with TPD simultaneously, of course. With this in view, the following experiments were carried out.

To a test tube containing 0.5 ml of red blood cells, 0.5 ml of plasma and 1 ml

TABLE II

THE RELATIONSHIP BETWEEN AMOUNT OF THIAMINE IN BLOOD CELLS AND THIAMINE CONTENT IN THE REACTION MEDIUM

To test tubes containing 0.5 ml of blood cells, various amounts of thiamine were added and the final volume adjusted with isotonic saline to 7.0 ml. Samples were incubated at 37° for 30 min.

Amount of thiamine added (mg)	*Thiamine content in blood cells (μg)*
0.75	1.27
1.50	1.68
3.00	3.19

TABLE III

ABILITY OF CELL MEMBRANE GHOSTS AND HEMOLYSATE TO REDUCE TPD

Tubes containing cell membrane ghosts or hemolysate (separated from 0.5 ml of blood cells), 1.0 ml of TPD solution and isotonic saline (total volume 7.0 ml) were incubated at 37° for 1 h.

	Amounts of free thiamine (nmoles)	
Free thiamine formed by membrane ghosts	344	
Free thiamine in supernatant		205
Free thiamine in membranes		139
Free thiamine formed by hemolysate	1279	
Total of free thiamine	1623	

TABLE IV

ABILITY OF BLOOD CELLS TO REDUCE TPD IN THE PRESENCE OF PLASMA OR WITHOUT IT

Values represent an average of 10 subjects (mean ± S.E.). Both in blood cells and in plasma, the difference between free thiamine values with plasma and without plasma is statistically significant by the student "*t*" test ($P < 0.01$).

	Amounts of free thiamine (nmoles)	
	With plasma	*Without plasma*
Blood cells	1891 ± 130	1153 ± 50
Supernatant	2035 ± 233	699 ± 59

of TPD solution was added and then isotonic saline to a final volume of 7.0 ml. Control tubes were identical to experimental samples except that the plasma was omitted. Tubes were incubated at 37° for 1 h. As shown in Table IV, the reduction ability in red blood cells in the presence of plasma was significantly higher than the control.

The ability to reduce TPD was not decreased in boiled red blood cells as shown in Table V. This fact suggests that this ability originated in a heat stable factor or factors and not in an enzymatic effect.

MATSUKAWA AND YURUGI[18] showed that TPD reacted with cysteine as the chemical equation in Fig. 3. From their finding it can be presumed that the SH group plays an important part in reduction of TPD in blood cells and the following experiments were carried out.

To 0.25 or 0.5 ml of red blood cells 1 ml of PCMB solution (inhibitor of SH groups) was added and the final volume made up to 7.0 ml with isotonic saline (pH: 7.4,

TABLE V

ABILITY TO REDUCE TPD IN BOILED BLOOD CELLS

To 0.5 ml of blood cell, 5.5 ml of isotonic saline was added, the mixture boiled 15 min and then 1.0 ml of TPD solution was added, and the mixture incubated at 37° for 30 min. For the control, the boiling process was omitted.

	Free thiamine content (nmoles)
Boiled blood cells	2098
Intact blood cells	2039

TABLE VI

THE EFFECT OF PCMB ON THE ABILITY OF BLOOD CELLS TO REDUCE AND INCORPORATE TPD

The reaction conditions are given in the text.

Volume of blood cells (ml)	*Pre-treatment*	*Free thiamine in blood cells (nmoles)*	*Free thiamine in supernatant (nmoles)*
0.25	PCMB	2	21
	Control	546	332
0.50	PCMB	154	326
	Control	1217	680

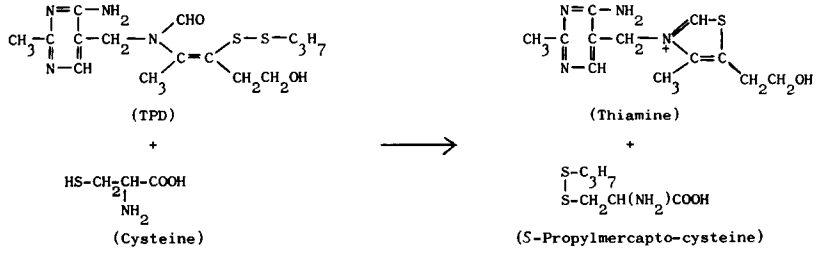

Fig. 3. Reduction of thiamine propyl disulfide with cysteine.

final concentration of PCMB was 1 mM). The mixture was incubated at 37° for 10 min, red blood cells were separated by centrifugation, washed twice, and then TPD was added to them, and the reaction was performed for 30 min. As shown in Table VI, the ability to reduce TPD and the amounts of thiamine which accumulated in red blood cells were decreased markedly in PCMB treated blood cells.

DISCUSSION

It is a well known fact that penetration of a substance into human blood cells is related to its lipid to water partition coefficient at pH 7.4 when transport is performed passively. Drugs of high lipid solubility enter rapidly and drugs of low lipid solubility enter slowly[19]. As the transport of TPD into blood cell is mainly passive[20] and TPD is more fat soluble than ordinary thiamine, it is reasonable that TPD penetrates into blood cells more easily than thiamine. Once incorporated in blood cells TPD is reduced rapidly to thiamine and the free thiamine produced stays in the blood cells because of the difficulty with which it crosses the membrane. Consequently these mechanisms cause an accumulation of thiamine in blood cells.

The role of SH groups in blood cells on the reduction mechanism was clarified by the SH inhibitor experiments (Table VI). The fact that a marked decrease of TPD incorporation into blood cells occurs after blockage of the SH groups of blood cells suggests the following hypothesis. TPD penetrates into PCMB treated blood cells as usual, but since the SH groups in the blood cells are blocked by PCMB there is nothing to reduce the TPD to thiamine and the fat soluble TPD comes outside of the blood cells again with washing so that the accumulation of thiamine in blood cells is not observed. The main SH group involved in TPD reduction may be that of glutathione or perhaps that of hemoglobin as discussed by several other investigators[21,22]. Although KUROKI[23] suggested the possibility of the involvement of some enzyme in the mechanism of the reduction of TPD, the result of the boiled blood cell experiment (See Table V), must be considered to show that the SH group plays a major role but that enzymatic factors have little influence on the reduction of TPD in blood cells.

The other curious fact in the current study is the effect of plasma on the ability of blood cells to reduce TPD. In spite of the weak ability to reduce TPD in plasma itself, when plasma is added to blood cells, accumulation of thiamine and the ability to reduce TPD increase markedly. This fact may be explained by assuming that the SH group which has once reacted with TPD has its reducing ability restored in the presence of plasma.

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