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MECHANISM OF METHYLMERCURY RELEASE FROM BOUND TYPE IN BLOODSTREAM OR TISSUES

Kimiaki Sumino, Ryoji Yamamoto, and Shoji Kitamura
Department of Public Health, Medical Faculty, Kobe University,
Ikuta-ku, Kobe, 650, Japan

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Summary - Reductants involving selenite freed methylmercury from bound protein without breaking the mercury-carbon bond in conjunction with sulfhydryls such as cysteine or homocysteine. The free methylmercury was transfered through an organic layer from the original -SH radicals to the other -SH radicals. The same effect occurred in normal physical reactions resulting from oxidoreductases, alcohol or lactic dehydrogenase. The oxidation-reduction system may play an important role in transfer of methylmercury from blood to tissues, or vice versa.

The authors previously reported that selenite caused methylmercury to be released from being bound with proteins, and that this may have a role in decreasing methylmercury toxicity (1). Selenite was effective only in conjunction with some components from blood cells or tissue homogenates, and not blood plasma, and the effect of selenate was one-tenth that of selenite. However, it is still unknown why selenite acts as a releasing factor for this liberation of methylmercury. On the other hand, methylmercury absorbed into bodies can easily become bound to blood cells in the bloodstream, and it is carried as a bound type and finally lodged in different organs, through blood cell membrances, as it is released from blood cells. In this ocasion, the methylmercury must be liberated from its linkage with blood proteins, but this mechanism is not clear. We discovered that some reductants involving selenite caused the oxidation-reduction potential to become greatly reduced, when acting with some SH-radicals such as cysteine or homocysteine, and the methylmercury bound with human plasma consequently became free type without breaking of the mercury-carbon bond, and also that similar reactions from enzymes, without selenite, can occur in vitro.

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MATERIALS AND METHODS

Release of methylmercury by selenite - A mixture of 50 µl human plasma taken from normal Japanese male, and 4 nmol of methylmercury chloride and sodium selenite in 2 ml of phosphate buffer (pH 7.5) was incubated at room temperature for various periods up to 60 min. with cysteine or homocysteine which was prepared 15 min. before use. Then free methylmercury was extracted and the benzene layer was analysed by a gas chromatograph with an electron capture detector (1). The other different combinations of redox substances were also examined (see Results). To confirm the transfer of methylmercury from the cysteine to different cysteine through the organic membrane (Benzene) using an H-shaped tube modified from Shinbo's report (2), the experimental method is described in the legend of Fig. 1. A platinum redox electrode (Orion) was used to measure the oxidation-reduction potential.

Release of methylmercury by enzyme reactions - Methylmercury (100 nmol) and 300 µl of human plasma were mixed, and 250 µmol of ethanol with 450 units of alcohol dehydrogenase (Boehringer) or 250 µmol of lactic acid with 530 units of lactic dehydrogenase (SIGMA), 5 mg of NAD, 10.5 units of diaphorase and 1 mg of methylene blue were added in 25 ml of phosphate buffer (pH 7.5). Free methylmercury was separated from 1-ml aliquot with 1 ml of benzene.

RESULTS

All experiments were repeated 3 times and each point in the figures was presented at mean value. The effect of selenite on the liberation of methyl mercury by cysteine or homocysteine is shown in Fig. 1. The ratio of free methylmercury to original amounts added increased in the early stages of increased incubation time. In the case of higher concentration of sulfhydryls, (100 times that of methylmercury at molar base), the rate of release tended higher than in lower concentrations of -SH radicals, and its maximum rate reached 80-90 %. More excess cysteine or homocysteine (1000 times more than methylmercury added at molar base) conversely made the liberation rate decrease. The rate of methylmercury transfer (solid line in Fig. 1) was lower than indirect extraction (broken lines), but it is clear that free methyl mercury was transfered through the benzene layer from the original side (R) containing selenite and cysteine linkaged methylmercury, to the other side (L) containing only cysteine.

While the other different combinations of redox substances were thought to present similar effects on the release of methylmercury, none of the other sulfhydryls, such as N-acetyl cysteine or penicillamine instead of cysteine or homocysteine, nor other reductants such as stannous chloride, or hydroxy-

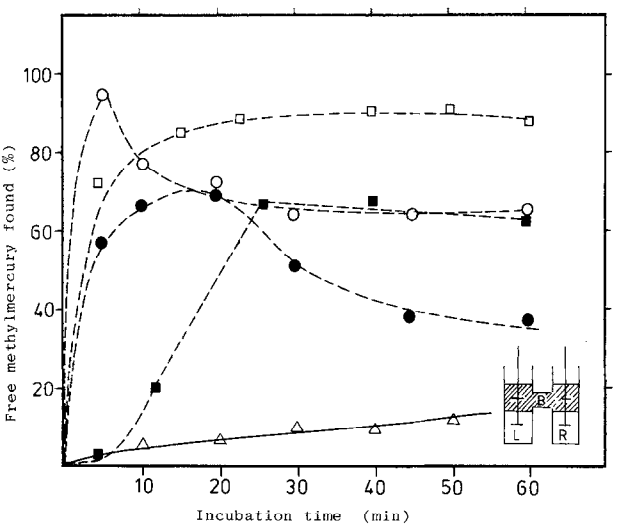

Fig. 1. Liberation rate of methylmercury from human plasma by selenite and -SH amino acids. ●— , 100 nmol cysteine; ○— , 400 nmol cysteine; ■— , 100 nmol homocysteine; □— , 400 nmol homocysteine. Solid line (△—) shows the rate of methylmercury transfer. R side of the tube included 80 nmol of methylmercury, 5 µmol cysteine and 160 nmol selenite in 20 ml of phosphate buffer. B was 20 ml of benzene. The reaction was performed at room temperature. Methylmercury transfered was measured from 1-ml aliquot in L tube with 2 ml of benzene after acidification with HCl. The amount of methylmercury determined each time was subsequently summed up to determine the rate of methylmercury transfer. GC conditions: 3 mm x 1 m glass column of Thermon 1000 on Chromosorb W at 80 ml/min. of N_2 flow rate and 180°C.

amine in lieu of selenite, had any effect in action, but glutathione with selenite and ferrous chloride with cysteine had moderately similar effects. When the methylmercury was released from the bound type, it was observed by a platinum redox electrode that the original redox potential fell phenomenally by a range of -150 to -200 mV.

In the enzymic reactions, using oxido-reductase (ADH or LDH), substrate (ethanol or lactic acid), coenzymes (NAD and diaphorase) and a hydrogen transmitter (methylene blue), about 10 % of the original amounts of methylmercury could be found as free type as shown in Fig. 2. The rate of the liberation tended to be higher, when both enzymes were mixed together (pyruvic acid must be used instead of lactic acid) in the same system, and lower at the lower temperature of incubation. On this ocasion, it was also observed that the redox potential was reduced.

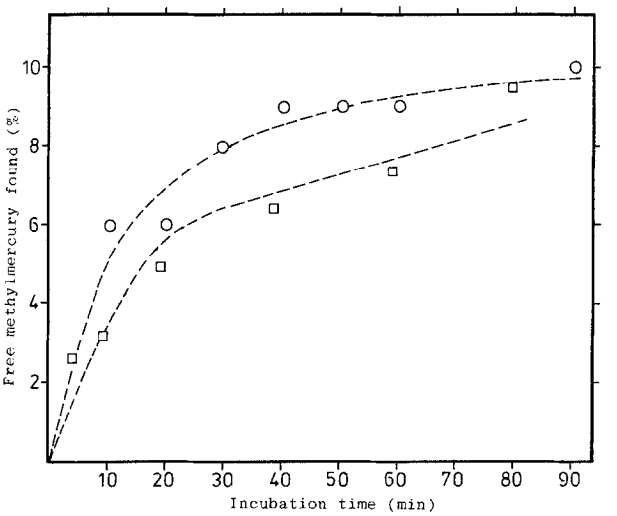

Fig. 2. Liberation rate of methylmercury from human plasma by enzyme reaction. -O- , ADH system; -□- , LDH system.

DISCUSSION

When methylmercury chloride was added to human plasma in a phosphate buffer, the methylmercury quickly combined with proteins in blood plasma, becoming bound to the proteins, and never changed while in that condition to a benzene-extractable type, which would have shown free methylmercury. Addition of selenite alone or cysteine alone to this solution had no effect on release of methylmercury from the bound type. However, when excess cysteine or homocysteine were added to this system, containing selenite and methyl mercury-plasma in a buffer, surprising liberation of methylmercury occurred. This phenomenon is really strange because cysteine, which has a strong affinity for methylmercury (3), on the other hand has a releasing ability when associated with selenite. It seemed as if methylmercury once liberated would lose the affinity for proteins or -SH radicals in that solution. However, methylmercury in the benzene layer, extracted from the neutral phosphate buffer, was identified as the methylmercury chloride originally added in our experiments, using thin layer chromatography and gas chromatography-mass spectromtry.

White and Rothstein (4), from their kinetic study on the interaction of methylmercury with erythrocytes, have found that cysteine alone induced a transient release of some methylmercury from erythrocytes to become bound with the cysteine, but did not find that liberation of methylmercury from the bound type occurred. They have stated that the equilibrium of methylmercury between the cells and the medium suggests a simple competition amoung sulfhydryls though, our findings cannot be explained on the basis of this idea. Levander et al have reported that selenite might catalyse the transfer of electrons from glutathione to cytochrome c (5), and also cysteine was a more effective reductant of cytochrome c than glutathione (6). In a similar way, when the electron transfer from sulfhydryl radicals to methylmercury-protein bonds (mostly Hg-S bonds) is caused by the addition of cysteine etc., the methylmercury may be released from the bound type. Selenite may act as the catalyser for the electron transfer and liberation of the methylmercury.

Masukawa and Iwata (7) reported that selenite catalyser the reduction of methemoglobin by glutathione but reduction reaction was inhibited by sulfhydryl inhibitors, such as $HgCl_2$ and CH_3HgCl. This inhibitory effect was greater when glutathion, that was added at molar concentration of 10-100 times of that of inhibitors, was preincubated with selenite. They explained that inhibitors were thought to form a cpmplex with the intermediate, which was formed during the process of the catalytic reaction by selenite and glutathione. However, far from being a complex, methylmercury became free in our experiment. At present it seems that the bound type of methylmercury may possibly be reduced by the catalytic effect of selenite with sulfhydryls.

It is not yet certain whether there is a direct causal relationship between the release of methylmercury and the decrease of redox potential, but it is suggested that it is important to change the oxidation-reduction system in the liberation of methylmercury under neutral conditions. If those circumstances occurred in living bodies, the transfer of methylmercury from a different radical among tissues might be caused. In human bodies, trace

amounts of selenium may also play a role in transfer of methylmercury from blood to tissues. It is probable that the reduction of redox potential in various enzyme reactions is creating the same effect. Therefore, it was decided to determine whether or not the liberation of methylmercury can be recognized in normal physical reactions without selenite. The results showed a clear observation of methylmercury release even in usual oxido--reductase reactions.

Schottel (8) has recently found the mercuric reductase and organo-mercurial hydrolase from Escherichia coli, and reported that the latter enzyme could break the mercury-carbon bond under the presence of EDTA and a sulfhydryl compound for activity. But our results have quite a different meaning from the biotransformation caused by the breakage of the mercury--carbon bond. New findings obtained here suggest that methylmercury could be liberated from the bound type by the action of various enzymes during the transfer from blood to tissues, or vice versa, in capillaries. For the present, it has not been confirmed that the liberation occurred by specific or non-specific enzyme reaction in living bodies, but the oxidation-reduction system, namely the electron transfer system, intra- and extra-cellular, may play an important role.

These findings, that releasing factors are present in the transfer of methylmercury, may have significance for the study of the carrying of all chemical substances by carrier-mediated transport, such as many kinds of heavy metals, drugs, pigments and nutrients in bloodstream, and thier liberation from the carrier proteins in blood.

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