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A novel and automated assay for thiol/disulphide homeostasis

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ABSTRACT

Objectives: To develop a novel and automated assay determining plasma thiol/disulphide homeostasis, which consists of thiol–disulphide exchanges.

Design and methods: Native thiol and total thiol concentrations were synchronously measured as a paired test. In the first vessel, the amount of native thiol groups was measured by a modified Ellman reagent. At the parallel run, first, dynamic disulphide bonds were reduced to free thiol groups by NaBH₄. The unused reductant remnants were completely removed by formaldehyde. Thus, the total thiol amount could be accurately measured. Mercaptoethanol solutions were used as calibrators. The half value of the difference between total thiol and native thiol amounts gave the disulphide bond amount.

Results: No separation step for the assay was needed. All processes were performed using an automated analyser within about 10 min. Plasma disulphide levels were 17.29 ± 5.32 $\mu\text{mol/L}$, native thiol levels were 397 ± 62 $\mu\text{mol/L}$ and disulphide/native thiol per cent ratios were 4.32 ± 1.49 in healthy subjects. Plasma disulphide levels were higher in patients with degenerative diseases and lower in patients with proliferative diseases.

Conclusion: An easy, inexpensive, practical, fully automated and also optionally manual spectrophotometric assay can be used to determine plasma dynamic thiol/disulphide homeostasis.

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Introduction

Thiols, also known as mercaptans, are a class of organic compounds that contain a sulphydryl group (–SH) composed of a sulphur atom and a hydrogen atom attached to a carbon atom [1]. The plasma thiol pool is mainly formed by albumin thiols, protein thiols and slightly formed by low-molecular-weight thiols such as cysteine (Cys), cysteinylglycine, glutathione, homocysteine and γ -glutamylcysteine [2].

Thiols (RSH) can undergo oxidation reaction via oxidants and form disulphide (RSSR) bonds [3]. A disulphide bond is a covalent bond; the linkage is also called a SS-bond or disulphide bridge. Under conditions of oxidative stress, the oxidation of Cys residues can lead to the reversible formation of mixed disulphides between protein thiol groups and low-molecular-mass thiols. The formed disulphide bonds can again be reduced to thiol groups; thus, dynamic thiol–disulphide homeostasis is maintained [4].

Dynamic thiol disulphide homeostasis status has critical roles in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and transcription factors and cellular signalling mechanisms [5,6]. Moreover, dynamic thiol disulphide homeostasis is being increasingly implicated in many disorders. There is also a growing body of evidence demonstrating that an abnormal thiol disulphide homeostasis state is involved in the pathogenesis of a variety of diseases, including diabetes [7], cardiovascular disease [8], cancer [9], rheumatoid arthritis [10], chronic kidney disease [11], acquired immunodeficiency syndrome (AIDS) [12], Parkinson's disease, Alzheimer's disease, Friedreich's ataxia (FRDA), multiple sclerosis and amyotrophic lateral sclerosis [13–15] and liver disorder [16]. Therefore, determination of dynamic thiol disulphide homeostasis can provide valuable information on various normal or abnormal biochemical processes.

The plasma thiol level is most commonly measured using the classical Ellman reagent, 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). This compound is stoichiometrically reduced by free thiols in an exchange reaction, forming a mixed disulphide and releasing one molecule of 5-thionitrobenzoic acid, which can be measured at 412 nm [17]. An alternative reagent to DTNB is 4,4'-dithiodipyridine (4-DPS) [18]. Reduction of 4-DPS leads to 4-thiopyridone tautomer, and this can be measured at 324 nm, which is a near ultraviolet wavelength. This wavelength cannot be used by automated analysers because the lowest wavelength is 340 nm in all automated analysers used in clinical chemistry laboratories.

Abbreviations: NaBH₄, sodium borohydride; DTNB, 5,5'-dithiobis-2-nitrobenzoic acid; GSH, reduced glutathione; GSSG, oxidised glutathione; –SH, RSH, sulphydryl group; RSSR, S–S–, disulphide bonds; Cys, cysteine; CySS, cystine; 4-DPS, 4,4'-dithiodipyridine; TCEP, Tris(2-carboxyethyl)phosphine.

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To the best of our knowledge, there is no automated colourimetric measurement method for plasma/serum dynamic disulphide levels [19]. In a few recent studies, the disulphide and thiol levels of low-molecular-weight disulphide compounds of plasma have been determined using high-performance liquid chromatography (HPLC) [20,21], fluorescence capillary electrophoresis [22] and bioluminescent systems [23]. In these sophisticated systems, separation processes such as the removal of the remaining reductants, which are NaBH_4 , Tris(2-carboxyethyl)phosphine (TCEP) and tributylphosphine, as well as precipitation of proteins, are also needed [19]. These pretreatment applications and measurement procedures are time-consuming, labour-intensive and costly, and require complicated techniques.

In this study, a novel and automated assay determining dynamic thiol/disulphide homeostasis is described and a new test cluster concept containing $-\text{S}-\text{S}-$, $-\text{SH}$, $-\text{S}-\text{S}-/-\text{SH}$, $-\text{S}-\text{S}-/(-\text{SH} + -\text{S}-\text{S}-)$ and $-\text{SH}/(-\text{SH} + -\text{S}-\text{S}-)$ is introduced.

Materials and methods

Chemicals

Reduced glutathione (GSH), oxidised glutathione (GSSG), albumin, 2-mercaptoethanol, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), hydrogen peroxide (H_2O_2), chloramine-T, ethylenediaminetetraacetic acid (EDTA), NaBH_4 , TRIS, NaOH, methanol and formaldehyde were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI) and Merck Co. (Darmstadt, Germany). All chemicals were ultrapure grade, and type I reagent-grade deionised water was used.

Samples

The new assay can be applied to a wide range of complex biological fluids, including plasma, serum, semen plasma, cerebrospinal, pleural and ascite fluids, urine, haemolysate and simple and heterogeneous solutions of pure chemicals.

Plasma samples

Venous blood samples were collected in tubes containing EDTA. Plasma samples were separated from cells by centrifugation at $1500 \times g$ for 10 min. The samples were run immediately or stored at -80°C . The study was approved by the Research Ethics Committee.

Apparatus

A Shimadzu UV-1800 spectrophotometer with a temperature controlled cuvette holder and a Cobas c501 automated analyser (Roche) were used.

Assays

The new assay

Principle of the new assay. Dynamic disulphide bonds ($-\text{S}-\text{S}-$) in the sample are reduced to functional thiol groups ($-\text{SH}$) by NaBH_4 . The unused NaBH_4 remnants are completely removed by formaldehyde. Thus, this prevents the extra reduction of the DTNB and further reduction of the formed disulphide bond, which are produced after the DTNB reaction. The total thiol content of the sample is measured using modified Ellman reagent. Native thiol content is subtracted from the total thiol content and half of the obtained difference gives the disulphide bond amount.

Assay reagents. The assay included two parallel vessels. Three reagents were used in the individual vessels. The first reagents of the vessels were different, while the others were the same reagents. One vessel measured total thiol content, consisting of native thiol plus reduced thiol, and the other measured only the native thiol content of the sample.

Reagent 1 (for total $-\text{SH}$). Reagent 1 (R1) was prepared by dissolving 378 mg of sodium borohydride in 1000 mL of water-methanol solution (50% v/v). The final concentration of sodium borohydride was 10.0 mM. The reagent was prepared freshly and used daily. This reductant solution was used to determine the total thiol content.

Reagent 1' (for native $-\text{SH}$). Reagent 1' was prepared by dissolving 585 mg of sodium chloride in 1000 mL of water-methanol solution (50% v/v). The final concentration of sodium chloride was 10.0 mM. This reagent is stable for at least 6 months at 4°C . The solution was used to determine the native thiol content.

Reagent 2 (and 2'). R2 (2') was prepared by dissolving 0.5 mL of formaldehyde (final concentration: 6.715 mM) and 3.8 g EDTA (final concentration: 10.0 mM) in 1000 mL TRIS buffer, 100 mM and pH 8.2. This reagent is stable for at least 6 months at 4°C . It was used for both of the vessels.

Reagent 3 (and 3'). R3 (3') was prepared by dissolving 3.963 g of DTNB in 1000 mL of methanol. The final concentration of DTNB was 10.0 mM. The reagent was prepared freshly and used daily. It was used for both of the vessels.

Automated measurements of total/native thiol concentrations and disulphide amounts

After manual spectrophotometric optimisation studies, parallel tests were applied using an automated analyser (Cobas c501, Roche). The assay formats for total and native thiol tests are given below.

R1 volume (for total $-\text{SH}$)	10 μL (R1: NaBH_4 , 10 mM in methanol-water solution, 50 v/v)
R1' volume (for native $-\text{SH}$)	10 μL (R1': NaCl, 10 mM in methanol-water solution, 50 v/v)
Sample volume	10 μL
R2 (2') volume	110 μL , (R2 [2']: 6.715 mM formaldehyde and 10.0 mM EDTA in Tris buffer, 100 mM, pH: 8.2)
R3 (3') volume	10 μL , (R3 [3']: 10 mM DTNB in methanol)
Wavelength	Main wavelength 415 nm, secondary wavelength 700 nm (optionally bichromatic)
Reading point	End-point, increasing measurement; the first absorbance is taken before the mixing of R2 and R3 and the last absorbance is taken when the reaction trace draws a plateau (assay duration is about 10 min).
Calibration type	Linear (2-mercaptoethanol is used as calibrators for the assay pair).

The disulphide parameter is a value which can be calculated automatically as half of the difference of the two measured values.

The assays can also be performed by manually using spectrophotometers or multiwell readers. All volumes of the samples and reagents must be increased at the same ratio. Use of a second (side) wavelength is optional.

Monitoring of thiol-disulphide exchanges

Native thiol, total thiol and disulphide amounts were measured in various pure chemical solutions and biological materials containing sulfhydryl groups. Reduced glutathione, 2-mercaptoethanol and albumin solutions were oxidised with hydrogen peroxide solutions at increasing concentrations (from 0 to 300 μM) for 10 min. Chloramine-T was used as oxidant instead of hydrogen peroxide for the oxidation of thiol groups of plasma, because it contains catalase, which has a high turnover number for hydrogen peroxide. Native thiol, total thiol and disulphide concentrations of the pretreated samples were then determined.

Statistical analyses

The paired Student's *t* test, variance analysis, correlation analyses and linear regression analyses were performed using IBM SPSS Statistics (Version 20) computer program (IBM, USA, 2011). Deming regression analyses and difference analyses were performed using the MedCalc (Version 12.7) computer program (MedCalc Software, Belgium, 2013).

Results

Optimisations of NaBH₄ and formaldehyde concentrations

Pure sodium borohydride solution reduced DTNB to TNB and led to false positive results in the assay. It also reduced the formed disulphide bonds, which are produced after DTNB reduction to TNB, to free sulphydryl groups; this vicious circle proceeded until NaBH₄ was consumed. After the reduction of dynamic disulphide bonds to free sulphydryl groups, the unused NaBH₄ remnants were completely removed using formaldehyde. After the removal of NaBH₄ by formaldehyde, no false positive result interference was seen; thus, the extra reduction of DTNB and further reduction of the formed disulphide bonds were completely prevented. Optimal NaBH₄ concentration was found to be 10 mM. The lower concentrations showed insufficient reduction capacity for disulphide bonds, while the higher concentrations showed false positive results. Optimal concentrations of the chemicals used were determined by changing the concentration of one chemical while the concentrations of the other chemicals were kept constant. The optimal formaldehyde concentration was found to be a mmolar concentration of 6.715.

Modification of Ellman's reagent

The classical Ellman reagent was modified by adding formaldehyde solution. Addition of the formaldehyde was not interfered with the assay (Fig. 1A). There was an important correlation ($r = 0.99$, $p < 0.001$; $n = 185$) between the results (Fig. 1B). There was no difference between the results of the original and the modified Ellman reagents (Fig. 1C). Thus, the modified Ellman reagent can be confidently used instead of the classical Ellman reagent to determine the sulphydryl group.

Reaction kinetics of blank and various plasma samples

The reaction kinetics of deionised water (as a blank) and plasma pool samples from healthy individuals, which were carried out in the automated analyser, are given in Fig. 2.

Monitoring of thiol–disulphide exchanges of 2-mercaptoethanol

A 500 μ M 2-mercaptoethanol solution was prepared; equal and separate portions of the solution were obtained, then they were oxidised with H₂O₂ solution at serially increasing concentrations. Oxidation–reduction reactions were monitored up to 300 μ M oxidant concentrations. As seen in Fig. 3A, the linear decrease in native thiol concentrations correlated with the linearly increasing oxidant concentrations ($r = 0.99$, $p < 0.001$). While thiol concentration decreased, disulphide amounts increased in a correlated fashion; no change was observed in the total thiol levels.

Monitoring of thiol–disulphide exchanges of glutathione

A 480 μ M GSH solution was prepared; equal and separate portions of the solution were obtained and then oxidised with H₂O₂ solution with serially increasing concentrations. Oxidation–reduction reactions were monitored up to 300 μ M oxidant concentrations. As seen in Fig. 3B, the linear decrease in native thiol concentrations correlated with the linearly increasing oxidant concentrations ($r = 0.99$, $p < 0.001$). While thiol concentration decreased, disulphide amounts increased in a correlated fashion; no change was observed in the total thiol levels.

Monitoring of thiol–disulphide exchanges of albumin

A 430 μ M albumin solution was prepared; equal and separate portions of the solution were obtained, then oxidised with H₂O₂ solution of serially increasing concentrations. Oxidation–reduction reactions were monitored up to 300 μ M oxidant concentrations. As seen in Fig. 4A, the linear decrease in native thiol concentrations correlated with the linearly increasing oxidant concentrations ($r = 0.99$, $p < 0.001$). While the thiol concentration decreased, disulphide amounts increased in a correlated fashion; no change was observed in the total thiol levels.

Monitoring of thiol–disulphide exchanges of plasma samples

A plasma pool was prepared; equal and separate portions of the solution were obtained and then oxidised with chloramine-T solution of serially increasing concentrations. Oxidation–reduction reactions were monitored up to 300 μ M oxidant concentrations. As seen in Fig. 4B, the linear decrease in native thiol concentrations correlated with the linearly increasing oxidant concentrations ($r = 0.99$, $p < 0.001$). While thiol concentration decreased, disulphide amounts increased in a correlated fashion; no change was observed in the total thiol levels.

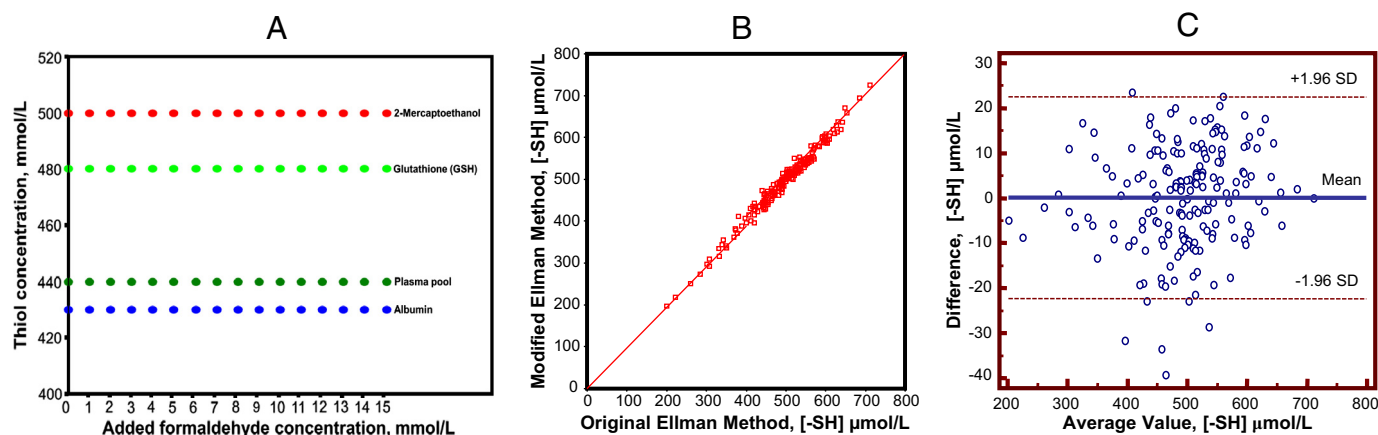


Fig. 1. Comparison of the original and modified Ellman methods. A: Effects of formaldehyde concentrations on the Ellman method. B: Correlation between two methods. C: Difference plot, with mean values as abscissa and the difference between original and modified Ellman methods as ordinate.

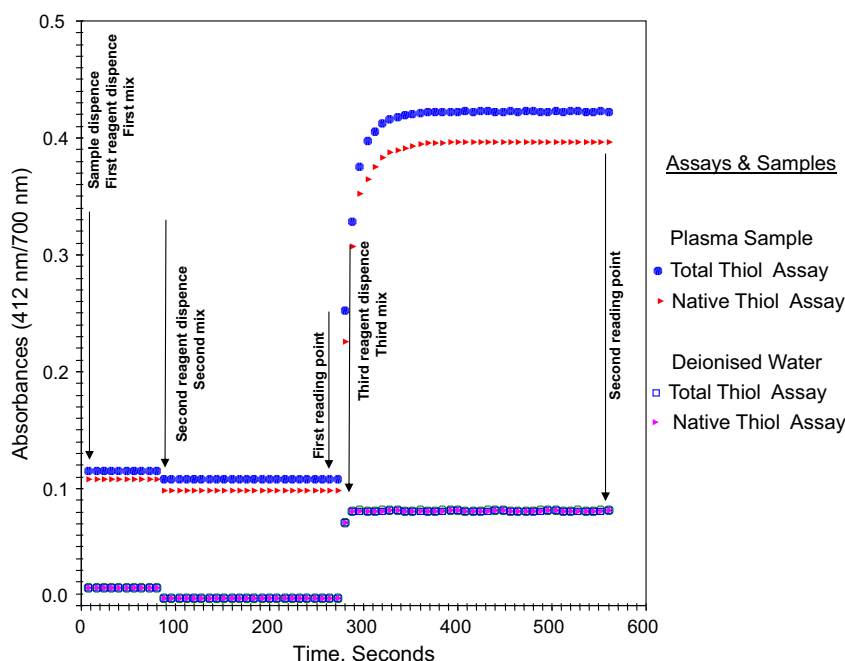


Fig. 2. Reaction kinetics of the assay pair of deionised water and plasma pool samples. Blue dots represent total thiol assays (native thiol + reduced thiol) and red dots represent native thiol assays. The half value of the difference between total thiol and native thiol amounts gives the disulphide bond amount. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Analytical recovery

The per cent recovery of the novel method was determined via the addition of 200 μM oxidised glutathione to plasma samples. The mean percent recovery was 98–101%.

Linearity

The linearity of the native thiol measurement was the same with that of Ellman's reagent assay. Serial dilutions of the glutathione solution were generated. The upper limit of the linearity for the native thiol measurement was 4000 μM . Linearity of the total thiol measurement was also dependent on the amounts of NaBH_4 and formaldehyde concentrations. Serial dilutions of the oxidised glutathione solution

were also generated. The upper limit of the linearity for the disulphide measurement was 2000 μM . Dilution of plasma samples did not affect the novel assay.

Lower detection limit

The detection limit of the assay was determined by evaluating the zero calibrator 10 times. The detection limit, defined as the mean value of zero calibrator + 3 standard deviations (SDs), was 2.8 μM .

Analytical sensitivity

As the slope of the calibration line, analytical sensitivity was found to be 7.9×10^{-4} Absorbance/Amount, $[\text{A} \times (\mu\text{M})^{-1}]$.

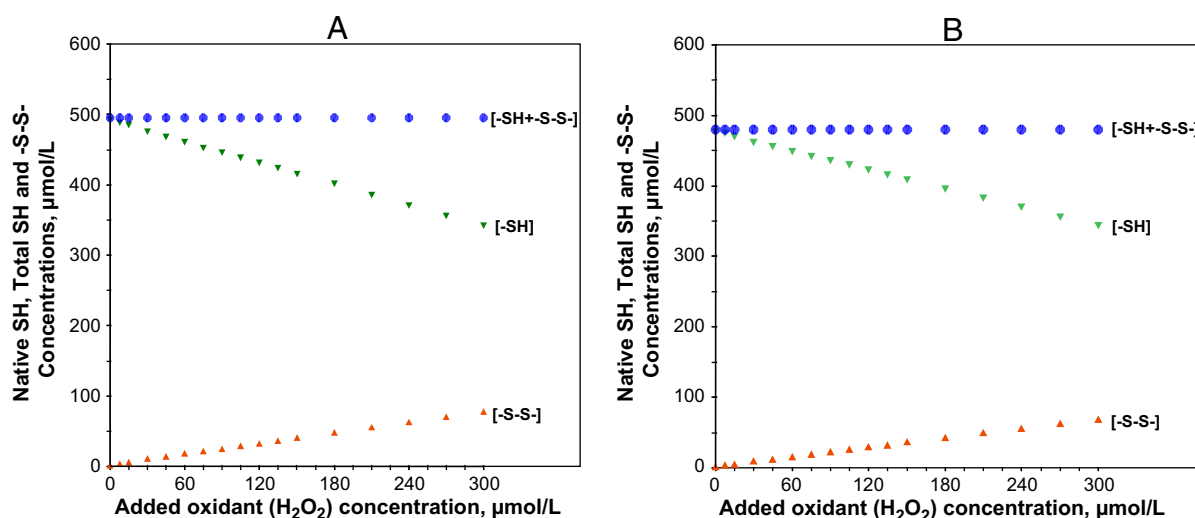


Fig. 3. A: The changes in native thiol, total thiol and disulphide concentrations of 2-mercaptoethanol solution by increasing the oxidant, hydrogen peroxide. B: The changes in native thiol, total thiol and disulphide concentrations of glutathione solution by increasing the oxidant, hydrogen peroxide.

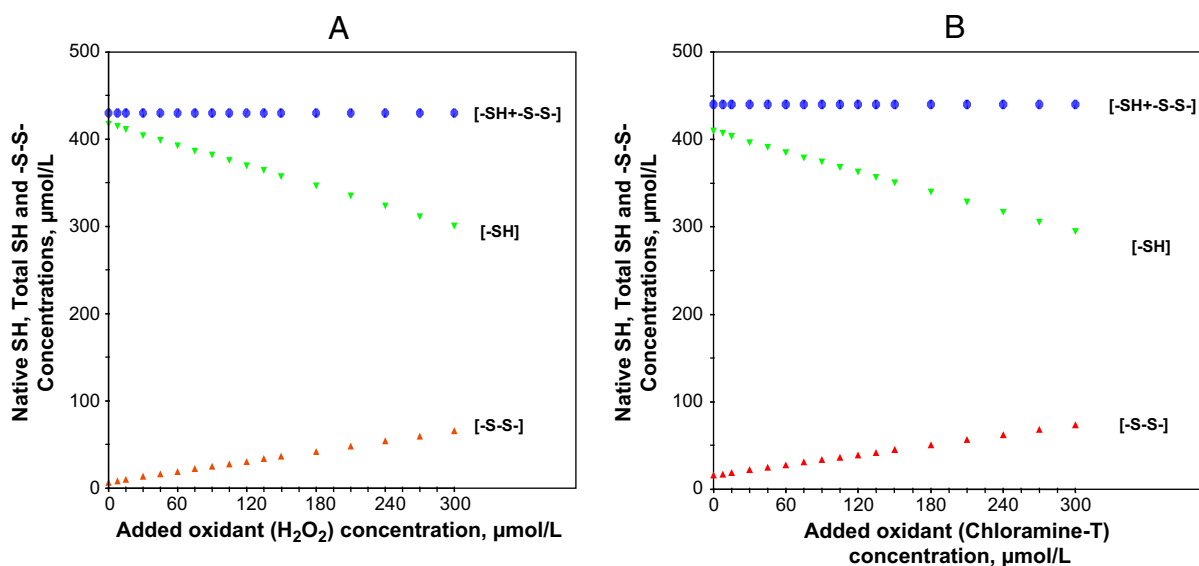


Fig. 4. A: the changes of native thiol, total thiol and disulphide concentrations of albumin solution by increasing the oxidant, hydrogen peroxide. B: the changes of native thiol, total thiol and disulphide concentrations of the plasma pool by increasing the oxidant, chloramine-T.

Interference

It was found that haemoglobin, EDTA, citrate and oxalate did not interfere with the assay developed, but bilirubin did negatively interfere with the assay. Lipaemic and uraemic plasma samples did not interfere with the assay. Plasma and serum samples can be used as samples.

Precision

To determine the precision of the novel assay, we assayed three levels of a plasma pool. A plasma pool that had high disulphide levels was obtained from the samples of patients with diabetes mellitus. The plasma pool with medium disulphide levels was obtained from the samples of healthy persons. The plasma pool with low disulphide levels was obtained from the samples of patients with urinary bladder cancer. Per cent coefficient variation (%CV) was 4 ($\bar{X} = 29.12$ and $\sigma_X = 1.2$) for high levels, 5 ($\bar{X} = 16.03$ and $\sigma_X = 0.79$) for medium levels and 13 ($\bar{X} = 7.15$ and $\sigma_X = 0.98$) for low levels.

Storage

Storage at 4 °C for 1 day led to a 7% decrease in the native thiol amount and 170% increase in the disulphide amount (total thiol, native thiol and disulphide levels of fresh and stored plasma samples were 391 $\mu\text{mol/L}$, 357 $\mu\text{mol/L}$, 17 $\mu\text{mol/L}$ and 391 $\mu\text{mol/L}$, 333 $\mu\text{mol/L}$, 29 $\mu\text{mol/L}$, respectively). Plasma native thiol, total thiol and disulphide concentrations were not affected by storage at -80 °C for 3 months.

Comparison of plasma and serum thiol and disulphide amounts

There were no significant differences between native thiol, total thiol and disulphide levels of plasma and serum sample pairs ($p > 0.05$).

Reference interval

To determine the reference interval for plasma disulphide levels, plasma specimens from 120 healthy individuals (60 women, 60 men, 16–64 years old) were assayed. The reference range was 6.65–27.93 $\mu\text{mol/L}$. Plasma disulphide levels were higher in patients with degenerative diseases such as inflammation, smoking, diabetes and

obesity, and were lower in patients with neoplastic diseases such as renal cancer, colon cancer, urinary bladder cancer and multiple myeloma.

Discussion

Thiol chemistry is a rapidly growing field in basic and applied bioscience, but there has been no report evaluating plasma dynamic thiol disulphide homeostasis. All of the published reports are experimental, manual, sophisticated studies, and they generally relate to the determination of thiol and disulphide amounts of low-molecular-weight thiol compounds such as Cys, CySS, GSG and GSSG [20–23]. Moreover, there is no published manual or automated method for the measurement of plasma dynamic thiol disulphide status [19]. In this study, thiol disulphide homeostasis was determined by measuring mainly native thiol and reducible dynamic disulphide amounts. Other related parameters were generated by calculations.

In previous advanced experimental studies, disulphide bonds were reduced to thiol groups using TCEP [20], tributylphosphine [22] and NaBH_4 [21]. However, the remaining reductants hampered the measurements. For this reason, the remaining reductants must be absolutely removed before the measurement. Different techniques have been used to remove reductant remnants, such as filtration, chromatography and dialysis, but none of these are appropriate for fully automated measurement. Glowacki et al. [20] used a dialysis system, Carru et al. [22] precipitated proteins via 5-sulphosalicylic acid and Chen et al. [21] acidified the reaction medium to remove the remaining NaBH_4 . The dialysis system and precipitation of proteins cannot be applied to automated analysers. On the other hand, acidification of the medium leads to abundant gas and bubble formation, which prevents spectrophotometric measurement and inhibits colour formation of DTNB because the acid medium leads to decolourisation of the formed 5-thio-2-nitrobenzoic acid [18].

In the novel method, the remained, unused reductant NaBH_4 was completely consumed and removed using formaldehyde solution. Moreover, there was no gas and bubble formation, and thus, no light scattering and no light transmission instability were observed in the spectrophotometric measurements. On the other hand, it was shown that the used formaldehyde did not interfere with the measurements (Fig. 1A). There was no significant difference between the original Ellman reagent and the modified reagent results (Fig. 1C). As seen in Fig. 1B, the correlation coefficient was also excellent ($r = 0.99$ and

$p < 0.0001$). According to linear regression analysis ($y = ax + b$), a was 1.0 and b was 0.0. It was decided that the modified reagent can be confidently used in the assay pair. Formaldehyde solution, which was shown not to affect the thiol measurement, was used in the reagents of the assay pair. Thus, maximal similarity and parallelity of the reagents of the assay pair were obtained.

In the first step of the assay, only dynamic and reducible disulphide bonds were completely reduced to free functional thiol groups. Static and structural disulphide bonds were not. In the second step, the remaining, unused reductant NaBH_4 , was completely consumed, and in the last step, all thiol groups – consisting of native thiol groups plus reduced thiol groups – were reacted with DTNB (Fig. 2). Half of the difference between assay pairs gave disulphide amounts which were calculated automatically. In addition to the measurement of plasma native thiol and disulphide amounts, $-\text{S}-\text{S}-/-\text{SH}$, $-\text{S}-\text{S}-/(-\text{SH} + -\text{S}-\text{S}-)$, $-\text{SH}/(-\text{SH} + -\text{S}-\text{S}-)$ ratios were calculated automatically and synchronously.

GSH, 2-mercaptoethanol, albumin solutions and plasma samples were used to monitor thiol–disulphide exchange reactions as low and the high molecular weight and thiol compound species (Figs. 3–4). Each sample containing thiol was oxidised with an oxidising agent at serially increasing concentrations. Before the oxidation processes, the native thiol contents were measured; thus, the native thiol contents of the samples were determined. By adding the oxidant at increasing concentrations, native thiol capacities decreased linearly and disulphide amounts increased in a correlated fashion. The increase was positively correlated with the added oxidant amounts ($r = 0.99$, $p < 0.0001$) and negatively correlated with decreases in the native thiol amounts ($r = -0.99$, $p < 0.0001$). Total thiol amount did not change and it maintained a plateau trace.

GSH, 2-mercaptoethanol and albumin molecules have only one free functional sulfhydryl group. Plasma albumin contains 17 static disulphide bridges which preserve its native structural conformation [24]. These static and structural disulphide bonds were not reduced to the free functional sulfhydryl group in this assay. This does not take place in dynamic thiol disulphide exchange reactions, and its role is related to the maintenance of the static and conformational status of the globular structure of albumin. In our experiments, we observed that while the free thiol group of albumin was implicated in the oxidation reduction reactions, the mentioned structural disulphide bridges did not affect the reduction reactions in the assay. As seen in Fig. 4A, the measured total thiol amounts drew a plateau line, while oxidant concentrations changed from zero to the highest concentration. If the static and structural disulphide bonds were part of the reduction reaction, we would have obtained a total thiol concentration which was 34 times higher than that of the measured free thiol concentration when the oxidant concentration was zero in the reaction medium. We determined only one thiol amount as a response to presenting one albumin molecule, while reductant NaBH_4 was present in the medium. These findings show that only functional dynamic disulphide bonds are involved in the reduction to functional sulfhydryl groups. In biological and physiological processes, only the free thiol group of albumin is involved in oxidation reactions, but the static and structural disulphide bridges do not operate in the reduction reactions during thiol–disulphide homeostasis processes.

Various experimental studies reported that alteration of the thiol/disulphide ratio leads to changes in cellular status. It has been shown that the increase in the GSH/GSSG redox state leads to proliferation, while the decrease in the GSH/GSSG redox state leads to apoptosis [25,26]. It has also been shown that cellular GSH/GSSG and Cys/CySS redox potentials decrease as a result of ageing, alcohol abuse, diabetes mellitus, cigarette smoking, increased carotid intima media thickness, reversible myocardial perfusion defects, chemotherapy and lung transplantation [25].

Our first preliminary results (Fig. 5) showed that plasma disulphide levels were higher in patients with degenerative diseases such as

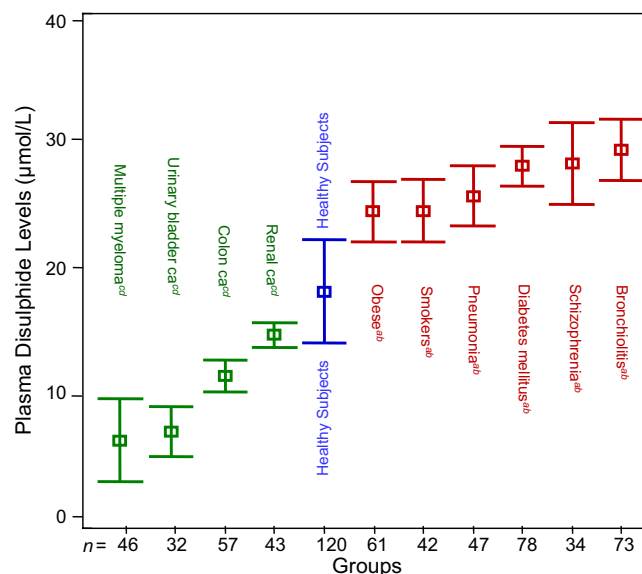


Fig. 5. Plasma disulphide levels ($\bar{X} \pm \sigma_M$) of patients with proliferative diseases and degenerative diseases and healthy subjects (a: higher than healthy subjects, b: higher than proliferative diseases, c: lower than healthy subjects, d: lower than degenerative diseases, $p < 0.05$).

smoking, diabetes, obesity and pneumonia, and were lower in patients with proliferative diseases such as multiple myeloma, urinary bladder cancer, colon cancer and renal cancer. Aggressively growing tumours showed the lowest disulphide levels, while slowly growing ones showed subnormal values. In previously performed studies, only GSH/GSSG and Cys/CySS redox parameters have been determined at the cellular level. In our study, plasma dynamic thiol/disulphide homeostasis was determined totally by using an automated method.

Conclusions

Plasma dynamic thiol/disulphide homeostasis can be fully automated, as determined by the described assay. In this study, plasma disulphide levels showed different, original and interesting patterns for various disease spectra. The developed assay has high and wide usage potential in research studies and clinical biochemistry laboratories, and has high potential to generate new knowledge and concepts.

Conflict of interest

There are no conflicts of interest.

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