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Mixed results with mixed disulfides

Regina Brigelius-Flohé

German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany

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ABSTRACT

A period of research with Helmut Sies in the 1980s is recalled. Our experiments aimed at an in-depth understanding of metabolic changes due to oxidative challenges under near-physiological conditions, i.e. perfused organs. A major focus were alterations of the glutathione and the NADPH/$NADP^+$ system by different kinds of oxidants, in particular formation of glutathione mixed disulfides with proteins. To analyze mixed disulfides, a test was adapted which is widely used until today. The observations in perfused rat livers let us believe that glutathione-6-phosphate dehydrogenase (G6PDH), i.a. might be activated by glutathionylation. Although we did not succeed to verify this hypothesis for the special case of G6PDH, the regulation of enzyme/protein activities by glutathionylation today is an accepted post-translational mechanism in redox biology in general. Our early experimental approaches are discussed in the context of present knowledge.

Introduction

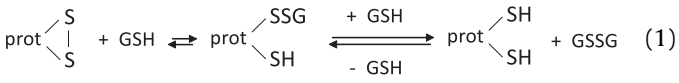
Helmut Sies and myself, we both studied in Tübingen in the late 1960s. However, we only saw from a distance, since he soon left to Munich to work on his MD thesis. When I spent an exchange year in Munich in 1971/72 at the Institute of Physiological Chemistry headed by Theodor Bücher, Helmut was there already busy with his habilitation and I cannot remember whether we said more than 'hallo' and 'how are you'? I returned to Tübingen in 1972 to finish my education in biochemistry but came temporarily back to Munich to work for my diploma thesis at the Gesellschaft für Strahlenforschung (GSF; now Helmholtz Zentrum München) together with Wolf Bors, Manfred Saran and Edmund Lengfelder on the SOD activity of low molecular weight copper chelates measured by pulse radiolysis, still with little contact to Helmut. Thereafter, I disappeared to Münster for my PhD. In 1977, I moved back to Munich to join the Institute of Veterinary Pharmacology. There I was working on the toxicity of redox cyclers as inducers of oxidative stress in perfused rat livers. At that time, an "oxygen club" had been established, where all oxygen and radical freaks of Munich met. Thus, after about 10 years of separate ways at same places, I finally got the chance to regularly talk with Helmut about oxidative stress, glutathione, the Alpes (see Fig. 1) and horses, and this talk is alive until today. In 1979, Helmut accepted a call to Düsseldorf. We kept contacts, and when I spend some days in Düsseldorf to give a talk, Helmut asked me whether I might be interested in a position at his institute. I said "yes" and moved to Düsseldorf in 1980.

At that time, Helmut was interested in processes affecting the glutathione status in liver and peripheral organs. It was known that peroxides caused an increase in GSSG due to the GPx reaction and that the GSSG was either reduced by glutathione reductase (GR) or secreted into the bile [1–3]. In sum, these reactions led to a loss of GSH. It was also already known that mixed disulfides of GSH with protein thiols existed [4]. However, the questions remained whether oxidative stress enhanced mixed disulfide formation and whether this had consequences for the activity and/or function of affected proteins/enzymes.

Here, I will focus on these questions, recall what we found, and briefly summarize how the present state-of-the-art in the field evolved.

1. The crux to correctly quantify protein-GSH-mixed disulfides

In the early 80s mixed disulfides were described to be formed by the reaction of GSH with protein disulfides or of GSSG with protein free thiols (eq (1)).



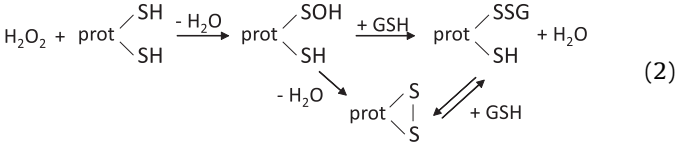
(1)

E-mail address: flohe@dife.de.

http://dx.doi.org/10.1016/j.abb.2015.11.011
0003-9861/

Fig. 1. Members of the Oxygen Club Munich climbing mountains in the Alpes.

An alternative way was also already at the horizon, since the formation of sulfenic acid intermediates in proteins by H_2O_2 had been demonstrated [5] and their reaction with GSH to form mixed disulfides appeared straightforward (eq. (2)).



(2)

Apart from eqs. (1) and (2), mixed disulfides can be formed via sulfenylamide, thiosulfinate, S-nitrosyl and, less likely, thiyl radical intermediates [6]. Analytical methods to quantify mixed disulfides had been reported. They essentially followed the same principle: reduction of the disulfide bridges and determination of the GSH thus released. What differed was i) the way of disulfide reduction, ii) precipitation of protein, iii) test for GSH and iv) sample preparation in general. For GSH determination essentially 4 methods were at hand:

1. Chemical reaction of reductively released thiols by group-specific reagents.

O-phtalaldehyde (OPT) [7] or 5,5'-dithiobis(2-nitro-benzoid acid) (DTNB) [8] were used as reagents and the reaction products estimated by optical methods with GSH as external standard.

2. Kinetic assays of GSH-dependent enzyme reactions.

Here mainly the coupled DTNB-GR test [9,10] was used. The test determines total glutathione (GSH and GSSG) and results in the quantification of GSH-equivalents.

3. Endpoint assays with GSH-consuming enzymes, *e.g.* glutathione-S-transferase [11].

4. HPLC analysis after GSH derivatization with iodoacetic acid and 1-fluoro-2,4-dinitrobenzene [12].

We started with the OPT method, since this was widely considered the method of choice. I first was excited, because we indeed found mixed disulfides in amounts of about 500 nmol/g liver! But the pleasure did not last very long. The amounts of mixed disulfide were not strikingly increased after treatment of livers with tBOOH, nitrofurantoin (NF) or paraquat (PQ), whereas GSSG starting from a level of 20 nmol/g liver was at least doubled under oxidative stress [13,14]. After fierce discussions with Helmut, he even sent me to the lab of Harisch in Hannover to learn the OPT method. However, results remained unchanged and I finally decided to try alternative methods and to compare the outcome with that obtained with OPT. I adopted the DTNB-GR method from Theo Akerboom (method 2), adopted a new one based on the GSH-S-transferase-catalyzed conjugate formation of CDNB and GSH (method 3), which can be monitored at 330–360 nm, and estimated borohydride-released and iodoacetic acid and fluoro-2,4-dinitrobenzene derivatized GSH by HPLC (method 4). All alternative methods gave a basal mixed disulfide level of 20–30 nmol/g liver and an increase after treatment with oxidants to 40–180 nmol/g liver, which perfectly corresponded to the increase of GSSG (see Table 1). In contrast, levels measured by OPT were much higher and remained unaffected by oxidants. Obviously, OPT was not specific for GSH and detected all kinds of SH groups as later confirmed by others [15]. Furthermore, serious problems resulted from the way of sample preparation.

With the novel results on a poster entitled 'Glutathione in hepatic protein mixed disulfides', we started to the 5th Karolinska Institute Nobel Conference in Skokloster in May 1982. I was quite nervous, since all the mixed disulfide levels so far reported were those obtained with the OPT method and accordingly higher than ours. Many people assembled in front of my poster, among them Nechama Kosower, the most famous GSH-lady at that time. While others critically pointed out that so far mixed disulfides levels were