Quantification and Localisation of SH-Groups in Human Blood Serum Proteins

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Z. Naturforsch. **33 c**, 803 (1978);
received August 4, 1978

Human Blood, Serum Proteins, SH-Groups

The thiol groups of human blood serum proteins were determined after 24 hours interaction with dithionitrobenzoic-acid (DTNB) to an average of 538 ± 60 μmol/l serum. After treatment of the serum with [^{35}S]DTNB, autoradiograms of the protein elpherograms revealed two main peaks: The first with 63% of total activity, in the albumin region, corresponding to 0.60 SH/mol, the second with 23% of total activity, in the γ-globulin range, corresponding to 2.2 SH/mol. After 30 minutes incubation with DTNB, or with *p*-chloromercuribenzoate (CMB), in freshly prepared pools of IgG only 0.2 SH/mol were found which is the expected value already known from the literature.

Autoradiograms taken from serum protein elpherograms after interaction with [^{14}C]CMB only show the main SH-peak in the albumin range. Thus ist is concluded that the SH-peak in the γ-globulin region after 24 hours incubation with [^{35}S]DTNB is due to one highly labile S-S-bond which easily undergoes a disulfide exchange with DTNB.

Blood serum freshly taken from 50 healthy male probands (20 to 45 years old) is diluted 1:10 with 0.1 M phosphate buffer pH 7.0 and subsequently mixed with the tenfold volume of phosphate buffer containing 1×10^{-2} M 5.5′-dithio-bis-nitrobenzoic-acid (DTNB).

After 24 hours reaction time at 4 ° in the dark the absorbance of the samples were measured at 412 nm. From the values corrected for blank absorbance of DTNB and serum, respectively, it was computed that human serum contains an average of 538 ± 60 μmol protein SH-groups per liter.

Similarly, the serum samples were treated with ^{35}S-labeled DTNB and separated electrophoretically on cellulose acetate strips (buffer pH 8.4, 110 V, 2 h). The autoradiograms prepared from the strips by 4 to 8 weeks exposure to an X-ray film exhibited a distinct main band in the albium region and another weaker one in the γ-globulin region. The quantitative densitometry revealed that (in the average) 63% of total radioactivity was present in the albumin peak, 23% in the γ-globulin peak and 14% scattered rather uncharacteristically in the region of α- and β-globulin. Based on these values and the (simultaneously estimated) content of albumin, γ-globulin and total SH-groups in the serum it was calculated that albumin (mole weight 69,000) has an SH content of 0.60 mol per mole and γ-globulin (mole weight 160,000) 2.2 mol per mol.

The value found for albumin is in excellent agreement with the already known SH content of isolated serum albumin and may thus be used as an internal standard for the method.

The SH content found for γ-globulin, however, appears surprisingly high, as an SH content of only 0.2 reactive SH/mol IgG which comprises the major part of the γ-globulin fraction, is reported in the literature [1].

This value was also confirmed by own experiments with freshly prepared pools of IgG. When these samples were incubated for 30 min with DTNB the developed yellow colour corresponded to 0.2 mol SH/mol IgG. Additional experiments using *p*-chloromercuribenzoate (CMB) as SH reagent yielded similar values. Furthermore, autoradiograms of electrophoretically separated serum samples labeled with [^{14}C]CMB instead of [^{35}S]-DTNB showed only one mayor peak (70% of total radioactivity) in the albumin region, but no distinct peak in the γ-globulin area.

These different results in respect to the SH content of the γ-globulin fraction suggest, that the γ-globulins contain in addition to 0.2 mol of reactive SH/mol also one highly labile disulfide bond, which reacts during long time incubation with DTNB in a disulfide exchange reaction [2].

$$\text{Prot} - \text{SH} + \text{XSSX} \qquad \text{Prot} - \text{SX} + \text{HSX}$$

$$\text{Prot} \begin{matrix} \text{S} \\ \text{S} \end{matrix} + \text{XSSX} \qquad \text{Prot} \begin{matrix} \text{SSX} \\ \text{SSX} \end{matrix}$$

The experiments are being continued for further clarification of the suggested secondary reaction of DTNB and in collaboration with W. List * and coworkers for investigations of pathalogical serum samples.

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[1] C. I. Luks and G. E. Connel, Canad. J. Biochem. **46**, 961–964 (1968).

[2] M. Friedman, Chemistry and Biochemistry of Sulfhydrylgroups in Amino Acids, Peptides and Proteins, Pergamon Press, London 1973.