Measurements of Human Epidermal Keratin SH Groups: a New Micromethod

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A study of the physicochemical characteristics of keratin, the principal epidermal protein which acts as the first and main barrier protecting from external influences, including the penetration of pathogens, is of crucial importance in many diseases, diabetes being one of them. It has been previously revealed that the development of experimental diabetes is associated with a tenfold reduction of SH groups in murine epidermal prekeratin in comparison with that in intact animals, this being paralleled by a corresponding increase in the number of SS groups [1]. These changes implied drastic shifts in keratin structure in diabetes. Unfortunately, current methods for measuring protein SH groups are labor-consuming and require large quantities of protein. We have attempted to develop a micromethod for measuring keratin SH groups using an original noninvasive method for obtaining keratin from the horny layer of the epidermis. The technique of keratin SH group determination is based on the effect of free cysteine SH groups on sulfanilic acid oxidation with ammonium persulfate in the presence of silver and bipyridyl ions. The role of cysteine SH groups consists in binding silver ions, this resulting in inhibition of sulfanilic acid oxidation with ammonium persulfate (indicator reaction). The cysteine content in the reaction mixture may be evaluated from the degree of indicator reaction inhibition [2]. The indicator reaction rate is assessed spectrophotometrically at wavelength 495 nm. The increment of reaction mixture absorption (ΔA) at a given wavelength corresponds to the increment of the concentration of chromophore, the indicator reaction product. The relationship between solution absorption and the time characterizes the rate of the indicator reaction, which remains unchanged for about 20 min and is inversely proportional to the cysteine content.

The present research was aimed at elucidating the possibility of using reduced glutathione as a standard for subsequent measurements of the SH groups of cysteine in the polypeptide chain and at developing conditions for SH group measurements in keratin isolated from the horny layer of human epidermis.

MATERIALS AND METHODS

Glutathione solution in a concentration of 1.5 mg/20 ml was used as a cysteine-containing reference solution. The reaction mixture contained (in %): 1) 0.1 M acetate buffer solution, pH 4.35; 2) fresh bipyridyl solution (15.6 mg/6 ml water) containing 5 μl of silver solution (0.01 M); 3) cysteine-containing protein solution; 4) sulfanilic acid (17.3 mg/10 ml water); 5) ammonium persulfate (288 mg/10 ml water). For the measurement of cysteine, glutathione, and protein SH groups, solutions 1-5 were incubated at 40°C for 15 min. The solutions were mixed in a cuvette in the following ratio: 0.5 ml of solution 1,

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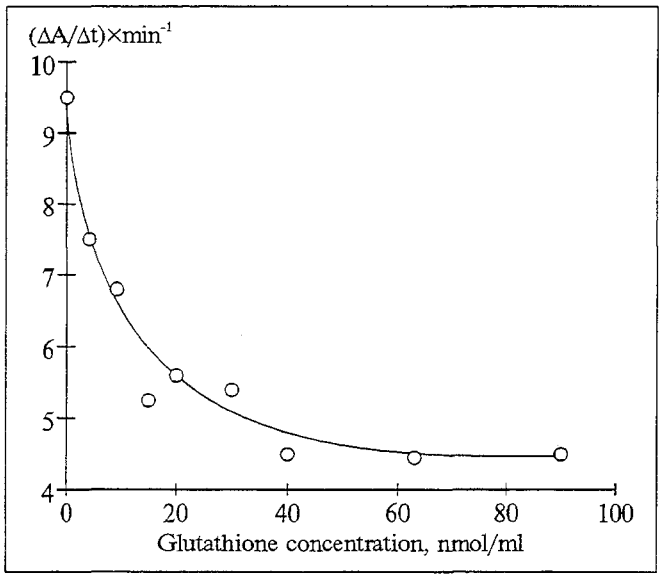

Fig. 1. ΔA/Δt as a function of glutathione concentration.

0.100 ml of solution 2, 0-50 μl of solution 3, and 0.200 ml of solution 4 and 5 per ml. The reaction mixture was placed in a termostatic (40°C Shimadzu spectrophotometer cell and the direct relationships between solution absorption and time were recorded for 5-10 min at wavelength 495 mn. Epidermal horny layer keratin was obtained using special films, this being followed by protein extraction and measurement [3].

RESULTS

Measurements of the indicator reaction rates as a function of glutathione added to the reaction mixture (Fig. 1) showed that a linear dependence is observed within the range of glutathione concentrations from 0 to 15 nmol/ml. Analysis of the linear segment of this curve (Fig. 2) showed an inversely proportional relationship between the solution absorption increment rate (ΔA/Δt) and the concentration of glutathione SH groups. These data prove that the suggested test for measuring SH groups of cysteine within the glutathione peptide chain may used to measure cysteine within the protein molecule and that glutathione may be used as a calibration standard. The sceme also demonstrates that the maximal deviation from the straight line along the glutathione concentration axis does not exceed 13.8% of the maximal glutathione concentration, this permitting measurements of SH groups within the said range with an accuracy up to ±1.9 nmol/ml.

Nevertheless, bearing in mind the possibility of side effects occurring during protein SH group measurements, depending first of all on the protein concentration in the reaction mixture and distorting the value of the absorption increment rate (ΔA/Δt), we tested the permissible concentrations of protein solution. For this purpose a protein (crystalline insulin) containing no free SH groups was used. The ΔA/Δt value remained constant when up to 0.2 mg/ml insulin was added to the reaction mixture (Fig. 3). A higher concentration of protein in the reaction mixture resulted in an increase of ΔA/Δt value. Hence, the permissible protein concentrations in the reaction mixture for measuring SH groups should not surpass 0.2 mg/ml.

SH groups were measyred in a solution of keratin isolated from epidermal horny cells of two healthy volunteers. The protein concentrations in the reaction mixture were the range of 0.05 to 0.07 mg/ml. The content of SH groups thus measured was 0.11±0.03

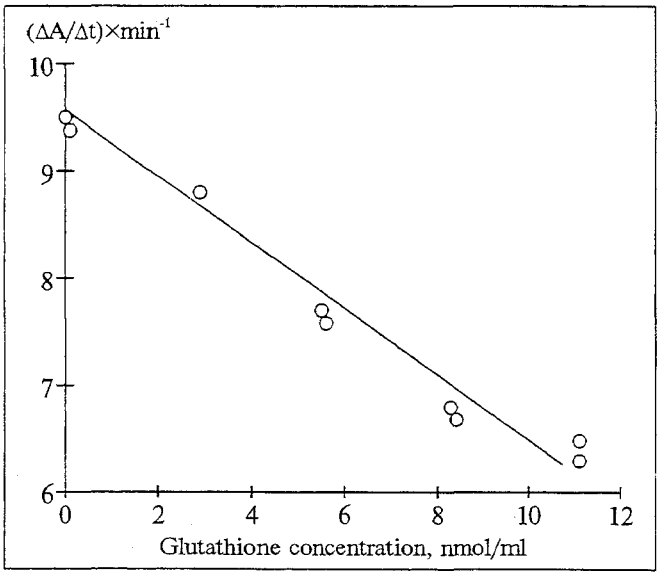

Fig. 2 ΔA/Δt as a function of glutathione concentration (region of linear dependence).

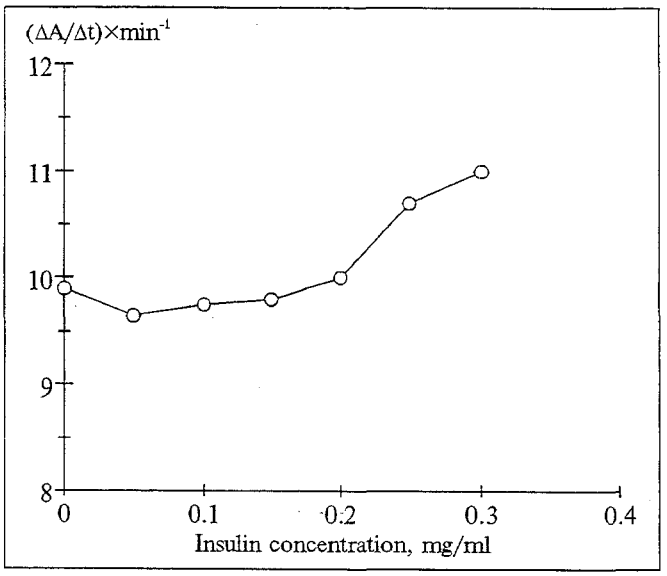

Fig. 3. ΔA/Δt as a function of insulin concentration.

μmol/mg protein for one donor and 0.10±0.04 μmol/mg protein for the other donor.

Thus, the suggested modified method may be used to measure cysteine SH groups in keratin and glutathione may be used as a reference reagent for quantitative characterization of peptide chain cysteine SH groups.

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