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Note

Determination of Sulfhydryl Groups in Proteins and Enzymes by Indirect Amperometric Titration*

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R. Hamm and K. Hofmann[1,2] described in 1965 a method for the determination of sulfhydryl groups by indirect amperometric titration of proteins with silver nitrate. This method is very useful because it can be applied to turbid solutions, suspensions or homogenates of miofibrills, muscle extracts or enzymes. We modified the above mentioned method using phenylmercuric acetate instead of $AgNO_3$ for the preincubation. The principle of the method is shown diagramatically in Fig. 1.

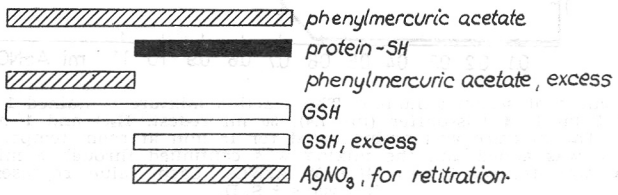

Fig. 1. Principle of the determination of SH-groups in proteins. The protein was preincubated with a large amount of phenylmercuric acetate. To the reaction mixture was added glutathione (GSH) and the excess of GSH, corresponding to the amount of SH-groups in the protein was retitrated with $AgNO_3$. Schematic representation after Hamm and Hofmann[1].

The advantage of our modification consists in shortening the time needed for the incubation of proteins in comparison with the original method which used an incubation time of 60 minutes. We verified the method by determining sulfhydryl groups in glutathione, human serum albumin and various commercial samples of urease. By direct amperometric titration we found one SH-group per mole of glutathione. In serum albumin 0.63 ± 0.02 SH-group per mole was found by indirect amperometric titration. The reason for this discrepancy lies in the fact that the protein consists of two closely related proteins, of which only one has a free SH-group. Our finding is in agreement with the data reported by a number of investigators[3,4] (Fig. 2).

Using various commercial samples of urease (»Merck«, »Kemika«) we obtained reproducible results. The amount of the sulfhydryl groups per mole found was 109 ± 7 using the value of 483000 as molecular weight for urease[5].

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The number of SH-groups per mole found by other authors was 109—113[6] (Fig. 3).

In the meantime the paper[7] appeared reporting on the determination of thiol groups with $AgNO_3$, *N*-ethylmaleimide (NEM) and *p*-chloromercuribenzoate by the same technique. The application of *N*-ethylmaleimide, *p*-chloromercuribenzoate and phenylmercuriacetate is recommended for checking and completing the results obtained with $AgNO_3$.

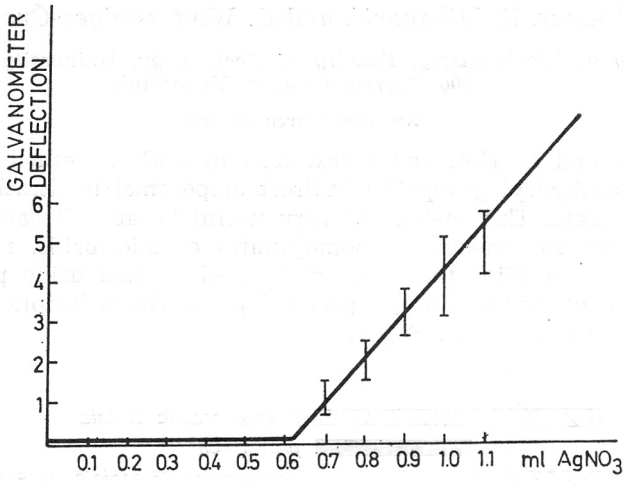

Fig. 2. Titration curve of serum albumine. The reaction mixture contained 1 ml 10^{-3} *M* human serum albumine 5 ml 1 *M* tris-buffer (*p*H 7.4), 30 ml redest. H_2O and 1 ml 10^{-3} *M* phenylmercuric acetate. The mixture was preincubated for 15 min at room temperature. Then, 1 ml 10^{-3} *M* glutathione was added and the mixing was continued through 5 min. After that the reaction mixture was retitrated with 10^{-3} *M* $AgNO_3$. Each value represents the mean of 30 assays ± S. D.

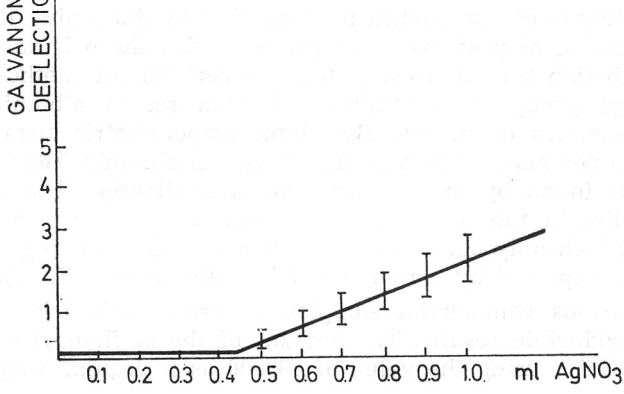

Fig. 3. Titration curve of Merck urease. The reaction mixture contained 2 ml urease (mg/ml), 5 ml 1 *M* tris-buffer (*p*H 7.4), 30 ml redest. H_2O and 1 ml 10^{-3} *M* phenylmercuric acetate. Experimental procedure was the same as mentioned in Fig. 2.

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IZVOD

Određivanje sulfhidrilnih grupa u proteinima i enzimima metodom indirektne amperometrijske titracije

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Razrađena je nova varijanta određivanja sulfhidrilnih skupina u proteinima i enzimima metodom indirektne amperometrijske titracije. Metoda je provjerena određivanjem sulfhidrilnih grupa u serum albuminu i ureazi.

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