The results for the dependence of the rate of the reaction on the concentration of sulfate at fixed concentration of Mg^{2+} in the range of all the concentrations of the ion are linearized satisfactorily in the Lineweaver–Burk coordinates (Fig. 1b). The maximum rate of the reaction, $V = K_{cat} \cdot E_0$ (where E_0 is the concentration of the enzyme and K_{cat} is the constant of the catalytic stage of the reaction) did not change with a variation in the amount of activator. Conversely, the value of the Michaelis constant (the intercept on the axis of abscissa, Fig. 1b) decreased with a rise in the concentration of magnesium ions, which, in its turn, indicates an increase in the role of the metal in the affinity of the enzyme and the substrate, since the smaller is the Michaelis constant the higher is the efficiency of an enzymatic reaction.

Thus, the facts that we have observed indicate that magnesium ions are an activator of cotton plant alkaline pyrophosphatase and there is no effect of inhibition by an excess of the metal.

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DETECTION OF CYSTINE AND ITS PEPTIDES ON TLC

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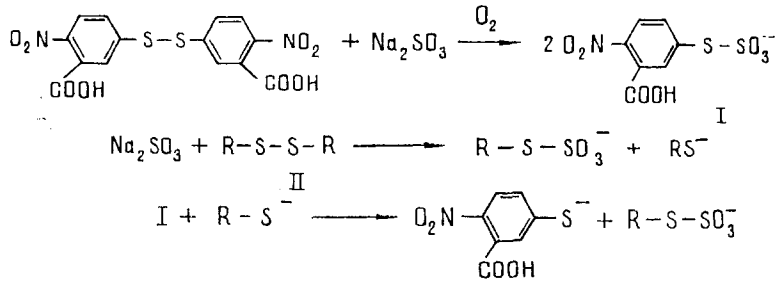
UDC 543.544:547.964

Cystine and its derivatives are detected on chromatograms by the use of the nitroprusside reagent. The plates are sprayed with sodium cyanide and dried and are then sprayed with a solution of sodium nitroprusside [1]. This method is inconvenient for two reasons: in the first place it is fairly lengthy and, in the second place, it requires the use of the highly poisonous sodium cyanide. In view of this, the search for safer reagents and for convenient procedures for revealing cystine and its derivatives is necessary. A quantitative spectrophotometric method of determining disulfide bonds in proteins and peptides in solution has recently been proposed [2]. The Ellman reagent [dithiobis(nitrobenzoic acid)] is converted into the S-sulfo derivative (I), the protein or peptide (II) is treated with sodium sulfite and then reagent (I) is added and the absorption at 412 nm is measured.



We have established that a similar method can be used for detecting cystine and its peptides on TLC. The spraying of the plate with a 1 M solution of sodium sulfite, drying, and spraying with reagent (I) permits cysteine and cystine to be detected on a chromatogram, but the sensitivity of this method is no better than 100 μg. This is probably connected with the rapid oxidation in air of the mercapto groups formed on the action of sulfite on a disulfide bond. However, since reagent (I) is obtained in an excess of sodium sulfite, the stage of the preliminary spraying with a solution of sulfite can be omitted.

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In actual fact, the use of reagent (I) alone led to considerably better results. A solution of nitrothiosulfobenzoate [2] was diluted 10-fold and a chromatogram was sprayed with it. Cysteine and cystine gave yellow spots on a white background. When silica gel or polyamide is used as the support, 2 μg of cystine can be detected in a spot, while when paper is used the amount is ≥ 10 μg. Cysteine appears rapidly. In the case of cystine, the coloration develops in 10-15 min. Other amino acids are not revealed by this reagent.

Cystine peptides with free amino and carboxy groups appear in the same way as cystine. When the amount of S–S bonds in a spot is ≥ 15 nmole, free peptides are readily detected on a chromatogram.

Protected cystine peptides appear considerably more slowly (time of development of the coloration 30-40 min) and in higher concentrations (20-30 μg) in a spot than in free ones. Protected cysteine peptides appear similarly.

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CIRCULAR DICHROISM SPECTRA OF THE PROTEIN FRACTIONS OF MAIZE

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An investigation of the circular dichroism (CD) spectra of plant proteins will permit the laws of their structural changes to be revealed [1]. We have previously [2] studied the nature of the absorption spectra of the protein fractions of maize in the ultraviolet and infrared regions, and the results of this can be used in practice for selection evaluation. In connection with this, it appeared of interest to determine the possibility of the CD method for investigating the albumins, globulins, zeins, and glutelins of maize.

The protein fractions were isolated from maize grain in the phase of full ripeness by the procedure described previously [3, 4]. The spectra were obtained on a DKhR-02 dichrograph and, for comparison of the preparations, the CD optical densities were calculated for the absorption of a 1% solution in a 1-cm cell.

A comparison of the CD spectra of the protein fractions revealed characteristic differences (Fig. 1). The CD band of the albumins was characterized by maxima at 216 and 220 nm,

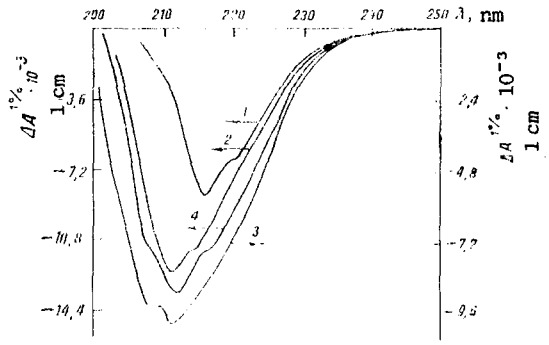

Fig. 1. CD spectra of maize albumins (1), globulins (2), zeins (3), and glutelins (4).

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