PHOTOMETRIC STARCH-IODINE DETERMINATION IN PLANT MATERIALS AS INFLUENCED BY ASCORBIC ACID. CRITICAL REMARKS

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ABSTRACT

Following the work of Sharma et al. (1990) on the interference of ascorbic acid (AA) with starch-iodine reaction, the present authors repeated it, giving a proper explanation of the influence of AA in this reaction. AA oxidizes iodine-iodide (I-KI) reagent which makes impossible to form the blue complex with starch. Three measures are suggested to overcome the interference of AA: 1) to titrate starch solution with I-KI reagent and thus work out an I-KI amount for development of blue coloration, 2) to remove from plant material AA (and other reducing soluble substances) by washing the material with cold water or ethanol, and 3) starch solution could be treated with alkali (pH about 9), which destroys AA in 15 minutes. After acidifying the solution and adding I-KI reagent the blue complex can be determined.

KEY WORDS: Starch-iodine complex, ascorbic acid, interference

INTRODUCTION

Owing to the classical work of McCready and Hassid (1943) starch can be estimated quantitatively using a colorimetric procedure. The procedure is simple and rapid provided that some physicochemical conditions of starch solution preparation and its determination are maintained. In a recent paper we have reported that: 1) the higher the temperature, the lower the absorbancy of the complex, 2) pH of the solution influences colour intensity: the same absorbancy was obtained between pH 1.5-2.5, but lower pH (1.0) diminished the absorbancy, 3) various starches (extracted from different plant materials) showed different absorbancy for the same starch concentration, 4) the removal of soluble constituent from the examined material before starch solubilization was not necessary (Samotus et al., 1993). In further experiments, however, we observed that in case of leafy materials, the removal of certain reducing substances was necessary before starch determination (unpublished data). Accordingly, the dried material (70 °C, using draft oven) was extracted with hot 80% ethanol and the residue was solubilized in concentrated CaCl₂ solution (boiling water bath, 30 min). Even after adopting this strategy, ceratin materials (the leaves of carrot, tomato, broad bean and beech) revealed some amounts of reducing substances, which interferred with the blue complex formation. To take care of such interference, it was necessary to add I-KI solution to the reaction mixture till the blue colour developed (starch in the solution served in this case as an indicator). In this manner, the amount of I-KI reagent to be used for starch estimation in a particular plant material was worked out.

Sharma et al. (1990) demonstrated a strong suppression of starch-iodine in the presence of ascorbic acid, a common

plant constituent; recovery being evident when I-KI level was increased. They explained that a complex between AA and I is formed, which could account for AA-induced depletion of iodine level and, in turn, lowered the magnitude of the reaction.

We repeated the experiments performed by Sharma et al. (1990) and examined the reasons for interference of AA with starch-iodine complex formation.

MATERIAL AND METHODS

Soluble starch (Polskie Odczynniki Chemiczne, Gliwice) was used to prepare the stock solution in distilled water (100 mg/100 ml). The I-KI stock solution contained 0.200 g I in 2.000 g KI per 100 ml of distilled water. Ascorbic acid (AA) solutions (chemically pure reagent) were prepared by dilution with 0.05 M HCl to prevent AA decomposition. Absorbancy of the blue starch-iodine complex was determined in 1 cm cell using the SPEKOL photometer at 620 nm.

For spectral studies different concentrations of AA solutions in 0.05 M HCl as well as iodine solution in 4% ethanol and that of KI in water were used. The absorbancy in UV region (200-300 nm) was performed in a 1 cm quartz cell using the SPECORD spectrophotometer (Carl Zeiss, Jena).

RESULTS

The influence of increasing concentrations of starch, while maintaining the iodine concentration constant, are presented in Fig. 1. As is apparent, the absorbancy increased linearly with an increase in starch concentration. In contrast, the increasing concentration of I-KI reagent applied to constant

Fig. 1. Absorbance of starch-iodine complex.

●-●-● = constant concentration of I-KI (1 ml of stock solution diluted to 100 ml), and different amounts of 2 mg/100 ml starch solution, ○-○-○ = constant starch solution (2 mg/100 ml) and different amounts of stock solution of I-KI diluted to 100 ml.

starch concentration followed a hyperbolic curve, which means that in the beginning (low I-KI concentration) starch molecules were not saturated with I-KI reagent, but they reached the saturation as soon as the concentration increased (Fig. 1). Obviously, it is necessary to work out a proper I-KI concentration to have reproducible results. The same conclusion was drawn by Sharma et al. (1990).

The above experiments were repeated with a double concentration of KI to check the interference of this reagent on blue complex absorbance and no difference in the absorbance was observed.

Ascorbic acid revealed a strong absorption peak in ultraviolet region at 245 nm (Fig. 2). After adding of a few drops of 1 M NaOH to the solution to obtain pH 9, the absorption peak moved to 265 nm and a rapid oxidation of AA started, which

completed in about 15 minutes. There was no absorbance due to the oxidation product(s) of AA (Fig. 2, curve 5).

Fig. 3. represents the absorption spectra in the near ultraviolet region of AA (curve 1), KI (curve 2) and iodine solutions (curve 3). As seen from curves 1, 2 and 3, one can expect an interference between I, KI and AA absorption spectra. To eliminate it, the iodine in 4% ethanolic solution was added to AA solution in stoichiometric amounts and a differential absorption spectrum was checked, using the expected KI concentration placed in the reference cell. The concentration of KI was established according to reaction: $I_2 + AA = 2 I^- + 2 H^+ + DA$ (dehydroascorbic acid). As seen from Fig. 3, the absorption was extinguished giving almost a flat curve 4, for DA does not show any absorption in UV region.

Fig. 2. Absorption spectra of AA solution (2.8 mg/100 ml of 0.05 M HCl) – curve 1, and after alkalization (pH about 9), checked in 2, 4.5, 9 and 15 minutes, respectively – curves 2, 3, 4 and 5.

Fig. 3. Absorption spectra of: AA solution (1.90 mg/100 ml in 0.05 M HCl) – curve 1, KI solution (1.36 mg/100 ml of water solution) – curve 2, and I solution (1.36 mg/100 ml in 4% ethanol) – curve 3. Curve 4 represents differential absorption spectrum against KI solution.

Fig. 4. Absorption spectra of the titrated AA solution: curve 1 = AA solution alone (1.19 mg/100 ml in 0.03 M HCl), curve 2-6 = 18 mg/100 ml of I in 2% ethanol added in 0.05 ml portions. The last curve 6 represents stoichiometric iodine amount added to the AA solution.

In the next experiment, the titration of AA with iodine solution in 4% ethanol was performed (Fig. 4). The reaction was followed by checking the absorption in the UV region. Curve 1 represents the absorption of AA alone. Curves 2, 3, 4, 5 and 6 were produced by successive iodine solution adding. During AA oxidation the peak of curve 1 was shifted from 245 nm to the shorter region (227 nm) and simultaneously was enhanced, because of KI formation. After complete AA oxidation with iodine, the produced peak corresponded to that of KI solution (compare Fig. 3., curve 2).

DISCUSSION

A standard amount of I-KI reagent (1 ml of stock solution per 100 ml of starch solution) protects the proportionality between starch-iodine complex and its absorbancy in the range from 1 to 7 ml of starch added (Fig. 1). I-KI solution used in the present study in a ratio: 0.2 g I and 2 g KI per 100 ml of water solution is commonly used in this kind of analysis (e. g. McCready and Hassid, 1943; Lau, 1988; Ruehl, 1989). The intensity of blue colour of the complex depends on iodide concentration (Ono et al., 1953), too.

The present data indicate that it is important to keep iodine concentration at an appropriate level. A lower iodine concentration impedes the formation of complex between spiral chains of amylose (and partly (and partly of amylopectin) with iodine, lowering the absorbance, whereas high iodine concentration can hasten the precipitation of starch-iodine complex. We observed such a precipitation in our experiments (unpublished data).

Ascorbic acid shows the strong reducing power because of the endiol system present in the molecule. The rate of aerobic oxidation of AA is pH-dependent, exhibiting the maxima at pH 5.0, corresponding to reaction with 1 equivalent of base, and at pH 11.5, corresponding to reaction with 2 equivalents of base. The oxidation products are different. A rapid change of pH of the solution shifted the absorption peak of AA from 245 to 265 nm (Fig. 2.). According to Herbert et al. (1993), it indicates the presence of a conjugated system carrying one or more enolizable hydroxyl groups. The deterioration of endiol group is the cause of absorption peak disappearance (fig. 2.).

The disappearance of the AA peak in ultraviolet region after iodine treatment can be explained in a similar manner. However, in this case the main structure of ascorbic acid molecule remains intact and can be regenerated from the dehydroascorbic acid by reduction with hydrogen sulfide (Hirst and Zilva, 1933). The reaction of AA with iodine can be summarized:

The evidence for a redox reaction between AA and I solutions is presented in Fig. 4. This reaction provides a simpler explanation for the disappearance of blue colour of starch-iodine complex after addition of AA to the reaction mixture than the mechanism proposed by Sharma et al. (1990). A shift in the absorption peak to the shorter wavelengths is not

necessarily a result of iodine-AA complex formation as suggested by Sharma et al. (1990). Our data indicate that the peak shift is due to the generation of iodide in the reaction between AA and I solutions.

On the basis of the present findings three measures are suggested to remove the interference of AA when starch is determined by blue iodine complex: 1) to titrate starch solution with I-KI reagent, and work out a proper amount of I-KI to develop blue colouration, 2) to remove AA from plant material either with cold water or ethanol and 3) starch solution can be treated with alkali, which destroys AA in a short time, subsequently the solution should be acidified before the complexation of starch with iodine.

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FOTOMETRYCZNE OZNACZANIE JODO-SKROBI W MATERIAŁACH ROŚLINNYCH W OBECNOŚCI KWASU ASKORBINOWEGO. UWAGI KRYTYCZNE

STRESZCZENIE

Analizując pracę Sharmy i in. (1990) nad negatywnym wpływem kwasu askorbinowego (AA) na reakcję jodo-skrobiową, autorzy powtórzyli tę pracę, podając poprawne wyjaśnienie wpływu AA na tę reakcję. AA utlenia odczynnik jodowo-jodkowy (I-KI) co uniemożliwia tworzenie się niebieskiego kompleksu z skrobią. Zasugerowano trzy możliwości uniknięcia zakłócenia tej reakcji przez AA: 1) można odmiareczkować roztwór za pomocą odczynnika I-KI i w ten sposób odtworzyć odpowiednią ilość I-KI celem wytworzenia niebieskiego zabarwienia, 2) można też usunąć AA (i inne substancje redukujące) z materiału roślinnego, przemycając go zimną wodą lub etanolem, 3) w końcu roztwór skrobiowy można potraktować ługiem przy pH ok. 9, rozkładając AA w przeciągu 15 minut. Po zakwaszeniu roztworu i dodaniu odczynnika I-KI oznacza się niebieski kompleks.

SŁOWA KLUCZOWE: kompleks jodo-skrobiowy, kwas askorbinowy.