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Application of square-wave adsorptive stripping voltammetry to the study of cysteine and its interaction with some monosaccharides

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The square-wave adsorptive stripping voltammetric behaviour of cysteine at the physiological pH (7.40) has been optimised with respect to accumulation time, accumulation potential, scan rate and drop size. This study describes the application of voltammetry to studies on the interaction of cysteine with monosaccharides.

Keywords: **square-wave adsorptive stripping voltammetry, chemical addition reactions, cysteine, monosaccharides, physiological pH**

Abbreviations: **$Hg(SR)_2$, mercuric cysteine thiolate; $Hg_2(SR)_2$, mercurous cysteine thiolate; RSH, cysteine; RS^-, cysteine thiolate anion**

Introduction

Adsorptive stripping voltammetry (AdSV) has received much attention in recent years as regards the determination of variety of inorganic and organic species. The species are able to undergo adsorptive accumulation at the electrode surface, either directly or by utilising suitable complexation reactions [1–6]. The technique has also been applied to studies involving biological molecules such as cysteine [7–9], human serum albumin (HSA) [10], immunoglobulin G (IgG) [11,12], concanavalin A (Con A) [13] and others [14,15].

Smyth and co-workers [16] have reported the results of investigations on the interaction of cisplatin with cystine and HSA. This study indicated the possibility of using AdSV to monitor directly the interaction of an antigen with its antibody in solution.

Monosaccharides participate in virtually all aspects of cellular life. They are basic constituents of glycoproteins and nucleic acids. It is known that monosaccharides bind covalently to amino acids [17]. The binding of an amino acid to a monosaccharide forms *S*-, *O*- or *N*-glycosides [18]. Sulfur containing volatile compounds thermally generated from cysteine in the presence of glucose have been reported [19,20]. The equilibrium constants of complexation and the stoichiometry of monosaccharide-amino acid complexes play fundamental roles in determining the free monosaccharide concentration in plasma.

Cysteine is an amino acid which occurs in proteins. Cysteine, which contains a thiol (-SH) group, plays a role in many functions of mitochondrial membranes [21], in membrane transport [22] and especially in enzyme catalysis [23]. Some sulfur-containing compounds can be advantageously followed by voltammetry. Thiols yield anodic waves corresponding to formation of mercury compounds and disulfides are reduced at the dropping mercury electrode [24].

No voltammetric references on the interaction of cysteine with monosaccharides could be traced in the literature. It was therefore considered important to report the use of square-wave adsorptive stripping voltammetry (SWAdSV) to monitor the interaction of cysteine with monosaccharides (glucose, galactose and fructose) at the physiological pH (7.4). The paper will represent an important methodological contribution that will allow study of the addition reactions of thiols to aldoses/ketoses.

Experimental

Reagents

L-Cysteine, D(+)-glucose, D(+)-galactose and D(−)-fructose were purchased from Merck. Inositol and 2-methyl-2-propanethiol were obtained from the Aldrich Chemical Co. All

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compounds were of analytical-reagent grade. Britton-Robinson buffer (pH 7.4) was used as supporting electrolyte and prepared from chemicals of analytical purity grade. All solutions were prepared in deionized triply-distilled water just before used and kept in the dark in order to minimise decomposition.

Apparatus

An EG&G PAR Model 384B polarographic analyser was used with an EG&G PARC Model 303A hanging mercury drop electrode (HMDE) as the working electrode. A Ag/AgCl/*sat.* KCl reference electrode was used and the auxiliary electrode was a platinum wire. PAR Model 305 magnetic stirrer was used. The voltammograms were recorded with a Houston Instrument DMP-40 plotter (Austin, TX, USA).

Procedure

Prior to each investigation, the buffer was purged with oxygen-free nitrogen for 8 min before adding the required amount of the analyte to the cell and purging for a further 2 min. Following a systematic study of the voltammetric response with various purge times, a time of 2 min was chosen for each experiment. After the purging step, the electrode potential was set at a equilibrium time of 5 s and the required accumulation potential (E_{acc}) for the required accumulation time (t_{acc}) with stirring, before the voltammetric experiments were carried out. All experiments were carried out at room temperature in the dark, with cell completely wrapped in Al foil at all times.

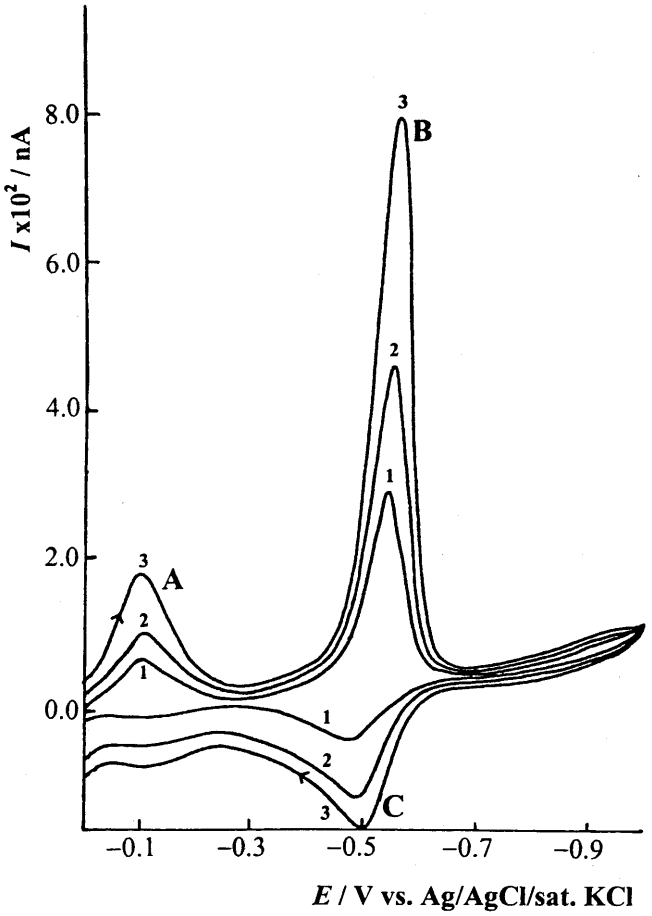

Figure 1. Cyclic voltammograms of 1×10^{-5} M cysteine solution in pH 7.4 at scan rates ($mV\,s^{-1}$) v = (1) 285.7; (2) 500; (3) 1000. A, the reduction of mercuric cysteine thiolate; B, the reduction of mercurous cysteine thiolate; C, the anodic formation of mercurous cysteine thiolate. Experimental conditions: equilibrium time, 5 s; medium drop size; initial potential, 0.00 V. Scan direction denoted on the curves.

Results and Discussion

Cyclic Voltammetry of Cysteine

The cyclic voltammogram of cysteine, preceded by a scan rate of 500 $mV\,s^{-1}$, produced two cathodic peaks at −0.10 V (peak A) and −0.55 V (peak B) on forward scan and an anodic peak at −0.49 V (peak C) on the reverse scan. The influence of scan rate on the peak current of peaks A, B and C was investigated. The response of peak current for the peaks A, B and C was linear with respect to scan rate, indicating that the processes were adsorption controlled (Fig. 1). A graph of log (peak current) versus log (scan rate) for the peaks A and C was found to be linear in the range 200–1000 $mV\,s^{-1}$ with a slope of 0.80. This value is similar to that obtained by Wang et al. [25] for riboflavin, and the process occurring might well agree with that of Laviron's model [26], which predicts a slope of unity at high and low scan rates and a smaller slope at moderate scan rates.

The peaks B and C can be attributed to the anodic and cathodic reactions of adsorbed mercurous cysteine thiolate ($Hg_2(SR)_2$) complex [27,28].

$$2Hg \rightleftharpoons Hg_2^{2+} + 2e^- \quad (1)$$

$$2RS^- + Hg_2^{2+} \rightleftharpoons [Hg_2(SR)_2]_{ads} \quad (2)$$

The peak A (−0.10 V) can be attributed to the formation and reduction of the mercuric cysteine thiolate ($Hg(SR)_2$). However, the electrode process for the peak at −0.10 V is more complex than those of peaks B and C (−0.55 and −0.49 V, respectively). At low coverages of the electrode surface by $Hg_2(SR)_2$ (at low cysteine concentration), $Hg(SR)_2$ is formed at high scan rates (ie. 500 $mV\,s^{-1}$) by a non-faradaic surface process of disproportion of the adsorbed mercurous

cysteine thiolate; in consequence, only one faradaic step (peaks B and C) is then observed. As soon as the potential (−0.10 V) corresponding to the step Hg(I) → Hg(II) is reached, a disproportion occurs of the adsorbed mercurous cysteine thiolate which is formed at the electrode surface (eq. (3)) [27,28].

$$[Hg_2(SR)_2]_{ads} \rightleftharpoons [Hg(SR)_2]_{ads} + Hg \qquad (3)$$

The replacement of mercurous by mercuric cysteine thiolate at the electrode surface is accompanied by a pronounced change of the electrode capacity at −0.10 V. However, at higher cysteine concentrations the electrode surface is covered by mercurous cysteine thiolates which hinder the non-faradaic disproportion; instead of it

$$[Hg_2(SR)_2]_{ads} + 2RS^- \rightleftharpoons 2[Hg(SR)_2]_{ads} + 2e^- \qquad (4)$$

occurs at the positive potentials (ie. −0.10 V), which is the cause of the second anodic step [27,28].

The proposed interpretation of the process in the peak at −0.10 V is only one of the possibilities. This peak corresponds to the formation or presence of another adsorbate than the adsorbate formed at the potential of the peak B at −0.55 V. But, the question is, how does it differ. Difference in the oxidation state of Hg as proposed here (and as have recently been proposed by Heyrovský et al. [27,28]) is one possibility. Another is different stoichiometry of the two adsorbates formed. However, it can also be: monolayer as opposed to multilayer coverage or island formation, or it can be two adsorbates with a different orientation of monomers of the mercury salt, or monomeric and polymeric mercury compounds. The interpretation of processes involved is complicated by the fact, that products of controlled potential electrolysis, after electrolysis has been interrupted, change composition with time, indicating either a relatively slow organization of the adsorbate or solid state reactions. As the nature of the electrode process involving cysteine is not the principal problem dealt with in this contribution, it can be said that two adsorbates are formed at −0.10 V.

Adsorptive Stripping Voltammetry (SWAdSV) of Cysteine

The square-wave adsorptive stripping voltammetric behaviour of cysteine is shown in Figure 2. With an accumulation potential of −0.30 V (*versus* Ag/AgCl/*sat*. KCl), an equilibrium time of 5 s, a frequency of 100 Hz, a pulse amplitude of 20 mV and a scan rate of 200 mV s^{-1}, a 5×10^{-8} M cysteine gave rise to one peak at −0.55 V, which increased with increasing accumulation time. This peak used in SWAdSV corresponds to the desorption of the adsorbate of $Hg(SR)_2$ or $Hg_2(SR)_2$, accompanied by a reversible reduction of Hg_2^{2+} ions.

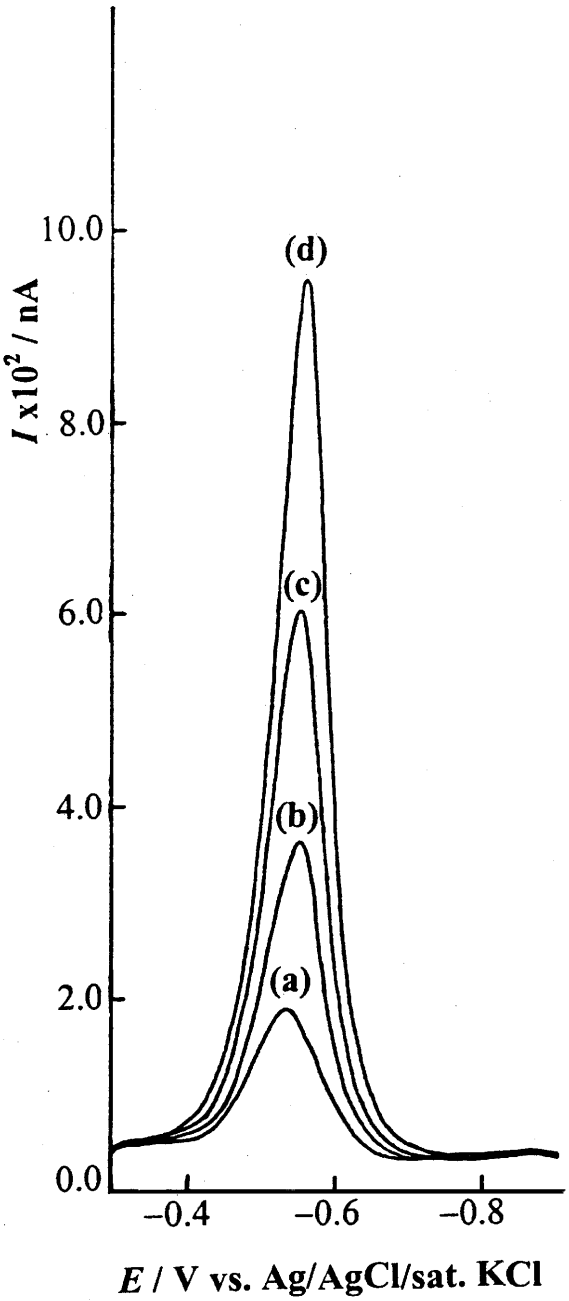

Figure 2. The dependence of the square-wave adsorptive stripping voltammetric signal of 1×10^{-7} M cysteine on accumulation times (t_{acc}): (a) 30, (b) 60, (c) 120, (d), 240 s. Experimental conditions: frequency, 100 Hz; pulse amplitude, 20 mV; scan rate, 200 mV s^{-1}; equilibrium time, 5 s; medium drop size; accumulation potential, −0.30 V.

The effects of accumulation time, accumulation potential, equilibrium time, scan rate and drop size on the square-wave peak current and peak potential of this peak have been studied. Hence the optimum conditions for the determination of cysteine are the following: E_{acc}, −0.30 V; equilibrium time, 5 s; frequency, 100 Hz; pulse amplitude, 20 mV; scan rate, 200 mV s^{-1}; drop size, medium; and accumulation time, 120 s.

Obviously a greater sensitivity can be achieved by using a longer accumulation time (>120 s), but this leads to longer determination times. Under the optimum conditions, a linear calibration curve was obtained for cysteine in the range 1×10^{-8}–2×10^{-7} M. The calibration curve had the following equation: $I_p(nA) = 59.18 \times 10^{-8}\ C + 1.73$ (r = 0.998). The calibration curve loses its linearity at cysteine concentrations greater than 2×10^{-7} M. This deviation from linearity indicates a saturation of the electrode surface with the adsorbed molecules which prevent further material from being accumulation. However, it is possible that a wider range towards higher concentration of cysteine can be achieved by using shorter accumulation time.

Titration of Cysteine With Some Monosaccharides

Firstly, the square-wave adsorptive stripping voltammetric behaviours of glucose, galactose and fructose have been studied at pH7.4. These compounds yield a single cathodic peak at almost −1.77 V (Fig. 3). It was reported that kinetic currents were observed for aldoses (like glucose and galactose), but ketoses (like fructose) gave diffusion controlled waves [29–33]. The relations between the peak currents and the concentrations of these monosaccharides are non-linear under our experimental conditions.

In order to confirm that cysteine binds to glucose, galactose and fructose, amperometric titrations of 1×10^{-7} M and 5×10^{-8} M solutions of cysteine or higher concentration (5×10^{-7} M) at a shorter accumulation time (30 s) with each of these monosaccharides ($1 \times 10^{-8} - 3 \times 10^{5}$ M) was performed. For this purpose, the mercurous cysteine thiolate reduction peak at −0.55 V was used. Plots of peak current (I_p) as a function of logarithm of concentration of monosaccharide are shown in Figures 4, 5 and 6. With the addition of gradually increasing amounts of glucose and galactose, the I_p decrease slow and almost linear initially. Then I_p begins to decrease sharply until attaining a constant value (Figs. 4 and 5). The

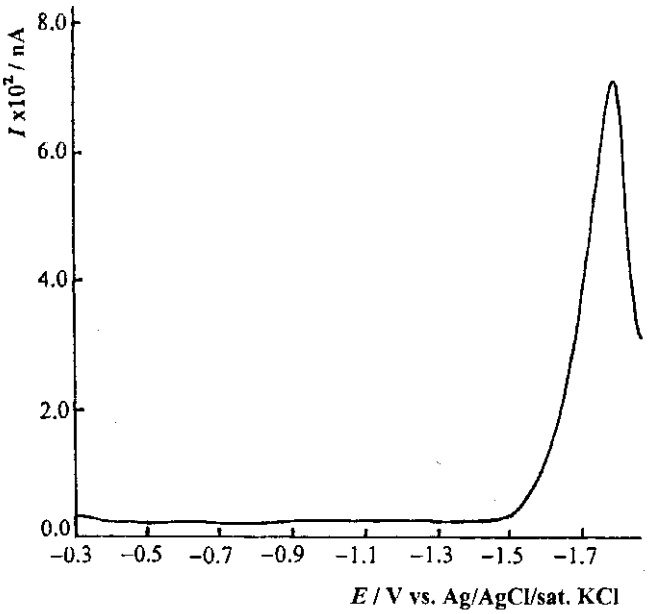

Figure 3. Square-wave adsorptive stripping voltammogram of 1×10^{-5} M glucose solution in pH 7.4; accumulation time, 120 s; accumulation potential, −0.30 V. Other conditions as in Figure 2.

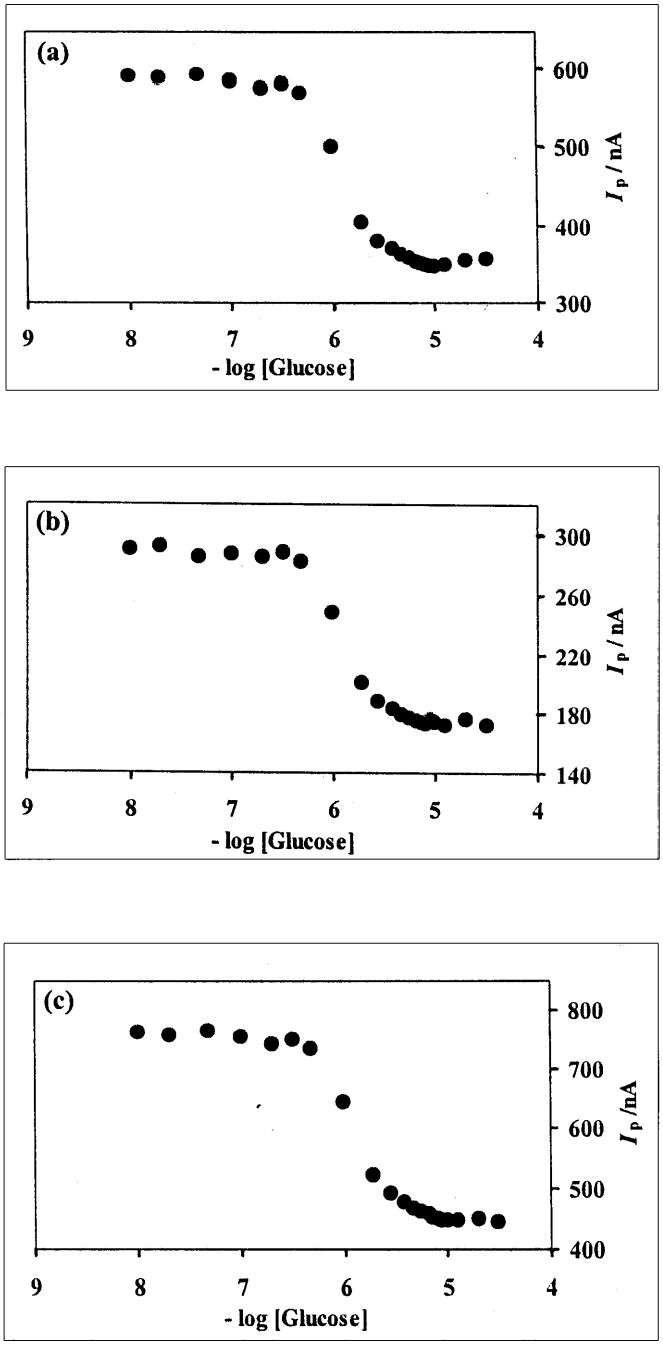

Figure 4. The dependence of the peak current of cysteine on the logarithm of glucose concentration (a) 1×10^{-7} M cysteine; accumulation time, 120 s; (b) 5×10^{-8} M cysteine; accumulation time, 120 s; (c) 5×10^{-7} M cysteine; accumulation time, 30 s. Other conditions as in Figure 2.

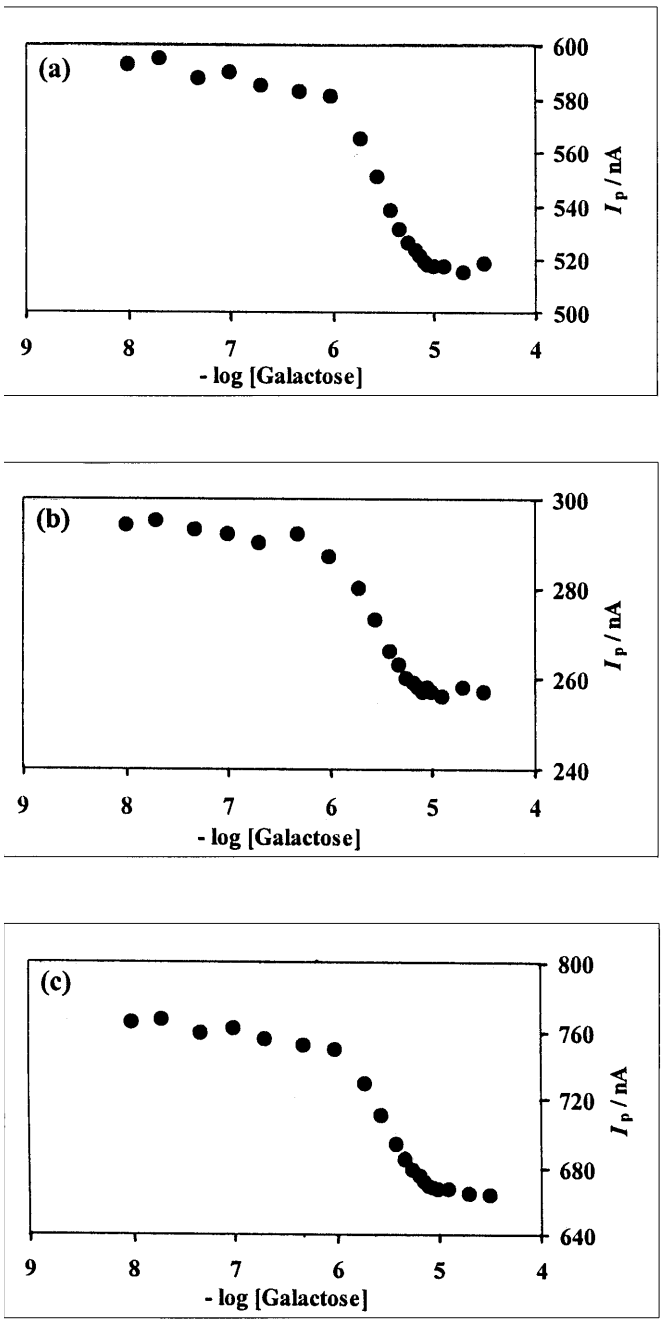

Figure 5. The dependence of the peak current of cysteine on the logarithm of galactose concentration (a) 1×10^{-7} M cysteine; accumulation time, 120 s; (b) 5×10^{-8} M cysteine; accumulation time, 120 s; (c) 5×10^{-7} M cysteine; accumulation time, 30 s. Other conditions as in Figure 2.

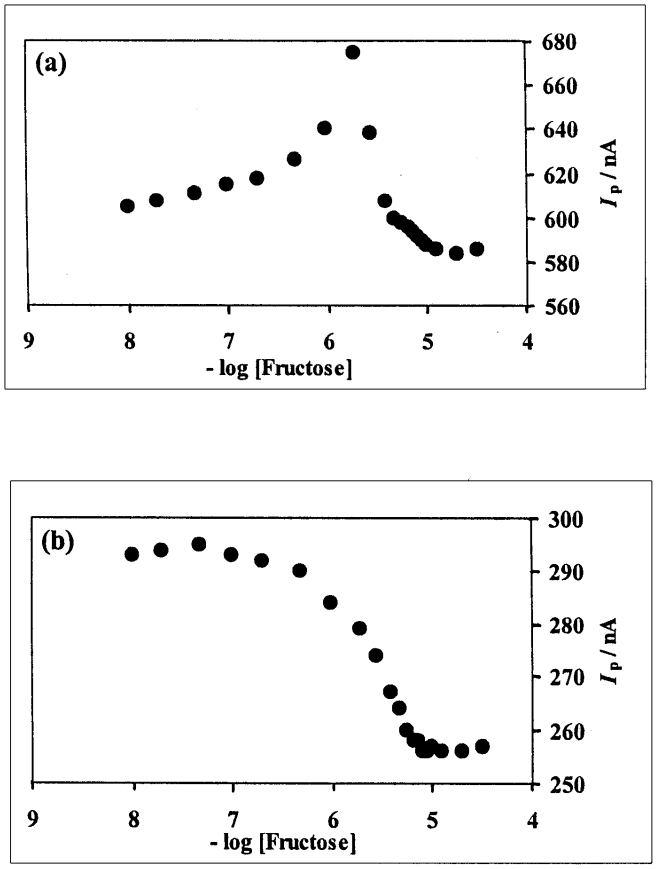

Figure 6. The dependence of the peak current of cysteine on the logarithm of fructose concentration (a) 1×10^{-7} M cysteine; accumulation time, 120 s; (b) 5×10^{-8} M cysteine, accumulation time, 120 s. Other conditions as in Figure 2.

essential question is, whether the decrease in the peak current of cysteine is due to a chemical reaction (e.g. thioketal formation) or competitive adsorption. Absence of current decrease in the presence of fructose seems to indicate that competitive adsorption is not the main factor (Figure 6a). Moreover, good fit of the $I_p = f$ (log[monosaccharide]) plots to a theoretical curve indicated establishment of an equilibrium. It was obtained that the inflection points of $I_p = f$ (log[monosaccharide]) plots were independent of initial cysteine concentration. When monosacharides, like other aldehydes and ketones, react with an alcohol (ROH) they readily form hemiacetals and acetals or hemiketals and ketals, respectively [17]. Analogous reaction can be between the carbonyl groups of monosaccharides and thiol group of cysteine (RSH). In order to verify that the reactive center on these saccharides most likely to react with cysteine is the carbonyl group, inositol (an alcohol of a comparative size) was added to the cysteine solution.

A considerable change in the peak current of cysteine was not observed by adding inositol (1×10^{-8}–3×10^{-5} M). Analogous experiments were carried out with a simple thiol (2-methyl-2-propanethiol). The obtained results are similar to those with cysteine.

It was observed that glucose caused a much larger decrease in the peak current than galactose. This behaviour could not only be explained by the addition of cysteine to the open-chain aldehyde form of glucose which is a very minor constituent of the equilibrium mixture but this is also involved with the ring structures.

The effect of fructose on the peak current of cysteine is different from that of glucose and galactose. Fructose increases the peak current at high cysteine concentration first and then decreases (Figure 6a). However, the effect of fructose is similar to the behaviours of glucose and galactose at low cysteine concentration (Figure 6b). As can be seen in Figure 6a, the initial increase which is followed by a decrease in the peak current may be explained by a precursor state formed during the addition reaction between cysteine and fructose. It may be concluded that the last products are the most stable species available in the reaction environment.

Biological Analogy

Some glycoxidation compounds are formed by reactions between sugars and proteins. These compounds accumulate in tissue collagen and at an accelerated rate in diabetes. The glycoxidation products are biomarkers of more extensive damage to the protein. Possible sources of damage to proteins in diabetes include free radicals generated by sugar adducts to protein. This may be amplified by continuing tissue damage and cell death [34]. The data presented here support the hypothesis that the reaction of free glucose with biological species such as proteins may be important in leading to some of the complications seen in diabetes. Therefore, the results demonstrating the ability of cysteine to react with monosaccharides may be of great interest.

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