

THE INTERFACIAL BEHAVIOUR OF MONO-, DI-, AND TRIPHOSPHATE OF INOSINE AT THE CHARGED INTERFACE

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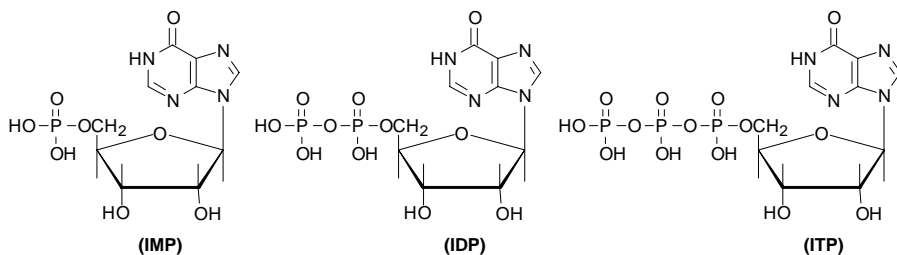
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The interfacial behaviour and adsorption equilibria of mono-, di-, and triphosphate of inosine (IMP, IDP, and ITP) were carried out in different buffer solutions by phase-sensitive *ac* voltammetry at HMDE. The characteristic properties and adsorption parameters of dilute and compact layers were evaluated from the obtained Frumkin isotherm at different pH values. The effect of some divalent metal ions on the adsorption stage and association of the investigated compounds has been studied.

Key words: Hanging mercury drop electrode; Adsorption; Inosine phosphates.

Important processes of nucleic acids as bases, nucleosides and nucleotides in the living cell and in organisms involve their interactions with charged biological interfaces¹. For the elucidation of their interactions with the usual biological interfaces in the living cell, the adsorption behaviour of some bases, the monomeric nucleosides and nucleotides was investigated previously at the charged model mercury/aqueous electrolyte interface²⁻¹⁴. It was found that the adsorbed nucleosides and nucleotides interact principally via vertical stacking of the bases⁶⁻¹⁴. The forces, which govern the formation of the intermolecular complexes in the film, are of considerable interest not only from the theoretical point of view but also with respect to their significance in stabilizing the structures of the polynucleotides and of polynucleotide-nucleotide complexes^{15,16}.



The interactions of nucleic acids and their constituents with transition metal ions have been widely reported in recent years¹⁷⁻²⁰. Conformational changes, mediated by metal ions, of nucleic acids and synthetic polynucleotides are of particular interest.

Metal ions are required for virtually all biological processes in which nucleic acids are engaged. This, together with the recent finding that certain transition metal complexes have been found to be potentially useful in cancer chemotherapy^{21,22}, created a renewed interest in the study of the interaction of heavy metal ion with some inosine nucleotides.

The present paper is focused on the investigating the surface activity and adsorption equilibria of mono-, di-, and triphosphate of inosine in solutions of varying pH at the mercury/solution interface by using phase sensitive *ac* voltammetry. The influence of complexation on the stacking interactions of the investigated compounds has been elucidated.

EXPERIMENTAL

Chemicals and Solutions

Mono-, di-, and triphosphate of inosine were obtained from Sigma and were used without further purifications. Solutions containing different concentrations of inosine phosphates were prepared by dissolving a known amount of the chemically pure product into a definite volume Britton–Robinson (BR) buffer. This buffer brought to a constant ionic strength of 0.5 mol l⁻¹ by the addition of NaCl or NaNO₃ and adjusted to the desired pH was used as supporting electrolyte. All chemicals were reagent grade. The pH was measured with a digital Radiometer pH meter, Model pH M64.

Apparatus and Methods

A Princeton Applied Research (PAR) Model 174 polarographic analyzer coupled with a PAR Model 174/50 *ac* polarographic interface and a PAR Model 510 (lock-in-amplifier) phase detector were employed for *ac* voltammetric measurements. Phase-sensitive *ac* voltammograms were recorded with a phase angle adjusted to 90°, corresponding to the out-of-phase component of the *ac* current (capacitive current component). The amplitude of the *ac* voltage was 10 mV peak-to-peak. The scan rate of the *dc* ramp of the mean electrode potential (*E*) was 2 mV s⁻¹ and the *ac* frequency had a value of 330 Hz, unless stated otherwise. The time dependence of the *ac* component of the capacitive current at an adjusted constant mean electrode potential was obtained as described earlier⁸.

A thermostated PAR cell equipped with a three electrode system was used for the voltammetric studies. The three electrode system contained a HMDE as the working electrode, an Ag/AgCl saturated KCl reference electrode and a platinum wire counter electrode. The solution was deaerated with pure nitrogen before contact with mercury and the commencement of electrochemical experiments.

RESULTS AND DISCUSSION

Phase sensitive *ac* voltammograms corresponding to the out-of-phase component of the total *ac* response provide an overall picture of the adsorption behaviour of the studied mono-, di-, and triphosphates of inosine (Fig. 1). The recorded capacitive *ac* component is proportional to the differential double layer capacitance in potential regions where no faradaic process occurs²³. First, the pH of the solution was adjusted to such a value (pH 2.3) that the protonated form of inosine phosphate prevailed. At low bulk concentrations

one observes around the potential of maximum adsorption (-0.5) a progressive decrease of the capacitive ac signal due to increasing adsorption of mono-, di-, and triphosphates of inosine. This decrease corresponds to the progressive coverage of the electrode surface by the "dilute" adsorption layer.

At more elevated bulk concentrations above the threshold value for the respective mono- and diphosphates a pit is observed. The pit occurs for IMP at a significantly lower threshold value than for IDP. As has been shown by Vetterl²⁴ the pit reflects the formation of a "compact" adsorption film due to pronounced lateral interactions of adsorbed nucleotide species in a certain potential range. As will be discussed later⁹ the adsorption of all inosine derivatives in the "dilute" stage takes place via the most hydrophobic moiety of the molecule, i.e. the purine base. The π -electron interaction with the electrode will favour for the range of positive charge or zero charge a flat adsorption of the purine moiety. In the compact stage of the adsorption the purine and phosphate moieties interact with the electrode depending on their charge and the sterical arrangement of the molecule.

The tendency for interfacial association diminishes in the order $\text{IMP} > \text{IDP} > \text{ITP}$ corresponding to the decreasing the effective charge on the protonated N(1) inosine due to the effect of the compensating negative phosphate groups. For mono-, di-, and tri-

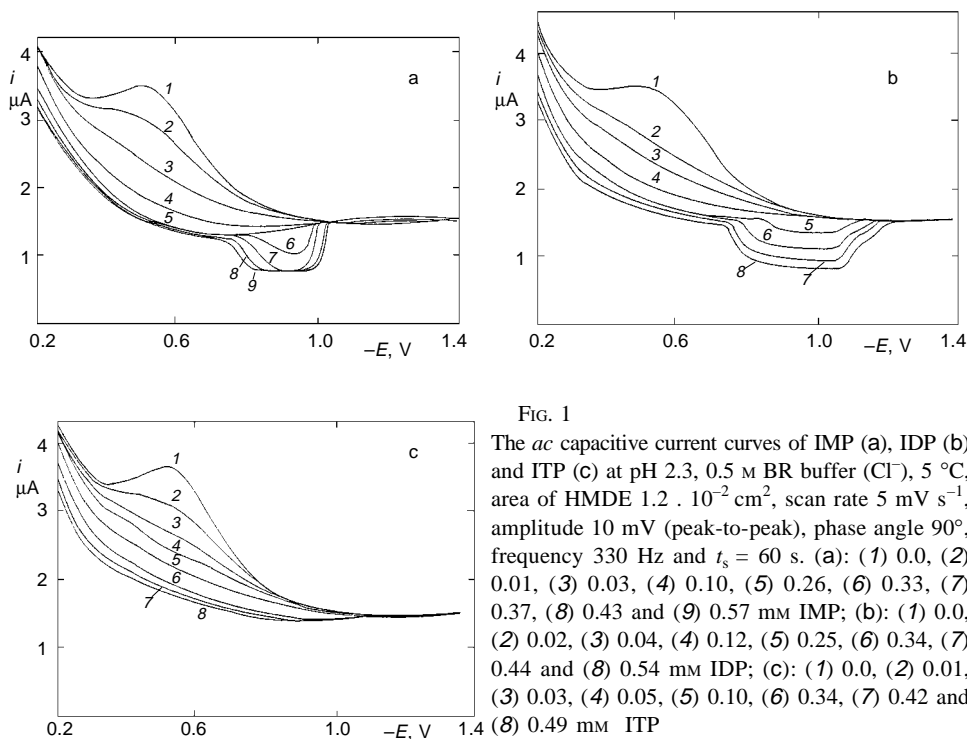


FIG. 1

The ac capacitive current curves of IMP (a), IDP (b) and ITP (c) at pH 2.3, 0.5 M BR buffer (Cl^-), 5 °C, area of HMDE $1.2 \cdot 10^{-2} \text{ cm}^2$, scan rate 5 mV s^{-1} , amplitude 10 mV (peak-to-peak), phase angle 90°, frequency 330 Hz and $t_s = 60 \text{ s}$. (a): (1) 0.0, (2) 0.01, (3) 0.03, (4) 0.10, (5) 0.26, (6) 0.33, (7) 0.37, (8) 0.43 and (9) 0.57 mM IMP; (b): (1) 0.0, (2) 0.02, (3) 0.04, (4) 0.12, (5) 0.25, (6) 0.34, (7) 0.44 and (8) 0.54 mM IDP; (c): (1) 0.0, (2) 0.01, (3) 0.03, (4) 0.05, (5) 0.10, (6) 0.34, (7) 0.42 and (8) 0.49 mM ITP

phosphates of inosine different degrees of compensation of the positive charge on the protonated N(1) are connected with sterical reasons determining the distance between the protonated N(1) and negative phosphates. In this context, ITP does not exhibit a pit in various buffer solutions with NaCl presumably because of significant electrostatic repulsion between adjacent negatively charged phosphate groups. However, upon replacing the chloride anion by oxyanions such as nitrate, a sharply defined pit is observed (Fig. 2). This indicates that halide anions are present at the interface and that their specific adsorption hinders the formation of a compact film for ITP and disturbs the stacking interactions between adsorbed molecules.

In moderately acid and alkaline solutions ($\text{pH} \geq 4$) and at more elevated bulk concentrations of IMP or IDP, the association of the adsorbed molecules on the electrode surface is hindered and no compact film formation is reached. This indicates that the reorientation in the pit is restricted to the protonated IMP or IDP and it is obviously connected with the influence of pH of buffer solution.

The effect of some divalent metal ions, such as Cd(II), Cu(II), Co(II), Ni(II), and Ca(II), on the adsorption stages and association of mono-, di-, and triphosphates of inosine has been investigated. The influence of Cd(II) on the condensed film formed by adsorption of IMP or IDP at pH 2.3 indicates a systematic and large decrease in the bulk solution concentration, C_{th} , which is required to cause formation of the compact layer. Thus, a bulk solution concentration of about $3 \cdot 10^{-4}$ M IMP or IDP is required to observe the capacitive pit, while a concentration of $1 \cdot 10^{-4}$ M IMP or IDP is required in the presence of $6 \cdot 10^{-5}$ M Cd(II). The *ac* voltammograms are characterized also by a pseudocapacitive peak at ca -0.7 V split at higher frequencies, assigned to reduction of Cd(II) bound to the adsorbed Cd(II)-IMP (or IDP) complex.

The *ac* capacitive current of ITP in presence of Cd(II) at pH 2.3, where ITP does not associate at the electrode surface, was recorded as shown in Fig. 3. The *ac* voltammograms indicate a well-defined pit of adsorbed state in the potential range from -0.35 to -0.7 V. The existence of this pit, in which the association of Cd(II)-ITP complexes

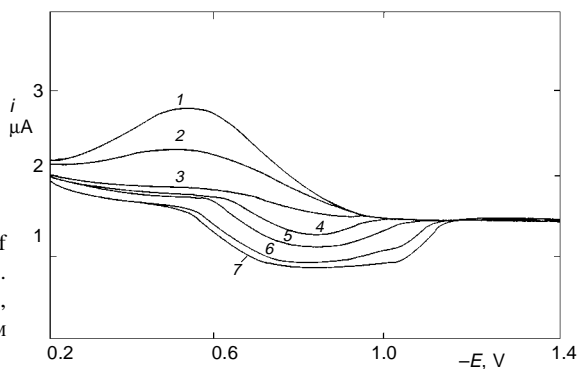


FIG. 2

The *ac* capacitive current curves of ITP pH 2.3. 0.5 M BR buffer (NO_3^-). (1) 0.0, (2) 0.01, (3) 0.04, (4) 0.06, (5) 0.08, (6) 0.16 and (7) 0.32 mM ITP. Other conditions as in Fig. 1

takes place, is explained by the surface rearrangement and reorientation of the adsorbed complex at the mercury/solution interface.

The aforementioned results indicate that complexation of the mono-, di-, and triphosphates of inosine molecules enhances the stacking interactions and, hence, would be expected to facilitate the formation of a perpendicularly stacked layer of the Cd(II)-IP complex on the electrode surface. It has been shown previously²⁵ that the conformation of polynucleotides is strongly influenced by divalent metal ions at very low concentration. In this context, some metal ions, by being able to bind to both base and phosphate sites, display a remarkable selectivity and subtlety in producing different concentrations of the various polynucleotides by binding sometimes to one site, sometimes to the other.

The adsorption behaviour of inosine nucleotide in the presence of Cu(II), Co(II), Ni(II), and Ca(II) was also investigated. By addition of 1 mmol l⁻¹ of these metal ions, the *ac* voltammograms of inosine nucleotide indicated that the decrease of the capacitive *ac* signal, but no compact film formation is obtained. This reveals that these metal ions only weakly interact with the investigated compounds.

Additional information about the course of association of inosine nucleotide is offered by the time dependence of the *ac* capacitive current, as shown in Fig. 4. The capacitive *ac* current measured at a constant mean electrode potential (E_{\max}) at first slowly decrease at low bulk concentrations of IMP or IDP to the first equilibrium value, corresponding to the dilute adsorption layer. For bulk concentration larger than the threshold value for IMP or IDP, the current rapidly decreases to the second equilibrium value characteristic of compact film formation. With ITP, the capacitive *ac* current decreases slowly with increasing bulk concentration to the first equilibrium value, and no compact film formation is obtained. However in presence of Cd(II) ions the second equilibrium value characteristic of compact film formation is obtained.

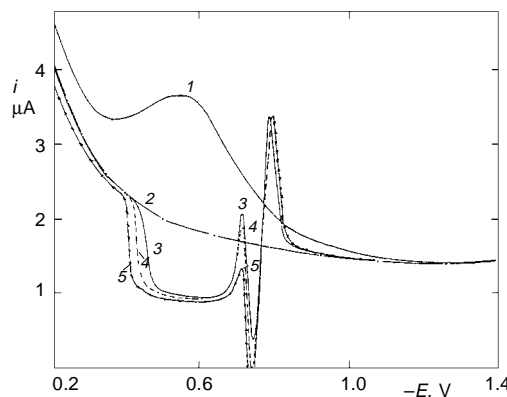


FIG. 3
The *ac* capacitive current curves of 0.3 mM ITP at different concentrations of Cd(II). (1) background, (2) 0.0, (3) 0.122, (4) 0.133 and (5) 0.156 mM Cd(II), pH 2.3. Other conditions as in Fig. 1

Further quantitative studies on the adsorption and association of inosine phosphate at different pH values could be elucidated by plotting Δi_{ac} (the decrease of the capacitive ac current for a given bulk concentration with respect to the i_{ac} value of the blank supporting electrolyte) as a function of bulk concentration (C) of the investigated substances (Fig. 5). The plot of Δi_{ac} vs C at the maximal adsorption potential indicates a two-steps isotherm at pH 2.3, but only one step at pH ≥ 3.2 for IMP and IDP. This corresponds to two adsorption stages of the adsorbed molecules on the electrode surface. The first stage reflects the flat adsorption stage for relatively low bulk concentration. Above the threshold concentration value, the second stage of adsorption is seen, which is due to strong lateral interaction and high interaction coefficients of adsorbed molecules giving rise to a vertical stacked adsorption stage.

However, the course of the concentration dependence of the ac capacity current decreases Δi_{ac} or the surface coverage θ for ITP has the form of one step isotherm at different adsorption potentials and in various buffer solutions. This behaviour indicates that a rather dilute adsorption layer is formed, whereas a compact film, which would be reflected by a double-step isotherm, is not observed. This is connected with the increas-

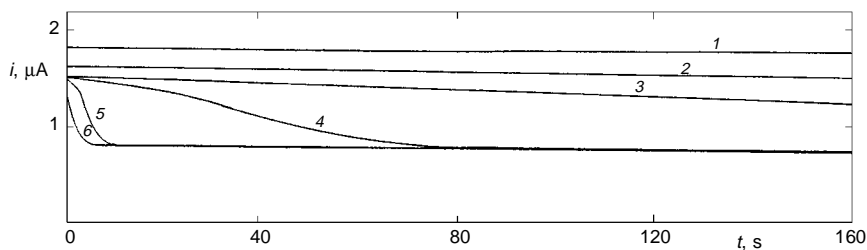


FIG. 4

Time dependence of the out-of-phase component of the ac current for IMP, pH 2.3 (BR buffer + Cl⁻).

(1) 0.0, (2) 0.07, (3) 0.2, (4) 0.26, (5) 0.3 and (6) 0.41 mM IMP. Other conditions as in Fig. 1

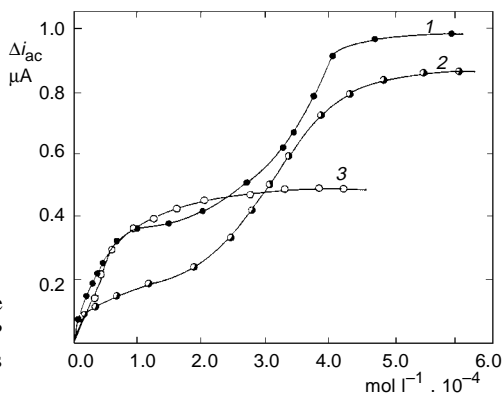


FIG. 5

Dependence of the capacity current decrease Δi_{ac} on the concentration at pH 2.3 for IMP (1), IDP (2) and ITP (3). Other conditions as in Fig. 1

ing number of hydrophilic phosphate groups and also with increasing lateral repulsion of the negatively charged phosphate groups. Obviously the electrostatic repulsion between adjacent negatively charged phosphate groups decrease the stabilization by base stacking, such stacking causes the interfacial association of adjacent adsorbed nucleotide molecules and consequently the formation of the compact layer.

In order to calculate the adsorption parameters for inosine phosphate at various pH values, the experimental results were fitted to several adsorption isotherms. From a comparison of the experimental results with the theory it seems that the isotherm is of a Frumkin type given by the equation: $\theta(1 - \theta)^{-1} \exp(-2a\theta) = \beta C$, where θ is the degree of coverage, a is the interaction coefficient, β the adsorption coefficient and C the bulk concentration of inosine phosphate. The interaction coefficient a was determined from the slope of the logarithmic plot of the Frumkin isotherm and the adsorption coefficient β from the value at the half coverage. The standard Gibbs energy of adsorption ($-\Delta G^0$) was then calculated from the adsorption coefficient β using the equation: $\beta = (1/55/5) \exp(-\Delta G^0/RT)$. The calculated values of the adsorption parameters of IMP, IDP and ITP at various pH values are given in Table I.

Values of adsorption parameters for both the dilute and compact layers of inosine nucleotides are seen to depend on the pH values. The magnitude of the adsorption coefficient, β , of IMP or IDP for compact layer is significantly lower than that in the dilute stage as the same pH. This significantly lower adsorptivity of the compact film

TABLE I

Adsorption parameters of dilute (A) and compact (B) layers of mono-, di-, and triphosphate of inosine calculated from the results of 90° out-of-phase *ac* voltammetry at various pH values

Compound	pH	C_{th} mol l ⁻¹	a		β , l mol ⁻¹		$-\Delta G^0$, kJ mol ⁻¹		$-E$, V
			A	B	A	B	A	B	
IMP	2.3	$1 \cdot 10^{-4}$	0.98	2.08	$1.33 \cdot 10^4$	$3.60 \cdot 10^2$	$3.12 \cdot 10^4$	$2.28 \cdot 10^4$	0.85
	7.2	–	0.47	–	$3.10 \cdot 10^3$	–	$2.78 \cdot 10^4$	–	0.50
	9.2	–	0.43	–	$2.20 \cdot 10^3$	–	$2.71 \cdot 10^4$	–	0.50
IDP	2.3	$2 \cdot 10^{-4}$	0.58	2.01	$3.80 \cdot 10^3$	$4.05 \cdot 10^2$	$2.83 \cdot 10^4$	$2.30 \cdot 10^4$	0.90
	7.2	–	0.50	–	$5.30 \cdot 10^2$	–	$2.37 \cdot 10^4$	–	0.50
	9.2	–	0.35	–	$5.07 \cdot 10^2$	–	$2.36 \cdot 10^4$	–	0.50
ITP	2.3	–	0.48	–	$5.40 \cdot 10^2$	–	$2.37 \cdot 10^4$	–	0.50
	7.2	–	0.45	–	$4.50 \cdot 10^2$	–	$2.33 \cdot 10^4$	–	0.50
	9.2	–	0.32	–	$3.80 \cdot 10^2$	–	$2.29 \cdot 10^4$	–	0.50

correlates with its sensitivity to larger alterations of the adsorption potential. This reflects the tendency of the compact film to collapse suddenly at certain values of the interfacial electric field at the positive and negative side of the region of maximum adsorption around the operative potential of zero charge. Nevertheless, the interaction coefficient a increases in the compact stage, due to enhanced possibilities for intermolecular attractive interactions resulting from the perpendicular orientation and the greater population of adsorbed molecules in the compact stage.

On comparison of the adsorption parameters of IMP, IDP, and ITP shows that the surface activity of ITP is significantly smaller. The adsorption coefficient β decreases by a factor 3.5 from IMP to ITP at the same pH. This lowering adsorptivity corresponds to a decrease in $-\Delta G^0$ of 3.1 kJ mol^{-1} , and this is due to the increasing number of hydrophilic phosphate groups and also with increasing lateral repulsion of the negatively charged phosphate groups. Such an effect is reflected also in the pattern of the a values.

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