Mid-point redox potentials of multiheme cytochromes determined after deconvolution of cv and dpv experimental data in different electrode systems

Cytochrome $\underline{c}_3$	Method	pH	E_1	E_2	E_3	E_4
$\underline{D.baculatus}$	CV (carbon-edge)	4.9	-60	-180	-300	-360
		6.3	-130	-190	-320	-360
		7.6	-140	-200	-320	-360
		8.0	-150	-210	-330	-380
		9.2	-150	-210	-330	-380
		10.3	-200	-270	-330	-380
$\underline{D.baculatus}$	CV (Indium oxide)	7.6	-150	-270	-340	-370
$\underline{D.baculatus}$	DPV (Au)	5.0	-70	-170	-300	-345
		7.0	-155	-260	-310	-355
		9.0	-170	-260	-320	-370
Estimated error (mv)			$E_1 \pm 10$	$E_2 \pm 20$	$E_3 \pm 15$	$E_4 \pm 10$

These redox potentials were determined by independent methods (namely EPR) with a good agreement, giving confidence on the deconvolution method used.

We plan to apply our method to other systems, namely hexaheme proteins (nitrite reductase) where multiredox reaction occurs in paralell with high-spin / low-spin equilibrium that is a determining factor of protein reactivity.

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REACTION OF PYRIDOXAL AND PYRIDOXAL-5'-PHOSPHATE WITH HEXYLAMINE. COMPARATIVE ELECTROCHEMICAL AND SPECTROPHOTOMETRIC STUDIES

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ABSTRACT

A comparative study of the Schiff bases formed by reaction of the pyridoxal (PL) and pyridoxal-5'-phosphate (PLP) with hexylamine in basic media was carried out. Acid-base dissociation constant of the imine group was obtained by spectrophotometric measurements. Apparent formation constant of the Schiff base was obtained as a function of the pH. Electrode processes were considered on the basis of polarographic and kinetic parameters.

INTRODUCTION

PLP is a cofactor of numerous enzymes which catalyze reactions of transamination, decarboxylation, α-β elimination, etc. The binding to protein is via formation of a Schiff base with a lysil residue[1]. Studies of the reaction of PLP with primary amines or amino acids confirmed that the formation of the Schiff base is coupled with multiple acid-base and tautomeric equilibria[2,3]. The Schiff base of PLP and hexylamine was proposed as a simple model to estimate polarities of the microenvironment of the coenzyme on the basis of spectrophotometric properties[4]. Indeed, combined spectrophotometric and electrochemical studies allowed a quantitative characterization of equilibria in solution of this Schiff base[5,6].

The present work deals with a comparative study of the Schiff base of PL and PLP with hexylamine by DC and DP and cyclic voltammetric in basic media. In addition, a study of the UV-visible spectra of the reaction mixture has been carried out to characterize the protonation equilibrium involved in the electroreduction of the Schiff base.

EXPERIMENTAL

PL and PLP were purchased from Sigma. The Schiff bases were obtained by adding known amounts of hexylamine to PL and PLP solutions. In all cases measurements were carried out after the reaction reaches equilibrium. All other conditions were similar to those described in refs. 7 and 8.

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RESULTS AND DISCUSSION

Our results indicate that the reaction mixture shows two reduction waves under most of the conditions. First wave corresponds to the reduction of the Schiff base and the second wave to the reduction of the free carbonyl (PLP or PL) . The ratio of the limiting current of the waves is a function of the

PLP   PL
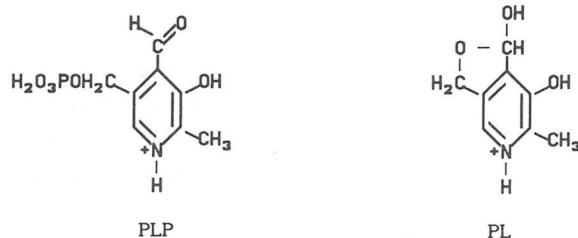
pH and the molar initial ratio amine:aldehyde. In the PLP Schiff base appear additional waves in strong basic media corresponding to the coenzyme. From a comparative analysis of the limiting current a competition between formation of the hemiacetal of PL and Schiff base is shown. This fact is observed a pH lower than the protonation pK of the heterocycle of this aldehyde.

The electrochemical behaviour is in agreement with the scheme 1, corresponding to electroactive species coupled by a pseudofirst order chemical reaction:

Scheme 1
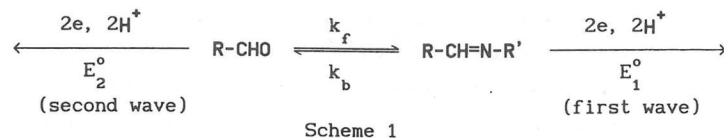
The analysis of the current-time curves on the limiting zone of the first wave shows a diffusion control in a wide pH range. However in strong basic media, a kinetic control is observed in the electroreduction of the Schiff base.

Under diffusion control and assuming similar diffusion coefficients the eqn (1) express the relationship between the limiting current of the first and overall process, the amine concentration and the equilibrium ratio, K_{pH}. This ratio is defined taking into account species with different protonation stages of Schiff base, PLP and amine.

$$(1/i_{L1}) = (1/i_D) + [(1/i_D K_{pH})(1/c_A)] \qquad (1)$$

The Schiff base of PLP shows a higher stability at pH close to 10. Two inflections due to protonation reactions of the nitrogen of the heterocycle and the imine are observed. In the Schiff base of PL, a plateau is obtained in strong basic media corresponding to unprotonated species in solution. The inflection at low pH should be related to the protonation of the nitrogen of PL.

In strong basic media the value of K_{pH} should be taken as an estimate because of the influence of the chemical reaction on the top of the wave. This effect is related to the presence of the carbinolamine intermediate in solution and its fast dehydration (Scheme 2). This is a difference in behaviour with a weak basic medium. Under such conditions carbinolamine is negligible and the dehydration reaction is the rate determing step[3].

By LSCV the kinetic effect is eliminated. The current function of the first peak corresponding to the Schiff base decreases as the scan rate increases. At v > 10 v/s the term $i_p/v^{1/2}$ is v-independent and proportional to the equilibrium concentration of the Schiff base. The value of K_{SO} corresponding to the reaction between unprotonated species can be obtained.

HP   Carbinolamine   HS

Scheme 2
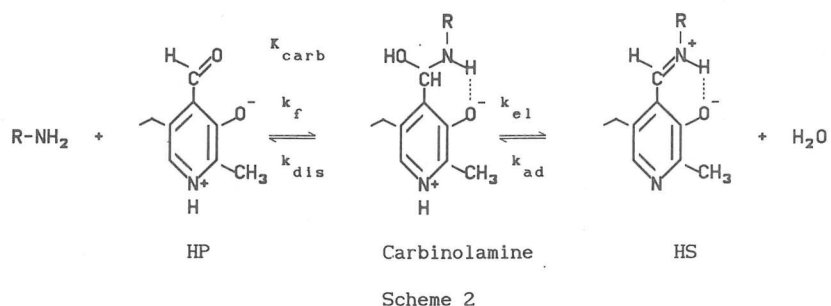
The protonation pK_{SH} of the imine group was obtained by UV-visible absorption spectra. Values of 10.7 and 13 were obtained for PL and PLP Schiff base, respectively. The influence of a proton bonding between the conjugate acid of the imine and o-hydroxi group is responsible of this difference.

In the table, polarographic and kinetic results are collected for both Schiff bases. The results are in agreement with a two electron, two proton reduction according to the general behaviour of the Schiff bases[9,10]. The electroactive species is the conjugate acid of the imine. At $pH > pK_{SH}$ a previous recombination of the species S^- with H^+ ion is observed. This reaction is observed even at $pH < pK_{SH}$ in the PLP Schiff base because of the kinetic control by dehydration of the carbinolamine.

Polarographic and kinetic parameters of the Schiff base

	PLP:hex		PL:hex	
	8.5<pH<11.3	pH>11.3	pH<10.4	pH>10.4
$\dfrac{\partial E_{1/2}}{\partial pH}$ /mV	-60	-90	-58	-88
$\dfrac{\partial E_p}{\partial pH}$ /mV	-	-	-54	-83
$\partial E/\partial \log \dfrac{i}{i_L - i}$ /mV	-29	-29	-29	-29
$\dfrac{\partial E}{\partial \log i}$ /mV	-28.2	-	-29	-29
$\dfrac{\partial \log i}{\partial \log c_H}$	2.2	-	2.1	3.0
$\dfrac{\partial \log i}{\partial \log c_S}$	-	-	-	0.9

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THE EFFECT OF Ta ION IMPLANTATION ON THE AQUEOUS CORROSION RESISTANCE OF M50 BEARING STEEL

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Corrosion of the localized type of steel bearings used in gas turbine engines has been found to be a serious problem. This is mainly due to the accumulation of salt-spray condensates in the engine lubricant, probably, during non-working periods.

A large percentage of bearings can be removed from service due to corrosion problems and even minor corrosion pitting of the receways has been proven to reduce life significantly.

The present work reports on results regarding corrosion resistance of ionically implanted substrates of M50 steel with Ta. Implantation with other ions is being implemented.

Measurements on non implanted samples were made on M50 steel disks supplied by RHP Bearings Ltd. Nominal composition is given elsewhere[1].

Potentiostatic determination of breakdown potentials (Eb) together with examination of surface morphology by Scanning Electron Microscopy were also conducted.

Experimental details are given elsewere[1].

Implanted surface with Ta at nominal concentrations from 5×10^{16} ions.cm^{-2} to 4×10^{17} ions.cm^{-2} were studied using the same techniques as for the base metal (see Fig.1).

An overall view of the effect of implantation is better analyzed when studying the peak current as a function of the scan rate in a log-log plane. The relationship is found to be linear (Fig. 2) with no significant difference between doses in the range from 5×10^{16} to 2×10^{17} ions.cm^{-2} probably due to sputtering and saturation[2,3]. A kinetic analysis is the subject of further publication.