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Role of Nitric Oxide in Thiol Mediated Ion Migration

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Nitric oxide is recognised as a signalling molecule. It contributes to blood pressure regulation, neuronal communication, immune defence and vasodilator, etc. Recent studies have shown that blood serum levels of NO are on the higher side in enclampsia. Also it is observed that thiols in presence of superoxides form disulphide bonds between interacting systems affecting embryo implantation and electrolyte transport across placental membranes. The author has therefore attempted to study the effect of thiols on electrolyte (NaCl, KCl and $CaCl_2$) migration and modifications, if any, of the same on addition of $NaNO_2$ and sodium nitroprusside (Na-Np). It is observed that membrane ion equilibria, conductance and flow rates of solutions of electrolytes are influenced by thiols (bovine serum albumin BSA, glutathione, cysteine) and that addition of $NaNO_2$ and Na-Np causes change in these. Turbidity of the thiol solutions is increased on addition of these nitro compounds. These observations indicate that thiols probably form some aggregates in presence of NO which affect their mechanism of ion transport resulting in pressure changes.

INTRODUCTION

Nitric oxide is a double-edged sword. Synthesized in appropriate amounts by endothelial cells, neurons or macrophages, it contributes to blood pressure regulation, neuronal communication and immune defence. It also acts as vasodilator. On the other hand exclusive and uncontrolled production of NO is associated with several diseases[1]. It is also known to be carcinogenic[2] and involved in hypercholesterolemia[3].

Elevated serum nitrite levels have been observed in women with preeclampsia[4]. With glutathione and other thiols NO is shown to affect embryo implantation and plays very significant role in early pregnancy[5, 6]. Relation between uterine nitric oxide synthase activity and free radical generation during early pregnancy has been reported[7]. Binding of glutathione to NO and its role in transport and catabolism of NO is observed[8]. Charge transfer and coupling to thiol disulphide redox reactions, diffusibility of NO and chromosomal abberations by glutathione with bleomycin with superoxide dismutase etc., have been discussed[9, 10]. Thus the role of thiols and NO appears to be a significant factor in pregnancy and related hypertension etc.

The author has therefore attempted to see if the thiols bovine serum albumin

(BSA) glutathione (gl) and cysteine show any tendency of cluster formation in presence of compounds donating NO and whether this affects the migration of common electrolytes like NaCl, KCl and $CaCl_2$ using simple physico-chemical methods namely turbidity measurement, equilibrium dialysis, flow rates and conductivity.

EXPERIMENTAL

Deionized water (obtained using ion-exchange resins of Ion-exchange India Ltd.) was used for all experiments. All reagents used were of AnalaR grade from reputed companies like Qualigen. bovine serum albumin (BSA), glutathione and cysteine were procured from SRL, SDS Fine chemicals and Ioba Chemic India, respectively.

Turbidity of BSA, glutathione and cysteine solutions alone and on addition of $NaNO_2$ and sodium nitroprusside (Na-Np) was measured by reading the absorbance at 600 nm[11] on Spectrionic-21 of Bausch and Lomb.

The membrane equilibria of electrolytes were measured by allowing the electrolyte solution to diffuse through a dialysis membrane (Hi-media 70; width 29–31 cm; diameter 17.5 mm; capacity approx. 2.4–1 mL/cm) in water for about 24 h.[12] The conductance (λ) of the dialysand was measured on equiptronics digital Conductometer. The λ was calculated per mL of the solution. The procedure was repeated in presence of thiols, $NaNO_2$ and Na-Np.

Flow rates of electrolyte solutions separately and in presence of BSA, $NaNO_2$ and Na-Np were read using Ostwald's two bulb type viscometer at constant temperature. Average of at least 8 readings was taken for each sample[13].

For observing the effect on conductance of electrolytes, which is measure of ionic mobility[14], λ of a series of dilutions of the electrolyte (0.5 M to 0.1 M) was read on Equiptronics conductometer. The same was repeated after the addition of thiols as well as $NaNO_2$ and Na-Np.

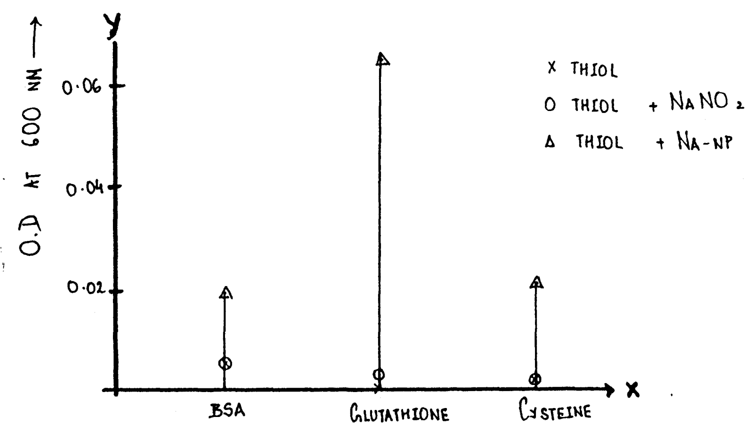

Fig. 1. Turbidity, X Thiol, O Thiol +$NaNO_2$, Δ Thiol +Na – Np

RESULTS AND DISCUSSION

The observation of turbidity (Fig. 1) indicates that a dilute solution of the thiols (10 mg/mL) has practically no turbidity. On addition of $NaNO_2$ it is slightly increased. However, on addition of Na-Np it is considerably increased indicating its tendency to induce cluster formation. The effect is maximum on glutathione, lesser on cysteine and still less on BSA.

The study of flow rates (Fig. 2) shows that in case of NaCl the flow time is reduced on addition of BSA and further on addition of $NaNO_2$ and Na-Np. While in case of KCl the flow rate progressively increases on addition of BSA. $NaNO_2$ and Na-Np. In case of $CaCl_2$ no significant changes are observed on addition of BSA and $NaNO_2$ or Na-Np to the $CuCl_2$ + BSA solution. Thus it is seen that the effect is different for each electrolyte which can have implications on migration of the electrolytes in the capillaries causing same changes in exerted pressure.

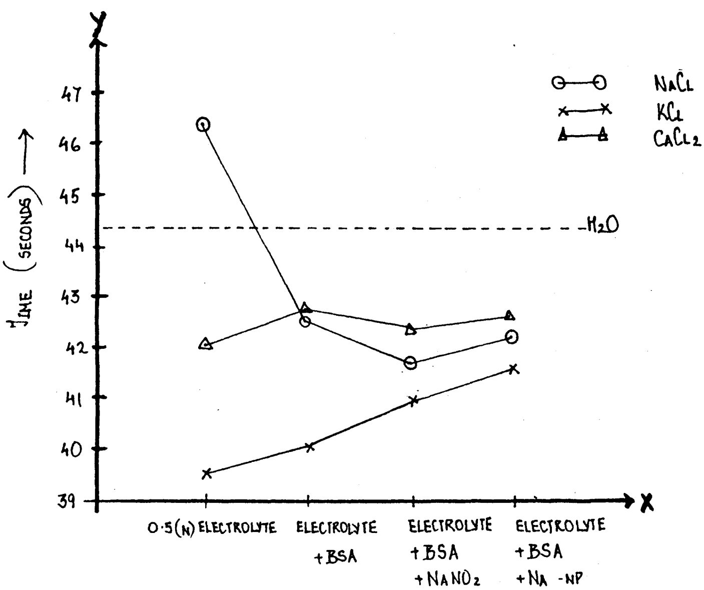

Fig. 2. Flow Rates O—O NaCl, X—X RCl, Δ—Δ $CaCl_2$

It can be seen (Table-1) that addition of thiols and further addition of $NaNO_2$ or Na-Np has varied but considerable effect on the migration of an electrolyte through a semipermeable membrane.

It is also observed (Table-2) that addition of thiols and further addition of $NaNO_2$ or Na-Np have in general the effect of lowering the conductance of electrolyte suggesting decrease in mobility of ions.

Thus, based on these simple physico-chemical observations it appears that an addition of NO in the form of $NaNO_2$ or Na-Np the thiols show some tendency towards cluster formation probably through disulphide bridges promoted by free radical and this has considerable effect on free flow of electrolytes across membranes and capillaries and may influence blood flow and embryo transplant.

TABLE-1
IONIC EQUILIBRIA OF SALTS CONDUCTANCE VALUES (mhos/mL)

	Electrolyte alone	In presence of thiol		Thiol + $NaNO_2$	Thiol + Na-Np
KCl (0.5 M)	2.50×10^{-1}	BSA	3.50×10^{-1}	5.20×10^{-2}	4.75×10^{-2}
		Glutathione	1.37×10^{-1}	5.60×10^{-2}	5.60×10^{-2}
		Cysteine	1.13×10^{-1}	6.00×10^{-2}	6.10×10^{-2}
NaCl (0.5 M)	2.40×10^{-2}	BSA	2.20×10^{-2}	4.85×10^{-2}	6.00×10^{-2}
		Glutathione	1.45×10^{-1}	5.40×10^{-2}	5.50×10^{-2}
		Cysteine	1.16×10^{-1}	5.60×10^{-2}	5.50×10^{-2}
$CaCl_2$ (0. 5 M)	3.75×10^{-2}	BSA	3.55×10^{-2}	5.56×10^{-2}	8.50×10^{-2}
		Glutathione	7.80×10^{-2}	8.00×10^{-2}	8.30×10^{-2}
		Cysteine	1.32×10^{-1}	7.40×10^{-2}	8.20×10^{-2}

TABLE-2
THE CONDUCTANCE VALUES OF ELECTROLYTE SOLUTIONS
(λ mhos $\times 200 \times 10^{-3}$)

KCl (0.1 M–0.5 M)	KCl + BSA	KCl + BSA + $NaNO_2$	KCl + BSA + Na-Np
13.5–55	11–55	9.5–39	4.5–36
	KCl + glutathione	KCl + gl + $NaNO_2$	KCl + gl + Na-Np
	11.5–50.5	9–35.5	9.5–36
	KCl + cysteine	KCl + cyst + $NaNO_2$	KCl + cyst + Na-Np
	13–50	9.5–30.5	10–37
NaCl (0.1 M–0.5 M)	NaCl + BSA	NaCl + BSA + $NaNO_2$	NaCl + BSA + Na-Np
10.5–44	10–40	7–29	8–30
	NaCl + glutathione	NaCl + gl + $NaNO_2$	NaCl + gl + Na-Np
	10–40	7.5–31	7–30
	NaCl + cysteine	NaCl + cyst + $NaNO_2$	NaCl + cyst + Na-Np
	10–41.5	8–30	8–30
$CaCl_2$ (0.1 M–0.5 M)	$CaCl_2$ + BSA	$CaCl_2$ + BSA + $NaNO_2$	$CaCl_2$ + BSA + Na-Np
7.5–37	6.5 × 27.5	6–22	6.5–23
	$CaCl_2$ + glutathione	$CaCl_2$ + gl + $NaNO_2$	$CaCl_2$ + gl + Na-Np
	8.0–25.5	6–20	6–20
	$CaCl_2$ + cysteine	$CaCl_2$ + cyst + $NaNO_2$	$CaCl_2$ + cyst + Na-Np
	8–28	6–20	6.5–21.0

gl: glutathione

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