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Osmotic Fragility of Human Erythrocytes is Altered by Porphyrin Compounds

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Abstract: *The chemical specificity of Porphyrin derivatives, Tetraphenylporphinesulfonate (TPPS), 5, 10, 15, 20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron (III) Chloride (FeTPPS) and 5, 10, 15, 20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron (III) nitrosyl Chloride (FeNOTPPS), on osmotic fragility of erythrocytes was examined in vitro. TPPS increased the osmotic fragility of erythrocytes more effectively in hypotonic PBS solution. Incorporation of iron and nitroso group in the Porphyrin ring decreased the effectiveness. The results showed that the size, form and elements introduced into the Porphyrin compound affected its affinity to the cell membrane, and changed the osmotic resistance in erythrocytes.*

Keywords: *Osmotic fragility, Porphyrin compounds, erythrocytes, hypotonic solution, hemolysis*

1. INTRODUCTION

Osmotic fragility is the degree of hemolysis that occurs when erythrocytes are subjected to osmotic stress by being placed in a hypotonic solution. Osmotic fragility is a useful indicator for evaluating interactions of various substances with the cell membrane *in vitro*. The osmotic fragility of erythrocytes depends upon the movement of water into the cells and is related to cellular deformability. It has been demonstrated that general [1,2,3] and local anesthetics [4,5], certain drugs [6,7], toxins [8,9], inorganic [10,11] and organic compounds [12,13], natural products isolated from plants [14,15] and crude plant extracts [16,17] affect the osmotic fragility of erythrocytes. The amount of cholesterol, which is one of major components of the cell membrane, is also known to affect membrane fragility [19-21].

The degree of resistance of erythrocytes to lysis as a result of a decrease in the NaCl concentration of their environment is the basis of the osmotic fragility test. The experimental basis for this measure has been the fact that when any red cell, of a population of red cells, reaches the hemolytic volume [22], the hemoglobin of that cell diffuses to equilibrium inside and outside the cell. The osmotic fragility test is common in hematology and is often performed to help in diagnosis of diseases of erythrocyte membrane abnormalities.

In erythrocytes, any mechanism which controls the total solute content can also regulate the cell volume [23]. Since K^+ and Na^+ represent the principal cations in erythrocytes, control of cell volume depends on the concentrations of K^+ and Na^+. The osmolality of mammalian blood plasma is in the 270–310 mosmol range. The substances regulating the osmotic properties are cations, such as, K^+ and Na^+, and anions, such as, chloride and hydrogen carbonate. The plasma concentrations of other metabolites such as urea, glucose, and proteins are very low and they

account for only 8–12 mosmol colloid oncotic pressure [24]. In this study, we compared the effects of Porphyrin derivatives on the osmotic fragility of isolated human erythrocytes.

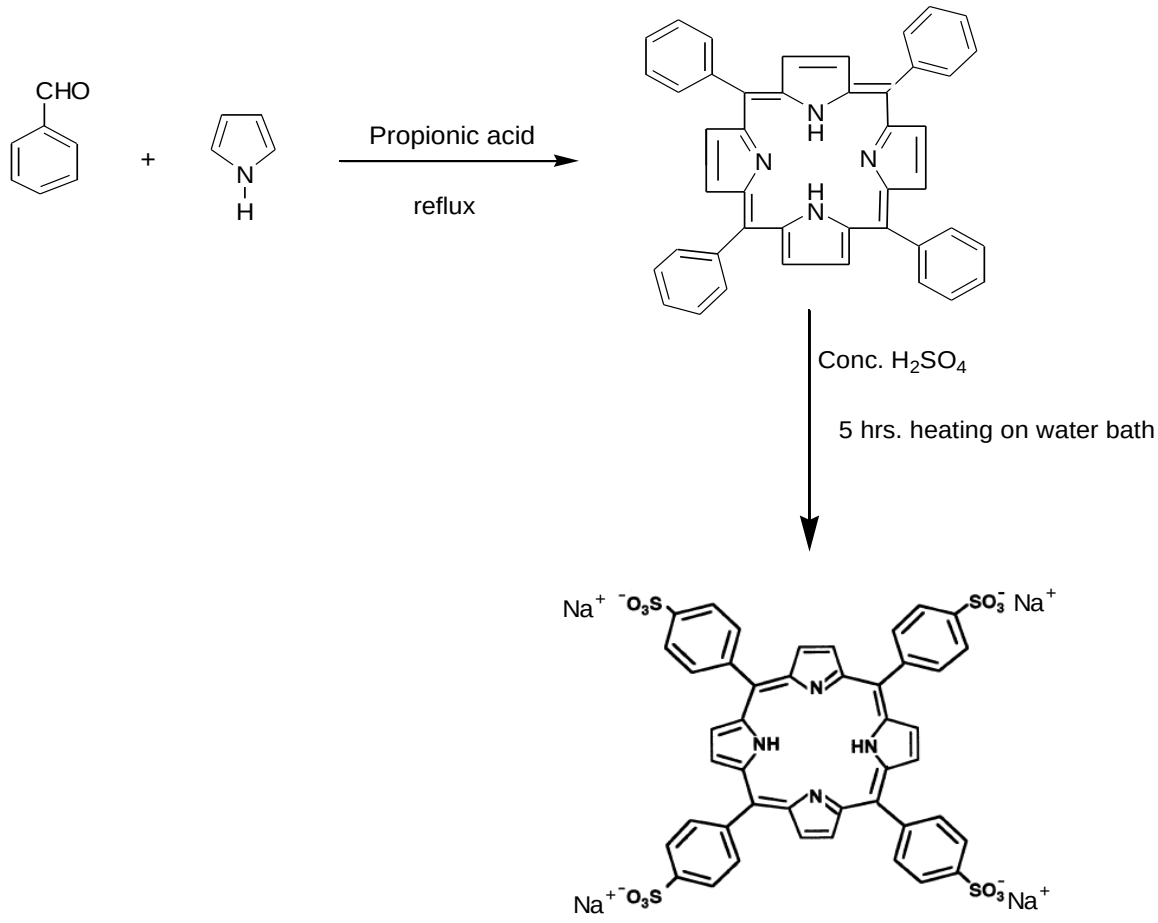
2. MATERIALS & METHODS

2.1. Chemical Synthesis of Porphyrin Compounds

A single equivalent each of pyrrole and benzaldehyde were mixed and 1mL boron trifloroetherate was then added. p-chloranil was added as an oxidant and the solution was refluxed for an hour. The solvent was evaporated and the product was separated using column chromatography (Scheme 1).

Supplementary Material

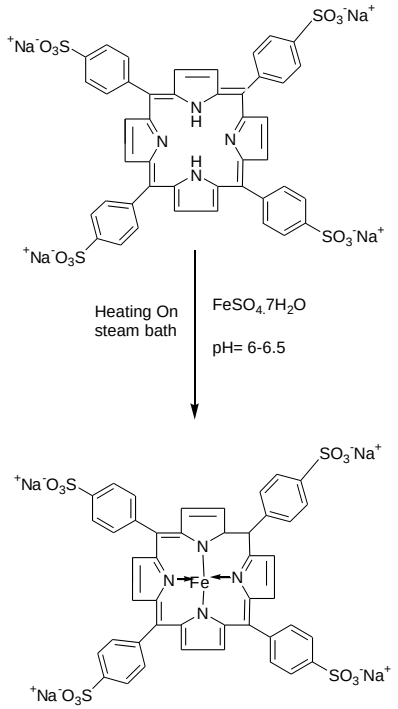
Chemical Characterization: $C_{44}H_{30}N_4$; C, 85.90%; H, 4.92%; N, 9.12%; ^{1}H NMR (400 MHz) ($CDCl_3$) δ 2.5-2.62 (br s, 2H, NH), 8.1-8.2 (dd, 8H, pyrrole), 7.6-7.7 (s, 20H, phenyl); UV-Vis (max.) 417, 513, 546, 589, 642 nm.



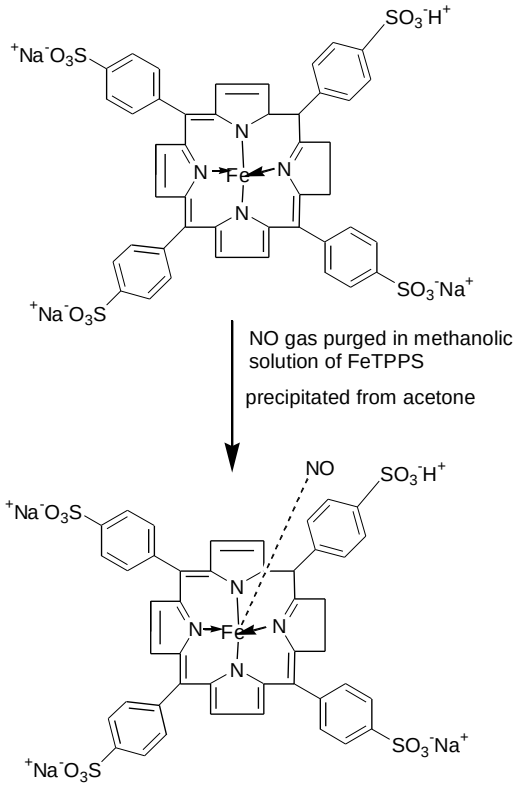
Scheme1. *Synthesis of TPP and TPPS.*

TPPS was synthesized according to the published literature procedure (Scheme 1) **[25]**. Pure TPP and concentrated H_2SO_4 were ground into a homogenous paste and 50 ml of concentrated H_2SO_4 was added. The mixture was heated in a steam bath for 4 hours and allowed to stand at room temperature for 48 hours. The filtrate was diluted with two volumes of distilled water and salt was precipitated with addition of acetone. FeTPPS was also prepared by using published methods **[24]**. But a modification was made during the purification of the final product. Instead of following the purification process by cation-exchange method, the classical method of the removal of iron using phosphate salt was adopted. Excess sodium sulfate and chloride present as impurities could be removed by dissolving the crude product in methanol and precipitating FeTPPS from solution by acetone (Scheme 2). FeNOTPPS was then synthesized by using the laboratory-synthesized FeTPPS as the starting material (Scheme 3). Nitric oxide was bubbled in a

solution of FeTPPS. The completion of the reaction was confirmed by the shift in Sǒ ret band of the UV-Vis spectrum from 394 to 420 nm.



Scheme2. *Synthesis of FeTPPS.*



Scheme3. *Synthesis of FeNOTPPS.*

2.2. Collection of Blood Samples

The study used blood samples from normal healthy subjects of both sexes that were divided into young (18-35 years; 32 subjects), middle (36-60 years; 31 subjects) and old (> 60 years; 26 subjects) groups. The osmotic fragility determinations were made by following the method of Dacie and Lewis **[26]**.

3. RESULTS & DISCUSSION

The intracellular fluid of erythrocytes comprises salts, glucose, various proteins and hemoglobin **[27]**. In a 0.9% NaCl solution, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane **[28]**. But in a *hypotonic* solution (e.g. 0.4% NaCl or distilled water), an influx of water molecules occurs and hemolysis of erythrocytes takes place. When exposed to a hypertonic solution, the erythrocytes give up intracellular fluid, shrink and then break up **[29]**. Hemolysis of erythrocytes begins in 0.39% to 0.45% salt solution and is completed in 0.30% to 0.33% salt solution over a period of 24 hours at 37° C.

This study investigated the effect of synthetic analogues (TPPS, FeTPPS, FeNOTPPS) on the osmotic fragility of human erythrocytes as a function of increasing NaCl content of the PBS solution. The results show that TPPS increases fragility of the erythrocytes more readily. The erythrocytes exhibit 52.08% hemolysis in the presence of TPPS in 0.75% w/v% NaCl in PBS solution. FeNOTPPS, on the other hand, had little or no effect. Similar to the control solution, more than half of the erythrocytes underwent hemolysis in the presence of FeNOTPPS in 0.65% w/v% NaCl in PBS solution. The effect displayed by FeTPPS, however, was intermediate between the effect of TPPS and FeNOTPPS on osmotic fragility (Table 1).

Table1. *%Hemolysis of Erythrocytes is shown versus w/v% of NaCl in phosphate buffered saline solution in the presence of TPPS, FeTPPS and FeNOTPPS.*

%PBS*	% Hemolysis			
	Normal	TPPS	FeTPPS	FeNOTPPS
0.85	0	0	0	0
0.75	24.5	52.08	37.4	24.5
0.65	55	81.6	69.2	58.56
0.55	76.8	88.08	80.8	76.8
0.45	83	92.88	88.56	84
0.35	86.6	94.43	89.8	87.6
0.2	90.8	96.77	96.17	90.8
0.1	100	100	100	100

**% PBS represents w/v% of NaCl in phosphate buffered saline solution.*

4. CONCLUSION

It is difficult to analyze the exact mechanism by which the three Porphyrin derivatives (TPPS, FeTPPS and FeNOTPPS) increase osmotic fragility of eythrocytes. One mechanism that can be invoked is that these compounds increase the activity of Na^+/K^+-ATPase and Ca^{+2}-ATPase which in turn, alters the intracellular solute content and thus affects the osmotic fragility. The results show that TPPS decreases the osmotic fragility more efficiently. On the other hand, FeTPPS has a smaller effect on osmotic fragility; while, FeNOTPPS shows almost no effect.

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