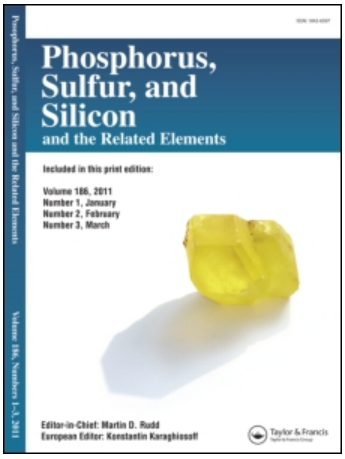

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Synthetic Polyphosphonates, Polyphosphates, and Phosphonocarboxylates as Allosteric Effectors of Hemoglobin

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SYNTHETIC POLYPHOSPHONATES, POLYPHOSPHATES, AND PHOSPHONOCARBOXYLATES AS ALLOSTERIC EFFECTORS OF HEMOGLOBIN

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Abstract Synthetic phosphonocarboxylates, diphosphonates, polyphosphates, and some triphosphonic acids were studied as effectors in the binding of dioxygen to human hemoglobin.Ethane-1,2-diphosphonic acid, and triphosphate anion were strong effectors.

INTRODUCTION

The binding of dioxygen to hemoglobin is modulated in human red blood cells by many factors including pH, chloride ion concentration, and the concentration of the natural allosteric effector 2,3-diphosphoglycerate (2,3-DPG)[1] and is interpreted for this latter as due to preferential binding of 2,3-DPG to deoxyhemoglobin.The site of binding to deoxyhemoglobin has been demonstrated by X-ray crystallographic studies to be between the amino termini of the beta chains. It is also known that adenosine triphosphate, ATP, is an effector of oxygen binding to hemoglobin. In this article we describe the preparation of synthetic hemoglobin effectors which are models or analogs of the natural effectors 2,3-DPG, and ATP. The *in vitro* activities of the synthetic effectors have been evaluated. While phosphonocarboxylates are not powerful effectors, some simple polyphosphonates are. The simple inorganic ions diphosphate and triphosphate also show effector ability. The results may be used to refine the model of how dioxygen binding to hemoglobin is modulated by external polyionic compounds.

EXPERIMENTAL

Starting materials for the syntheses were commercial samples purified by distillation or crystallization, as appropriate. Nitrilotris(methylene)triphosphonic acid was purchased from Aldrich Chemical Co. Purity of starting materials and products was checked by infrared and both proton and ^{31}P nuclear magnetic resonance spectroscopy.

Diphosphonic acids $H_2O_3P(CH_2)_nPO_3H_2$. Compounds where n = 1 through 4 and n = 6 were prepared by literature methods[2] involving Arbuzov reactions[3], followed by ester hydrolyses under strongly acidic conditions.

Phosphonocarboxylic acids $HO_2C(CH_2)_nPO_3H_2$ Compounds with n = 0,1,2 or 3 were prepared by published procedures[3].

Tris(phosphonomethyl)phosphine oxide, TPPO, $(H_2O_3PCH_2)_3PO$. This compound was made by a literature method[3].

Determination of binding constants for hemoglobin-dioxygen binding. Hemoglobin was prepared from fresh whole human blood by the method of Rossi Fanelli et al.[4] and then stripped of ions bound to the protein by passing the solution through a column of mixed-bed ion exchange resin (Bio-Rad AG501X8). The oxygen affinity was measured by a spectrophotometric method[5] and is expressed as p_{50} which is defimed as the pressure of dioxygen im mm of mercury at which the hemoglobin is half-saturated. Workup of the raw data was by means of the empirical Hill equation.Conditions for all p_{50} determinations were as follows: bis-tris buffer, 0.05 M;pH 7.3; chloride 0.05 M; T = 20°C; Hb concentration 5×10^{-5} M (tetramer).

RESULTS AND DISCUSSION

The $\log p_{50}$ values for the effectors tested are given in Tables I to IV.

Table I The effect of alkanediphosphonic acids on the oxygen affinity of human hemoglobin.

$H_2O_3P(CH_2)_nPO_3H_2$	M	$\log p_{50}$	Hill parameter
n = 2	1.0×10^{-4}	0.36	3.0
	1.0×10^{-2}	0.86	2.0
n = 3	1.0×10^{-4}	0.46	3.0
	1.0×10^{-2}	0.68	2.8
n = 4	1.0×10^{-4}	0.45	1.9
	1.0×10^{-2}	0.58	2.9
n = 6	1.0×10^{-4}	0.26	2.7
	1.0×10^{-2}	0.36	2.7
----	none	0.52	2.4

Table II The effect of alkane phosphonocarboxylic acids on the oxygen affinity of human hemoglobin.

$H_2O_3P(CH_2)_nCOOH$	M	$\log p_{50}$	Hill parameter
n = 0	1.0×10^{-4}	0.24	2.5
	1.0×10^{-2}	0.57	2.5
	1.0×10^{-1}	0.78	2.9
n = 1	1.0×10^{-4}	0.24	2.5
	1.0×10^{-2}	0.52	2.8
	1.0×10^{-1}	0.81	2.8

n = 2	1.0×10^{-4}	0.30	2.8
	1.0×10^{-2}	0.52	2.8
	1.0×10^{-1}	0.87	2.8
----	none	0.65	3.0

Table III The effect of triphosphonic acids on the oxygen affinity of human hemoglobin.

Compound	M	$\log p_{50}$	Hill parameter
$N(CH_2PO_3H_2)_3$	1.0×10^{-4}	0.75	2.8
	1.0×10^{-2}	1.0	2.8
$OP(CH_2PO_3H_2)_3$	1.0×10^{-4}	0.24	3.0
(TPPO)	1.0×10^{-3}	0.61	3.0
--	none	0.52	2.4

Table IV Comparison of the effect of organophosphates with those of inorganic diphosphate and triphosphate on the oxygen affinity of human hemoglobin.

Compound	M	$\log p_{50}$	Hill parameter
5' ATP	1.0×10^{-4}	0.45	3.0
	1.0×10^{-1}	0.75	3.0
5' ADP	1.0×10^{-4}	0.39	2.5
	1.0×10^{-1}	0.67	3.0
2,3-DPG	2.5×10^{-4}	1.05	2.5
$Na_4P_2O_7$	1.0×10^{-4}	0.42	2.8
	1.0×10^{-1}	0.93	2.8
$Na_5P_3O_{10}$	1.0×10^{-4}	0.80	2.8
	1.0×10^{-1}	1.1	3.0
---	none	0.52	2.4

The results in Table I indicate that for the diphosphonic acids the order of effector ability was n = 2 > n = 3> n = 6 > n = 4. Ethane 1,2-diphosphonic acid, where n = 2, was about half as active an effector as 2,3-DPG (Table IV).In all cases the value of the Hill parameter was near 2.8 indicating no change in cooperativity. None of

the phosphonocarboxylic acids was particularly active as an effector (Table II).Inorganic triphosphate and diphosphate were more effective than ATP and ADP respectively, suggesting that for the nucleotides the essential binding to hemoglobin is solely through the phosphate moiety.

These studies have begun to pinpoint the factors necessary for compounds to be active as hemoglobin effectors. These include at least two phosphate-like groups as binding sites; and an optimum distance between these groups. If a method to deliver ethane-1,2-diphosphonic acid to red blood cells can be developed, it should turn out to be an active effector in vivo.

The compound TPPO (Table III) increased substantially the oxygen affinity of hemoglobin, even at a concentration of 10^{-5} M. This was an unexpected finding, since the other polyphosphonates tested acted as 2,3-DPG analogs, that is, they decreased the oxygen affinity of hemoglobin By contrast nitrilotris(methylene)triphosphonic acid, which differs from TPPO only in the substitution of a nitrogen atom for a PO group, produced the decrease in oxygen affinity expected for a polyphosphonic acid.It may be that because of steric factors and/or its large negative charge or, more probably, some specific binding interaction of its PO group, TPPO binds to a quite different site in hemoglobin than do 2,3-DPG and other polyphosphonates. Experiments with site-blocked hemoglobin derivatives are currently under way to test this hypothesis. If TPPO could be introduced into intact red blood cells, it might be an effective agent against the effects of sickle cell anemia, but it must be stressed that such a conclusion is highly speculative at the present time.

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