KETO-ACIDS AS REGULATORS OF REVERSIBLE OXYGENATION OF HEMOGLOBIN

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The efficiency of oxygen transport by artificial carriers based on hemoglobin (Hb) is determined by interaction between the hemoprotein and regulators of reversible oxygenation. Regulation of oxygen release in the erythrocyte is effected by means of 2,3-diphosphoglycerate [5]. However, this regulator cannot be used for an extraerythrocytic oxygen carrier, for it cannot be irreversibly added to a protein molecule and, consequently, it is rapidly eliminated from the blood stream during circulation [1].

Accordingly as intensive search is being conducted for compounds of functional analogs of 2,3-diphosphoglycerate capable of reducing the affinity of Hb for oxygen and of attaching itself irreversibly to a protein molecule sufficiently effectively. Compounds with these properties include organic phosphates [6] and, in particular, pyridoxal-5'-phosphate [4]. The attention of investigators has been attracted to regulator molecules containing phosphate groups. The regulatory action of carboxylic acids when added to Hb solutions has also been described in the literature [2, 3], in the absence of any covalent binding with protein molecules. However, interpretation of the results is difficult because the functional characteristics of the test solutions were measured at pH values below physiological.

In the investigation described below interaction of Hb with a number of carboxylic acids irreversibly added to a protein molecule was studied. Functional characteristics of acid-modified Hb and also its isoelectric properties were assessed.

EXPERIMENTAL METHOD

Keto-acids which, because of the presence of the keto-group, can be added to a protein molecule, were chosen as modifying agents. To modify Hb, monocarboxylic keto-acids — pyruvic (PA) and glyoxalic (GA) — and a dicarboxylic keto-acid — ketoglutaric (KGA) — were used.

Addition of the keto-acids to Hb was carried out by the method described for glyoxalic acid [6].

Proof of modification of Hb was obtained by ion-exchange chromatography on DEAE-Toyoperl-650. Elution was carried out with 0.05 M Tris-HCl, pH 8.6, using an NaCl gradient from 0 to 0.3 M.

To remove the excess of modifying agents, the samples were dialyzed on Hl-P-5 fibers (from Amicon, USA) against 0.85% NaCl, adjusted to pH 7.5 with Tris-buffer.

Oxygen dissociation curves were investigated under near-physiological conditions (pCO_2 40 mm Hg, pH 7.4, 37°C, 12-14% of hemoglobin, Cl^- concentration 0.15 M) on Hem-O-Scan (Aminco) and IL-217 (USA) instruments. Concentrations of met-Hb and oxy-Hb were measured on an IL-280 Co-Oximeter (USA).

EXPERIMENTAL RESULTS

The results of ion-exchange chromatography on DEAE-Toyoperl-650 showed that irreversible addition of keto-acids to Hb was reflected in a longer retention time of the resulting com-

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TABLE 1. Effect of Keto-Acids on Functional Properties of Hb (M ± m)

Test substance	Met-Hb, %	p_{50}, mm Hg	AV, vols. % O_2	Δ lg p_{50}/Δ pH	Δ lg p_{50}/Δ lg $[Cl^-]$
Hb-PA	2,8±0,3	25,8±0,9	4,4±0,4	—0,19±0,01	0,81±0,12
Hb-GA	6,7±0,5	29,6±1,5	5,1±0,5	—0,22±0,02	1,10±0,08
Hb-KGA	1,1±0,5	19,0±0,6	1,7±0,3	—0,27±0,02	1,15±0,05
Native Hb	<3%	18,0±0,7	1,8±0,1	—0,25±0,01	1,18±0,06
Normal whole blood	<1%	27,0±1,8	4,4±0,6	—0,42±0,03	—

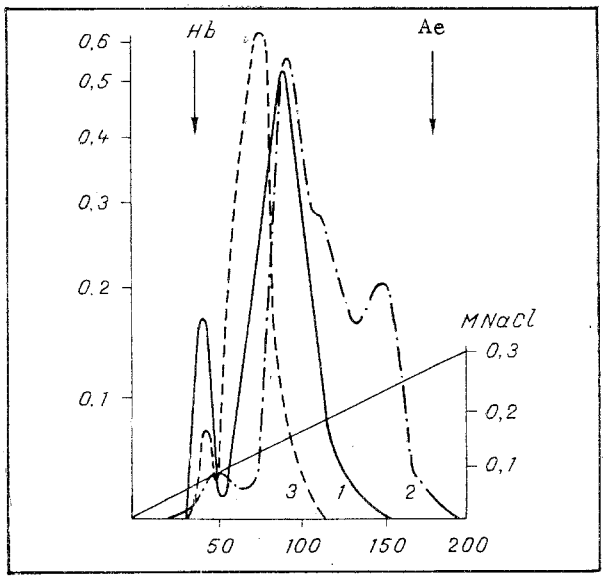

Fig. 1. Ion-exchange chromatography of Hb derivatives. Abscissa, volume of eluate (in ml); ordinate, optical density at 408 nm (in relative units). 1) Hb modified by GA, 2) Hb modified by PA, 3) Hb modified by KGA. Conditions of elution: DEAE-Toyoperl-650, K12/27, eluting systems: linear gradient, duration 2 h; elution rate 100 ml/h, volume of sample 100 µl of 15% solution. Al) Albumin.

pounds on the anion-exchange resin than of the initial Hb (Fig. 1). This fact is evidence of a change in the isoelectric properties of these Hb derivatives as a result of the modifications induced. The greatest degree of modification was observed when PA was used; moreover, the reaction product possessed considerable polydispersity for charge. The least changes in the total charge of Hb were induced by addition of KGA.

The results of investigation of the gas-transport characteristics of the modified Hb are given in Table 1.

It will be clear from Table 1 that GA is the most effective regulator of reversible oxygenation. Addition of GA leads to an increase in the value of p_{50} (partial pressure of oxygen at 50% saturation of Hb solutions) — a parameter characterizing the affinity of the hemoprotein for oxygen — by more than 10 mm Hg. This significantly increases the efficiency of oxygen release, and this is expressed quantitatively by the value of the arteriovenous difference (AV): the quantity of oxygen (in vols. %) given up by Hb during a drop of the partial pressure of oxygen from 90 mm Hg in the artery to 40 mm Hg in the vein. Hb modified by PA, with respect to its gas-transport characteristics (p_{50} and AV), is close to freshly prepared normal whole blood. The fact will be noted that addition of the dicarboxylic acid KGA caused virtually no change in the functional properties of Hb, as is clear by comparison of the values of p_{50} and AV given in Table 1 for native Hb and for Hb modified by KGA.

To study the effect of changes in the concentrations of H^+ and Cl^- ions on the gas-transport characteristics of the resulting Hb derivatives, their interaction with these ligands was investigated. Investigations of this kind can also explain whether the modifications carried out extend to those protein groups that are responsible for interaction with these allosteric effectors.

It will be clear from Table 1 that GA and KGA had virtually no effect on the magnitude of the Bohr effect ($\Delta \lg p_{50}/\Delta pH$) and that only PA caused a statistically significant decrease in this value. Reduction of the Bohr effect by about 20% is evidence that addition of PA partly involves the groups responsible for the Bohr effect (α-terminal amino groups, β = 146 histidine and β = 122 histine). PA also had the strongest effect on interaction with Cl^- ions and KGA the weakest. On the whole, by analyzing the functional properties of these Hb derivatives it can be concluded that modification by KGA leads to no significant changes in the functional characteristics of native Hb, whereas addition of PA and GA causes changes in these characteristics, which bring them close to values obtained for normal whole blood.

These experiments thus show that irreversible addition of keto-acids to Hb affects the total charge on the protein, and this is reflected in its isoelectric properties, and also changes the character of interaction of Hb with allosteric effectors (O_2, Cl^-, H^+). This fact may be used to obtain modified Hb derivatives with affinity for oxygen similar to that of normal whole blood.

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PROSTAGLANDIN SYNTHETASE ACTIVITY IN LAYERS OF THE KIDNEY OF YOUNG RATS RECEIVING POLYENE ANTIBIOTICS

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Among the extensive group of drugs which cause severe injury to the structure and functions of the kidneys, the polyene antibiotics (PA) deserve special attention [1, 9, 11]. The mechanisms of the nephrotoxicity of PA remain virtually unexplained, although data recently obtained in the writers' laboratory suggest that the damaging effects of PA may be based on a disturbance of prostaglandin (PG) biosynthesis in different parts of the kidneys [6].

To test this hypothesis we studied the effect of PA on PG-synthetase (PGS) activity in the cortex, medulla, and papillary layer of the kidneys in young rats.

EXPERIMENTAL METHOD

Male rats weighing 180-200 g were used. The animals were divided into four groups. Rats of the control group received an intravenous injection of physiological saline in 5%

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