THE EFFECTS OF ORGANIC PHOSPHATES ON THE OXYGEN EQUILIBRIA OF TWO DISTINCT HEMOGLOBINS OF THE EEL, Anguilla japonica

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SUMMARY

The effects of organic phosphates, such as adenosine triphosphate, 2,3-diphosphoglycerate and inositol hexaphosphate (phytic acid), on the oxygen equilibria of two major hemoglobins isolated from eel erythrocytes and freed of phosphate were investigated. One of these hemoglobins (E_1) was found to be greatly affected on its oxygen affinity and pH dependency by added organic phosphates, while the other (E_2) was almost insensitive to these phosphates. Results were described under considerations of the possible role of organic phosphates in regulating the function of eel hemoglobins in situ.

It has been reported that the Japanese eel, Anguilla japonica, possesses at least two major hemoglobin components which are very distinct from one other in their structural and functional properties (1, 2, 3). Yoshioka et al. (2) have demonstrated that one of these hemoglobins (E_1) has a relatively high oxygen affinity, an intense heme-heme interaction (n=2.4) and no Bohr effect in the binding reaction with oxygen, while the other (E_2) has a low oxygen affinity, a weak heme-heme interaction (n=1.2) and a large Bohr effect. It has also been evidenced that E_1 migrates cathodally upon electrophoresis, whereas E_2 migrates anodally (1). Such striking differences forced us to investigate how the function of these hemoglobins could be modulated by organic phosphates, which have been known to be a regulator of the hemoglobin function in situ in a variety of animals.

Recently, some effects of ATP on the oxygen affinity of hemoglo-

Abbreviations; ATP; adenosine triphosphate, DPG; 2,3-diphosphoglycerate, IHP; inositol hexaphosphate.

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bins of the European (4) and the American eels (5) have been reported. In the present study, we have evidenced with the Japanese eel hemoglobins that the function of E_1 but not E_2 is markedly affected by organic phosphates.

MATERIALS AND METHODS

Animals were purchased from Nozawaya Fish Company, Tokyo, Japan. Two major hemoglobins were isolated from hemolysates by a column chromatography on DEAE cellulose as described previously by Hamada et al.(1). The hemoglobin components eluted at a low concentration of phosphate was designated as E_1 and that eluted at a high concentration of phosphate as E_2, as done by the previous authors (1,2). Each hemoglobin was then passed successively through two columns of Sephadex G-25 (2.5 X 50 cm) to remove phosphate (to strip), one of which had been equilibrated with 0.1 M NaCl and the other with 0.05 M tris-HCl buffer of appropriate pH. The stripped hemoglobins thus obtained were completely free of phosphates.

Oxygen equilibria were determined photometrically as previously described (2), at 20°C in 0.05 M tris-HCl buffer of pH 7.0 to 9.0. 0.05 M bis-tris buffer, below pH 7.5, was also used. No significant difference in the results obtained with different buffers was observed. All spectral measurements were made with a Cary Model 14 recording spectrophotometer. Oxygen affinity was expressed in terms of P_{50} which is assigned to the partial pressure of oxygen at half saturation. The Hill coefficient, n, was calculated by plotting log $y/(1-y)$ versus log pO_2, in which y is the fractional saturation of hemoglobin with oxygen and pO_2 is the partial pressure of oxygen.

Concentrations of ATP and DPG in erythrocytes were determined enzymatically (6), and IHP was determined according to the method of Ohshima et al. (7), except that phosphorous concentration was estimated by the method of Ames and Dubin (8).

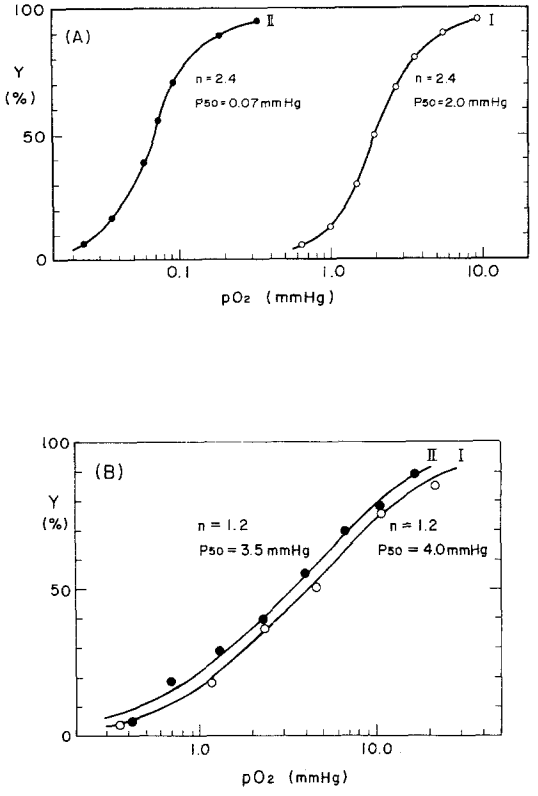

Fig. 1. Oxygen equilibrium curves of non-stripped and stripped E_1 (A) and E_2 (B) hemoglobins determined in 0.05 M tris-HCl buffer, pH 7.5, at 20° C. Hemoglobin concentration; 1 X 10^{-5}M. Open circles; non-stripped, and closed circles; stripped hemoglobins.

RESULTS AND DISCUSSION

Fig. 1, (A) and (B), shows the oxygen equilibria of E_1 and E_2 hemoglobins. As seen, P_{50} of E_1 markedly decreased when it was freed of phosphate. The values of P_{50} were estimated to be 2.0 and 0.07 mmHg for non-stripped and stripped E_1, respectively, under conditions described in the figure. On the other hand, decrease in P_{50} of stripped E_2 was a little as compared with that of stripped E_1. P_{50} was estimated to be 4.0 and 3.5 mmHg for non-stripped and stripped E_2, respectively. It should be noticed that the stripping procedure gave no effect on the n value of both E_1 (n=2.4) and E_2 (n=1.2) hemoglobins.

The effect of organic phosphates on P_{50} of stripped E_1 and E_2

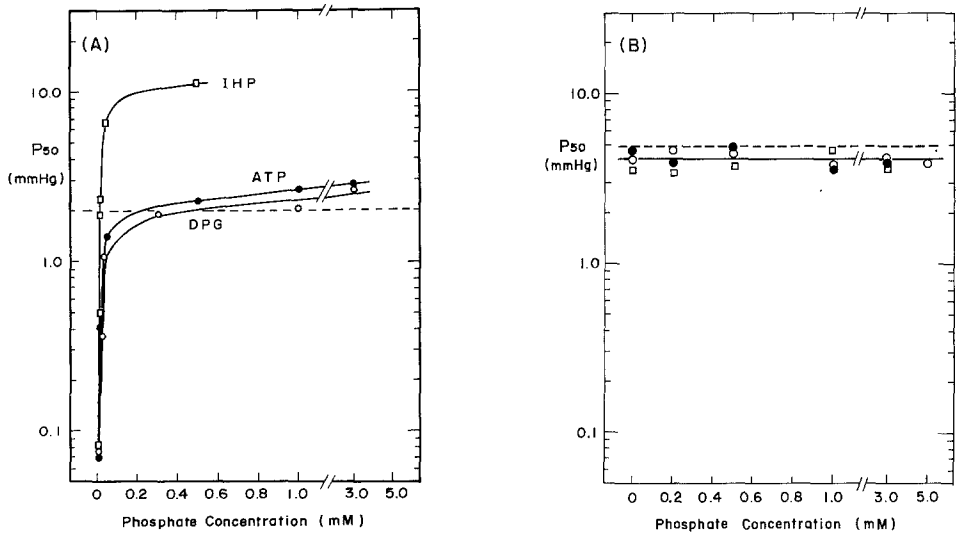

Fig. 2. Effects of organic phosphates on P_{50} of stripped E_1 (A) and stripped E_2 (B) hemoglobins measured in 0.05 M tris-HCl buffer, pH 7.5, at 20° C. Hemoglobin concentration; 1 X 10^{-5}M. Broken line indicates the level of P_{50} of the non-stripped hemoglobin, E_1 or E_2, at pH 7.5.

were then tested. As seen in Fig. 2 (A), the addition of anyone of these phosphates resulted in an increase in P_{50} of stripped E_1. This fact may imply that each of these organic phosphates binds preferentially to the deoxygenated E_1 hemoglobin, as has been proved in mammalian hemoglobin (9). The results also show that the maximal level of P_{50} was attained by the addition of 0.5 mM of ATP or DPG to the stripped E_1 and was slightly higher than that of non-stripped E_1. On the other hand, the effect of IHP on the oxygen affinity of E_1 was somewhat different from the effect of other phosphates in that IHP caused a marked increase of P_{50} in its extremely low concentration and the maximal level observed was far beyond the level attained by the addition of ATP or DPG. The reason for the extreme increase in P_{50} in the presence of IHP remains obscure.

Contrary to these characters of E_1, it is noteworthy that P_{50} of stripped E_2 was not affected by the addition of anyone of organic phosphates tested, as shown in Fig. 2 (B).

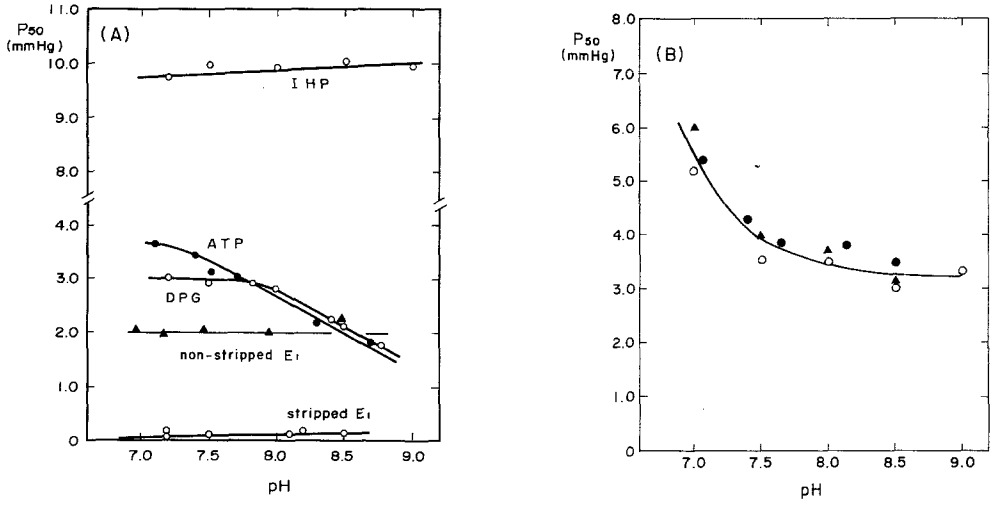

Fig. 3. Dependence of P_{50} of E_1 (A) and E_2 (B) on pH in the absence and presence of organic phosphates measured at 20° C. ATP; 1 mM, DPG; 1 mM, and IHP; 0.1 mM. Hemoglobin concentration; 1 X 10^{-5}M. Symbols used in (B) are ; non-stripped, o ; stripped, and o ; stripped E_2 in the presence of 1 mM ATP, respectively.

As mentioned above, one of the striking functional differences between E_1 and E_2 hemoglobins is that E_1 has no Bohr effect, whereas E_2 has a large one. It appears from Fig. 3 (A) that E_1 showed little Bohr effect even when it was stripped.* It is noted, however, that an apparent Bohr effect emerged, when 1 mM of ATP or DPG was added to stripped E_1 hemoglobin. No such effect was observed by IHP, although it was most effective in increasing P_{50}. The occurence of a latent Bohr effect which becomes evident by the addition of organic phosphates has been reported in tadpole hemoglobin (10). It would be interesting that ATP and IHP but not DPG are effective in the case of tadpole hemoglobin, whereas ATP and DPG but not IHP are effective in the case of E_1 hemoglobin.

In contrast to these behaviors of E_1, E_2 which shows a marked Bohr effect in the non-stripped state was not received any change in

* A reversed Bohr effect, such as reported by Gillen et al. (5), was actually observed in stripped E_1 but very slightly.

Table I. Concentrations of the organic phosphates in erythrocytes

Species		ATP	DPG	IHP
Eel		5.0	0.1	less than 0.1
Rio Grande cichlid *		2.8	0.2	——
Bullfrog**	Adult	3.0	1.5	0.3
	Tadpole	6.0	3.1	0.4

Concentrations in μmoles/ml packed cell
* Determined by Gillen et al. (11)
** Calculated from the data of Araki et al.(12)

the pH dependency of P_{50} by the stripping and also by the addition of organic phosphate (Fig. 3 (B)).

In order to evaluate which organic phosphate is physiologically significant to regulate the hemoglobin function, the content of organic phosphates in the eel erythrocytes was determined. The result is shown in Table I. The contents of phosphates in erythrocytes of the Rio Grande cichlid fish (11) and of bullfrog and tadpole (12) are also presented for a comparison. From the result, it could be deduced that in eel erythrocytes ATP is an only organic phosphate which may have a physiological significance in regulating the hemoglobin function.

Wood et al. (4) have recently observed that hypoxia caused a marked decrease of ATP in erythrocytes of the eel, Anguilla anguilla, and this in turn resulted in an increase of the blood oxygen affinity. Our present data indicate that E_1 but not E_2 hemoglobin in eel erythrocytes may be subjected to the effect of ATP. It is tempting to speculate that, when ATP in erythrocytes is sufficient, E_1 behaves like E_2 hemoglobin serving to transport oxygen, whereas in hypoxia, as the level of ATP decreases, E_1 becomes to have a myoglobin-like function by which it serves to store oxygen. On the other hand, E_2 hemoglobin

which is not affected by ATP may retain its function to transport oxygen during the change of the level of ATP. Thus, the presence of two distinct hemoglobins in the erythrocytes might be advantageous to the eel for surviving in an environment of low oxygen pressure, to which the animal may encounter during its life history.

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