ADAPTATION TO HIGH ALTITUDE: EFFECTS OF SMALL CHANGES IN THE REGULATORY BEHAVIOR OF THE ANDEAN CHICKEN HEMOGLOBIN.

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RESUMEN: Adaptación a la Altura: Efectos de Pequeños Cambios en la Conducta Reguladora de Hemoglobina de Gallinas Andinas

Se ha descubierto en los Andes Peruanos un grupo de gallinas de altura (*Gallus gallus*) con alta afinidad de la hemoglobina por el oxígeno. Hemos estudiado el posible mecanismo molecular subyacente de esta adptación a la altura. Se ha postulado pequeños cambios en la concentración intracelular de inositol pentafosfato (IPP), el principal efector alostérico de la hemoglobina en los eritrocitos de aves. Hemos estudiado la sangre de gallinas andinas y de nivel del mar. Las afinidades de suspensiones frescas de eritrocitos están significativamente incrementadas en gallinas andinas comparadas con las de nivel del mar. Los valores de los coeficientes de Hill a saturación 50% (n50) son mayores para suspensiones de gallinas que para las de mamíferos. Esto podría sugerir la existencia de un proceso de agregación molecular dentro de las células deoxigenadas, altamente concentradas de ambos tipos de aves. Para las soluciones "desnudas" de hemolizados de ambos tipos de gallinas, las afinidades son idénticas enel buffer libre de fosfato, indicando que las afinidades intrínsecas de las Hbs de gallinas andinas y de nivel del mar son las mismas. Con la adición de inositol hexafosfato (IHP), un fuerte efector alostérico de hemoglobinas de aves, observamos un incremento pequeño pero significativo (aproximadamente 20%) en la afinidad de los hemolisados de altura en relación a los de nivel del mar. Nuestros resultados también sugieren que la diferencia en la afinidad entre sangres de altura y de nivel del mar puede deberse a un proceso adaptativo posiblemente relacionado con una ligera disminución en la concentración y/o actividad del principal efector celular IPP, más bien que a una anormalidad estructural de la hemoglobina.

Palabras claves: Adaptación a la altura, Hipoxia, Hemoglobina, Unión al oxígeno, Eritrocitos, Sangre de Gallinas.

RÉSUMÉ: Adaptation à l'altitude : effet de légères variations du comportement régulateur de l'hémoglobine des poules andines.

Un groupe de poules natives des zones d'altitude (*Gallus gallus*) dont l'hémoglobine présente une très grande affinité pour l'oxygène a été découverte dans les Andes du Pérou. On a étudié le mécanisme moléculaire qui pourrait être à l'origine de leur adaptation à l'altitude et on a émis l'hypothèse du rôle de légères variations de la concentration intracellulaire d'inositol pentaphosphate (IPP), principal effecteur allostérique de l'hémoglobine des érythrocytes des oiseaux. On a étudié le sang des poules andines et celui des poules du niveau de la mer. L'affinité pour l'oxygène de l'hémoglobine des suspensions d'érythrocytes (RBC) augmente de façon significative chez les poules andines, en comparaison avec celles du niveau de la mer. Pour les RBC des poules, les valeurs des coefficients de Hill à 50 % de saturation (n_{50}) sont supérieures à celles des RBC des mammifères. Cela peut suggérer l'existence d'un processus d'agrégation moléculaire à l'intérieur des cellules désoxygénées et fortement concentrées des deux types de poules. Pour les solutions d'hémolysat purifié et dépouillé de ces deux types de poules, les affinités sont identiques en *buffer* libre de phosphate, indiquant que les affinités intrinsèques des Hb sont les mêmes dans les Andes et au niveau de la mer. Après adjonction d'IPP, puissant effecteur allostérique des hémoglobines des mammifères, on observe une légère mais significative augmentation (environ 20 %) de l'affinité des hémolysats d'altitude comparés à ceux du niveau de la mer. Les résultats obtenus suggèrent également que la différence d'affinité entre le sang d'altitude et celui du niveau de la mer pourrait être dû à un processus adaptatif qui pourrait lui-même être lié à une légère augmentation de la concentration et/ou de l'activité du principal effecteur IPP, plutôt qu'à une anormalité structurelle de l'hémoglobine.

Mots-clés : Adaptation à l'altitude, Hypoxie, Hémoglobine, Phosphates organiques, Affinité de l'oxygène, Erythrocytes, Poules.

SUMMARY: A strain of high altitude chickens (*Gallus gallus*) displaying a high oxygen affinity hemoglobin has been discovered in the Peruvian Andes. We addressed the question of the molecular mechanism possibly at the origin of the high altitude adaptation of these chickens. The role of small changes of the intracellular concentration of inositol pentaphosphate (IPP), the main allosteric effector of hemoglobin in avian erythrocytes, has been postulated. We have studied the blood from andean and sea-level born chickens. The oxygen affinities of fresh red blood cells (RBC) suspensions are significantly increased in andean compared to sea-level chicken blood. The values of the Hill coefficients at half-saturation (n50) are higher for chicken RBC than those observed for mammalian RBC. This may suggest the existence of a molecular aggregation process inside the deoxygenated, highly concentrated cells of both avian types. For the stripped purified hemolysate solutions of the two types of chickens, the oxygen affinities are identical in phosphate-free buffer, indicating that the intrinsic oxygen affinities of andean and sea-level Hbs are the same. Upon addition of inositol hexaphosphate (IHP), a strong allosteric effector of avian hemoglobins, we observed a small but significant increase (about 20%) of the oxygen affinity of the andean relatively to sea-level hemolysates. Our results also suggest that the difference in affinity between the sea-level and andean blood may be due to an adaptative process possibly related to a slight decrease in the concentration and/or of the activity of the main cellular effector IPP, rather than to a structural abnormality of the hemoglobin.

KEY WORDS: High Altitude Adaptation, Hypoxia, Hemoglobin, Organophosphates, Oxygen Binding, Red Blood Cells, Chicken Blood.

INTRODUCTION

A strain of chickens (*Gallus gallus*) adapted to high altitude hypoxia and displaying a high oxygen affinity hemoglobin (Hb) has been discovered in the Peruvian Andes (1,2).

These birds have proved to be extremely resistant to common avian diseases and to life at high altitudes. In contrast, sea-level chickens, transported to high altitudes, are particularly sensitive to the low oxygen pressure in the high altitude atmosphere and suffer of chronic mountain sickness and low fertility.

The needs for oxygen at high altitude may be fullfilled by several adaptative mechanisms such as hyperventilation, insuring a low viscosity blood flow, varying density of the capillaries at the periphery etc... The oxygenation characteristics of hemoglobin also play an important role; they depend on the intrinsic oxygen affinity of the molecule and of its interactions with heterotropic cofactors such as pH, chloride anions and organic phosphate compounds such as 2,3, DPG. This compound is present in most mammalian adult species while inositol pentaphosphate (IPP) is the corresponding effector in avian species. Upon binding to hemoglobin these cofactors lower the affinity for oxygen thence contribute to increase the oxygen release to the tissues. In the present paper we addressed the question of which molecular mechanism could be responsible for the adaptation to high altitude. The possible role of small changes in the activity of inositol pentaphosphate (IPP) on the oxygenhemoglobin equilibrium has been postulated (2).In this work the allosteric effector used was inositol-hexaphosphate (IHP) whose hemoglobin binding properties are similar to those of IPP.(3).

Oxygen binding properties of Chicken fresh RBC suspensions

	P_{50} (torr)	n_{50}
Andean "Puno" Chicken RBC :	38.8	3.1
"Hybrid" Chicken RBC (andean chicken living at Lima since 6 years) :	45.7	3.0
Sea level (Lima) Chicken RBC :	56.8	3.1
Human Adult RBC :	30.0	2.4

Experimental conditions :
pH 7.4, NaCl 0.14 M, bis-Tris 0.05 M, 41°C.

Table 1

MATERIALS AND METHODS

We have studied the oxygen binding properties of blood from andean (from Puno, 4 000 m) and sea-level (from Lima) born chickens. We have also studied the blood of chickens born at Puno and living in Lima for 6 years and referred to as "hybrid" RBC's .

Oxygen equilibrium curves were recorded by a continuous method using the Hemox Analyzer system (TCS, Huntington Valley, PA,USA): simultaneous recordings of the PO_2 were made with a Clark type electrode and the light transmission at 560 nm in a 1 cm optical cuvete (4). P_{50} and n_{50} values were calculated by linear regression from the Hill equation for oxygen saturation levels between 40% and 60%.

For the fresh red blood cells (RBC) suspensions, the experimental conditions were: pH 7.4, NaCl 0.14 M, bis-Tris buffer 0.05 M at 41 ºC.

For stripped hemolysate, the experimental conditions were: pH 7.2, NaCl 0.1 M, bis-Tris buffer 0.05 M at 41 ºC(1).

The organophosphate-effect on the oxygen affinity of hemoglobin was estimated after addition of 1mM of inositol hexaphosphate (IHP) as $\Delta\log P_{50}\pm IHP$.

The DNA sequence of the high altitude chicken b chain globin has been verified. A 600bp DNA fragment containing the ß69 codon was amplified by PCR and sequenced.

RESULTS

Oxygen equilibrium curves for the fresh red blood cells suspensions

The oxygen affinities of RBC suspensions were significantly increased in adean compared to sea-level chicken blood: P_{50} were 38.8 and 56.8 mmHg respectively, at 41°C, pH 7.4, NaCl 0.14M (Table 1 and Figure 1). For the "hybrid"chicken RBC, the P_{50} value was intermediary (45.7 mmHg) syggesting a time dependent decrease of the oxygen affinity.

The value of the Hill coefficient at P_{50} (n_{50}) was increased to 3.1 instead of 2.5 in mammalian RBC (Figure 2). This suggests an aggregation process inside the cells of these different types of avian RBC suspensions. This is confirmed by the lower n_{50} values found for hemoglobin solutions studied in the obsence of organophosphates (Table 2)(5).

Oxygen equilibrium curves for stripped hemolysate

For the stripped purified hemolysate solutions of the two types of chicken, the oxygen affinities were identical in phosphate-free bufer : P_{50} = 5.0 mmHg at 41°C, pH 7.2, NaCl 0.1M (Table 2). These results indicate that the intrinsic oxygen affinities of andean and sea-level Hbs are the same. Upon addition of inositol hexaphosphate to stripped hemolysates, we observed a small but significant increase (about 20%) of the oxygen affinity of the andean relatively to sea-level hemolysates: P_{50} were 52 and 62 mmHg respectively at 41°C with lmM IHP (Table II).

DNA sequence

A point mutation was previously described in the Andean chicken ß chain sequence resulting in the replacement of the amino acid residue ß 69 Serine by a Threonine. We have confirmed the existence of this substitution.(6) This structural change cannot be related to the high oxygen affinity of the andean relative to the sea-level blood as this position does not correspond to a known organophosphate binding site (7).

Oxygen binding properties of Chicken stripped hemolysate

		P_{50} (torr)	n_{50}
Andean Chicken hemolysate :			
NaCl 0.1M		5.0	2.0
+ IHP 1mM		52.0	2.6
Δ log P_{50} ±IHP 1mM :	*1.0*		
Sea level Chicken hemolysate :			
NaCl 0.1M		5.0	2.0
+ IHP 1mM		62.0	2.6
Δ log P_{50} ±IHP 1mM :	*1.1*		
***Human Adult hemoglobin* (Hb A) :**			
NaCl 0.1M		12.8	2.1
+ IHP 1mM		96.1	2.1
Δ log P_{50} ±IHP 1mM :	*0.9*		

Other experimental conditions :

pH 7.2, bis-Tris 0.05 M, [Heme] = 60 µM, 41°C.

Table 2

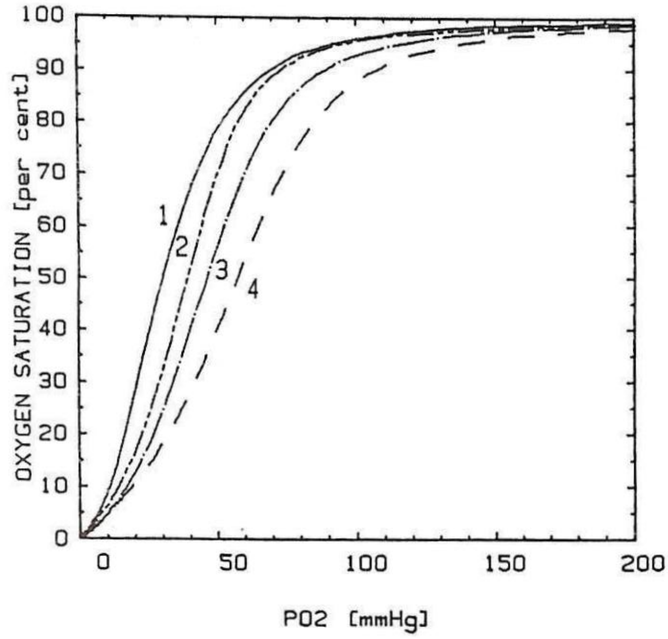

Figure 1. Oxygen Equilibrium Curves of Red Blood Cells suspensions: 1) normal human RBC's; 2) andean chicken RBC's; 3) "hybrid" (partially deadaptated) chicken RBC's; 4) sea-level chicken RBC's. Experimental conditions were: pH 7.4, NaC1 0.14M, bis-Tris buffer 0.05M at 41¦C.

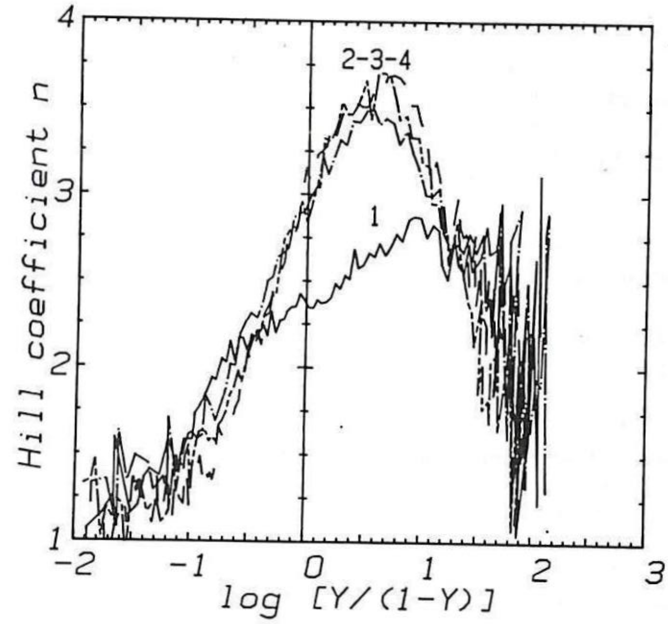

Figure 2

Figure 2. Cooperativity Curves of Red Blood Cells suspensions versus log[Y/(1-Y)] (corresponding to the first derivative of those illustrated in Figure 1): 1) normal human RBC's; 2) andean chicken RBC's; 3) "hybrid" (partially deadaptated) chicken RBC's; 4) sea-level chicken RBC's. Experimental conditions as in Figure 1.

DISCUSSION AND CONCLUSIONS

Our results therefore suggest that the difference in oxygen affinity (P_{50}) between the sea-level and high altitude andean chicken blood may be due to an adaptative mechanism: it may be related to a modification of the concentration and/or of the activity of the main cellular effector IPP rather than to a structural abnormality. Similar mechanisms have been reported in the past in other species (7,8,9). The slow reversibility of the blood oxygen affinity towards the sea-level values observed in the "hybrid" chicken group does not favor either a genetic origin. We have also performed preliminary measurements of the intra-erythrocytic phosphates content of these red cells by ^{31}P-NMR analyses (Pr. Canioni et al., Bordeaux, France)(8). Up to now, it did not revealed important changes in the intracellular components of these red cells.(9) It will be also important to consider that birds have nucleated erythrocytes which may account for more complicated metabolic changes; such as hydration, pH, ionic activity; induced by intracellular hypoxia. We cannot exclude either a change on the intracellular hemoglobin concentration. All these phenomena, alone or associated, could be responsible for the observed so-called-adaptation to hypoxia.

ACKNOWLEGMENTS

This research was supported in part by funds from INSERM, the Faculté de Médecine de Paris-Sud and the Direction des Recherches, Etudes et Techniques (contract No. 92/177).

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