The Interaction of Aldolase with the Cytoplasmic Domain of Human Erythrocyte Band 3 Inhibited by Lanthanum Ions

Yujuan Zheng
Key Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China

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The effect of lanthanum ions on the activity of the cytoplasmic domain of human erythrocyte band 3 (CDB3), which was measured according to the inhibition to aldolase, was studied. In the presence of low concentration of lanthanum ions, the function of CDB3 to inhibit aldolase activity decreased significantly. It indicated that lanthanum ions in the erythrocyte would change the conformation of CDB3 and influence the control on aldolase activity.

Band 3 is the predominant integral membrane protein present in human erythrocyte, consisting approximately 25% of the total membrane proteins.[1–3] It has two independent domains with distinct structures and functions. The highly conserved membrane domain of approximately 55 kDa is responsible for anion-exchange activity.[4] The 45 kDa cytoplasmic domain of band 3 (CDB3) consisting of 403 amino acids functions primarily as an anchoring site for many membrane-associated proteins including ankyrin,[5] band 4.1,[6,7] band 4.2,[8] and it also has binding sites for enzymes, which participated in erythrocyte glycolysis, such as aldolase,[9] glyceraldehyde-3-phosphate dehydrogenase[10] and phosphofructokinase.[11] Most of these glycolytic enzymes were partially or completely inhibited when they associated with the 58 amino acids fragment of the N terminal of CDB3. It is one of the mechanisms for erythrocyte to regulate its glycolysis rate.

In China, lanthanides have been widely used as a kind of addictives of fertilizer because of the function in promoting the growth of plant and animal. As a result lanthanides were detected in both plant and animal. Through the food chain lanthanides were ingested by human and accumulated major in lung, liver and kidney etc. The accumulation leads to damage such as reducing the membrane fluidness, increasing the fat content in liver cells, influence enzymes activity, cell lysis, and so on. It had been reported lanthanides had already exist in healthy human blood plasma.[12] Our previous work also showed that lanthanides could enter human erythrocyte through membrane[13] and change the cell morphometrics. However it is unclear whether lanthanides influence the interactions among the proteins existing in erythrocyte. In this paper we intend to study the effects of lanthanum ions on the interaction between CDB3 and aldolase, one of the key enzymes involved in glycolysis catalyzing a reversible reaction of D-fructose-1,6-diphosphate (FDP) to glyceraldehydes-3-phosphate (GAP) and dihydroxyacetone phosphate (DAP).

It should be stressed here the highly elongated cytoplasmic region of band 3 display nearly identical structural properties before and after cleavage from the membrane.[14] In this paper human CDB3 gene was amplified by standard PCR method from plasmid pHB3 and constructed into recombinant plasmid pCDB with an expression vector pET28b. CDB3 protein corresponding to the N-terminal 403 amino acids of band 3 was expressed as soluble protein in *Escherichia coli* BL21(DE3) at a high level after the transformant was induced by IPTG. CDB3 was purified by using DEAE-Sepharose A 50 and Sephacryl S-200 column. As shown in Figure 1, only one single band was detected by SDS-PAGE corresponding to the 45 kDa purified CDB3.

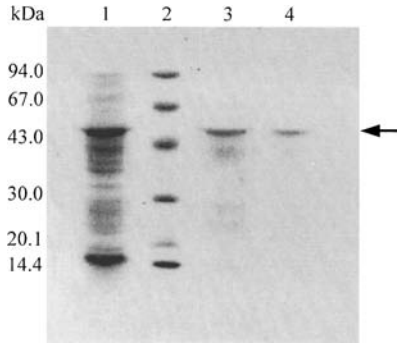

Figure 1. SDS-PAGE analysis of the purification of recombinant CDB3 from *E. coli*. The arrow indicates the cytoplasmic domain of human band 3 protein. Lane 1, total protein from the *E. coli* (harboring the recombinant plasmid pCDB) cell lysate; Lane 2, standard molecular weight markers; Lane 3, partially purified CDB3 by DEAE-Sepharose A50 Column; Lane 4, purified CDB3 by Sephacryl S-200 column.

The CDB3 activity was assayed according to the method of inhibition to aldolase activity.[15] The reaction mixture contained 30 μl of 70 mM hydrazinium sulfate, 100 μl of 0.3 M Tris-HCl buffer (pH 7.5), 100 μl of 1 mM zinc sulfate, 100 μl of 40 mM D-fructose-1,6-bisphosphate and distilled water. After incubation at 30 °C for 5 minutes, 5 μg aldolase and 5 μg purified CDB3 was added into 1 ml of the reaction mixture. The time course of absorbance at 240 nm was monitored for 2 minutes and the slope of the reaction curve represented the aldolase activity. In the control experiment, 5 μg BSA was used as substitute for CDB3. Table 1 shows that about 70% aldolase activity was inhibited by pure recombinant CDB3, which was almost the same result as that

Table 1. Aldolase activity inhibited by purified CDB3

Proteins in the reaction system	K[a]	Inhibition/%
Aldolase	0.3596	No inhibition
Aldolase + BSA	0.3578	No inhibition
Aldolase + soluble E. coli. proteins[b]	0.3581	No inhibition
Aldolase + CDB3	0.1053	70.7

[a]K is the slope of the time course curve monitored at 240 nm and represents the aldolase activity. [b]Soluble proteins from E. coli. BL21(DE3) without harboring the recombinant plasmid pCDB.

of the CDB3 in human erythrocyte,[9] while no inhibition was detected in the control. It suggested that the recombinant CDB3 was expressed in the correct conformation form and could have the identical function like that in erythrocyte.

To investigate the effect of lanthanum ions on the activity of aldolase and the interaction of aldolase with recombinant CDB3, some experiments were done with the conditions that were the same as described above except that the metal ions exist in the reaction. Figure 2 shows the effect of lanthanum ion on the aldolase activity. $LaCl_3$ was added into the reaction mixture (without Zn^{2+} or Ca^{2+}) at the final concentration from 1 μM to 100 μM. At the low final concentration of La^{3+} from 1 μM to 4 μM, the aldolase activity continually increased and exhibited higher activity than the case of absence of La^{3+}. When the La^{3+} concentration ranged from 4 μM to 10 μM aldolase activity was steadily held at the high level. In the higher concentration of La^{3+} from 10 μM to 100 μM, the activity of aldolase was inhibited dramatically. In contrast, no inhibition was detected when Zn^{2+} or Ca^{2+} were present. It indicates that effect of lanthanum ion on aldolase obey the rule of Hormesis effect.[16]

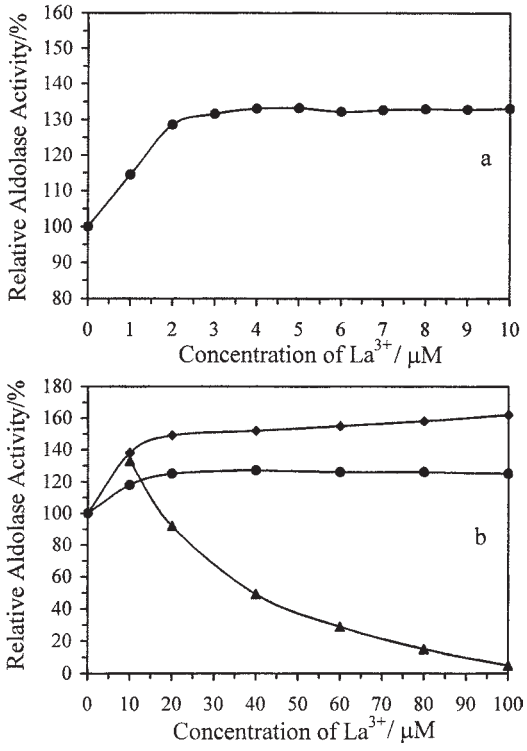

Figure 2. The effect of different metal ions on aldolase activity. a. the aldolase activity measured in low concentration of $LaCl_3$. b. the effect of Zn^{2+} (●), Ca^{2+} (♦) and La^{3+} (▲) on the aldolase activity.

Because lower concentrations of La^{3+} shows no inhibition to aldolase activity, we can investigate the effect of lanthanum ion on the interaction of CDB3 with aldolase in the range of 1 μM to 10 μM. Figure 3 shows the effect of lanthanum ions on the function of CDB3. In the absence of lanthanum ion, the activity of aldolase would be inhibited significantly, only 30% of aldolase activity can exhibit. In the presence of lanthanum ions, there was a steep rise in aldolase activity up to 5 μM La^{3+}. Then there is little change in the range from 5 μM to 10 μM La^{3+}, the relative activity of aldolase can get to 130%, identical with the activity in the absence of CDB3. It suggests the inhibitory function of CDB3 to the aldolase is almost lost completely. Comparing the two curves in Figure 3, the CDB3 was more sensitive to lanthanum ion than aldolase. It indicated the presence of La^{3+} could change CDB3 conformation and influence its inhibition to aldolase. The result would lead the acceleration of glycolysis in erythrocyte. The major carbohydrate source of erythrocyte is glucose obtained from blood plasma. As a result of accelerated glycolysis, the glucose concentration in blood may be decreased or the converting of hepatin into glucose in liver may be increased to keep the normal blood glucose concentration. In both cases the normal human physiological functions may be influenced.

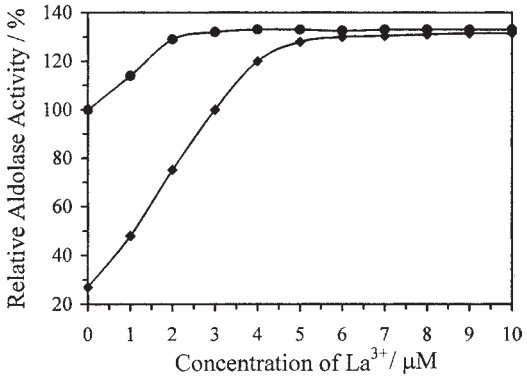

Figure 3. The interaction of aldolase with the cytoplasmic domain of human band 3 inhibited by lanthanum ions. (●) aldolase activity influenced by La^{3+}. (♦) the interaction of aldolase with the cytoplasmic domain of human band 3 influenced by La^{3+}.

The cytoplasmic domain of band 3 is such an important protein for its regulation on the glycolysis rate in erythrocyte. The presence of lanthanides would influence the inhibition of CDB3 to aldolase and then change the exhibition of aldolase activity. The result would enhance glycolysis in erythrocyte and then lead the disorder of carbohydrate metabolism.

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