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Purification and Characterization of Carbonic Anhydrase from Human Erythrocyte Plasma Membrane

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Abstract: Carbonic anhydrase is the basic enzyme in inhalation function. Untill now no research had been done to determine whether CA is in the human erythrocyte plasma membrane or not. Carbonic anhydrase (CA) was purified from human erythrocyte plasma membrane and described in this study. For this purpose, the blood samples taken from young human test subjects were hemolyzed, then the membrane fraction was separated, and this fraction was repeatedly washed. The enzyme (CA) was removed from the membrane with buffered TritonX-100 (1%), which was purified with a factor of 119.19 by affinity chromatography. The CA obtained from the erythrocyte membrane has esterase activity as well as hydratase activity. The V_{max} and K_M of the enzyme for the substrate (p-nitrophenyl acetate) are $1.517*10^{-1}$ µM/L*min. and 1.78 mM, respectively. The purification degree of the enzyme was controlled by SDS-PAGE (3-10%), which showed one distinct band. It was determined that the enzyme was active within the pH range of 4-10, and that the optimal pH was 7.5. The temperature at which it showed activity was 5-70ºC, and optimal temperature was 35ºC. The molecular weight of CA was found to be ~ 36,600 by gel filtration. On the other hand, sulphanilamide, KSCN and NaN_3 inhibited the enzyme. Finally, CA was shown to be present in human erythrocyte plasma membrane and this enzyme is optimized.

Key Words: Carbonic anhydrase, Plasma membrane, sulfanilamide, KSCN and NaN_3

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