pH EFFECTS IN Na,K-ATPase: CATALYTIC ACTIVITY, FLUORESCENCE AND CIRCULAR DICHROISM ANALYSIS

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The Na,K-ATPase consists of protomers containing one α (~100 kDa) catalytic subunit and one β (~50 kDa) subunit of regulatory function. The αβ protomers may be organized as dimers (solubilized form) or higher oligomers as in the plasmatic membrane. We have studied the effect of the change of pH on the secondary and tertiary structure of the enzyme, by circular dichroism and fluorescence spectroscopy. The $(\alpha\beta)_2$ form of Na,K-ATPase rabbit kidney outer medulla was purified as described by Santos et al. (2002) *Braz. J. Med. Biol. Res*., 35:277. The ATPase activity of the enzyme was assayed discontinuously at 37ºC, and correlated with circular dichroism (Jasco 810 spectrophotopolarimeter) and fluorescence (Spectronic SLM 8100) over a pH range of 4-10. The ellipticity at 222 nm showed a minimum value at a pH value of approximately 7.5, which correlated well with the optimum pH of the catalytic activity observed for the hydrolysis of ATP. These results were also correlated with the profile of the intrinsic tryptophan fluorescence intensity changes, which showed a maximum value over the pH range of 6.0-8.5. These results indicate that the loss of only 10 to 35% of secondary and tertiary structures of the Na,K-ATPase at extreme pH values are responsible for the total enzyme inactivation. CAPES, CNPq and FAPESP

Key words: solubilized Na,K-ATPase, CD, fluorescence.