The effect of aspirin on the protein binding of ascorbic acid

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Tukamoto, Ozeki, Hattori & Ishida (1974) demonstrated the binding of ascorbic acid (AA) to bovine serum albumin (BSA) at 5°C and 20°C by the method of dynamic dialysis developed by Meyer & Guttmann (1968). Using this procedure, AA-BSA binding is shown to occur at 37°C. The presence of acetylsalicylic acid (ASA) is found to reduce this binding. The method of dynamic dialysis involves measurement of the rate of diffusion of AA from a dialysis sac in the presence and absence of BSA. The concentration of free and bound AA in the sac was calculated by measurement of AA diffusion rate. 100 ml aliquots of the Sorenson buffer, at pH 7.38, surrounding the sac were sampled and replenished with fresh buffer at 30 min intervals. AA was analysed by taking 0.5 ml of the sample and adding 4 ml of 0.2 M hydrochloric acid and reading the absorption on an ultra-violet spectrophotometer at 243mμ and compared with a standard curve. Initial sac concentrations of AA, BSA, and ASA were 2×10^{-2} M, 2×10^{-4} M, and 1×10^{-2} M, respectively. All experiments were carried out under nitrogen to prevent the oxidative degradation of the AA. The molecular weight of BSA was taken as 69,000.

Typical results are normally shown in the form of a semi-log plot of total drug concentration against time. In the presence of AA alone, there is a rapid rate of diffusion. When BSA is also present, the rate of diffusion of AA is decreased over time. The slower rate of diffusion indicates that binding of AA with BSA is taking place within the dialysis sac. The degree of separation of these plots is an estimate of the extent of binding of AA to BSA.

The results of these experiments show that AA-BSA binding occurs at 37°C. The introduction of ASA into the sac containing AA and BSA causes the semi-log plot of total drug-concentration against time to approach the line obtained in the absence of BSA. This shows that ASA causes a reduction in the binding of AA to BSA.

By the method of Scatchard (1949) two types of binding sites were isolated for AA binding to BSA. There are ~6 sites per mole of protein of one type. The other sites are non-specific, low-affinity sites of lesser importance.

With ASA present the number of binding sites decreases to ~8 with no change being observed in the non-specific type.

References

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Synaptosome transmitter release and ATPase activity

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It has been proposed that acetylcholine release at the periphery (Paton, Vizi & Zar, 1971) and in the central nervous system (Vizi, 1972) is triggered by inhibition of sodium, potassium-activated, magnesium-dependent adenosine triphosphatase (Na^+, K^+-ATPase). A stringent test of this hypothesis should include simultaneous measurements of acetylcholine release and ATPase activity. We have determined the effects of phenytoin, an inhibitor of nerve terminal Na^+, K^+-ATPase, and electrical stimulation on the release of acetylcholine and inorganic phosphate from synaptosomes during the same period to determine any relationship between the two.

Rat cerebral cortex synaptosomes were prepared as described previously (Gilbert & Wyllie, 1976). The synaptosomes were layered on filters and maintained at 37°C in an oxygenated medium (pH 7.4) containing (mM): NaCl (153.5); KCl (5.65); $MgSO_4 . 7H_2O$ (2.8); $CaCl_2 . 6H_2O$ (2.1); $NaHCO_3$ (1.8); glucose (8.3); sucrose (64.3); physostigmine (0.02). Phosphate and/or acetylcholine release were measured over 10 min periods with and without supramaximal electrical stimulation by platinum electrodes (100 Hz, 1 ms, 10 V). Phosphate was determined by the method of Bonting, Simon & Hawkins (1961) and acetylcholine by bioassay using the leech dorsal muscle.