Haemostasis 7: 97 (1978)

Standardization of Chromogenic Synthetic Peptide Substrates for Proteolytic Enzymes

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Key Words. Chromogenic substrates · Proteolytic enzymes

Methods using synthetic substrates for assaying enzyme activities are easy and comfortable to perform. Results obtained may, however, differ from place to place and time to time, due to differences of the substrate preparations and of their use.

(1) The enzymatic hydrolyzable substrate content in the so-called 'substrate preparations' have to be known. Only then a correct concentration of digestable substrate can be made. This is of fundamental importance when K_m and V_{max} are established for a special enzyme-substrate system. But it is also of great importance to know the exact assaying conditions with respect to the correct substrate concentration, temperature, pH, ionic strength as well as the composition of the buffer used, to obtain comparable results from different laboratories.

(2) The impurities or ballast of the substrate preparations should not have any influence on the enzymatic katalytic properties, neither activate nor inhibit.

(3) The products formed from the substrate, due to the enzymatic cleavage of the bond between the peptide and the *p*-nitroanilide group, i.e. the amine derivative P_1 (*p*-nitroanlinie) and the acid derivative P_2 (peptide acyl derivative) should not be involved in the enzymatic activity as they are formed during the enzymatic hydrolysis. To be able to obtain substrate solutions with correct concentrations, we have to know the percentage of hydrolyzable substrate in the commercial preparations, or we have to know the exact number of μmoles of convertable substrate per vial.

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