Kinetic Properties of Urease Immobilized to Merrifield-Resin

P. Grunwald and C. Brandt

Institut für Physikalische Chemie der Universität Hamburg,
D-2000 Hamburg 13, Laufgraben 24, F.R.G.

Up to now Merrifield resin has been used for solid phase peptid synthesis [1]. Lately it could be shown, that it is possible to fix proteins directly to this carrier [2]. This immobilization technique is of advantage because of its mild reaction conditions.
This paper describes the preparation of urease immobilized to Merrifield resin and its kinetic properties. The activities of immobilized urease run through a distinct maximum depending on enzyme supply. This effect is interpreted that way, that there are different orientations of the enzyme molecules on the carrier-surface depending on enzyme concentration [3]. The kinetics of preparations from three characteristic areas of enzyme supply - i.e. before the maximum at low enzyme supply, at the maximum, and behind the maximum at high enzyme supply - have been examined. The kinetic behaviour of immobilized urease cannot be described by the simple Michaelis-Menten equation which is valid for the native enzyme, because with increasing substrate concentration the activities of the three preparations mentioned above run through plateaus, that are typical for allosteric enzymes. Moreover, the samples prepared at high enzyme supply yield turnover/time - plots with two distinguishable areas of slope, for which two different K_m-values are obtained. This finding supports the thesis already advanced by other authors [4] that urease has at least two active sites with different substrate affinity.
Furthermore, the paper in question shows the practical utility of immobilization technique. The thermostability of urease is increased about 20 degrees Celsius up to 80 degrees Celsius when it is immobilized to Merrifield resin.

1. Merrifield, R.B. (1969) Adv. Enzymol. 32, 221 - 296.
2. Grundwald, P., 12th IUPAC Intern. Sympos. on the Chemistry of Natural Products, Sept. 1980.
3. Grunwald, P. and Gunßer, W., Heiker, F.R. and Roy, W. (1979) Anal. Biochem. 100, 54 - 57.
4. Lynn, K.R. and Yankwich, P.E. (1964) Biochim. Biophys. Acta 81, 533 - 547.