Enzyme-Polymer Adducts of Aldolase, Glyceraldehydephosphate Dehydrogenase, and Fructosediphosphatase

Insolubilized enzymes can be useful in a large number of applications involving enzyme-controlled processes. The enzyme-polymer adducts have properties differing significantly from those of the native enzymes, the most significant being enhanced stability[1,2]. We report here that complex subunit-containing enzymes can be stabilized by attachment to polymeric matrices, while retaining much of their catalytic properties. Aldolase, glyceraldehyde-3-phosphate dehydrogenase (GAPD) and fructose-1, 6-diphosphatase (FDPase) have been covalently attached to aminoethyl cellulose (AEC) via glutaraldehyde. In addition, some aldolase-polymer adducts were prepared from ethylene maleic anhydride copolymer (EMA) according to the procedure of LEVIN and KATCHALSKI[3], and from *p*-aminobenzyl cellulose (PAB) by the procedure of CAMPBELL et al.[4].

The glutaraldehyde technique has been used to attach single-chained trypsin to AEC[5], and we have now obtained adducts of more complex enzymes to AEC. The following details a procedure for preparing a GAPD adduct. AEC was washed successively with deionized water, phosphate buffer (pH 7.5), deionized water, methanol and then air dried. The AEC (500 mg) was suspended in 6.0 ml of sodium phosphate buffer (0.05 *M*, pH 7.5) containing dithioerythritol (10^{-3} *M*) and glutaraldehyde (1%). After stirring 5 min at room temperature, 2.0 ml of GAPD (30.0 mg/ml, Worthington) was introduced into the suspension. This suspension was stirred at room temperature for 1 h, followed by stirring overnight at 4 °C. The resulting light brown GAPD-AEC adduct was collected by filtration. It was washed with cold phosphate buffer solution (500 ml, 0.5 *M*, pH 7.5 containing 0.5 *M* NaCl), then with the phosphate buffer containing no NaCl until no GAPD activity could be detected in the washings, and stored at 4 °C. The enzyme assay systems for aldolase, GAPD, and FDPase were adapted respectively from the procedures by REJKUMAR et al.[6], ALLISON[7], and PONTREMOLI[8].

Experiments indicated that the protein content of the adducts are high. Table I shows the actual amounts bound. The insolubilized aldolase derivatives had about 10% the specific activity of unmodified aldolase, and GAPD and FDPase derivatives had about 1% that of unmodified enzymes.

The enhanced stabilities of the insolubilized enzymes suggest that these complex enzymes may derive their in vivo stability from being associated with membrane-like materials. Aldolase-EMA retained its original activity for 30 days at 4 °C, while unmodified aldolase lost about 25% of its original activity. Aldolase-AEC retained about 95% of the original activity at room temperature for a period of 3 weeks, but unmodified aldolase lost more than 70% of the original activity in $2^1/_2$ weeks. GAPD provides a dramatic demonstration of the increased stability of this enzyme resulting from its attachment to the polymer matrix. Unmodified GAPD became deactivated overnight at room temperature, but GAPD-AEC retained about 95% of its original activity.

Insolubilized enzymes exhibit some altered properties as a result of their attachment to various types of polymers[9]. For example, dependent upon the type of polymer used, the pH optimum for the enzyme can be

Table I. Protein content and specific Activity of insolubilized aldolase GAPD, and FDPase

Enzyme-polymer adduct	mg enz/mg Carrier[a]	Maximum specific activity, moles NADH/min/mg Protein)
Aldolase-EMA	4.9×10^{-2}	6.0×10^{-7}
Aldolase-PAB	1.4×10^{-2}	4.1×10^{-7}
Aldolase-AEC	4.2×10^{-2}	4.5×10^{-7}
GAPD-AEC	3.6×10^{-2}	2.0×10^{-8}
FDPase-AEC	7.0×10^{-2}	5.5×10^{-9}

[a] Protein was determined by the ninhydrin method[10,11] after the adduct was hydrolized in 6 *N* HCl under reduced pressure for 40 h.

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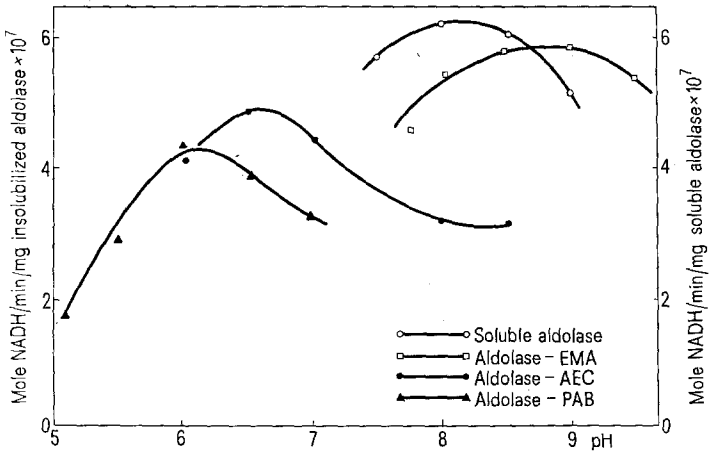

The effect of pH on the activity of aldolase and insolubilized aldolase derivatives.

shifted to either higher or lower values. As shown in the Figure, the approximate pH optimum of aldolase is 8.5; aldolase-EMA 9.0, aldolase-AEC 6.5 and aldolase-PAB 6.0. For the aldolase system, attachment to polymers bearing positive charges, such as AEC, shifts the pH optimum to the acidic side. Thus, when GAPD is attached to AEC, the pH optimum is shifted from 9.0 for GAPD to about 7.0 for GAPD-AEC. When FDPase is attached to AEC, the pH optimum is shifted from about 9.3 to 8.5. It can be concluded that polymers definitely exert vicinal effects on the pH around the catalytic site of the enzymes.

The apparent K_m for substrates can be affected as shown in Table II. When the substrates are negatively charged, polymers bearing negative charges increase the apparent K_m, as in the case of aldolase-EMA. Polymers bearing positive charges decrease the apparent K_m, as in the case of aldolase-PAB and GAPD-AEC.

It is possible to link sequential reactions by appropriate column techniques. Two columns in series containing aldolase-AEC and GAPD-AEC were packed to demonstrate the principle of sequential synthetic reactions. Into the first column, containing aldolase-AEC, was percolated a solution of fructose-1,6-diphosphate and nicotinamide adenine dinucleotide (NAD). The effluent solution contained dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (GAP) and unchanged NAD. This mixture was passed through a second column packed with GAPD-AEC while sodium arsenate was added. GAPD catalyzed oxidation of GAP to 3-phosphoglyceric acid in the presence of NAD and sodium arsenate, and the NAD was reduced to NADH. The effluent from the second column flowed into a solution of methylene blue and diaphorase which were used as specific indicators for NADH. The decolorization of methylene blue proved the presence of NADH which could only have been formed by the projected two-step sequential enzyme-catalyzed synthesis.

Table II. Apparent K_m values for different enzyme-polymer adducts

Enzyme	Substrate	K_m values (mM)
Aldolase	Fructose-1,6-diphosphate	0.04
Aldolase-PAB	Fructose-1,6-diphosphate	0.006
Aldolase-EMA	Fructose-1,6-diphosphate	3.6
GAPD	Glyceraldehyde-3-phosphate	6.4
GAPD-AEC	Glyceraldehyde-3-phosphate	0.66

Résumé. On a preparé des enzymes polymères dérivés de l'aldolase, de l'aldéhyde glycérique-3-phosphate déhydrogénase et de la fructose-1, 6-diphosphatase. On peut, semble-t-il, tirer parti de la technique de liaison transversale par l'aldéhyde glutarique pour augmenter la teneur en protéine et pour obtenir des enzymes polymères adducteurs. Les enzymes traités sont plus stables que les enzymes intacts. La direction des changements du pH optimal et du K_m apparent change selon les polymères auxquels on lie les enzymes.

R.D. Falb, J. Lynn and J. Shapira

Batelle Columbus Laboratories, Biology and Medical Sciences Section, 505 King Avenue, Columbus (Ohio 43201, USA); and Ames Research Center, NASA, Moffett Field (California 94035, USA), 5 February 1973.

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Presence of Lipid Bodies in Rice Leaves and their Discolouration During Pathogenesis

Lipids are considered as the third important class of plant constituents after proteins and carbohydrates, and are widely distributed in plant parts. In vegetative parts they are usually in the form of small droplets or globules dispersed through the cytoplasm of the cell[1]. Although the lipid content of the grains of rice (*Oryza sativa* L.) have been investigated[2,3], neither pure anatomical investigations nor pathological studies[4–17] have previously resulted in reports of the presence of these globules in rice leaf tissue.

While observing free-hand sections of rice leaves for anatomical changes during pathogenesis, we found abundant lipid globules in cells throughout the chlorophyll-containing parenchyma tissue of several varieties (Figure). These spherical globules averaged 3.3 µm (range 1.6–4.8 µm). Both healthy and diseased cells contained the lipid globules. Usually 2 or more globules were visible per cell. In healthy tissue the globules appear colourless or light green to yellowish green, presumably because of their location close to chloroplasts. In diseased tissue, the globules become reddish brown to dark brown depending on the nature and stage of disease, and distance from the site of infection. Discoloration of lipid bodies is an early primary symptom of leaf spots resulting from

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