Substrate Effect on Riboflavin-Sensitized Photoinactivation of Taka-Amylase A

It is known that the presence of substrate considerably protects Taka-amylase A from heat- and UV-inactivation[1,2]. The protein structure of Taka-amylase A composed of a single polypeptide chain is held by 4 –S–S– links[3] and hydrogen bridges between peptide chains. These links may be broken by the thermal vibration at higher temperatures to bring about the destruction of enzyme structure. Such destruction leads to heat-denaturation and -inactivation. Irradiation with the UV-light longer than about 250 nm mainly gives rise to the cleavage of –S–S– bond in cystine to –S··S– radicals and the oxidation of the other amino acid residues[4]. These events yield the photo-inactivation. In the case of UV-inactivation, the substrate inhibits not only the photochemical process of inactivation, but the thermal processes due to the microscopic local heating by the internal conversion of excitation energy into heat and due to the macroscopic temperature of the system[2].

The photo-inactivation sensitized by riboflavin is also inhibited by the presence of substrate. The effect of substrate (soluble starch) concentration on the ratio of the initial velocity of photo-inactivation at 20° in the presence of substrate (R) to that in the absence of substrate (R_0) is shown in Figure 1. This ratio decreases strongly even in the presence of small amounts of substrate. Figure 2 shows the effect of substrate on heat- and photo-inactivation at various temperatures. Heat treatment in the dark or irradiation with visible light absorbed by 445 nm-band of riboflavin was carried out for 10 and 60 min under aerobic conditions. The irradiating light was isolated from a 500 W projection lamp by means of appropriate glass filters. The activity was measured at 40° by the blue value method[5]. The photo-inactivation observed is caused by the effects of both heat- and photochemical processes. Riboflavin is unstable under illumination with visible light and, in the presence of oxygen, is decomposed irreversibly to lumiflavin or lumichrome[6–8] and ribose side chain[9]. Lumiflavin is converted to lumichrome finally. These derivatives as well as riboflavin in their triplet states may form 'triplet-triplet complexes' with the solvated oxygens in the triplet ground states. The allowed multiplicity of the complex is singlet, triplet and quintet according to the spin conservation law. The singlet complex is allowed to dissociate into singlet dye and singlet oxygen, and the triplet one into singlet dye and triplet oxygen. The latter process stands for the quenching of triplet state of dye by oxygen. The excited singlet state of oxygen created by the former process is metastable and highly reactive. The reactive oxygen may react with water to produce hydrogen peroxide which oxidizes the amino acid residues or may directly oxidize them. This is the main mechanism for the photo-inactivation of Taka-amylase A sensitized by riboflavin. Such photochemical process is temperature-dependent, while that in the UV-inactivation in the absence of riboflavin is independent of temperature. As shown in Figure 2, curve (1) and (5), the temperature-dependent photochemical process gives rise to a gradual decrease of activity with increasing the temperature in the region below about 40° where no appreciable heat-inactivation occurs as shown in curve (3) and (7). The activity decreases rapidly to zero at higher temperatures in both cases of photo- and heat-inactivation, as the result of heat-denaturation. But, in the case of photo-inactivation, the activity begins to decrease at lower temperature than in the case of heat-inactivation owing to the combined effect of heat-denaturation and photo-oxidation.

The substrate inhibits partly the thermal inactivation by the formation of substrate- and product-enzyme complexes which highly stabilizes the enzyme structure and prevents the thermal destruction of enzyme[1], and partly

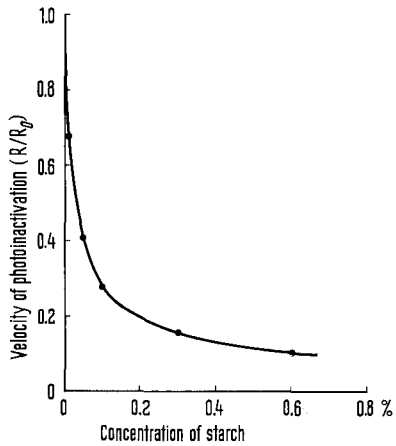

Fig. 1. Effect of substrate on velocity of photo-inactivation sensitized by riboflavin. R and R_0 are respectively initial velocities of photo-inactivation in the presence and in the absence of substrate at 20°, pH 5.6. Concentration of Taka-amylase A: 0.005%. Concentration of riboflavin: $3.3 \cdot 10^{-5} M$. Exciting light: light absorbed by 445 nm band of riboflavin.

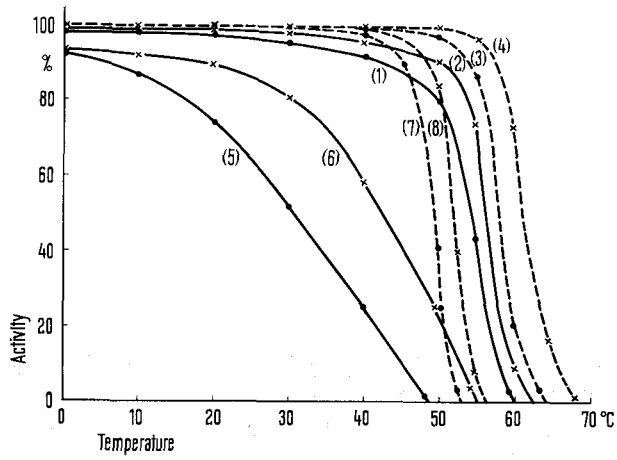

Fig. 2. Effect of substrate on heat- and photo-inactivations at various temperatures. Dashed line, heat-inactivation; solid line, photo-inactivation; •, in the absence of substrate; ×, in the presence of substrate; (1), (2), (3), (4), treated for 10 min; (5), (6), (7), (8), treated for 60 min; concentration of Taka-amylase A, 0.005%; concentration of starch, 0.6%; pH 5.6. Exciting light: light absorbed by 445 nm band of riboflavin.

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the photo-oxidation process of amino acid residues by decreasing the collision frequency between active oxygen or hydrogen peroxide and enzyme, increasing the viscosity of medium. Furthermore, these oxidizers may be destroyed by the reaction with substrate or product around the enzyme, creating a favourable condition for enzyme protection.

Zusammenfassung. Durch sichtbares Licht wird die Taka-Amylase A in Gegenwart von Riboflavin inaktiviert. Die Inaktivierung der Amylase ist durch photochemische und thermische Einwirkung verursacht. Das Substrat verhindert in gewissem Masse die Inaktivierung der Amylase. Der mögliche Mechanismus für eine solche Schutzwirkung des Substrats wird diskutiert.

G. TOMITA and S. S. KIM

Institute of Biophysics, Faculty of Agriculture, Kyushu University, Fukuoka (Japan),
December 7, 1964.

Distinction entre les variations phénotypiques sexuelles et génétiques chez le Rat, par l'analyse immunoélectrophorétique des protéines urinaires spécifiques[1]

Introduction. Nous avions antérieurement noté que les protéines urinaires possèdent une personnalité antigénique complexe, étant le siège de variations individuelles et sexuelles[2].

Nous avons poursuivi ces études de caractérisation des variations phénotypiques des protéines urinaires, dans le double but de voir s'il y a corrélation entre l'importance de ces différences phénotypiques et la pureté génétique et afin de distinguer entre elles les variations reliées au sexe et à la pureté génétique.

Matériel et méthodes. Nous avons soumis à l'ultrafiltration[3], 3 pools d'urine de 10 rats de race Sprague-Dawley adultes chacun, de même sexe et de sexe différent, afin d'obtenir des concentrations protéiques ajustées de 65 mg/ml d'urine. Nous avons fait l'analyse électrophorétique comparée, sur membrane d'acétate de cellulose[4], à l'aide de l'appareil microzone R-101 de Beckman, de ces urines concentrées solubilisées en buffer phosphate, pH 7.0. D'autre part, ces urines poolées ont été utilisées après lyophilisation, pour la production d'immunsérums, chez le lapin, selon la méthode de l'adjuvant complet de Freund; ces anticorps étant subséquemment utilisés dans

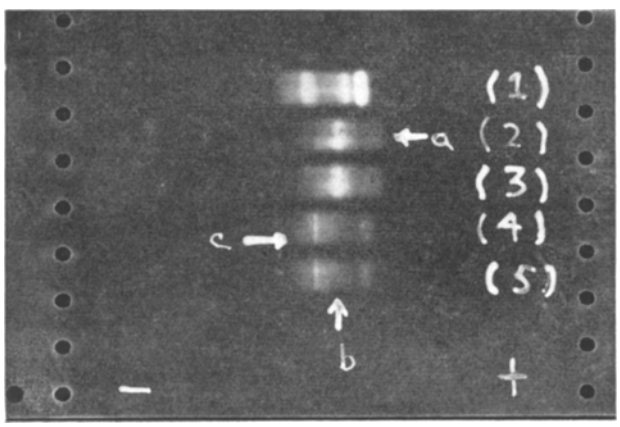

Fig. 1. Electrophorèse sur membrane d'acétate de cellulose des protéines urinaires concentrées par ultrafiltration (65 mg de protéines/ml de solution en buffer phosphate pH 7.0) de rats mâles (2 et 3) et de rats femelles (4 et 5); 1 représentant la migration de référence du sérum de rat normal. A noter les différences marquantes entre les protéines urinaires mâle et femelle (flèches). Ces différences sont surtout importantes au niveau des rho-protéines (flèche a) et de l'uroprotéine principale (flèche b).

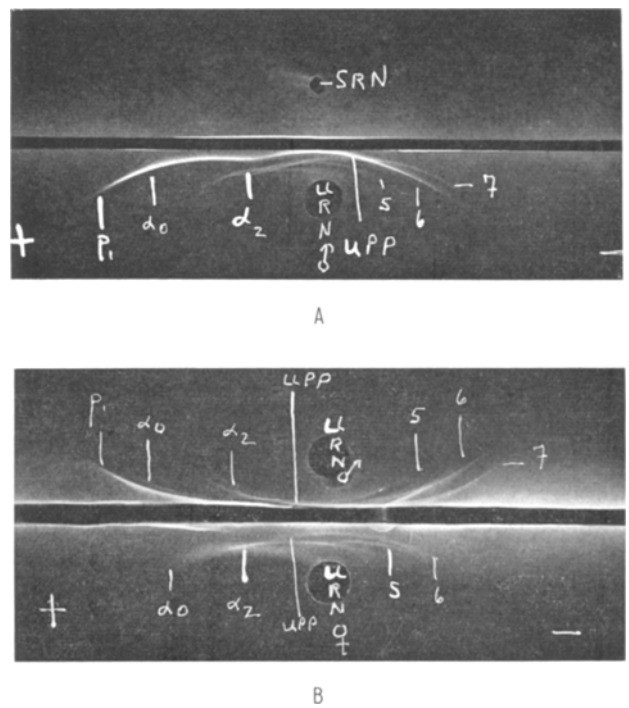

Fig. 2. Illustration des sites protéünes où se situent les variations phénotypiques reliées au sexe sur les protéïnes urinaires du rat. A: A.I.E.; montrant la richesse de l'anticorps de Lapin en protéïnes urinaires spécifiques (cet immunsérum permettant de dévoiler avec l'urine de rat mâle concentrée 7 constituants d'origine non-sérique). B: Analyse immunoélectrophorétique comparée de l'urine du rat mâle (haut) et femelle (bas) à l'aide de l'immunsérum antiurine de mâle (cuve longitudinale) démontrant l'absence d'une rho-protéïne dans l'urine femelle et la différence quantitative relative de *l'UPP.*

[1] Travail subventionné par l'octroi n° 362-10 du Conseil des Recherches médicales du Canada.
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