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Comparative Studies on Synthetic Chromogenic Substrates for Urokinase in Rabbit, Guinea pig, Dog and Human

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ABSTRACT

Two synthetic chromogenic substrates, pyro-Glu-Gly-Arg-pNA(S-2444) and H-Glu-Gly-Arg-pNA (S-2227) are available for urinary plasminogen activator, urokinase (UK). Although the structural difference between them is slight, the modes of catalysis were found to differ among different species. Guinea pig plasma was the most sensitive to the catalysis of S-2444, and human plasma, the least. On the other hand, in the case of the catalysis of S-2227, human plasma was the most sensitive, and dog plasma, the least. Regarding the response to added UK, human plasma was the most sensitive for S-2444, and guinea pig plasma, the least. In the case of the catalysis of S-2227, dog plasma was the most sensitive and the other three species showed almost identical sensitivities. Thus, a slight change in substrate structure led to different modes of action in different species.

INTRODUCTION

Use of synthetic chromogenic substrates has considerable advantages over conventional methods for measuring enzyme activity, viz. short and simple operation[1-9]. Two synthetic chromogenic substrates are produced for measuring urokinase activity : S-2444 (pyro-Glu-Gly-Arg-pNA) [10] and S-2227 (H-Glu-Gly-Arg-pNA)[11]. Although the difference in strucuture between the two is not great (pyroglutamyl for S-2444 and glutamyl for S-2227), it has remained unclear as to whether their modes of respones to urokinase are identical among different mammalian species. The present study was therefore undertaken to clarify the species specificity in UK response of the synthetic chromogenic substrates, S-2444 and S-2227.

MATERIALS AND METHODS

S-2227 and S-2444 syntheriged were kindly supplied from Dr. K.Sasaki (Zeria USA, Inc, New York). Determination of amidolytic activity was performed as follows [12] : 50 μl of 2 mM substrate, 50 μl of test material and 400 μl of buffer (0.05 M Tris-HCl, pH 8.8, I=0.05 for S-2444 ; 0.05 M Tris-HCl, pH 9.0, I=0.05 for S-2227) were incubated at 37 ℃ in a doublebeam photometer (Hitachi 202) and the increase in optical density at 405 nm was recorded. The plasma used was obtained from rabbits, guinea pigs, dogs and man. Blood was taken from the peripheral vein in the case of the rabbit, dog and man, and by heart puncture in the guinea pig. Sodium citrate was used as the anticoagulant (blood : 3.8% sodium citrate=9:1).

The plasma was mixed with various concentrations of urokinase (Uronase, Mochida pharm. Co. Ltd., Tokyo) (final concentrations : 0, 5, 25, 50 and 125 units/ml of plasma), for use in the experiments.

RESULTS

The increase in optical density (OD) which appeared after release of free p-nitroanilide was plotted against reaction time (min). In the rabbit, the OD for S-2444 increased with increasing reaction time, and a remarkable increase was observed with the addition of UK (see the line 0 in the left graph of

Fig.1). Addition of 5 units (IU) caused the OD to double approximately ; however, further addition of UK did not induce any clear extra increase. The change in OD for S-2227 was less than half of that with S-2444, and addition of UK did not lead to OD increases in a dose−response manner (right graph of Fig.1).

Among the various species studied, the greatest OD increase with S-2444 was observed in the case of the guinea pig, and the addition of UK caused the greatest increase in OD (left graph of Fig.2). However, the fluctuations in OD with S-2227 were relatively slight in the guinea pig, and the addition of UK did not induce any clear increse in OD (right graph of Fig.2).

In the dog, the OD increse with S-2444 was slight, but addition of UK caused OD increases in a dose-response manner over the range 0−50 units (left graph of Fig.3). The addition of 5 units UK doubled the OD increase, the addition of 25 and 50 units further increased the OD, but the addition of 125 units UK showed a reversal of this trend. On the other hand, S-2227 was not clearly cleaved (right graph of Fig.3) : addition of UK increased the OD, but only within a narrow range.

Among the various species studied, the least OD increase with S-2444 was observed in man (left graph of Fig.4). The addition of UK (final : 5, 25 and 50 units) increased the OD slightly, and 125 units UK increased the OD mildly. The OD increase with S-2227 was relatively slight, but increased as the UK concentration increased (right graph of Fig.4).

In order to compare the substrate specificities in the various species, the ratio of the S-2444 catalytic activity to the S-2227 catalytic activity was calculated for rabbit, guinea pig, dog and human plasma from the data between 10 and 20 min incubation (Table I). S-2444 was the most sensitive to guinea pig plasma.

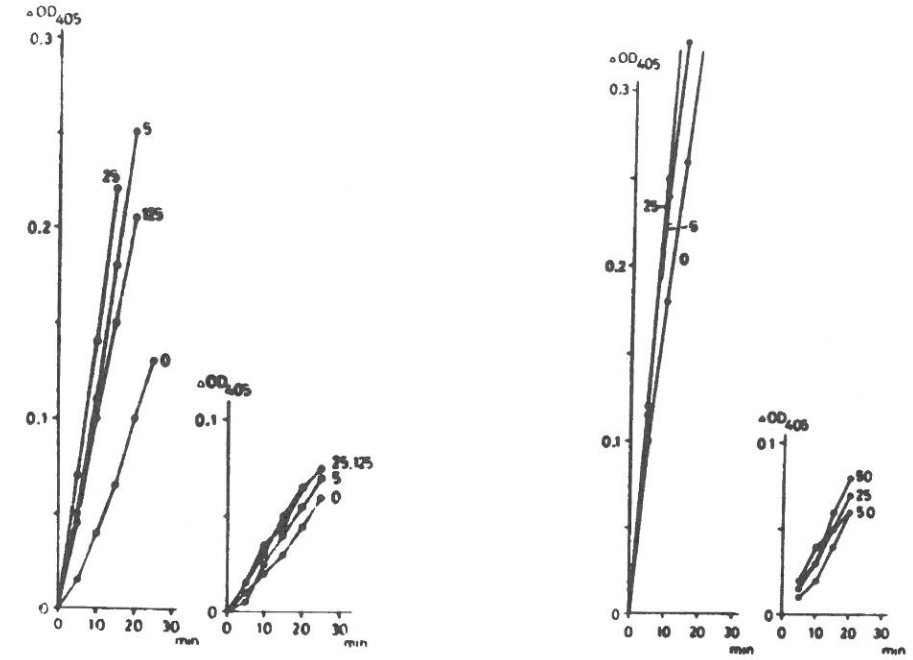
In order to compare the UK sensitivities of the substrate catalytic activities, the ratios of the substrate catalytic activity after UK addition to that before UK addition were calculated (Table II). Human plasma was the most sensitive to UK addition in the case of S-2444 catalytic activity (Table IIA), and dog plasma, in the case of S-2227 catalytic activity (Table IIB).

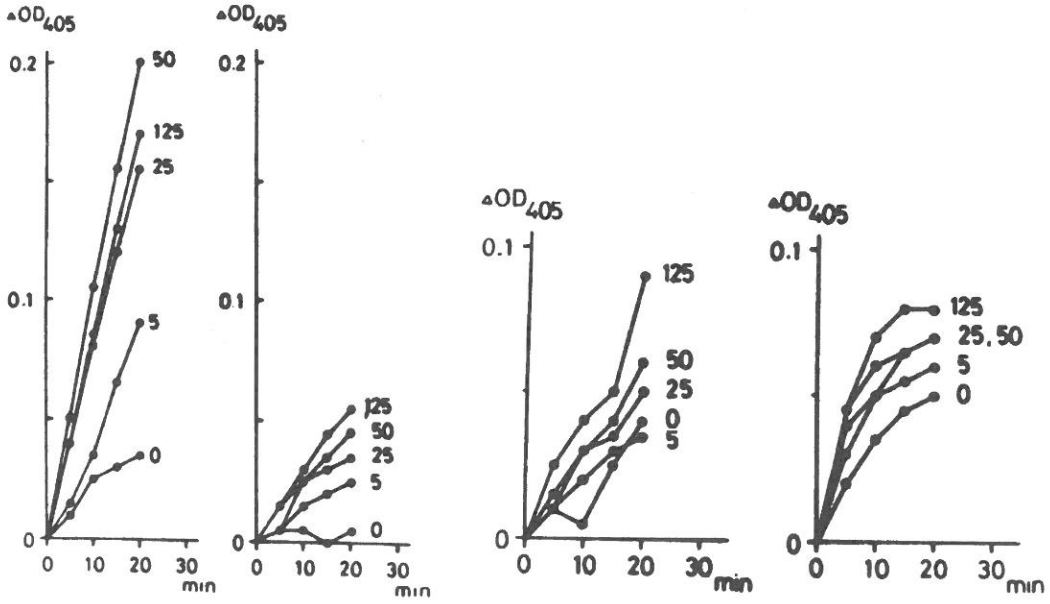
DISCUSSION

The synthetic chromogenic substrates, S-2444 and S-2227, are both urokinase sensitive substrates. The KmValues are 9×10^{-5} in S-2444 [11] and 2×10^{-4} in S-2227 [10] which were measured using urokinase of Leo Reagent, Melmö. Their structures are very similar, but they differ in the nature of their terminal amino acids —— pyroglutamyl in S-2444 and glutamyl in S-2227. The present study aimed to clarify the effect of such structure variation on species specificity.

The S-2444 to S-2227 catalytic activity ratio was the highest in guinea pig plasma and least in human plasma (Table I). This suggests that the structures of S-2444 and S-2227 are approprate for the catalytic action of the plasminogen activator in guinea pig and human plasma, respectively. When the responses of the substrate catalytic activity to human UK addition were compared, the increase in S-2444 catalytic activity was the largest in human plasma and least in guinea pig plasma (Table IIA), and that of S-2227 was the largest in dog plasma and least in human plasma. These substrates are designated by structure-activity correlations,[13] and not imitations of natural substrate (-Pro-Gly-Arg- ; heavy chain side of the activation bond in plasminogen). It is reported that S-2444 is the most effective among the substrates synthesized for UK[13]. As mentioned, when human UK was added to plasma of different species, human plasma responsed most in S-2444 and dog plasma in S-2227 activity (Table II). The structure-activity correlation were thus influenced by the enviroment (in this case, plasma).

The present study aimed to compare the responses to UK of the plasmas of different species in





FIGS. 1(upper left), 2(upper right), 3(lower left)and 4 (lower right)

The cleavage of S-2227 (right graph of each FIGS) and S-2444 (left graph of each FIGS) in the plasma of rabbit (FIG. 1), guinea pig (FIGS. 2), dog (FIG. 3) and human (FIG. 4). Ordinate : the increase of optical density at 405 nm. Abscissa : incubation time (min). The number in the figures indicates the absence (0) or presence of urokinase (final concentration 5, 25, 50, 125 units/ml of plasma).

TABLE I

Ratio of S−2444 catalytic activity to S−2227 catalytic activity in rabbit, guinea pig, dog and human plasma*.

	Rabbit	Guinea pig	Dog	Man
S−2444 catalytic activity (A)	4	18	2.5	0.5
S−2227 catalytic activity (B)	2	2	0.5	3.5
A/B	2	9	5	0.14

* Expressed in OD x 10^3/min, calculated from data between 0 and 10 min incubation.

TABLE II

Effect of UK addition on substrate catalytic activity*.

A. S−2444 catalytic activity

	Rabbit	Guinea pig	Dog	Man
Before UK addition (C)	4	18	2.5	0.5
25 units UK addition (D)	14	25	8	3
D/C	3.5	1.39	3.2	6

B. S−2227 catalytic activity

	Rabbit	Guinea pig	Dog	Man
Before UK addition (E)	2	2	0.5	0.5
25 units UK addition (F)	3	3	2.5	5
F/E	1.5	1.5	5	1.43

* For the calculation of catalytic activity, cf. Table 1.

common use in experimental reserch.

A slight change in substrate (glutamyl or pyroglutamyl in the present study) can thus lead to different modes of catalysis by urokinase, suggesting the critical nature of the structure in the active site[13].

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