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Short Communication

Fluorescent species produced by the interaction of streptomycin and glucose

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Introduction

Several studies have been made on the degradation of antibiotics in solutions containing carbohydrates. Alkaline solutions of cloxacillin and other penicillins in the presence of carbohydrates decompose rapidly [1–3].

The purposes of this study were to identify the fluorescent species that are formed during the degradation of streptomycin in the presence of glucose, to examine in detail the interactions between streptomycin and glucose in solutions at different alkaline pH values, and to examine the role of glucose in the generation of fluorescence.

Experimental

Apparatus

Fluorescence spectra were obtained using a Perkin–Elmer–Hitachi 204 spectrofluorimeter with synchronized recorder and with a Xenon lamp XBO of 150 W.

pH values were measured with a Spandomatic SS-2 pH meter.

A Selecta thermostatic bath was used.

Materials

All reagents were of analytical grade purity and were used without further purification.

Pure antibiotics for clinical use from different manufacturers were used for this study.

Methods

To study the degradation of antibiotics in the presence of glucose and the changes in fluorescence intensity with time, the following techniques were used for all antibiotics. The antibiotic (0.2 mg ml^{-1}) was dissolved in 0.1 M sodium hydroxide. Glucose was added to give a final concentration of 0.5%, 1% or 2% (w/v). The fluorescence intensity

Table 1
Variation of fluorescence intensities of various antibiotics in the presence of glucose at various concentrations (C_g) in alkaline solution

	Fluorescence intensity Time 0 min						Time 120 min			
	λ_{ex} nm	λ_{em} nm	$C_g = 0$	$C_g = 0.5\%$	$C_g = 1\%$	$C_g = 2\%$	$C_g = 0$	$C_g = 0.5\%$	$C_g = 1\%$	$C_g = 2\%$
Amoxicillin trihydrate	395	475	1	1	1	1	2	2	2	2
Ampicillin trihydrate	350	420	17	16	16	15.5	19	18	17	17
Ampicillin trihydrate pH = 7.7	350	420	65	70	72	70	69	68	70	68
Ampicillin trihydrate pH = 5.4	350	420	22	18	21	20	24	21	23	22
Sodium ampicillin	350	420	24	25	25	26	26.5	30	28	28
Chloramphenicol	350	430	—	—	—	—	—	—	—	—
Sodium cloxacillin	350	400	1	1	1	1	1	1	1	1
Dimethyl chlortetracycline	400	510	15	16	17	17	15	17	18	18
Gentamicin sulphate	350	390	2	2	2	3	2	2	2.5	3
Sodium novobiocin	270	450	3	3	3	2	4	4	4	4
Benzathine penicillin	350	420	15	12	14	17	17.5	14.5	15	15
Procaine penicillin G	320	350	25	28.5	31	27	120	96	71	29
Tetracycline phosphate	460	540	13	13	14	14	13	13	13	15
Streptomycin sulphate	360	430	1	1	1	1	1	11	13	15

of these solutions was measured immediately after preparation of the solution (time = 0), and again after various intervals of time. The results are shown in Table 1. From these results, streptomycin was selected for further study in order to investigate the interaction between the antibiotic and glucose.

Several experiments were carried out to determine the time required to produce the maximum fluorescence response and the maximum wavelength excitation.

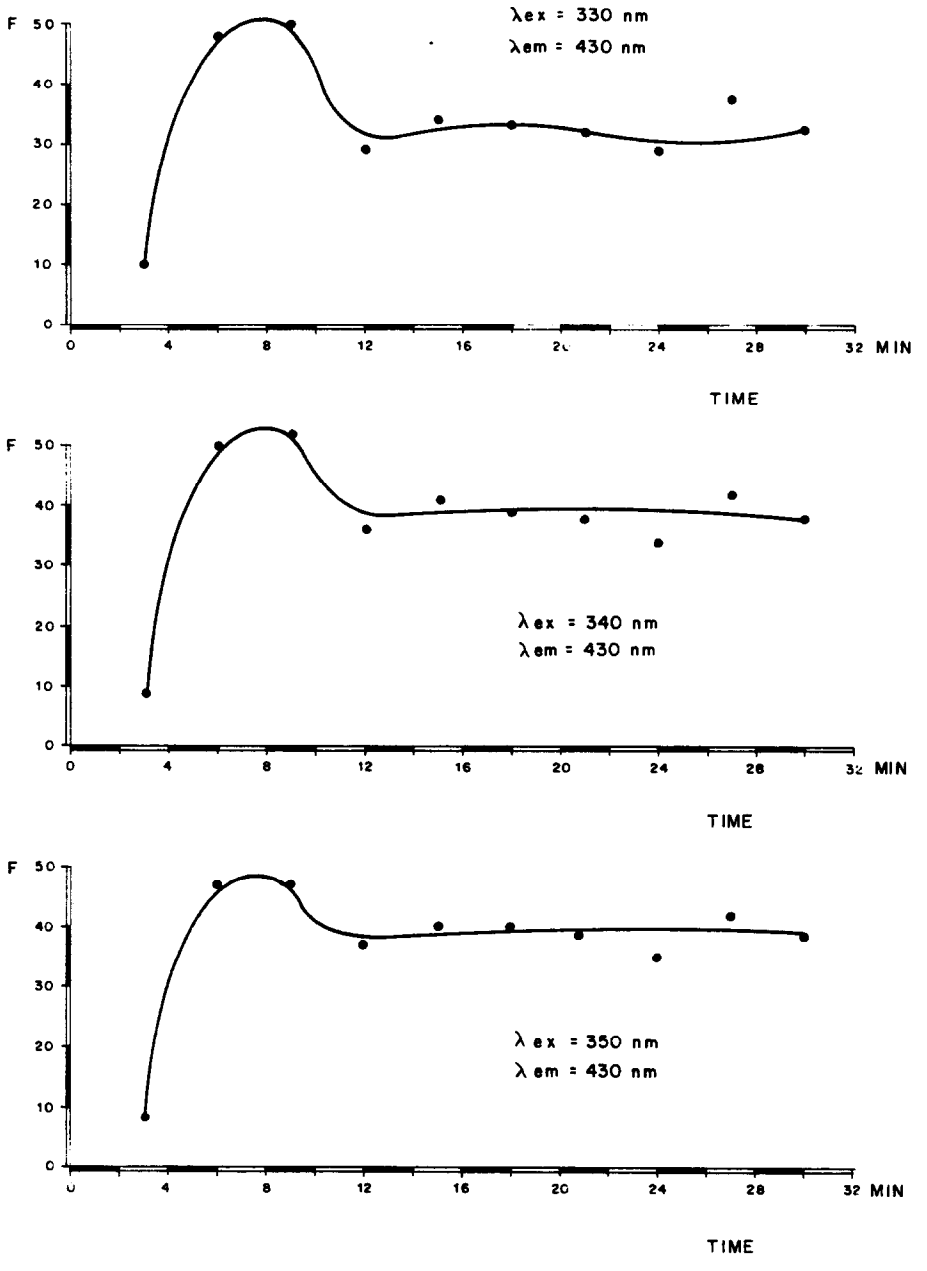

Figure 1
Relative fluorescence intensity of streptomycin and glucose after heating at 100°C.

A solution of streptomycin sulphate (0.5 mg ml^{-1}) was prepared in 0.1 M sodium hydroxide. Ten millilitres of this solution were added to 5 mg of glucose. Blank solutions were prepared at the same time, one containing streptomycin only and the other containing glucose only. The sample solution was heated in a boiling water bath to accelerate the reaction. Aliquots were removed every 3 min and cooled to stop the reaction. The results of the fluorescence measurements are shown in Fig. 1.

In a related experiment the formation of the fluorescent compound at room temperature was studied. As a result of the observations made, the fluorescence intensities in later experiments carried out at room temperature were measured at 2 h and again at 3 h after the start of the reaction.

Solutions of streptomycin sulphate at concentrations of 0, 2.5, 5.0, 7.5, 10 and 15 mg ml^{-1} in 0.1 M sodium hydroxide were prepared. Glucose was added to each solution to give a final concentration of 30 mg ml^{-1}. The reaction was stopped 2 h after the addition of the glucose, then aliquots of 4 ml of each solution were diluted to 50 ml with 0.01 M

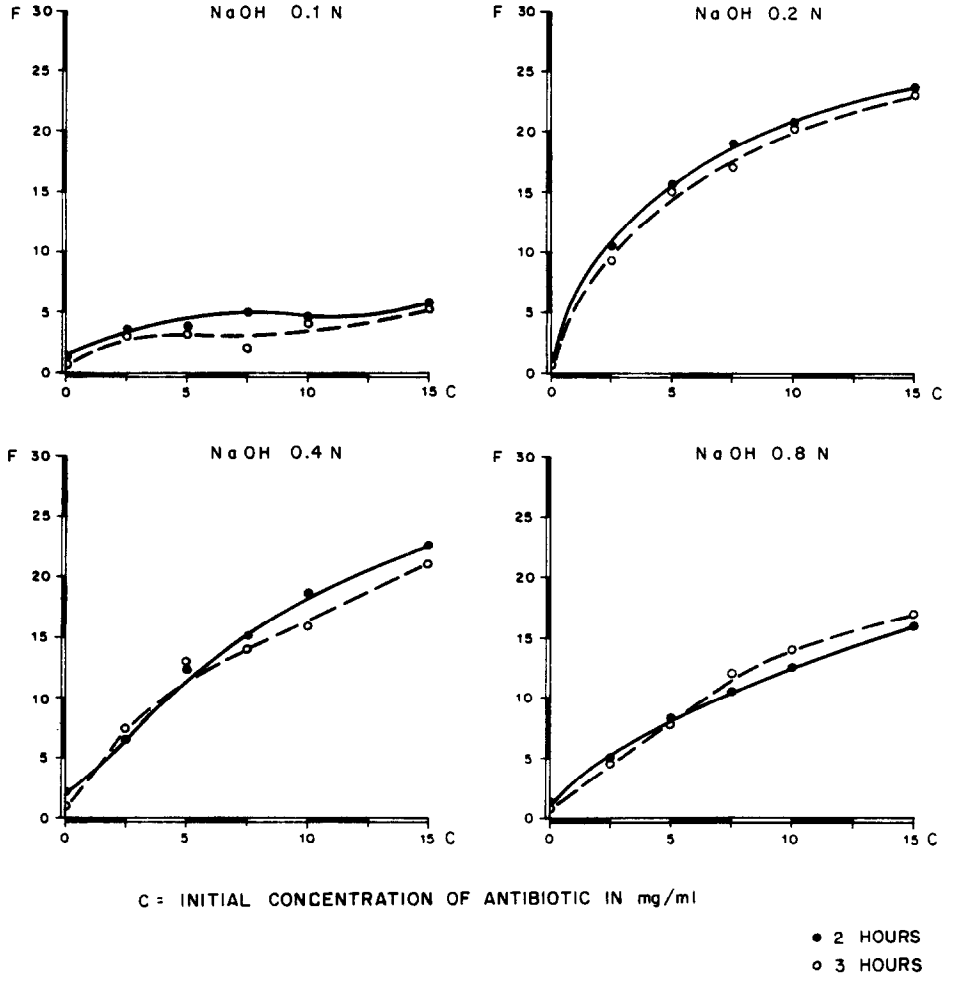

Figure 2
The relative fluorescence intensities at 2 and 3 h after mixing solutions of glucose at constant concentration and of streptomycin at different concentrations in the presence of various concentrations of sodium hydroxide.

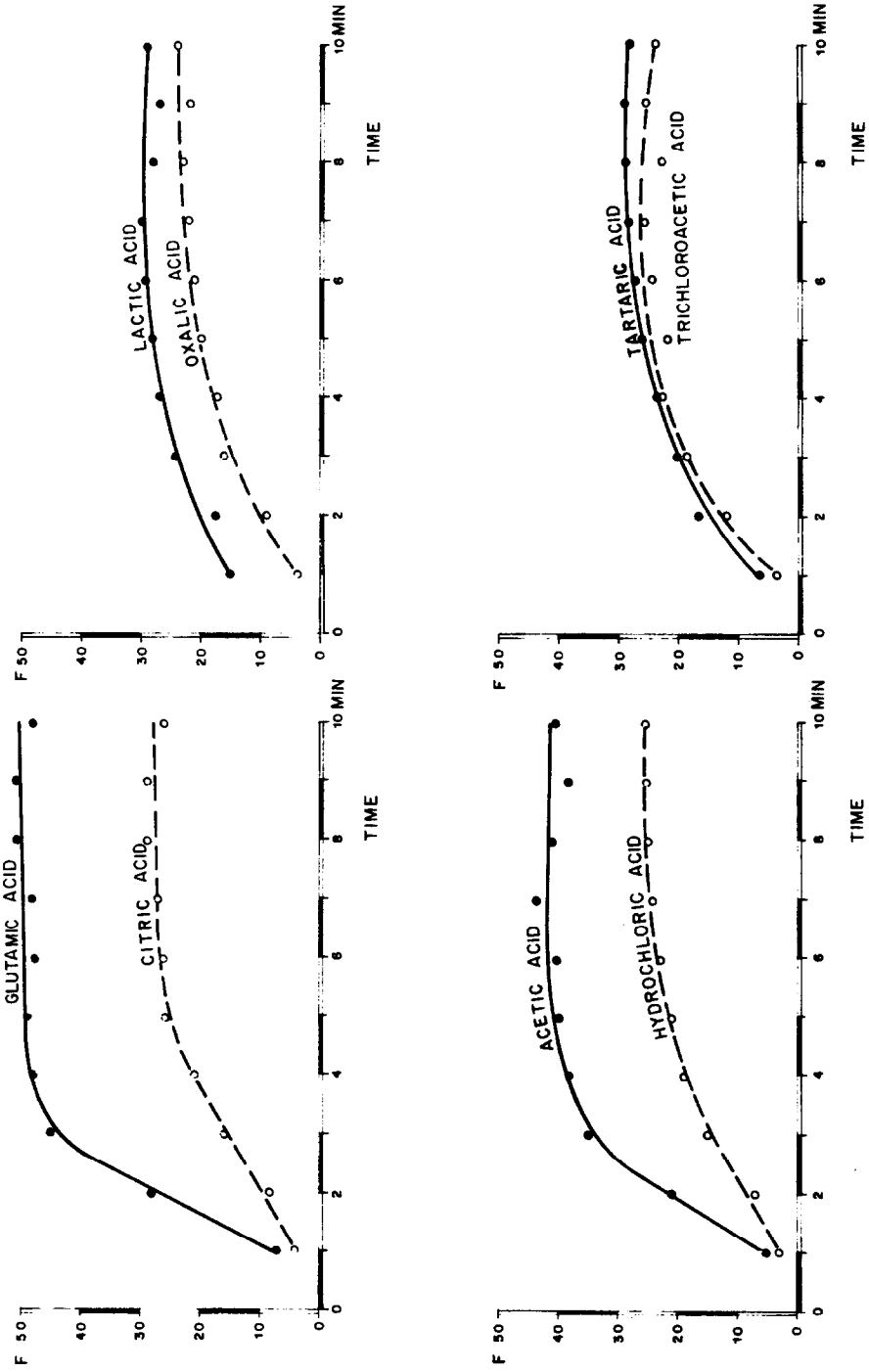

Figure 3
The relative fluorescence intensities of solutions of streptomycin and glucose, measured at different times after the addition of various acids.

acetic acid. The fluorescence intensity was then measured. A similar experiment was carried out with a 3 h reaction time.

These experiments were repeated with different concentrations of sodium hydroxide (0.2, 0.4 and 0.8 M). The results are shown in Fig. 2.

To investigate the effect of different acids on the reaction, 2 ml of a solution of glucose (10 mg ml^{-1}) in 0.1 M sodium hydroxide were mixed with 2 ml of a solution of streptomycin (10 mg ml^{-1}) in 0.1 M sodium hydroxide. The mixture was heated in a water bath and then the acid (0.01 M) was added to give a final volume of 50 ml. The fluorescence intensity was measured immediately. The results are shown in Fig. 3.

In order to identify the compound responsible for the fluorescence, streptomycin was hydrolyzed to streptidine and streptobiosamine [4] as shown in the following reaction:

$$C_{21}H_{39}N_7O_{12} + H_2O \xrightarrow{H^+} \underset{\text{streptidine}}{C_8H_{18}N_6O_4} + \underset{\text{streptobiosamine}}{C_{13}H_{23}NO_9}$$

$$\downarrow OH^-$$

$$\underset{\text{streptamine}}{C_6H_{14}N_2O_4} + 4\,NH_3 + 2\,CO_2$$

Streptidine (0.1 mg ml^{-1}) was dissolved in 0.1 M sodium hydroxide. To one aliquot of this solution glucose was added to give a concentration of 3 mg ml^{-1} and to another aliquot glucose was added after 30 min. The results are shown in Fig. 4. The fluorescence spectra of streptidine and streptobiosamine in the presence of glucose demonstrated that

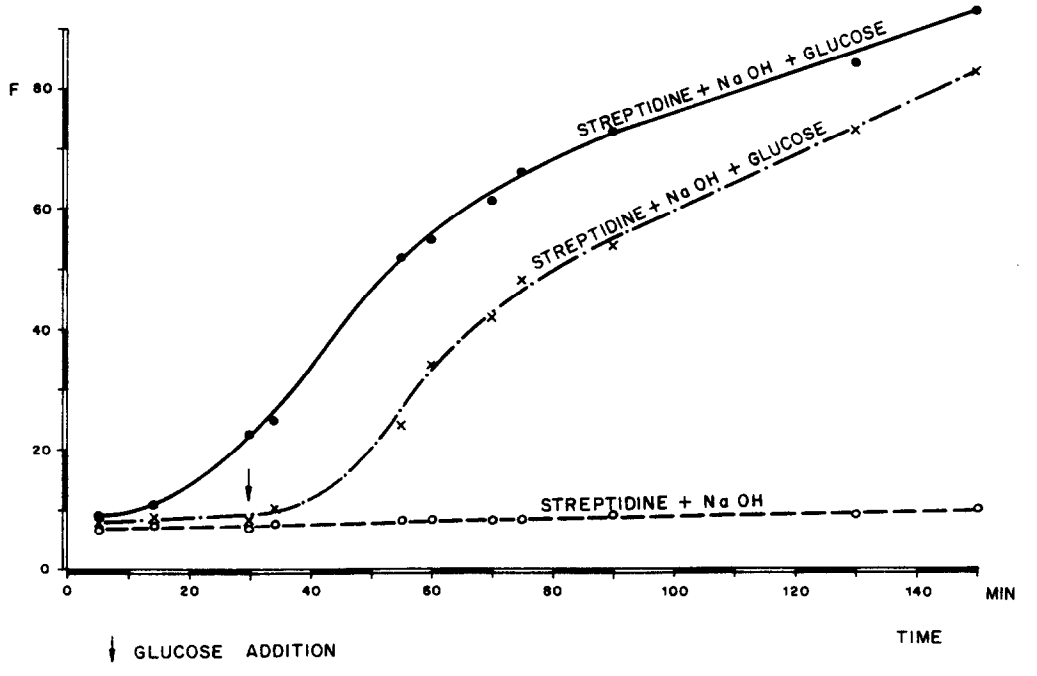

Figure 4
The relative fluorescence intensities at various times after the addition of glucose to streptidine.

streptidine–glucose shows the same fluorescence wavelength maxima as streptomycin–glucose (λ_{ex} = 340 nm, λ_{em} = 430 nm).

Results and Discussion

Table 1 shows that the fluorescence response of a number of antibiotics does not change when glucose is present. However, in alkaline solution after the addition of glucose, streptomycin, which is not fluorescent, develops fluorescence that increases with time. The relationships between fluorescence intensity, streptomycin concentration (Fig. 2) and glucose concentration (Table 1) were established. The interpretation of the results was carried out by numerical methods which permit the expression of the relationship between fluorescence intensity and other variables by means of a regression functions that were object of an earlier paper [5].

The fluorescence intensity was the same at 2 and 3 h although the response at these times was less than that obtained during the first few minutes of reaction. The stability of the fluorimetric response was useful for studying the fluorescent compound.

The increase in fluorescence stopped when various acids were added to solutions of glucose and streptomycin (Fig. 3). Of those acids studied acetic and glutamic acids were found to give the greatest effect. Thus the acid used to acidify the solution affects the fluorogen produced in the reaction.

The results also show that streptidine is responsible for the fluorescence that is produced and that the reaction proceeds only in basic solution. It was also established that the fluorogen produced in the reaction with streptidine does not form instantaneously (Fig. 4), and it is probable that further hydrolysis of streptidine to streptamine occurs in the alkaline solution before the interaction with glucose can take place.

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