Kinetic Analysis of Complex Formation between Penicillin and Sucrose

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Abstract □ The formation of 1:1 molar complexes between sucrose and the following penicillins was studied: potassium penicillin G USP; potassium phenoxymethyl penicillin USP; sodium dicloxacillin, monohydrate; and ampicillin, anhydrous. Kinetic parameters, based on a model system, are presented for each interaction. The degree of complexation was greatest with sodium dicloxacillin and least with ampicillin. The rate of degradation of complexed penicillin was 5–6 times the rate for the uncomplexed penicillin. The relatively high equilibrium constant obtained for sodium dicloxacillin was consistent with the strong effect of sucrose on the observed rate constant. Ampicillin, whose observed rate constant was only slightly affected, had a very low equilibrium constant. Complexation involves the intact penicillin and accelerates the degradation of penicillin but does not appear to change the degradation pathway.

Keyphrases □ Potassium penicillin G—complexation with sucrose, effect on degradation rate □ Potassium phenoxymethyl penicillin—complexation with sucrose, effect on degradation rate □ Sodium dicloxacillin, monohydrate—complexation with sucrose, effect on degradation rate □ Ampicillin, anhydrous—complexation with sucrose, effect on degradation rate □ Penicillins—complexation with sucrose, effect on degradation rate □ Sucrose—complex formation, effect on degradation rate of penicillins □ Complex formation—sucrose with penicillins, effect on penicillin stability

A number of reports (1–6) indicated that dextrose causes the inactivation of penicillin, although other workers (7, 8) concluded that dextrose has little effect on the chemical stability of potassium penicillin G USP. Moss and Cole (9) found that the degradation of 6-aminopenicillanic acid was accelerated in the presence of dextrose, maltose, or lactose. In that study, the *N*-glycosyl, *N*-maltosyl, and *N*-lactosyl derivatives of 6-aminopenicillanic acid were isolated by column chromatography and were found to contain approximately 1 mole of sugar/mole of 6-aminopenicillanic acid. The authors concluded that the amino group of 6-aminopenicillanic acid and the reducing group of the sugar were involved in the interaction, because they observed no reaction when benzylpenicillin or a nonreducing sugar was present.

Schneider and de Weck (10) recently reported a reaction between benzylpenicillin and a number of carbohydrates, including reducing sugars, nonreducing sugars, dextran, and simple glycols. They speculated that the reaction resulted in the formation of an α-ester of penicilloic acid. The reaction product of benzylpenicillin and raffinose was separated by Sephadex chromatography and contained equimolar amounts of raffinose and benzylpenicillin.

Preliminary experiments in these laboratories also indicated an interaction between sucrose and penicillin. The investigation reported here was undertaken to investigate this interaction through a determination of its kinetic parameters.

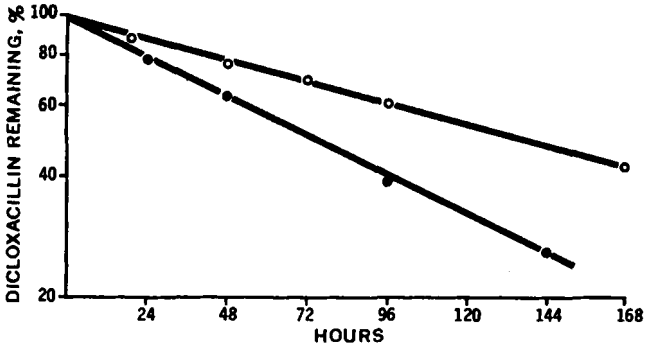


Figure 1—*Change in potency of sodium dicloxacillin, monohydrate, during aging in a 0.01* M *solution at pH 7, 45°. Key:* ○, *in absence of sucrose; and* ●, *in presence of 0.0175* M *sucrose.*

EXPERIMENTAL

Materials—Four penicillins were studied: potassium penicillin G USP; potassium phenoxymethyl penicillin USP; sodium dicloxacillin, monohydrate; and ampicillin, anhydrous. Each was taken from one lot of commercially prepared material. The sucrose[1] and other chemicals used were reagent grade.

Procedure—Solutions for the kinetic studies were prepared at pH 7.0 nsing a 0.06 *M* citrate buffer. The buffer solution containing the appropriate amount of sucrose was brought to the desired temperature in a constant-temperature bath before the penicillin was added. The pH of each solution was determined initially and at the end of the experiment. No significant changes in pH occurred.

Samples were withdrawn during the study and assayed for intact penicillin by a modified iodometric titration procedure (11) or by the microbiological cylinder-plate assay, with *Staphylococcus aureus*, ATCC 6538P, as the test organism.

The rate of hydrolysis of penicillin was also determined by following the acid produced with the aid of a Radiometer setup, consisting of a PHM 26 pH meter, a TTT-1 automatic titrator, a TTA3 titrator assembly, an SBR2 titragraph, and an ABU 12 autoburet.

Ampicillin solutions (0.01 *M*), buffered to pH 7.0 with 0.06 *M* citrate buffer, were examined by TLC after storage for 6 days at 45° both in the presence and absence of 0.029 *M* sucrose. Samples containing 20 mg. of ampicillin were diluted 1:1 with *n*-propanol, and 10 μl. was applied to a 250-μ silica gel G-HR/UV plate[2]. The plate was activated by heating at 100° for 15 min. and was developed in a saturated chamber containing *n*-propanol–water (70:30 v/v). The solvent front was allowed to travel 15 cm. Visualization was effected by: (*a*) exposing the plate to iodine vapors for 60 min., and (*b*) spraying the plate with 0.25% ninhydrin in ethanol and heating at 100° for 10 min.

RESULTS AND DISCUSSION

The four penicillins were found to degrade according to apparent first-order kinetics. Plots similar to Fig. 1 were obtained throughout the study when the logarithm of the penicillin concentration *versus* time was plotted. As seen in Fig. 2, at pH 7.0 and 45°, sucrose accelerated the degradation of 0.01 *M* penicillin solutions in 0.06

[1] Analyzed reagent, J. T. Baker Chemical Co., Phillipsburg, N. J.
[2] Analtech, Inc., Wilmington, Del.

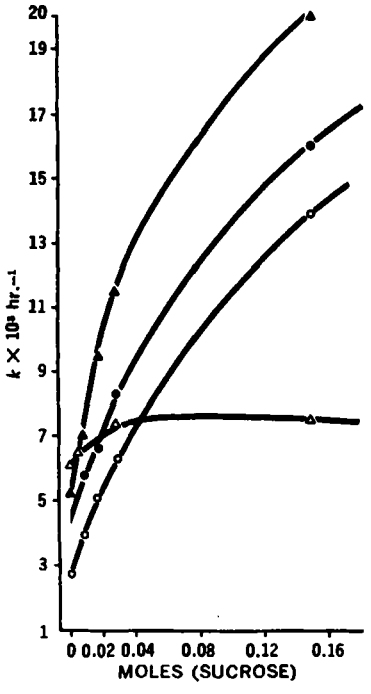


Figure 2—*Effect of sucrose on the apparent first-order rate constant for various penicillins at pH 7.0, 45°, in 0.06 M citrate buffer. Key: ○, potassium phenoxymethyl penicillin USP; ●, potassium penicillin G USP; △, ampicillin, anhydrous; ▲, sodium dicloxacillin, monohydrate.*

M citrate buffer. Potassium penicillin G, potassium phenoxymethyl penicillin, and sodium dicloxacillin showed a 3–5-fold increase in apparent first-order rate constant as the sucrose concentration was increased to 0.15 *M*. A significant but much smaller increase in the rate of degradation of ampicillin occurred in the presence of sucrose. The overall relationship between apparent first-order rate constant and sucrose concentration is nonlinear, although it approximates linearity for sucrose concentrations up to 0.03 *M* or 3 times the molar concentration of sucrose.

The observed effect of sucrose was not due to trace metal catalysis caused by impurities. Highly purified sucrose was used in this study, and its effect on the rate of degradation of sodium dicloxacillin was identical in the presence or absence of 0.05% disodium edetate USP.

TLC analysis of ampicillin solutions degraded in the presence and absence of sucrose (Fig. 3) indicated that sucrose has no effect on the degradation products. Several degradation products were observed in each solution. The R_f values of these products were unchanged in the presence of sucrose.

Schneider and de Weck (10) speculated that the reaction between benzylpenicillin and a number of carbohydrates occurred by esterification of the potential acid group of the β-lactam. They noted that the penicilloyl–dextran conjugate separated by Sephadex chromatography showed no antibiotic activity by the Oxford cup assay. This observation supports their esterification hypothesis since biological activity is only present in penicillins when the β-lactam is intact (12). To determine if esterification was responsible for the observed effect of sucrose on penicillin (Fig. 2), an experiment was run comparing biological activity to the iodometric assay results for a series of buffered sodium dicloxacillin solutions aged for 7 days at 25°. Three identical solutions (A, B, and C) were prepared using 0.025 *M* sodium dicloxacillin and citrate buffer. Three similar solutions (D, E, and F) which also contained 2 *M* sucrose were prepared. The biological and iodometric assay results for these solutions are presented in Table I. In all cases the biological activity agrees well with the iodometric assay results. The retention of biological activity in the presence of sucrose indicates that a different interaction is occurring than the probable esterification observed by Schneider and de Weck (10).

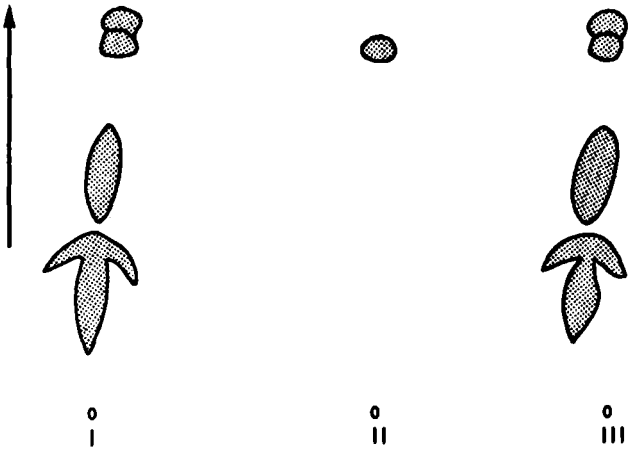


Figure 3—*TLC analysis of 0.01 M solutions of ampicillin, anhydrous, buffered at pH 7.0 with 0.06 M citrate. Key: I, preparation aged 6 days at 45°; II, fresh preparation; and III, preparation with 0.029 M sucrose, aged 6 days at 45°.*

Table I—Comparison of Potency of Buffered Sodium Dicloxacillin Solutions Determined by Biological and Iodometric Assay

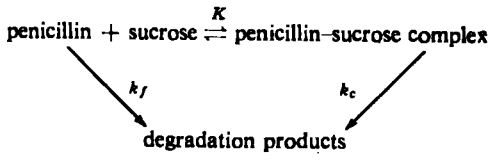
	Initial *M*		Aged 7 Days at 25°, % Initial	
	Iodometric	Biological	Iodometric	Biological
No sucrose				
A	0.0253	0.0244	95	105
B	0.0245	0.0250	96	101
C	0.0249	0.0244	96	98
Average			96	101
With 2 *M* sucrose				
D	0.0247	0.0243	90	89
E	0.0258	0.0236	87	84
F	0.0263	0.0253	91	83
Average			89	85

In addition, the rate of degradation of a pH 10 solution of 0.02 *M* potassium penicillin G at 45° in the presence of 0.29 *M* sucrose was determined by the pH-stat and iodometric methods. The results obtained show good agreement between these two methods (Table II). At pH 10, where the possibility of ester formation would be most likely to occur, there is good agreement for the degradation of sodium dicloxacillin between the pH-stat and iodometric methods both in the presence and absence of sucrose. The agreement in the rate of degradation as determined by pH-stat titration, which measures the release of protons upon cleavage of the β-lactam, and the iodometric assay, which is based on the consumption of iodine by the cleaved β-lactam, indicates that penicillin in the presence of sucrose does not degrade through the formation of an intermediate ester. The presence of any significant amount of ester would give differences in the observed penicillin degradation rates between the pH-stat and iodometric methods. These results, therefore, further confirm the postulation that esterification of the penicillins is insignificant in these systems.

As seen in Fig. 1, no induction period or change in slope was noted during the kinetic studies. This observation, in addition to the nonlinear dependence of the apparent first-order rate constant on sucrose concentration (Fig. 2), suggests that the interaction is based upon an equilibrium such as occurs in complexation. The

Table II—Comparison of Rates of Penicillin Degradation by pH-Stat and Iodometric Assay Techniques

System	Rate of Degradation, min.⁻¹ pH-Stat	Iodometric
0.02 *M* sodium dicloxacillin monohydrate, pH 10 and 45°	8.9×10^{-2}	8.8×10^{-2}
0.02 *M* sodium dicloxacillin monohydrate with 0.29 *M* sucrose, pH 10 and 45°	4.5×10^{-1}	4.9×10^{-1}



Scheme I

retention of biological activity indicates that the β-lactam is intact in the presence of sucrose. The R_f values of the degradation products were identical in the presence or absence of sucrose. Based on these observations and the conclusion of Moss and Cole (9) and Schneider and de Weck (10) that a 1:1 reaction occurs between carbohydrates and penicillin, the model shown in Scheme I was selected as the simplest system that completely satisfies the experimental observations; K is the equilibrium constant for the penicillin–sucrose complex, and k_f and k_c are the apparent first-order rate constants for the degradation of uncomplexed and complexed penicillin, respectively.

The formation of a penicillin–sucrose complex as proposed by the kinetic model is supported by a recent study of the interaction of penicillin and guanosine (13). NMR studies showed that penicillin G interacts with guanosine in dimethyl sulfoxide by forming a binary hydrogen-bonded complex. The binding sites were shown to be the carboxylate and carbonyl groups in penicillin G and the NH and NH_2 groups of guanosine. Although no attempts were made in the present study to elucidate the structure of the proposed penicillin–sucrose complex, the hydroxyl groups of sucrose may be able to act as hydrogen donors and form a binary hydrogen-bonded complex analogous to the penicillin–guanosine complex.

The complexing behavior of pharmaceuticals with various other materials has been reported by numerous workers. In most previous reports discovered by the authors, complexation contributed favorably to the stability of the drug. The rate of hydrolysis of benzocaine was significantly reduced in the presence of caffeine (14). Chelate formation by boric acid stabilized epinephrine (15). Guttman (16) and Wadke and Guttman (17, 18) demonstrated that molecular interactions can improve the stability of riboflavin and related compounds.

Although the effect of complex formation has been to inhibit the rate of reaction in pharmaceuticals, acceleration of the apparent rate also is possible and has been observed in the cyclohepta-amylose-catalyzed hydrolysis of penicillin (19), the acetolysis of

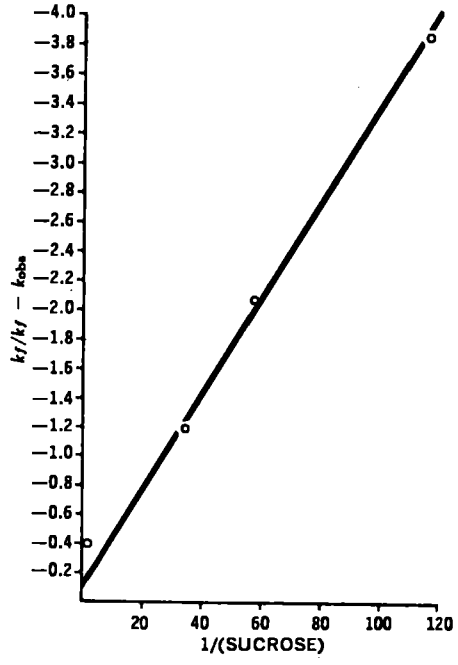


Figure 4—*Interaction between potassium penicillin G USP and sucrose. Plot based on equation of Connors and Mollica (25).*

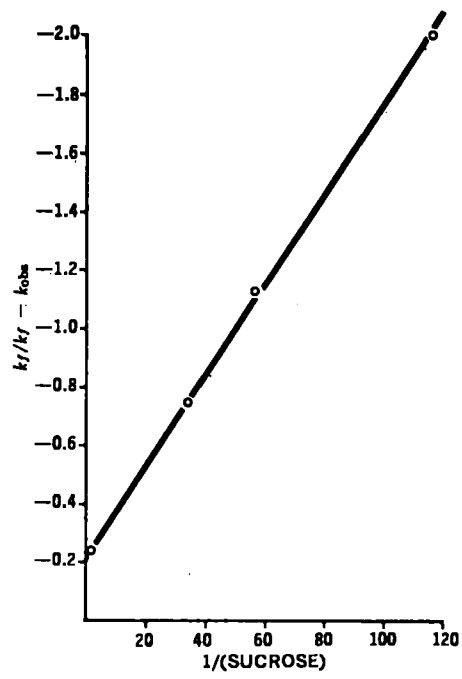


Figure 5—*Interaction between potassium phenoxymethyl penicillin USP and sucrose. Plot based on equation of Connors and Mollica (25).*

certain toluenesulfonates (20–23), and the racemization of optically active binaphthyl donors (24).

In the present study, the interaction between sucrose and penicillin was studied using the kinetic analysis for a 1:1 molar complex developed by Connors and Mollica (25). This analysis makes no assumptions regarding the relative magnitude of k_f and k_c. They derived the following equation to describe the effect of the formation of a 1:1 molar complex on the observed reaction rate:

$$\frac{k_f}{k_f - k_{obs}} = \frac{1}{q_{11}K(\text{sucrose})} + \frac{1}{q_{11}} \qquad \text{(Eq. 1)}$$

where k_{obs} is the observed rate constant at any sucrose concentration, and $q_{11} = [1 - (k_c/k_f)]$.

The rate of degradation of uncomplexed penicillin was determined in the absence of sucrose and confirmed by extrapolating

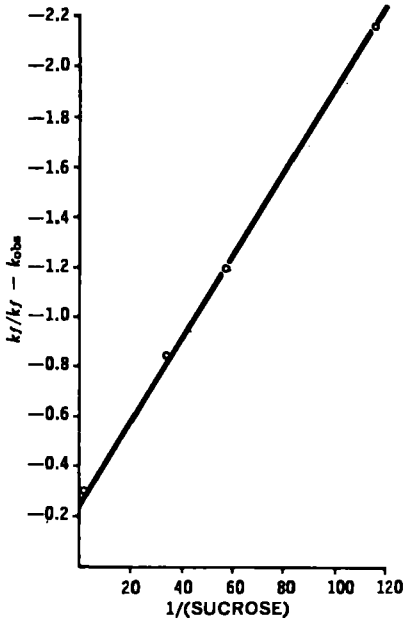


Figure 6—*Interaction between sodium dicloxacillin, monohydrate, and sucrose. Plot based on equation of Connors and Mollica (25).*

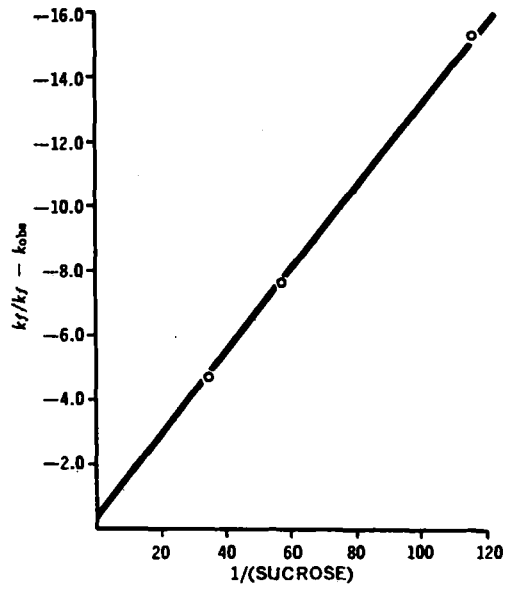


Figure 7—*Interaction between ampicillin, anhydrous, and sucrose. Plot based on equation of Connors and Mollica (25).*

the effect of sucrose on the observed rate constant to zero sucrose concentration.

The rate of degradation of complexed penicillin was obtained from the intercept of a plot of $[k_f/(k_f - k_{obs})]$ *versus* [1/(sucrose)] and the relationship between q_{11} and k_c/k_f. The equilibrium constant was calculated by dividing the intercept of this plot by the slope.

The data in Fig. 2 are plotted in Figs. 4–7 for each penicillin according to the variables in Eq. 1. An excellent linear relationship is obtained for potassium penicillin G (Fig. 4), potassium phenoxymethyl penicillin (Fig. 5), sodium dicloxacillin (Fig. 6), and ampicillin (Fig. 7). The excellent agreement between the experimental data and the behavior predicted by Eq. 1 also supports the kinetic model based upon the formation of a 1:1 molar complex. The equilibrium constant, k_f, and k_c were calculated from Figs. 4–7 and are given in Table III.

The data indicate that the complexed penicillin degrades 5–6 times as fast as the uncomplexed penicillin and results in an increased overall rate of degradation. These results indicate that the sucrose–penicillin complex that forms is degraded by the same mechanism, regardless of the penicillin tested. The differences in overall rate constants, however, are due to the differences in the equilibrium constants for the penicillins. The relatively high equilibrium constant obtained for sodium dicloxacillin was consistent with the strong effect of sucrose on the observed rate constant. Ampicillin, whose observed rate constant was only slightly affected by sucrose, had a very low equilibrium constant, indicating a much lesser degree of complexation. If the penicillin–sucrose complex has a structure analogous to the penicillin–guanosine complex, then the presence of the amine group in the ampicillin side chain, which is unique with ampicillin, may interfere with complex formation due to stearic or charge factors. Tutt and Schwartz (19) also observed that the effect of cycloheptaamylose on the hydrolysis of penicillin was dependent on the structure of the penicillin side chain.

The present study offers a unique example of a drug that is less stable in the complexed form. The complexes between penicillin and sucrose appear to form instantly. No induction period or change in the rate of degradation was observed in any case. Complexation thus appears to occur between the intact penicillin molecule and sucrose. Degradation of the complexed and uncomplexed penicillin then occurs by the usual pathway and results in an increased rate of degradation.

Table III—Kinetic Parameters for Penicillin–Sucrose Complexes at pH 7.0, 45°, in 0.06 *M* Citrate Buffer

	K, l./mole	$k_f \times 10^3$ hr.$^{-1}$	$k_c \times 10^3$ hr.$^{-1}$	k_c/k_f
Potassium phenoxymethyl penicillin USP	11.5	2.7	17.0	6.3
Potassium penicillin G USP	6.6	4.6	26.6	5.9
Ampicillin, anhydrous	1.5	6.1	38.1	6.2
Sodium dicloxacillin, monohydrate	15.5	5.2	25.4	4.9

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