The Exchange of C^{14} between Urea and Cyanate and between Cyanate and Carbonate

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The exchange of C^{14} between labeled urea and cyanate ion has been studied at 80°. The experimentally determined rate equation has the form $R = k_1(\text{urea}) + k_2(\text{urea})(\text{OCN}^-)$. At 80°, k_1 is 2.5×10^{-6} sec.$^{-1}$ and k_2 is 1.2×10^{-6} M^{-1} sec.$^{-1}$. Two possible mechanisms are given. Both mechanisms lead to a rate equation of the form found. The two mechanisms are discussed. No exchange occurs between urea and cyanate at room temperature. No exchange of C^{14} occurs between labeled carbonate and cyanate at temperatures up to 80°.

Introduction

The hydrolysis of urea,[1] the formation of urea from ammonium cyanate[2] and the hydrolysis of cyanate,[3] all have been widely studied in aqueous solution. It is generally accepted that in the non-enzymatic hydrolysis of urea, ammonium cyanate is an intermediate. The hydrolysis of cyanate probably is through an intermediate of carbamic acid, and thus the complete hydrolysis of the urea would be through cyanate and carbamic acid.

In the enzymatic hydrolysis of urea, the mechanism most consistent with experimental data appears to be the so-called carbamic acid mechanism[4] in which the urea goes to carbamic acid, which then reacts to form carbon dioxide and ammonia.

A study of the rates of exchange of carbon-14 between the various binary systems of urea, cyanate, carbonate and carbamate, combined with the present knowledge of the rates of decomposition and formation of these interdependent compounds, might help elucidate the mechanisms. In this paper the results of the exchange between urea and cyanate and between cyanate and carbonate are reported. The results of exchanges in the other systems will be reported later.

Experimental

Reagents.—C.P. potassium cyanate was recrystallized according to the procedure in "Inorganic Syntheses."[5] Tests for carbonate and ammonia on the recrystallized product were negative. Analysis for cyanate was made as previously described,[3a] and showed a minimum of 96% cyanate. C^{14}-labeled urea was purchased from Tracerlab with a specific activity of 1.46 millicuries per millimole. For these experiments the urea was diluted with C.P. urea which had been recrystallized from alcohol. The active urea was dissolved in ethyl alcohol, the inactive urea added, the total urea dissolved and then precipitated by evaporation of the alcohol at room temperature and reduced pressure. Melting point of the urea was 132°. This urea was used in all exchange reactions involving urea. The specific activity of this urea was determined by diluting a small weighed sample with a weighed sample of inactive urea and hydrolyzing this mixture to carbon dioxide. The carbon dioxide was frozen out in a trap immersed in liquid nitrogen and counted according to the procedures of Christman.[6]

Sodium carbonate labeled with C^{14} was prepared by exchanging labeled barium carbonate, purchased from Oak Ridge National Laboratories, in a solution of sodium carbonate, filtering off the barium carbonate and evaporating the solution to dryness at reduced pressure. The activity of the sodium carbonate was determined by treating a fraction of the sample with acid to liberate carbon dioxide and then counting the carbon dioxide in the same procedure as for the urea.

All other reagents were C.P. and taken without further purification. Distilled water was used throughout.

Experimental Procedure.—Weighed quantities of labeled urea (or sodium carbonate) and a neutral salt to maintain a constant ionic strength were dissolved in water in a glass stoppered erlenmeyer flask. A weighed quantity of potassium cyanate was dissolved in a second flask. Both flasks then were immersed in a constant temperature bath controlled to 0.02° until thermal equilibrium was established. The potassium cyanate solution then was transferred rapidly to the other flask to start the exchange reaction. Aliquots of the solution were withdrawn at various times for analysis.

In the carbonate-cyanate exchange studies the aliquot was added to a solution of barium chloride to precipitate the carbonate. This precipitate was removed by rapid filtration through a sintered glass filter, the filtrate flowing directly into a silver nitrate solution. The silver cyanate formed was filtered off and washed prior to counting.

In the urea-cyanate exchanges the aliquot was discharged directly into a silver nitrate solution and the silver cyanate filtered off and washed.

The silver cyanate from these procedures was hydrolyzed to carbon dioxide in a closed vacuum line and the carbon dioxide counted for C^{14} as in the urea or carbonate assays. Experiments performed with samples withdrawn immediately upon mixing showed no activity in cyanate in either type of experiment indicating that the separation of the cyanate was complete and that there was no exchange occurring during the separation procedure.

Results

Cyanate–Carbonate Exchange.—Since both urea and cyanate are hydrolyzed to carbonate during the exchange studies, it is necessary to know the exchange that may occur between carbonate and cyanate before the urea–cyanate system can be studied. The carbonate–urea exchange is not critical for these studies since the reaction is being followed by the appearance of carbon-14 in the cyanate.

In eleven experiments under varying conditions, no exchange was found to occur between cyanate and carbon-14 labeled carbonate. The experimental conditions are given in Table I. Ammonium ion was added to some experiments to determine any effect it might have since it is a product in both the hydrolysis of urea and of cyanate.

Urea–Cyanate Exchange.—Once it had been shown that no exchange takes place between cyanate and carbonate, any carbon-14 appearing in the cyanate must be due to an exchange between the urea and the cyanate, or intermediate products in the decomposition of these substances.

(1) W. H. R. Shaw and J. J. Bordeaux, THIS JOURNAL, 77, 4729 (1955).

(2) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, p. 257.

(3) (a) A. R. Amell, THIS JOURNAL, 78, 6234 (1956); (b) I. A. Kemp and G. Kohnstam, J. Chem. Soc., 900 (1956); (c) M. W. Lister, Can. J. Chem., 33, 426 (1955).

(4) J. H. Wang and D. A. Tarr, THIS JOURNAL, 77, 6205 (1955).

(5) "Inorganic Syntheses," Vol. II, McGraw-Hill Book Co., Inc., New York, N. Y., 1946, p. 86.

(6) D. R. Christman, N. E. Day, P. R. Hansell and R. C. Anderson, Anal. Chem., 27, 1935 (1955).

TABLE I

CONDITIONS FOR EXCHANGE STUDIES BETWEEN CYANATE
AND CARBONATE

$(OCN^-) = (CO_3^{-2}) = $ (Added salt) $= 0.1\ M$

Added salt	Time of exchange (hr.)	Temp., °C.
NaCl	0	35
NaCl	24	35
NaCl	18	60
NaCl	4 (2 separate expt.)	80
NaNO₃	4	80
NaCl + NaNO₃	3	80
NH₄NO₃	4	30
NH₄NO₃	12	30
NH₄NO₃	2	80
NaCl + dilute HCl to lower initial pH to 7	21	30

A series of experiments of varying conditions was run at 30°. No activity was found in the cyanate with one exception. Exchange was carried out for as long as 24 hours in some experiments. In one experiment at 30°, urease was added to the solution. In this experiment a very high activity was found in a single sample of cyanate taken at 16 hours. However, the amount of urea hydrolyzed in this time under these conditions would be great, and it is possible that the activity is due to carbonate coprecipitated with the cyanate. Some further work is planned on similar experiments.
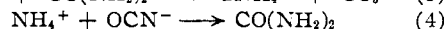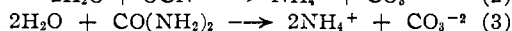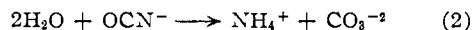
In experiments at 80°, exchange between urea and cyanate was found to occur. All remaining studies were carried out at this temperature.

Calculations.—The familiar equation for the rate of isotopic exchange is[7]

$$R = \frac{ab}{t(a + b)} \ln (1 - f) \qquad (1)$$

where R is the rate of exchange, a and b the constant concentrations of reactants undergoing exchange, t is the time of exchange, and f the fraction exchange at time t, calculated as the specific activity x at time t of the species originally isotopically normal divided by the specific activity x_∞ at isotopic equilibrium.

The urea–cyanate system is a complex one with at least three reactions occurring in addition to the exchange

$$2H_2O + OCN^- \longrightarrow NH_4^+ + CO_3^{-2} \qquad (2)$$
$$2H_2O + CO(NH_2)_2 \longrightarrow 2NH_4^+ + CO_3^{-2} \qquad (3)$$
$$NH_4^+ + OCN^- \longrightarrow CO(NH_2)_2 \qquad (4)$$

The rates of these reactions are all known or can be calculated from existing data. Using these rates, the concentrations of urea and cyanate in the solutions at any time t can be determined.[8] In most of the experiments performed, the time of exchange is short enough so that the change in concentration of the reactants was small (a few % of the original). For this reason, equation 1 was used to calculate the exchange rate with a and b

(7) G. Friedlander and J. W. Kennedy, "Nuclear and Radiochemistry," John Wiley and Sons. Inc., New York. N. Y.. 1955. p. 315.

(8) The rate of hydrolysis of cyanate is taken from (3b). that of urea from (1).

the concentration of urea and cyanate in the solutions at the time the measurements were taken, x the specific activity of the cyanate at time t and x_∞ the specific activity of the cyanate carbon when exchange is complete. This is determined from the total carbon content of the system and the amount of carbon-14 in the system.

With this limiting assumption, we can calculate the rate of exchange R from equation 1. Data and rates are given in Table II. The effects of varying concentrations upon R can be used to determine the form of the rate equation.

Dependence of Rate of Exchange upon Urea Concentration.—Experimental series A and B (Table II) were performed to determine the effect of urea concentration upon the exchange. From the ratio of the average rates and urea concentrations, all other conditions identical, the order in urea is calculated as being 0.88. We feel that this probably indicates a first-order reaction within experimental error.

TABLE II

Temp. 80°; ionic strength 0.500

Series	No. of expt.	$(OCN^-)_0$	$(Urea)_0$	Av. rate × 10⁷, M sec.⁻¹	$R/$ (Urea) × 10⁶, sec.⁻¹
A	6	0.100	0.0500	1.6 ± 0.1	3.2
B	4	.100	.100	2.9 ± .1	2.9
C	8	.110	.100	2.7 ± .4	2.7
D	5	.055	.100	2.0 ± .4	2.0
E	2	.252	.101	2.6 ± .1	2.6
F	2	.192	.111	2.7 ± .1	2.4
G	2	.072	.101	2.3 ± .1	2.3
H[a]	2	.110	.100	1.3 ± .1	..
I[b]	3	.110	.100	2.0 ± .2	..
J[c]	2	.110	.100	1.6 ± .5	..

[a] Reactions run for 24 and 26 hours. Because of the long time for these experiments the extent of hydrolysis of the reactants is large and equation 1 is not valid. However, even with this error, rate constants calculated from these rates using the concentrations at time t agree with the other values quite well. [b] Solution is 0.1 M in NH₄⁺; pH of solution is 6.6. [c] pH of solution lowered to 6.6 by addition of dilute HCl.

Dependence of the Rate of Exchange upon Cyanate Concentration.—Since it has been determined that the rate of exchange is first order in urea, dividing the experimental rate by the urea concentration will give a function which is dependent upon the cyanate concentration only. Inspection of the numerical values thus obtained shows that for all reasonable functions of cyanate concentration there is a non-zero intercept for $R/$ (Urea) as a function of (OCN^-), indicating a term in the rate equation which is zero order with respect to cyanate, i.e.

$$R/(\text{urea}) = k_1 + k_2(OCN^-)^a \qquad (5)$$

The value of "a" can be determined by plotting $R/$ (Urea) against $(OCN^-)^a$ for $a = 1,2, \ldots$, etc., and determining the best fit of the experimental data. This has been done. It is found that $a = 1$ gives the best fit. The graph is given in Fig. 1. A least squares determination of the equation of the straight line leads to the over-all rate equation for the exchange

$$R = 2.5 \times 10^{-6}(\text{urea}) + 1.2 \times 10^{-6}(\text{urea})(\text{OCN}^-) \quad (6)$$

The exact significance of the second term of the rate equation is doubtful due to the possible experimental error in the rates. However, we feel
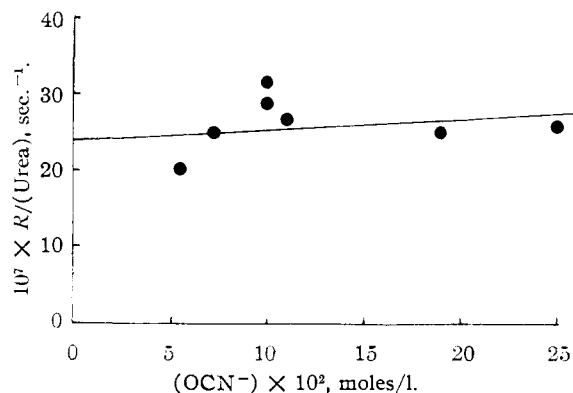

Fig. 1.—Dependence of rate of exchange, R/initial urea concentration, upon initial cyanate concentration.

that the data do show a dependence, even though small, upon the cyanate concentration and therefore leave this term in the rate equation.

Discussion

The most probable first step in the hydrolysis of urea is the dissociation into ammonium cyanate. There must be an appearance of C^{14} in the cyanate due to this step. It has been shown many times that the urea hydrolysis is first order in urea, and thus the exchange through the dissociation into ammonium cyanate should also be first order in urea, and independent of the cyanate concentration with the same rate constant as for the urea decomposition. Different workers have reported different values for this rate constant. The most recent value is that of Shaw and Bordeaux[1] who report 3.3×10^{-6} sec.$^{-1}$ at 80° under conditions approximately the same as for our exchange, the big difference being the presence of the cyanate in the exchange experiments.

It has been observed in most of the studies on the hydrolysis of urea that the first-order rate constant decreases as the extent of the hydrolysis increases. This has been attributed to the reverse reaction. the formation of urea from ammonium and cyanate ions. This should have no effect upon our measurements. If, however, the decrease is due, at least in part, to a reaction between cyanate and urea, we should expect this to have a marked effect upon the rate measured with the high concentration of cyanate present. The difference between our value of 2.5×10^{-6} sec.$^{-1}$ and the 3.3×10^{-6} sec.$^{-1}$ of Shaw and Bordeaux could be due to such a reaction.
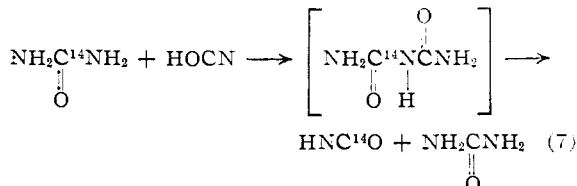
We are performing experiments at the present time on the exchange with labeled cyanate exchanging with isotopically normal urea. Under these conditions exchange due to the reversal of the hydrolysis of the urea should be small. This is analogous to the exchange of N^{15} between labeled ammonia and urea measured by Kistiakowsky and Thompson[9] during enzymatic hydrolysis. Since

(9) G. B. Kistiakowsky and W. E. Thompson, THIS JOURNAL, 78, 4821 (1956).

the exchange rate we have found shows a dependence upon cyanate, there must be another exchange path possible.

The formation of biuret during the hydrolysis of urea has been observed and postulated many times.[8b,10,11]

An exchange of carbon-14 between urea and cyanate could occur through the reversible formation of a biuret-like intermediate in the reaction

$$NH_2C^{14}NH_2 + HOCN \longrightarrow \left[\begin{array}{c} O \\ \| \\ NH_2C^{14}NCNH_2 \\ \| \; | \\ O \; H \end{array} \right] \longrightarrow$$

$$HNC^{14}O + NH_2CNH_2 \quad (7)$$
$$\| \\ O$$

The decrease in exchange rate found upon the addition of ammonium ion (Series I), parallel to the effect of ammonia found by Redemann[11] in the formation of biuret, might be due to a more rapid hydrolysis of the cyanate rather than a decrease in rate. It could, however, indicate that the exchanging species is cyanate ion rather than the acid. Because of the complexity of this reaction, we cannot distinguish. The lowering of the rate by the addition of dilute HCl does indicate that the effect is due to a change in pH (series J).

There is a second possible, although we feel less desirable, explanation of our results. While ammonium cyanate is accepted as the intermediate in the non-enzymatic hydrolysis of urea, ammonium carbamate is thought to be the first step in the enzymatic hydrolysis.[4] It is possible that exchange can occur by both paths, the carbamate formed in the initial step of urea hydrolysis exchanging with the cyanate. The form of the rate equation obtained from such a mechanism would be the same as for the first exchange mechanism.

There are objections to this mechanism which make the first mechanism preferable. If carbamate formation is the first step in the hydrolysis of urea it is difficult to explain the work of Shaw and Bordeaux in the absence of acid, since the carbamate should react quite rapidly even in the aqueous system without added acid and the ammonia formed should be greater than the 1:1 ratio of urea reacting.

The proposed intermediate in a cyanate–carbamate exchange could be of a form proposed previously to account for the hydrolysis of cyanic acid,[3a] but it is not symmetrical and it is not easy to see how a simple rearrangement could lead to exchange in this intermediate.

DURHAM, N. H.

(10) T. Mukaiyama and T. Matsunaga, ibid., 75, 6209 (1953).

(11) C. E. Redemann, F. C. Riesenfeld and F. S. LaViola, Ind. Eng. Chem., 50, 633 (1958).