

**N-DIAZO COUPLING OF 4-CHLOROBENZENEDIAZONIUM CHLORIDE WITH  $\alpha$ -AMINO ACIDS\***

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N-Diazo coupling of 4-chlorobenzenediazonium chloride with  $\alpha$ -amino acids has been studied in aqueous medium by amperometric method. Dependence of the reaction rate on concentration of hydroxonium ions, ionic strength of medium, temperature, and structure of the  $\alpha$ -amino acids has been investigated.

Reactions of arenediazonium ions with amino acids were given considerable attention. The first investigators were Eagle and Vickers<sup>1</sup> who studied the reaction of 4-sulfobenzenediazonium chloride with a series of  $\alpha$ -amino acids from the preparative viewpoint. Later on other authors dealt with isolation of products of the reaction of benzenediazonium chloride and 4-nitrobenzenediazonium chloride with glycine<sup>2</sup>. Zahn, Wolleman and Waschke<sup>3</sup> studied the reaction of 5-chloro-2-methylbenzenediazonium chloride with a series of aliphatic amino acids and found that the reaction products consist of compounds of the type of monoalkyltriazenes and pentazdiene besides a considerable amount of 1,3-diaryltriazenes. The most extensive work in this field was carried out by Howard and Wild<sup>4</sup> who proved that the reaction of benzenediazonium chloride with glycine in alkaline medium results in formation of the derivative of 1,5-diphenyl-1,4-pentazdiene,  $(C_6H_5N=N)_2NCH_2CO_2H$ . The reaction was accompanied by formation of decomposition products from benzenediazonium chloride, the amount of the decomposition products being greater with amino acids of higher molecular weight.

This paper deals with kinetics and mechanism of N-diazo coupling of 4-chlorobenzenediazonium chloride with  $\alpha$ -amino acids. The reaction rate was followed by amperometric method (polarometric method) with indication dropping mercury electrode.

This method is based on reduction of 4-chlorobenzenediazonium ion on the dropping mercury electrode, which gives two well developed waves: a one-electron one at  $-0.3$  to  $-0.4$  V and a four-electron one at  $-0.6$  to  $-0.7$  V (measured against S.C.E.). The gradually formed triazene and pentazdiene compounds are reduced outside the region of the limit current of 4-chlorobenzenediazonium chloride. A practically linear dependence between the limit current value and concentration of 4-chlorobenzenediazonium chloride was attained under the experimental conditions with the imposed voltage of  $-0.45$  V.

\* Part X in the series N-Diazocoupling; Part IX: This Journal 40, 2104 (1975).

Influence of hydroxonium ion concentration, ionic strength and temperature on the N-diazo coupling rate was studied, and also the effect of structure of  $\alpha$ -amino acid was given attention. The acid-base pre-equilibria of the both 4-chlorobenzenediazonium chloride and amino acids were taken into account in the measurements. The aim of the present work was to obtain information useful for simulation the electrophilic interaction of a biological substrate especially proteins and their structural units.

## EXPERIMENTAL

4-Chlorobenzenediazonium chloride was prepared from 4-chloroaniline of *p.a.* purity grade (Bayer, Leverkusen). Glycine, L-alanine, DL-leucine, L-lysine, L-histidine, L-tyrosine, DL-methionine, DL-phenylserine, L-valine, L-threonine and isoleucine were *p.a.* reagents (Loba-Chemie, Wien). The other chemicals used were also of *p.a.* purity grade (Lachema, Brno). Britton-Robinson buffer solutions having accurate ionic strength were used<sup>5</sup>. 0.1M and 0.01M 4-chlorobenzenediazonium chloride solutions were prepared by the known method<sup>6</sup>. Kinetic measurements were carried out with the apparatus described by Elofson and Mecherly<sup>7</sup> and modified in our laboratory<sup>8</sup>. Hydroxonium ion concentration in reaction mixtures was checked with "Precision pH-meter" apparatus (Radelkis, Budapest) using a high-ohm glass electrode (Labora, Prague) and a saturated calomel electrode (Labora, Prague). pK<sub>a</sub> Values of selected  $\alpha$ -amino acids and 4-chlorobenzenediazonium chloride were determined titrimetrically with 0.1M-NaOH with potentiometric indication using a "Universal pH-meter" apparatus (Radelkis, Budapest) connected with a line compensation recorder EZ2, the rate of dosing of sodium hydroxide from the used linear doser being 0.98522 ml/min. The indication electrode was a combined glass electrode OP 8071 (Radelkis, Budapest).

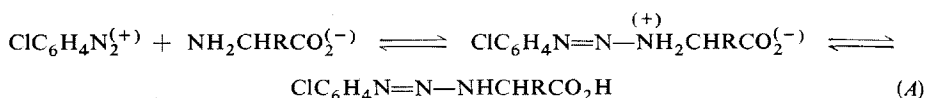
*Kinetic measurements.* 47.5 ml Britton-Robinson buffer solution of the required hydroxonium ion concentration and ionic strength was placed in a tempered reaction vessel. Then the solution was bubbled with a stream of nitrogen for 10 minutes, and 1 ml of equally pre-bubbled 0.01M 4-chlorobenzenediazonium chloride solution was added. After fixation of the limit current, 1.5 ml 0.2M solution of the respective  $\alpha$ -amino acid was pipetted quickly into the vessel, the solution being also pre-bubbled with nitrogen. Nitrogen was introduced into the reaction vessel for the whole reaction time, too. At definite time intervals concentrations of 4-chlorobenzenediazonium chloride were read from the obtained kinetic curve (*e.g.* in the case of glycine, pH 9.50,  $\mu$  0.5, 25°C: 1; 1.5; 2; 2.5; 3; 3.5 min) (these concentrations were proportional to the limit current values), and the corresponding rate constants were calculated.

*Measurements of acid-base equilibria.*  $5 \cdot 10^{-3}$  mol of the respective  $\alpha$ -amino acid was placed into the tempered titration vessel, the equivalent amount of hydrochloric acid was added thereto, and the solution was diluted with distilled water to 50 ml and titrated with 1M sodium hydroxide solution using potentiometric indication. The pK<sub>a</sub> value was read from the obtained potentiometric curves in the usual way. The value  $(pK_a + pK_2)/2$  of 4-chlorobenzenediazonium chloride was determined in analogous way<sup>9</sup>.

## RESULTS AND DISCUSSION

From the kinetic measurements it follows that the primary reaction of 4-chlorobenzenediazonium chloride with  $\alpha$ -amino acids leading to 1-aryltriazene derivative

is (under the chosen experimental conditions: pH 8 to 11, molar ratio of 4-chlorobenzenediazonium chloride to  $\alpha$ -amino acid 1 : 30, temperature 25 to 55°C, ionic strength 0.005 to 1.0) a bimolecular reaction of the second order. It is presumed that the reaction takes place between the arenediazonium cation and the non-protonated amino group of  $\alpha$ -amino acid (reaction (A)). Then the N-diazo coupling rate is given by relation (1).



$$-d[\text{ClC}_6\text{H}_4\text{N}_2^{(+)}]/dt = k_{02}[\text{ClC}_6\text{H}_4\text{N}_2^{(+)}][\text{NH}_2\text{CHR}\text{CO}_2^{(-)}]. \quad (1)$$

TABLE I

Dependence of Rate Constants  $k_2$  ( $1 \text{ mol}^{-1} \text{ min}^{-1}$ ) of N-Diazo Coupling of 4-Chlorobenzenediazonium Chloride with Glycine on Concentration of Hydroxonium Ions at 25°C at Ionic Strength  $\mu$  0.5

pH	$k_2$	pH - log $k_2$	pH	$k_2$	pH - log $k_2$
8.00	3.19	7.60	9.50	77.4	7.61
8.50	10.3	7.49	10.00	193	7.72
9.00	36.6	7.44	10.25	295	7.78

TABLE II

Comparison of N-Diazo Coupling Rate Constants of 4-Chlorobenzenediazonium Chloride with  $pK_a$  Values of the Studied  $\alpha$ -Amino Acids at pH 9.00 (ionic strength 0.5, temperature 25°C)

Amino acid	log $k_2$	$pK_a$	Amino acid	log $k_2$	$pK_a$
Glycine	1.563	9.62	L-Lysine	1.465	8.95
L-Valine	0.984	9.60	DL-Methionine	0.985	9.21
L-Alanine	1.063	9.69	L-Threonine	0.844	9.15 <sup>a</sup>
DL-Leucine	1.085	9.60	DL-Phenylserine	2.142	9.15
Isoleucine	1.167	9.68			

<sup>a</sup> The present paper.

The measurements carried out for the reaction of 4-chlorobenzenediazonium chloride with glycine indicate that the N-diazo coupling rate increases in the region pH 8 to 11. The dependence  $\log k_2$  vs pH is linear in the mentioned region, its slope being practically unity. This fact suggests that, in this region, the arenediazonium cation of  $(pK_1 + pK_2)/2 = 11.70$  is the real reactive form, the substrate being  $\alpha$ -amino acid with non-protonated amino group, the concentration of which increases with pH in the sense of the above acid-base equilibrium (Table I).

Effect of ionic strength on the rate of the abovementioned N-diazo coupling (pH 9.50, 25°C) follows from the experimental results:

$\mu$	0.005	0.02	0.1	0.5	1.0
$k_2, \text{l mol}^{-1} \text{ min}^{-1}$	118	84	126	135	62

The dependence  $\log k_2$  vs  $\mu$  is not clear-cut, but in spite of the relatively great scattering of the rate constants it can be stated that the measured reaction does not depend on ionic strength.

Dependence of the reaction rate on temperature was studied in the range 25 to 55°C (pH 9.50, ionic strength 0.5):

$t, ^\circ\text{C}$	25	35	45	55
$k_2, \text{l mol}^{-1} \text{ min}^{-1}$	135	256	527	896

The values obtained agree well with the Arrhenius relation, the found activation energy being 11.8 kcal/mol.

Table II gives the N-diazo coupling rate constants of nine  $\alpha$ -amino acids and their  $pK_a$  values<sup>10</sup>. Comparison of these rate and equilibrium constants shows no significant correlation. The differences in N-diazo coupling rates of the individual  $\alpha$ -amino acids will probably be caused by some other factors *e.g.* sterical, presence of other hetero atoms in the molecule of  $\alpha$ -amino acid *etc.* We intend to deal with these important problems further.

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