PROPERTIES OF THE ARSENATE-WATER O^{18} EXCHANGE REACTION*

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Published data (Slocum and Varner, 1958; Varner et al., 1958; Slocum et al., 1959) indicate that the half time of the nonenzymatic exchange of arsenate oxygen with water oxygen is sufficiently long to allow the use of O^{18}-arsenate in studies of enzymatic arsenolytic reactions. This note reports the direct determination of the half time of the arsenate-water exchange and also reports the energy of activation of this reaction.

In the experiments described here, arsenate labeled with O^{18} was prepared from water containing approximately 1.4 atom per cent excess O^{18}. Anhydrous di-sodium arsenate was dissolved in O^{18}-enriched water. The solution was placed in sealed ampules and heated at 100° C for 24 hours. The solution was freeze-dried, the salt redissolved in O^{18}-enriched water, sealed in an ampule and again heated at 100° C for 24 hours. This solution was freeze-dried and the salt used in this form.

For the equilibration experiments ordinary water was introduced into the main chamber of a side arm flask and the dry arsenate placed in the side arm. The system was evacuated and the flask tilted so that the water and arsenate were mixed. The arsenate dissolved almost instantaneously in the water, after which the reaction flask (still under vacuum) was immersed in a constant temperature water bath. Prior to collecting a sample, the system was

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Table 1

Conditions	Time min.	A. % excess x 1000	Theor. A. % excess x 1000	Half-time
32° pH 10.0	0	0	166	--
	42	68	166	55
	62	90	166	55
	180	161	166	--
32° pH 8.0	0	0	40	--
	16	9	40	44
	62	25	40	44
	180	38	40	--
32° pH 6.0	0	0	32	--
	6	6	32	20
	1440	32	32	--
	36	59	76	17
	49	63	76	19
	71	64	76	26
	1440	76	76	--
32° pH 2.0	0	0	40	--
	5	35	40	--
	16	36	40	--
	31	37	40	--
	103	36	40	--
	1440	39	40	--

Table 2

Conditions	Time Min.	A. % Excess x 1000	Theor. A. % excess x 1000	Half-time	Activation Energy (Calories)
4° C, pH 8.0	0	0	40	--	4° - 14° 4044
	60	17	40	75	
	72	19	40	78	
	90	22	40	78	
	95	22	40	82	
	121	25	40	85	
	151	31	40	70	
	360	40	40	--	
14° C, pH 8.0	0	0	40	--	14° - 32° 3874
	60	20	40	60	
	120	29	40	64	
	245	37	40	--	
32° C, pH 8.0	0	0	40	--	32° - 55° 3325
	16	9	40	44	
	62	25	40	44	
	180	38	40	--	
55° C, pH 8.0	0	0	37	--	
	19	13	37	31	
	29	19	37	28	
	1440	37	37	--	

"pumped out" for one minute. This was to insure that the aliquot taken was water from the arsenate solution undiluted by water from the vapor phase. The sample was condensed and frozen in an evacuated sample tube connected to the reaction vessel by a ground glass joint and cooled by dry ice. The sample tube was removed from the reaction vessel and dry gaseous CO_2 introduced. After equilibration (Cohn, 1953) the CO_2 was collected in a liquid nitrogen trap, then introduced into a Mass Spectrometer (Consolidated Electrodynamics Corp. Model 21-260) for analysis.

It can be seen (Table I) that at constant temperature the exchange between O-18 arsenate and water varies somewhat with the hydrogen ion concentration. At pH 10, the half time is 55 minutes; at pH 8, 44 minutes; at pH 6.0, 21 minutes;and at pH 2.0 less than 1 minute.

In Table 2 the hydrogen ion concentration was held constant and the temperature varied. The half time of exchange at 4° C is 80 minutes; at 14° C, 62 minutes; at 32° C, 44 minutes; and at 55° C, 30 minutes.

The activation energy calculated from these values is approximately 3700 calories. Thus, from the above data it is apparent that oxygen-18 labeled arsenate has a sufficiently long half time to be useful as a biological tool for the study of _in vitro_ and _in vivo_ enzymatic mechanisms.

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