catalytic constant toward any of the substrates is much lower. (2) It is remarkably unreactive toward *p*-nitrophenyl *N*-benzyloxycarbonyl-L-tyrosinate; in fact, it is doubtful if it has any enzymatic activity at all toward this substrate since the most pure elastase produced in this research has one-tenth the activity of soybean trypsin inhibitor and one-half the activity of bovine serum albumin toward this substrate. (3) The enzymatic specificity of elastase is very feeble compared with that of α-chymotrypsin. From the fastest to the slowest substrate for elastase there is a difference of 250 in second-order rate constants while for α-chymotrypsin there is a difference of one million. Thus elastase does not discriminate very much among the esters used as substrates in this study. The "specificity" of hydroxide ion is 80 from fastest to slowest. Thus the purely "enzymatic specificity" of elastase exclusive of the electronic and steric factors manifest in the hydroxide ion reaction is only a factor of 3. The purely enzymatic specificity factor for α-chymotrypsin is 10,000 and thus it is a much more discriminating and, therefore, a more sophisticated catalyst than either elastase or hydroxide ion.

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Biodehalogenation. The Pathway for Transhalogenation and the Stereochemistry of Epoxide Formation from Halohydrins*

E. W. Bartnicki and C. E. Castro

ABSTRACT: A partially purified protein obtained from a *Flavobacterium* sp. exchanges the halogen atoms of α-halo epoxides. The process has been shown to be reversible and its path delineated. The conversion of epibromohydrin into epichlorohydrin *via* the route: epibromohydrin + chloride $\rightleftarrows$ 1-bromo-3-chloropropanol $\rightleftarrows$ epichlorohydrin + bromide is illustrative. The conversion of halohydrins into epoxides by the enzyme is a stereospecific *trans* process.

The epoxidation of halohydrins (eq 1), hydrolytic epoxide opening (eq 2), and an unusual transhalogenation (eq 3) by a partially purified protein obtained from a *Flavobacterium* sp. have been described (Castro and Bartnicki, 1968). We now wish to outline the general biochemical paths of these enzymatic conversions and to report the stereochemistry of the epoxidation.

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Experimental Section

Materials. All substances employed had physical constants and spectra which checked with the literature, or they were freshly distilled, or preparatively gas chromatographed before

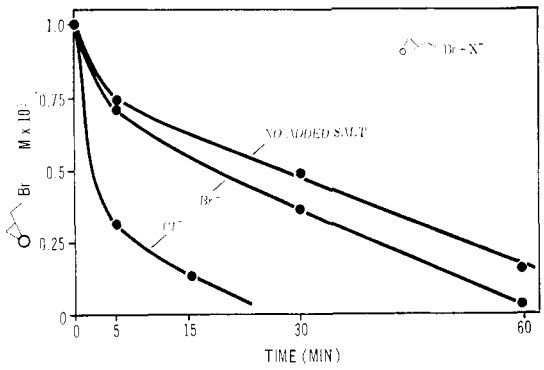


FIGURE 1: The rate of consumption of epibromohydrin in buffer, with added 0.1 M KBr and 0.1 M KCl.

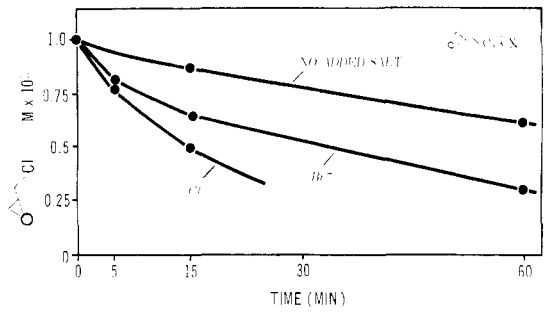
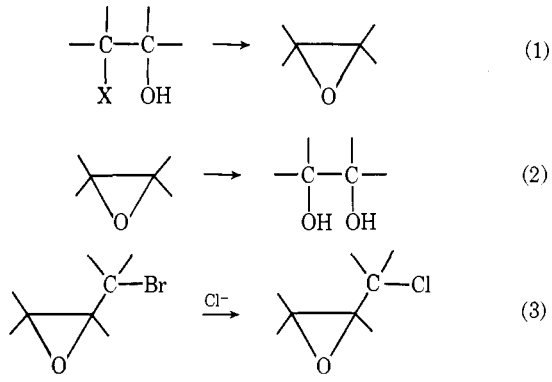


FIGURE 2: The rate of consumption of epichlorohydrin in buffer, with added 0.1 M KBr and 0.1 M KCl.



(1)

(2)

(3)

use. Final purity was established by gas chromatography. All conversions were effected with freshly fractionated protein that had an activity of 300 in the conversion of epibromohydrin into epichlorohydrin (*cf.* Castro and Bartnicki, 1968).

Products and substrates were directly analyzed by gas chromatography. Authentic samples of products, and derivatives obtained by basic hydrolysis, were coemergent with the substances obtained from the reaction. The salient new gas chromatographic information is given in Table I. The emergence times of other products and the derivatives employed for their characterization have been described. As before, bromide ion was monitored potentiometrically.

Enzymatic Conversions. Rate and product runs were conducted in identical fashion. Typically, 10 ml of 10^{-3} M substrate solution containing 0.01 M phosphate buffer (pH 7.0) and 0.25 mg of protein was thermostatted at 24° and magnetically stirred. Reactions in the presence of added halide (KCl or KBr) were 0.1 M in halide ion.

Results and Discussion

The rates of consumption of epibromohydrin and epichlorohydrin, each in the absence of added salt and in the presence of 0.1 M KBr and KCl, are depicted in Figures 1 and 2, respectively. It will be noted that for both epoxides the qualitative capacity of the enzyme(s) to transfer halide or water to the substrate lies in the order $Cl^- > Br^- > H_2O$ or HO^-. Product analyses from the halohydrin reactions that correspond to the rate runs of Figure 1 and 2 are given in Table II along with the results for other substrates employed in this study. It is to be reemphasized that no transformation of any substrate could be noted in these time periods either in the absence of enzyme nor in the presence of boiled enzyme. For those cases in which two or three products are listed, a quantitative analysis of all constituents was not made. Qualitatively, the substances noted account for all of the substrate consumed. The trace quantities of glycidol in the product from epibromohydrin may be due to a small on column conversion of 1-bromo-2,3-dihydroxypropane upon gas chromatography (*cf.* Castro and Bartnicki, 1968). Neither 1-chloro-2,3-dihydroxypropane nor 1-bromo-2,3-dihydroxypropane is converted into glycidol by the purified enzyme.

Taken together the results of Table II and the kinetics accord with the transhalogenation being an equilibrium process. The conversion of epichlorohydrin in the presence of chloride ion into 1,3-dichloro-2-hydroxypropane and the reverse closing of 1,3-dichloro-2-hydroxypropane to epichlorohydrin are illustrative. The data of Table II can be summarized by eq 4–9. Most instructive is an examination of the reversal of eq 4 and 8. The reaction of the mixed-halide 1-bromo-3-chloropropanol with the enzyme in the absence of

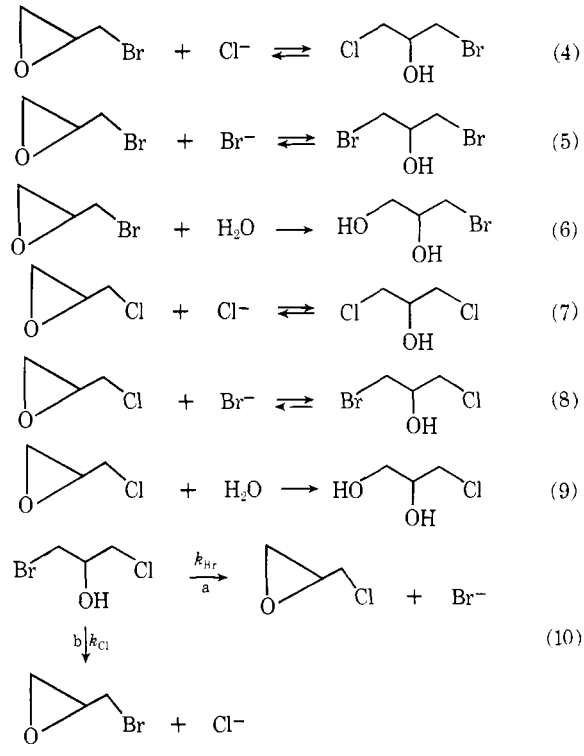


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TABLE I: Gas Chromatographic Analysis of Substrates, Products, and Derivatives.

Substance	Column		Temp (°C)	Time (min)	Hydrolysis Product
	Length (ft)	Packing			
1-Bromo-3-chloropropanol	2	Porapak P[a]	150	12	Glycerol
	1	Porapak P[a]	150	4.6	
1,3-Dichloropropanol	2	Porapak P	150	6.5	Glycerol
	1	Porapak P	150	3.0	
erythro-2-Bromo-3-butanol	5	Porapak P[a]	150	20	
	1	Porapak P	150	4	
threo-2-Bromo-3-butanol	5	Porapak P[a]	150	21	
	1	Porapak P	150	4	
cis-2,3-Epoxybutane	5	Porapak P[a]	150	3.2	DL-2,3-Dihydroxybutane
trans-2,3-Epoxybutane	5	Porapak P[a]	150	3.8	*meso*-2,3-Dihydroxybutane
meso-2,3-Dihydroxybutane	1	Porapak P	150	1.8	
DL-2,3-Dihydroxybutane	1	Porapak P	150	1.8	

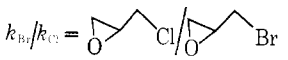
[a] Using 3% diethylene glycol succinate. Both materials obtained from the Varian-Aerograph Co., Inc.

TABLE II: Products from Enzymatic Conversions of the Halohydrins and Halo Alcohols.

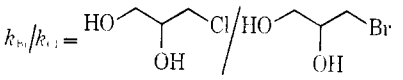
Substrate	Added Salt (0.1 M)	Time (min)	% Conversion	Products (% Yield)[a]
Epibromohydrin	KCl	1	7.4	1-Bromo-3-chloro-2-hydroxypropane[b]
	KCl	60	100	1,3-Dichloro-2-hydroxypropane, 1-chloro-2,3-dihydroxypropane, 1-bromo-2,3-dihydroxypropane
	KBr	1	2.0	1,3-Dibromo-2-hydroxypropane
	KBr	60	95	1-Bromo-2,3-dihydroxpropane, glycidol (trace)
	None	1	1.7	1-Bromo-2,3-dihydroxypropane
	None	60	81	1-Bromo-2,3-dihydroxypropane, glycidol (trace)
Epichlorohydrin	KCl	1	1.6	1,3-Dichloro-2-hydroxypropane
	KCl	60	100	1,3-Dichloro-2-hydroxypropane, 1-chloro-2,3-dihydroxypropane
	KBr	1	1.2	1-Bromo-3-chloro-2-hydroxypropane
	KBr	60	60	1-Chloro-2,3-dihydroxypropane, 1-bromo-2,3-dihydroxypropane, epibromohydrin
	None	1	0.4	1-Chloro-2,3-dihydroxypropane
	None	60	31	1-Chloro-2,3-dihydroxypropane
1-Bromo-3-chloro-2-hydroxypropane	None	5	30	Epichlorohydrin (85), epibromohydrin (15)
	None	120	100	1-Chloro-2,3-dihydroxypropane (86), 1-bromo-2,3-dihydroxypropane (14)
1,3-Dibromo-2-hydroxypropane	None	1	10	Epibromohydrin
1,3-Dichloro-2-hydroxypropane	None	10	50	Epichlorohydrin
		30	69	1-Chloro-2,3-dihydroxypropane, epichlorohydrin
erythro-2-Bromo-3-hydroxybutane		60	75	*trans*-2,3-Epoxybutane
threo-2-Bromo-3-hydroxybutane		60	65	*cis*-2,3-Epoxybutane

[a] Where yields are not given, products are listed in a decreasing order of significance. Yield = (moles of product/moles of substrate converted) 100. [b] Yields of all single products are 100%.

added halide ions should allow an internal assessment of the rates of C-Br (eq 10a) and C-Cl scission (eq 10b) to form the respective epoxides. Thus, during the early stages of the reaction (5 min)

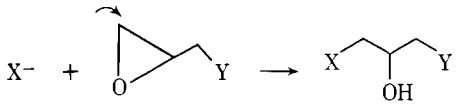


In the latter stages (2 hr) the epoxides themselves will have been converted into the corresponding dihydroxyhalopropanes according to eq 6 and 9. At these times



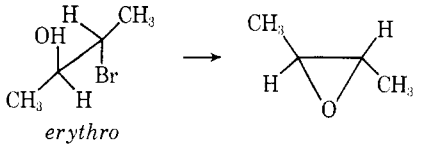
This is indeed the case, and we have calculated $k_{Br}/k_{Cl} = 6.0$ by both methods.

Thus we conclude the transhalogenation proceeds by attack on the terminal epoxide carbon (eq 11, X or Y = Cl^-, Br^-), and we presume the same path is involved in the hydrolytic opening.



(11)

The stereochemistry of epoxide formation by the protein was delineated with the isomeric 3-bromo-2-butanols the results given in Table II are sketched in eq 12 and 13. The epoxide formation step is a stereospecific *trans* elimination of HBr. Regrettably, the butene oxides were not attacked readily by the enzyme in either the presence of Br^- or Cl^- over a 6-hr period, and although a small amount of diol is produced enzymatically, we could not determine the stereochemical consequence of the reversal of eq 13. However, in light of the *trans*-hydrolases obtained from other systems



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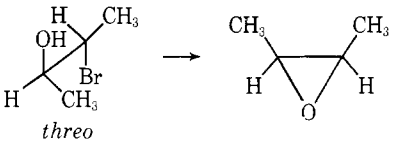
erythro

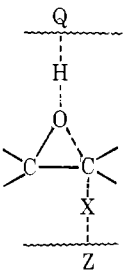


(13)

threo

(Jerina *et al.*, 1968), the *trans* character of the converse reaction (noted herein), and the path of opening observed for terminal oxides, a *trans* process would be implied.

Although our enzyme is not sufficiently pure to warrant any precise formulation for these reactions, it is clear that both epoxidation and transhalogenation could be explained by the same pattern of bond movements at the active site



in which Q and Z may be any of a variety of negatively and positively charged loci.

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