measuring as much as 0.8 mm. in greatest dimension were formed. Precession photographs of the zero and first levels taken with the X-ray beam perpendicular to the plate, showed the crystals to be hexagonal (Laue symmetry D_{6h}-6/mmm). Further examination revealed no systematic absences, so the probable space group is one of P622, P6mm, ($P\bar{6}m2$, $P\bar{6}2m$), or P6/mmm. Cell dimensions as found from the powder photograph are a = 8.96 Å. and c = 7.79 Å.

The preparation containing niobium is dark blue. The X-ray powder pattern is indexable on the basis of a hexagonal cell with a = 9.01 Å. and c = 7.81 Å.

An attempt to substitute Ge^{IV} for Nb^{IV} in reaction (2) was not successful. It was discovered, however, that mixing reactants according to equation (3) resulted in a product structurally similar to those of reactions (1) and (2).

$$0.75BaO + 0.25Ta_2O_5 + 0.50GeO_2 = Ba_{0.75}(Ge_{0.50}{}^{IV}Ta_{0.50}{}^{V})O_{3.0} \quad (3)$$

When a small sample of the preparation containing germanium was cooled slowly, clear hexagon-shaped crystals were formed. Precession photography showed these crystals to have the same Laue symmetry and space group possibilities as the crystals of reaction (1). Cell dimensions as found from the precession and powder photographs are a = 8.96 Å. and c = 7.79 Å. The product has not been analyzed chemically.

Work is being carried out to determine the structure of these compounds using the crystals prepared by reaction (1). The electrical properties are also being examined. From preliminary measurements on a pressed pellet of the niobium compound, it was found to be an n-type conductor and to have thermoelectric properties. A plot of resistivity versus temperature from 70 to 145° produced a straight line with the resistivity increasing with temperature.

This work has been supported by a contract between the Office of Naval Research and The University of Connecticut. Reproduction in whole or part is permitted for any purpose of the United States Government.

DEPARTMENT OF CHEMISTRY
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RECEIVED JANUARY 14, 1958

THE ACTIVE SITE OF THROMBIN

Sir:

In previous communications[1,2] we have reported the inactivation of thrombin by diisopropylphosphorofluoridate (DFP) and on the stoichiometry of the reaction. We now wish to report the isolation of a series of radioactive peptides from acid hydrolyzed P^{32}-labeled diisopropylphosphoryl (DIP) thrombin. These experiments identify serine as the site of the DFP binding and a sequence of Asp.-Ser.Gly as the amino acids about the serine residue.

Thrombin was inactivated by DFP^{32} and purified by column chromatography as previously described.[2] The enzyme was degraded by mild acid hydrolysis, and P^{32}-containing peptides were isolated by column chromatography on Dowex-50 as described by Schaffer, *et al.*[3] Qualitative and quantitative amino acid analysis of the peptides was performed utilizing the two-dimensional paper chromatographic system of Irreverre and Martin.[4] Compositions of these phosphopeptides are shown in Table I.

TABLE I

RADIOACTIVE PHOSPHOPEPTIDES ISOLATED FROM DIP^{32}-THROMBIN

Fraction of peptide	Amino acid composition
2	(Asp, Ser, Gly)
4	(Asp, Ser)
5	(Ser, Gly)
7	(Asp, Ser, Gly)
8	(Asp, Ser, Gly, Glu, Ala)

All amino acids present in the individual fractions 2 through 7 were in a molar ratio of 1; glycine in fraction 7, however, was present in a greater proportion. The yield of fraction 8 was too small to estimate proportions accurately. Quantitative column chromatography according to the method of Moore and Stein,[5] of fraction 2, performed by Dr. D. R. Kominz (this laboratory), gave a molar ratio of Asp:Ser:Gly = 1:0.96:0.93. End group analysis (N-terminal) of fraction 2, carried out by Dr. J. E. Folk, National Institute of Dental Research, yielded aspartic acid. Since serine is common to all fractions, it is evident that this amino acid residue contains the bound phosphate. The data establish the sequence Asp.Ser.-Gly for fraction 2.

The fact that the radioactive fractions which emerged from the Dowex-50 column corresponded identically in number, volume displacement, distribution and amino acid content to those observed by Schaffer, *et al.*,[3] for DIP^{32}-chymotrypsin, make it very likely that the position of the second glycine in fraction 7 is N-terminal, thus giving the sequence Gly.Asp.Ser.Gly. It is also noteworthy that the composition of each peptide is in excellent agreement with the over-all presumed sequence Gly.Asp.Ser.Gly.Glu.Ala. reported for chymotrypsin[3,6] and the Asp.Ser.Gly sequence found in trypsin.[6,7] The possibility that this agreement occurs by chance is exceedingly remote.

The amino acid compositions of the fractions are also in remarkable agreement with these reported by Koshland and Erwin for the active site of phosphoglucomutase.[8] We wish to postulate, therefore, the sequence Gly.Asp.Ser.Gly(Glu, Ala) as a portion of the active site of thrombin.

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(1) J. A. Gladner and K. Laki, *Arch. Biochem. and Biophys.*, **62**, 501 (1956).

(2) J. A. Gladner, K. Laki and F. Stohlman, *Biochim. et Biophys. Acta*, in press.

(3) N. K. Schaffer, L. Simet, S. Harshman, R. R. Engle and R. W. Drisko, *J. Biol. Chem.*, **225**, 197 (1957).

(4) F. Irreverre and W. Martin, *Anal. Chem.*, **26**, 257 (1954).

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 633 (1951).

(6) N. K. Schaffer, R. R. Engle, L. Simet, R. W. Drisko and S. Harshman, *Fed. Proc.*, **15**, 347 (1956).

(7) N. K. Schaffer, *J. Biol. Chem.*, personal communication, in press.

(8) D. E. Koshland and M. J. Erwin, THIS JOURNAL, **79**, 2657 (1957).

These findings are strongly indicative of a common "active center" in thrombin, chymotrypsin and trypsin, and phosphoglucomutase, enzymes of varying specificity from serum, pancreas, and muscle, respectively. Further sequence analysis of phosphopeptides obtained by acid and enzymatic hydrolysis are in progress and will be reported in a later publication.

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RECEIVED JANUARY 17, 1958

RATE AND MECHANISM OF THE ELECTROOXIDATION OF IODIDE[1]

Sir:

We wish to report the first known instance of an electrode reaction involving an electron transfer process which has substantiated second order kinetics. In a range of potentials between +0.6 and +1.1 v. (*versus* the normal hydrogen electrode), the anodic current component of the polarographic wave of iodide in 0.1 *M* perchloric acid at a conical platinum microelectrode corresponded to the reaction

$$2\mathrm{I}^- \xrightarrow{k''_{\mathrm{ox}}} \mathrm{I}_2 + 2\mathrm{e}^- \qquad (1)$$

and was found to obey the rate law

$$i_{\mathrm{a}} = -10^{-6}FAk''_{\mathrm{ox}}(a^0{}_{\mathrm{I}^-})^2 \qquad (2)$$

where i_{a} (expressed in amperes) denotes the anodic component of the current, F is the faraday (expressed in coulombs), A is the effective area (in sq. cm.) of the indicator electrode, k''_{ox} denotes a rate constant (expressed in cm.^{4}/mole sec.) referred to unit electrode area, and $a^0{}_{\mathrm{I}^-}$ denotes the molal activity of iodide at the electrode surface. In accordance with concepts of the absolute rate theory,[2] it was postulated that the second order rate constant in Eq. 2 was correlated with the relevant electrode potential E

$$k''_{\mathrm{ox}} = (k''_{\mathrm{ox}})^0 \exp\left[(1-\alpha)(E-E^0)\frac{2F}{RT}\right] \qquad (3)$$

where $(k''_{\mathrm{ox}})^0$ defines a "specific rate constant" which is operative at the standard potential E^0 of the iodine–iodide couple and $(1-\alpha)$ is the transfer coefficient. It was assumed that the converse process

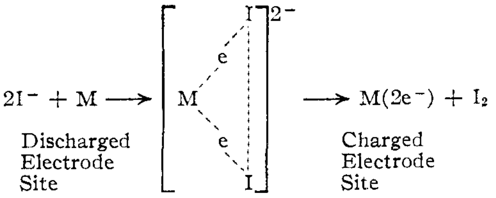
$$\mathrm{I}_2 + 2\mathrm{e}^- \xrightarrow{k'_{\mathrm{red}}} 2\mathrm{I}^- \qquad (4)$$

which yielded the cathodic component i_{c} on the current-voltage wave, was governed by first order electrode kinetics.[2] Using Eq. 2 and 3 as specific premises, the wave equation (5) was derived for anodic current–voltage curves of iodide

$$-i/(i-i_1)^2 = [(FAm^2{}_{\mathrm{Red}}/f^2{}_{\mathrm{Red}}k''_{\mathrm{ox}}) + (k'_{\mathrm{red}}f_{\mathrm{Ox}}FAm^2{}_{\mathrm{Red}}/k''_{\mathrm{ox}}f^2{}_{\mathrm{Red}}m_{\mathrm{Ox}})]^{-1} \qquad (5)$$

Eq. 5 is based on principles[3] generally applicable to steady state situations in which both mass transfer (by diffusion and forced convection) and electron transfer are current-controlling factors. The symbol m in Eq. 5 denotes "mass transfer coefficients" which depend on the prevailing conditions of diffusion and flow, f represents activity coefficients, i_1 is the anodic limiting current, and i (the net current) = $i_{\mathrm{a}} + i_{\mathrm{c}}$; the subscripts Ox and Red refer to iodine and iodide, respectively.

Under judiciously controlled experimental conditions, Eqs. 2, 3 and 5 have been verified for ten current–voltage curves of iodide obtained in flowing solutions, covering a range of flow velocities between 100 and 700 cm./sec. The values of the relevant specific rate constants were determined as $(k''_{\mathrm{ox}})^0$ = 0.03 cm.^{4}/mole sec. and $k'_{\mathrm{red}})^0$ = 60 cm./sec. The corresponding rate laws are accounted for by the reaction mechanism



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DEPARTMENT OF CHEMISTRY
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RECEIVED JANUARY 20, 1958

A NOVEL REARRANGEMENT OF TWO γ-BENZOYLOXYCYCLOALKANONES

Sir:

Treatment of 4-benzoyloxycyclohexanone,[1] m.p. 63–64.5°, $\lambda_{\max}^{\mathrm{CCl}_4}$ 5.80 μ, with 1.1 molar equivalents of potassium *t*-butoxide in *t*-butyl alcohol gave, in 53% yield, an *acidic isomer*, I, m.p. 67–68°, $\lambda_{\max}^{\mathrm{CCl}_4}$ 3.0–3.5, 5.84, 5.97 μ, $\lambda_{\max}^{\mathrm{EtOH}}$ 244 mμ (ϵ 16,700) (*Anal.* Calcd. for $\mathrm{C_{13}H_{14}O_3}$: C, 71.54; H, 6.46; equiv. wt., 218. Found: C, 71.68; H, 6.55; neutralization equiv., 219), which gave a 2,4-dinitrophenylhydrazone of the corresponding methyl ester, m.p. 161–162° and 172–173°, $\lambda_{\max}^{\mathrm{CHCl}_3}$ 5.78, 6.20, 6.29 μ, $\lambda_{\max}^{\mathrm{CHCl}_3}$ 384 mμ (ϵ 29,400) (*Anal.* Calcd. for $\mathrm{C_{20}H_{20}N_4O_6}$: C, 58.25; H, 4.89; N, 13.58. Found: C, 58.43; H, 5.09; N, 13.74). I is assigned a 2-benzoylcyclopropanepropionic acid structure on the basis of this evidence and these considerations: (i) it failed to add bromine and to reduce aqueous potassium permanganate, indicating the absence of ethylenic unsaturation; (ii) its infrared band at 5.97 μ bespoke the presence of a benzoylcyclopropyl system (*cf.* benzoylcyclopropane,[2] $\lambda_{\max}^{\mathrm{CCl}_4}$ 5.96 μ, with acetophenone, $\lambda_{\max}^{\mathrm{CCl}_4}$ 5.91 μ); although the position of this band could also be

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[1] From a thesis by R. A. Javick.

[2] S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Company, Inc., New York, N. Y., 1941, pp. 575–580.

[1] E. R. H. Jones and F. Sondheimer, *J. Chem. Soc.*, 615 (1949).

[2] Sample kindly supplied by Dr. W. J. Close, Abbott Laboratories.