Table I[a]

NaBH$_4$, *M*	Changes in expt	Glutamate formed: nmoles	Glutamate formed: Moles/50,000 g of enzyme
0.02[b]	None	132	1.65 ± 0.17[c]
0.02	2 mg of enzyme	82	2.05
0.02	0.5 mg of enzyme	14	1.40
0.55[d]	None	102	1.27 ± 0.42[c]
0.55	0.005 *M* α-ketoglutarate	48	0.60
0.55	0.001 *M* α-ketoglutarate	13	0.17
0.55	0.0005 *M* α-ketoglutarate	4	0.05
0.55	0.0001 *M* α-ketoglutarate	1.6	0.020
0.55	NaBH$_4$ added before α-ketoglutarate	0	0.00
0.55	Nonlabeled α-ketoglutarate added, then NaBH$_4$, then labeled α-ketoglutarate	0	0.00
0.02[b]	4×10^{-5} *M* added DPN	96	1.20
0.02	4×10^{-6} *M* added DPNH	332	4.15
None	20 mg of $Na_2S_2O_4$ in place of NaBH$_4$	64	0.90

[a] Experiments were conducted at 0°, with reagents added in the following order: glutamate dehydrogenase (Boehringer), 4 mg; ammonium acetate, 0.2 *M*, pH 9.3; α-ketoglutarate-5-^{14}C, 5×10^7 dpm/mmole, 0.02 *M*; NaBH$_4$, as noted below, or $Na_2S_2O_4$. The final volume was 1 ml. The mixture was incubated 10 min, and then protein was precipitated with 1 *M* $HClO_4$ and centrifuged. Glutamate was separated by ion-exchange chromatography on Dowex 50 and was assayed for ^{14}C by liquid scintillation. [b] NaBH$_4$ in 10^{-3} *M* NaOH, added in 20 portions over 10 min. [c] Average ± standard error of mean. [d] NaBH$_4$, 5 mg, added as solid.

the borohydride. The reaction was unaffected by addition of 0.02 *M* DL-glutamate or 0.02 *M* DL-α-hydroxyglutarate, or variation of pH from 8.1 to 10.3. Several experiments indicated that sodium dithionite could replace borohydride as reducing agent.

Kinetic studies with catalytic amounts of enzyme in a similar system, with 1.6×10^{-4} *M* DPNH and without borohydride, have shown that the rate of over-all enzyme reaction depended on the concentration of α-ketoglutarate in almost exactly the same manner as did the formation of α-iminoglutarate.

The enzyme was specifically assayed for bound DPN or DPNH by fluorometry of DPNH.[10] No bound coenzyme was detected, and the sensitivity of the method enabled the detection of 0.03 molecule per enzyme subunit. The nonmediation by coenzyme was corroborated by the effects on borohydride reduction of the addition of much larger amounts of DPN or DPNH (Table I).

Curiously, about one or two molecules of glutamate was formed per enzyme subunit of mol wt 50,000, regardless of reaction conditions, suggestive of a stoichiometric reaction. The reason for this is not immediately clear; however, we have found that the enzyme retained full activity under these conditions.

The product from borohydride reductions was identified as glutamate-^{14}C by gradient ion-exchange chromatography, recrystallization with carrier, and paper chromatography in three different solvent systems. In the latter, the radioactivity coincided with ninhydrin color of added glutamate. The glutamate-^{14}C was recrystallized with D- and L-glutamate·HCl after Graff, Rittenberg, and Foster,[11] and the results, shown in Figure 1, indicate that the glutamate formed from the presumed enzyme-bound α-iminoglutarate was predominantly in the L configuration. The dithionite product was identified as glutamate by ion exchange and paper chromatography; the stereochemistry has not yet been determined.

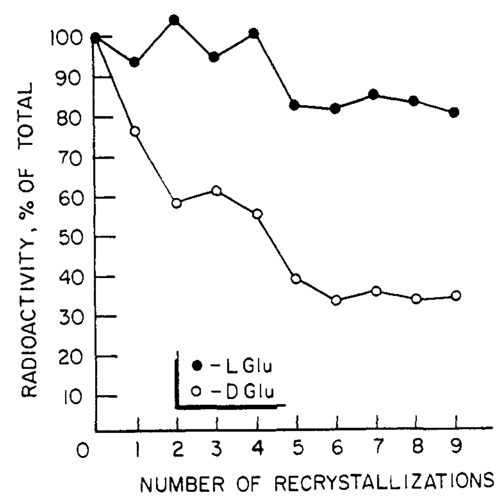

Figure 1. Carrier L- or D-glutamic acid hydrochloride was added to portions of the labeled glutamate produced as described in Table I, row 1. The carrier plus label was recrystallized from water-acetone, and samples from each recrystallization were assayed.

The stereochemistry of the glutamate from borohydride reduction, the dependence on active enzyme, and the correlation of over-all enzyme rate and extent of α-iminoglutarate formation with the concentration of α-ketoglutarate all suggest that the α-iminoglutarate formation by the enzyme is a part of the enzyme reaction rather than a side reaction or nonspecific reaction. Further studies are in progress. In particular the effect of ammonia concentration, analogs of ammonia, and other reductive trapping agents will be investigated, and attempts will be made to determine the reason for the near-stoichiometric amount of α-iminoglutarate that is produced.

Acknowledgment. We gratefully acknowledge the technical assistance of Mrs. Evelyn Connor.

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Received July 31, 1969

Spectral Evidence for the Existence of the Superoxide Ion in Molten LiF-NaF-KF

Sir:

We wish to report the existence of an unusual oxidant in molten LiF-NaF-KF (46.5-11.5-42.0 mole %) at 500°. We believe this oxidant to be the superoxide ion, O_2^-. This solute species can be added directly to the molten solvent (as NaO_2) or can be generated by the selective oxidation of oxide ion in the melt.

The addition of sodium superoxide (NaO_2) to molten LiF–NaF–KF yields a yellow solution. The spectrum of this solution consists of an absorption peak at 372 nm and a shoulder at 260 nm, which is on the side of an absorption edge in the ultraviolet region. The 372-nm peak agrees with the spectrum ascribed to superoxide ion in liquid ammonia by Thompson and Kleinberg.[1] Diffuse reflectance spectra of NaO_2 reported by Griffiths, Lott, and Symons[2] also place the superoxide absorbance in the region of 370 nm. The 260-nm shoulder was not reported in either investigation.

The superoxide ion can be generated by the addition of either of the fluorinating agents MnF_3 or CoF_3 to the molten solvent which contains oxide ion.[3] When MnF_3 is added to the melt, a spectrum attributable to Mn(III) is observed in the region of 525 nm. The melt spectrum of Mn(III) is similar but not identical with that reported in other media;[4] differences in the spectra will be described in a future paper. Along with the expected spectrum of Mn(III), the absorption spectrum of O_2^- is noted. The addition of CoF_3 to the molten solvent results in no spectral evidence for Co(III); instead the Co(II) spectrum similar to that seen by Young and White[5] is observed along with the 372-nm peak and the 260-nm shoulder. A previous study indicated that Co(III) decomposed under these conditions.[6]

In molten LiF–NaF–KF which contains oxide ion, spectral evidence indicates that O_2^- is formed by the reaction of the melt with elemental oxygen and, further, is generated electrochemically at a platinum anode. Electrochemical evidence for the stability of peroxide in molten LiF–NaF–KF at 600° was reported by Pizzini and Morlotti.[7] Zambonin and Jordan have reported the existence of superoxide in molten nitrate based on electrochemical studies.[8]

The molten solution of O_2^- in LiF–NaF–KF is quite stable if the melt is contained in a silica cell or platinum windowless cell.[9] In the former cell, the solution could be maintained under a pressure of *ca.* 5 μ for a period of 2 hr with no decrease in the absorbance of the 372 nm peak. The oxidation ability of the solution was demonstrated by the addition of Co metal with a resultant disappearance of the O_2^- spectrum and the appearance of the Co(II) spectrum. The O_2^- ion was found to be unstable in graphite. The absorbance at 372 nm gradually disappeared over a period of several hours probably owing to the formation of volatile CO or CO_2.

Based on spectral evidence, the superoxide ion can be generated by the addition of solid UO_2 to a solution of Mn(III) in molten LiF–NaF–KF. In this study an excess amount of MnF_3 was added to molten LiF–NaF–KF solvent in a graphite cell. Superoxide ion was generated, from oxide impurities in the solvent, and removed by reaction with the container as stated previously. It is interesting to note that the resultant melt at this point was markedly improved in clarity and exhibited only the spectrum of Mn(III). Upon the addition of UO_2, the reaction of Mn(III) with O^{2-} supplied by UO_2 resulted in the reappearance of the absorption peak at 372 nm. This generation of superoxide ion from oxide ion in the presence of MnF_3 could lead to a spectrophotometric method for the determination of oxide directly in molten salts. Further studies of this seemingly unusual solute species are underway to confirm its identity.

The spectral studies described herein were performed in a 1-cm^2 quartz cell, a $^3/_8$-in. i.d. graphite captive liquid cell,[9] or a 0.25-in. i.d. platinum captive liquid cell.[9] The captive liquid cells were contained in a vacuum furnace especially designed for spectrophotometric studies of molten salts.[9] A helium atmosphere was maintained within the furnace, and the absorption spectra were obtained with a Cary Model 14-M spectrophotometer. Additions of solute to the molten solvent were made through a metal ball valve. The solutes, as solids, were loaded into a compartment that is an integral part of the valve body; this operation was performed in an inert atmosphere box. The compartment was then sealed to avoid contact with the atmosphere.

Acknowledgment. The authors wish to acknowledge the help of D. E. LaValle, Oak Ridge National Laboratory, who supplied the MnF_3 and NaO_2 used in this study. This research was sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corp.

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Received May 21, 1969

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Perfluoro-1-methylpropenylsilver

Sir:

We wish to report the preparation of *trans*-perfluoro-1-methylpropenylsilver, the first example of a perfluorovinylsilver compound, by the nucleophilic addition of silver fluoride to perfluoro-2-butyne.

$$AgF + CF_3C{\equiv}CCF_3 \longrightarrow \textit{trans}\text{-}CF_3CF{=}C(CF_3)Ag$$

Unlike 1-methylpropenylsilver, which decomposes at room temperature,[1] perfluoro-1-methylpropenylsilver is a thermally stable compound and was isolated by sublimation *in vacuo* at temperatures up to 175°. Its structure was supported by cleavage reactions of the carbon–silver bond and by its absorption spectra and method of preparation.[2] Only the *trans* isomer appeared to be formed. It was found to be soluble in donor-type aprotic solvents, such as ethers and nitriles, and was attacked by oxygen and by water in solution.

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(2) The state of aggregation of *trans*-$CF_3CF{=}C(CF_3)Ag$ has not been determined in solution. A peak corresponding to $(CF_3CF{=}CAgCF_3)_4$ was found in the mass spectrum of the crystalline material.