most elements exhibit a higher tendency to form negatively charged complexes in hydrochloric acid media than in nitric acid media, these results may be useful for separations and purification of thorium with anion-exchange resins.

On the basis of ionic radius considerations alone, one would conclude that the tendency of the chloride ion to form complexes should be higher than that of the nitrate ion. It has been shown that reversal in this order can be expected as a consequence of the structure of the nitrate ion and resulting high polarizability.[7] However, it is interesting to observe that among the elements previously studied only Nd(III), Pr(III), Sm(III), Eu(III)[7] and the transuranic elements in the +3 and +4 state such as Pu(III), Pu(IV),[7] Am(III)[8] and possibly Np(IV)[7] have less tendency to complex formation with the chloride ion than with the nitrate ion.

(7) J. C. Hindman, "Ionic and Molecular Species of Plutonium in Solution," National Nuclear Energy Series, Vol. 14-A, pp. 333, 346, 463.

(8) G. N. Yakolev and V. N. Kosyakov, "Spectrophotometric Studies of the Behaviour of Americium Ions in Solution," Proceedings of the International Conference on Peaceful Uses of Atomic Energy, Vol. 7, p. 363, United Nations, 1956.

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RECEIVED SEPTEMBER 27, 1956

## UNEXPECTED FORMATION OF ANHYDRO COMPOUNDS IN THE SYNTHESIS OF ASPARAGINYL AND GLUTAMINYL PEPTIDES[1]

*Sir:*

It was of considerable interest to find that some peptide-forming reagents lead to what appear to be intramolecular dehydrations during the formation of asparaginyl peptide bonds and, to a minor degree, during the formation of glutaminyl peptide bonds. Carbobenzoxy-L-asparagine previously has been coupled with the benzyl and methyl esters of S-benzyl-L-cysteine by the *o*-phenylene chlorophosphite,[2] diethyl chlorophosphite,[3] tetraethyl pyrophosphite,[3] phosphorazo[4] and *sec*-butyl chlorocarbonate[4] methods of peptide synthesis. We wish to report that the use of the tetraethyl pyrophosphite or N,N′-dicyclohexylcarbodiimide reagents for the preparation of asparaginyl-S-benzyl-cysteine peptides leads to the formation of compounds having the composition of anhydrides of the expected products, in addition to the expected protected peptides. The coupling of carbobenzoxy-L-asparagine with S-benzyl-L-cysteine methyl ester by the pyrophosphite procedure in diethyl phosphite solution gave the expected product ($C_{23}H_{27}O_6N_3S$, m.p. 199–200°) in 35% yield, and 27% of a compound of m.p. 128–129°, $[\alpha]^{24}D$ −42.1° (*c* 1, acetic acid) (*Anal.* Calcd. for $C_{23}H_{25}O_5N_3S$: C, 60.6; H, 5.53; N, 9.22; S, 7.04. Found: C, 60.6; H, 5.73; N, 9.11; S, 7.08). The use of dicyclohexylcarbodiimide in tetrahydrofuran solution for this coupling gave 39% of the protected dipeptide ester and 26% of the anhydro compound. Treatment of tosyl-L-glutaminyl-L-asparagine and S-benzyl-L-cysteine benzyl ester with dicyclohexylcarbodiimide in dimethylformamide solution yielded 40% of the tosyl tripeptide ester, m.p. 228–229°, $[\alpha]^{23}D$ −30.7° (*c* 1, dimethylformamide) (*Anal.* Calcd. for $C_{33}H_{39}O_8N_5S_2$: C, 56.8; H, 5.63; N, 10.0; S, 9.17. Found: C, 56.6; H, 5.75; N, 10.0; S, 9.04), and 15% of its anhydro derivative, m.p. 210–211°, $[\alpha]^{23}D$ −33.5° (*c* 1, dimethylformamide) (*Anal.* Calcd. for $C_{33}H_{37}O_7N_5S_2$: C, 58.0; H, 5.40; N, 10.3; S, 9.43. Found: C, 58.0; H, 5.56; N, 10.3; S, 9.14). No by-product of the type described could be detected when carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteine methyl ester was prepared in tetrahydrofuran solution through the mixed anhydrides of carbobenzoxy-L-asparagine with carboxylic or alkyl carbonic acids. The best preparative procedure employed isovaleryl chloride to form the mixed anhydride, and gave a 58% yield of the protected dipeptide ester.

Dehydration took place to a lesser extent during the preparation of carbobenzoxy-L-glutaminyl-S-benzyl-L-cysteine methyl ester. Tetraethyl pyrophosphite in diethyl phosphite gave a 70% yield of the expected product, m.p. 201°, $[\alpha]^{23}D$ −28.0° (*c* 1, dimethylformamide) (*Anal.* Calcd. for $C_{24}H_{29}O_6N_3S$: C, 59.1; H, 5.99; N, 8.62. Found: C, 59.2; H, 6.07; N, 8.53), and only 5% of the anhydro compound, m.p. 103–104°, $[\alpha]^{23}D$ −35.0° (*c* 1, dimethylformamide) (*Anal.* Calcd. for $C_{24}H_{27}O_5N_3S$: C, 61.4; H, 5.75; N, 8.94. Found: C, 61.7; H, 5.90; N, 8.75). The dicyclohexylcarbodiimide coupling, in tetrahydrofuran solution, gave 76% of the protected dipeptide ester, and no detectable anhydro compound.

(1) Alteration of asparagine during the course of peptide synthesis using tetraethyl pyrophosphite has been independently demonstrated in this laboratory by C. Ressler (THIS JOURNAL, **78**, 5956 (1956)). In the synthesis of the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinamide, a side product was isolated in which it was found that the asparagine had undergone change.

(2) S. J. Leach and H. Lindley, *Aust. J. Chem.*, **7**, 173 (1954).

(3) R. A. Boissonnas, St. Guttmann, P.-A. Jaquenoud and J.-P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

(4) J. Rudinger, J. Honzl and M. Zaoral, *Collection Czechoslov. Chem. Communs.*, **21**, 202 (1956).

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RECEIVED AUGUST 15, 1956

## ON THE MOLECULAR WEIGHT, SIZE AND SHAPE OF THE MYOSIN MOLECULE

*Sir:*

Despite progress in several laboratories on the macromolecular characterization of myosin, no conclusive results have hitherto appeared. Part of the difficulty has recently been ascribed to spontaneous, strongly temperature dependent, side-to-side molecular aggregation.[1] Consequently, the molecular unit can be studied only by extremely rapid preparation and experimental analysis of the protein, working entirely in the cold up to the moment of actual measurement. We present here a summary of results obtained observing these precautions.

(1) A. Holtzer, *Arch. Biochem. & Biophysics*, in press.

Myosin was prepared in the standard way[2,3] and complete light scattering, viscosity and ultracentrifuge measurements were made within sixty hours after the rabbit's demise. Within this period the ultracentrifuge showed a single peak. Longer storage (at 4°) caused the appearance of a second, faster peak indicating some dimer formation. All the data reported here, therefore, refer to a monodisperse protein.

Results for three samples are given in the table.

TABLE I

| Sample | $[S_{20,w}] \times 10^{13}$ | $[\eta]$ (dl/g) | $M \times 10^{-3}$ | $(\overline{\rho^2})^{1/2}$ (Å.) |
|---|---|---|---|---|
| VII | 6.38 | 2.3 | 570 | 487 |
| VIII | 6.43 | 2.2 | 500 | 473 |
| IX | .... | 2.4 | 526 | 470 |

A typical angular scattering envelope, extrapolated to zero concentration, is shown in Fig. 1. The molecular weight, $M$, and r.m.s. radius of gyration, $(\overline{\rho^2})^{1/2}$, were determined from the curve in the usual way.[4] The intrinsic viscosity, $[\eta]$, and sedimentation constant, $[S_{20,w}]$, were obtained by extrapolation to infinite dilution. The solvent was 0.6 $M$ KCl and the temperature of measurement 25°.

The values for $[\eta]$ and $[S_{20,w}]$ found here are in good agreement with some of those in the literature.[5,6,7] The observed molecular weight, though lower than earlier values,[6,7] agrees well with the more recent estimate of Laki and Carroll.[8]

These data may be used to elucidate the molecular configuration. In particular, we can test the consistency of the most likely models, the rod and random coil.

To test the rod model we use the theoretical relations for intrinsic viscosity and sedimentation constant.[9,10] The equations relate these properties to the length, $L$, diameter, $d$, and mass of a rigid string of spherical beads. Since the diameter is the only parameter not directly measured, the consistency of this model depends upon whether a single value of this quantity fits the viscosity and sedimentation behavior.

The viscosity equation can be written[10]: $[\eta] = 24\bar{\nu}J^2/9000 \ln J$, where $J$ is the axial ratio and $\bar{\nu}$ the partial specific volume.[11] From this we get $J = 71$. Since the light-scattering radius corresponds to a rod-length of 1650 Å., we obtain 23.3 Å. for the diameter.

If Stokes' law is assumed for the spherical rod-segments, the sedimentation equation becomes[9]: $[S] = (1 - \bar{\nu}\rho_0)d^2 \ln (6M\bar{\nu}/N\pi d^3)/18\ \bar{\nu}\eta_0$ with $\eta_0$ the solvent viscosity, and $N$ Avogadro's number. This equation yields 27.4 Å. for the diameter.

(2) A. Szent-Györgyi, "The Chemistry of Muscular Contraction," Academic Press, New York, N. Y., 1951, 2nd edition, p. 146.

(3) W. F. H. M. Mommaerts and R. G. Parrish, *J. Biol. Chem.*, **188**, 545 (1951).

(4) B. H. Zimm, *J. Chem. Phys.*, **16**, 1093 (1948).

(5) A. Szent-Györgyi, ref. 2, p. 39.

(6) H. Portzehl, *Z. Naturforsch.*, **5b**, 75 (1950).

(7) R. G. Parrish and W. F. H. M. Mommaerts, *J. Biol. Chem.*, **209**, 901 (1954).

(8) K. Laki and W. R. Carroll, *Nature*, **175**, 389 (1955).

(9) J. G. Kirkwood and J. Riseman, *J. Chem. Phys.*, **18**, 512 (1950).

(10) J. G. Kirkwood and P. Auer, *ibid.*, **19**, 281 (1951).

(11) We use the value 0.728 as determined by W. F. H. M. Mommaerts and R. G. Parrish, ref. 7.

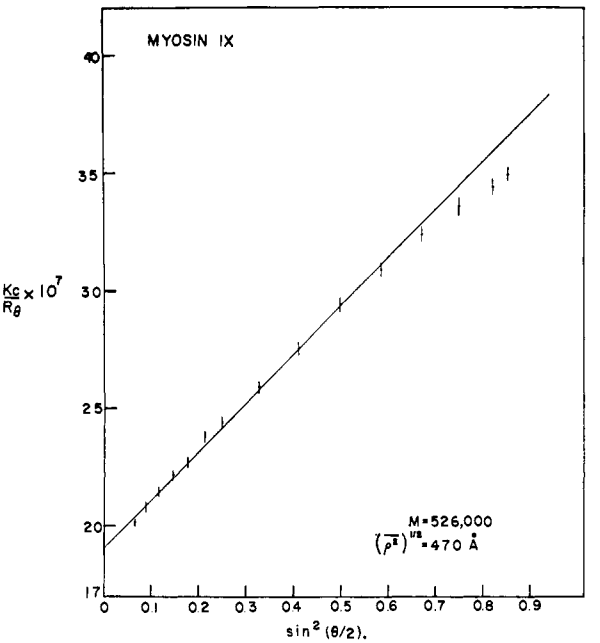


Fig. 1.—Extrapolated angular scattering curve for pure myosin.

A third estimate can be made from the definition of density: $1/\bar{\nu} = 6M/N\pi d^2L$, which gives 27.9 Å. for $d$.

The agreement, within error, of these three values of $d$ demonstrates the consistency of the rod model.

The inappropriateness of the random coil model may be shown by using the Flory–Fox relation for $[\eta]$ and the Flory–Mandelkern relation for [S].[12] These equations incorrectly predict values of 335 and 300 Å., respectively, for $(\overline{\rho^2})^{1/2}$.

We conclude that the appropriate model is a rod 1650 Å. long, 26 Å. thick, and of molecular weight 530,000.

(12) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 616 and 609.

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## SYNTHESIS OF POTASSIUM HEXACHLORORHENATE AND POTASSIUM HEXABROMORHENATE

*Sir:*

It has been shown that small amounts of perrhenate can be converted quantitatively to hexachlororhenate(IV) ion by chromium(II) chloride reduction in strong hydrochloric acid solution.[1] The same reaction may be used for the synthesis of macro amounts of potassium hexachlororhenate-(IV).

The synthesis described here may be accomplished in considerably less time than with the other preparations of this salt,[2,3] yet the purity remains at

(1) V. W. Meloche and R. L. Martin, *Anal. Chem.*, **28**, 1671 (1956).

(2) L. C. Hurd and V. A. Reinders, "Inorganic Synthesis," Vol. I, McGraw-Hill Book Co., New York, N. Y., 1939, pp. 178–180.

(3) C. L. Rulfs and R. J. Meyer, THIS JOURNAL, **77**, 4505 (1955).