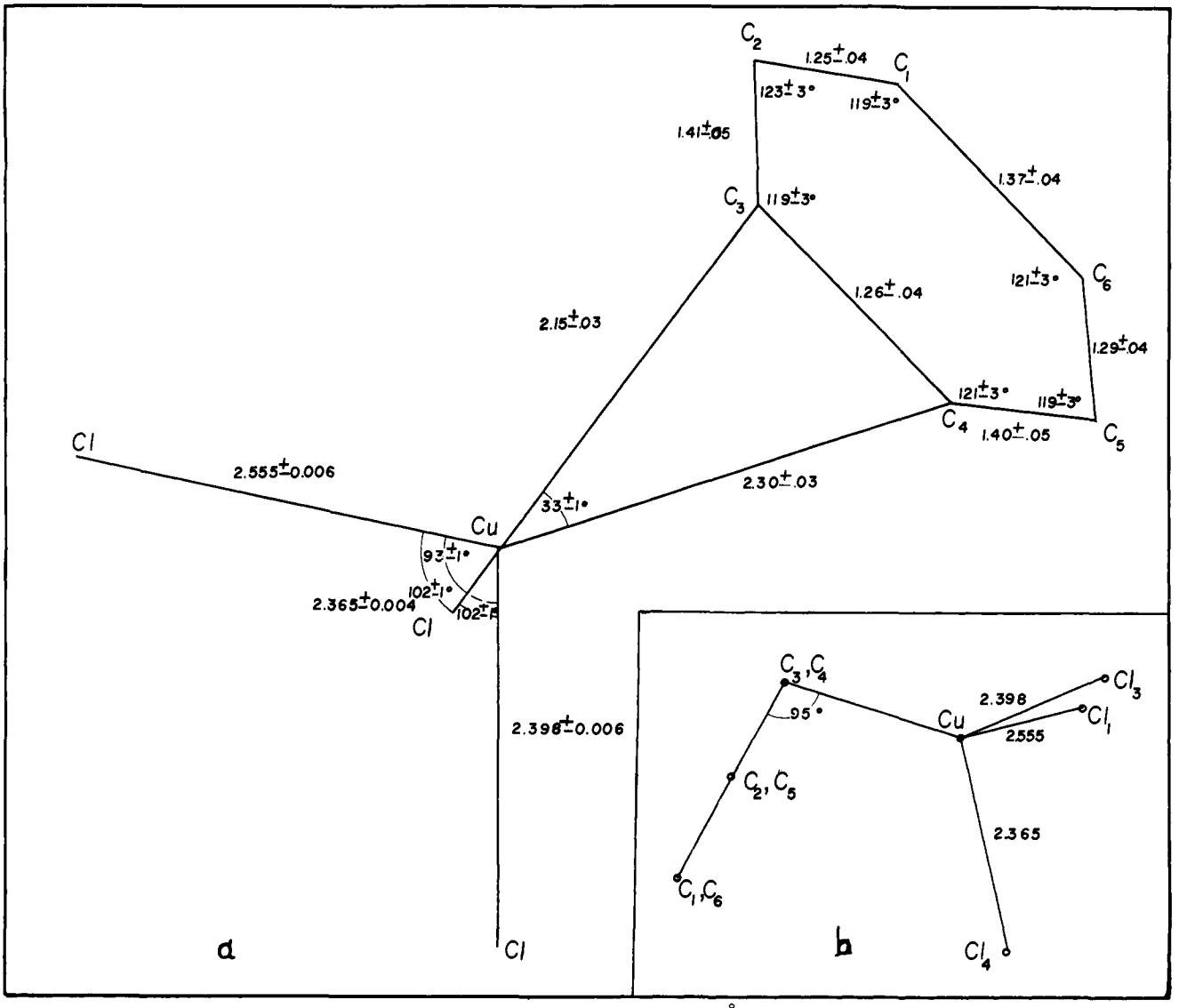

Fig. 1.—Molecular configuration of the $C_6H_6 \cdot CuAlCl_4$ com lex (all distances in Å.): a, view down the "c" axis; b, end on view of the benzene ring.

Å. with the shortest Al–Cl associated with the non-Cu coordinated Cl atom. The Al–Cl distances observed in $Co(AlCl_4)_2$[6] varied from 2.11 to 2.19 Å., but here none of the Cl atoms could be considered as being coordinated to only an aluminum atom.

Not only is the Cu(I) ion not placed on the benzene sixfold axis, but it is also not equidistant from the two nearest adjacent carbon atoms, 2.30 and 2.15 Å. (distance expected from sum of covalent radii is 2.12 Å.). A somewhat similar situation exists in $C_6H_6 \cdot AgClO_4$ (Ag–C, 2.49 and 2.63 Å.), but in this case the unequal metal to carbon distances manifest themselves in what appears to be a statistical disorder. The distortion of the benzene ring is toward a cyclohexatriene system with one of the short C–C bonds nearest the Cu(I). Although this ring distortion is just over the edge of statistical significance, it is worth noting that for $C_6H_6 \cdot AgClO_4$ the C–C bonds closest to the metal ion were 1.354 Å. and the others were 1.427 Å. in length. In both these cases this bond shortening is opposite to what one might predict from simple MO or valence bond theory, and polarization forces may be the dominant factor in the bonding.

To a first approximation the Cu(I) could be considered as tetrahedral with three chlorine and one benzene acting as electron donors.

(6) J. I. Ibers, *Acta Cryst.*, **15**, 967 (1962).

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The Polymerization of Bovine Pancreas Carboxypeptidase A[1]

Sir:

Only two proteolytic enzymes, chymotrypsin[2] and mercuripapain[3] are known to form a rapidly equilibrating, polymerizing system in which double boundary formation, due to the presence of polymers higher than the dimer,[4,5] can be observed in sedimentation experiments. This phenomenon has been observed only in *low* ionic strength buffers (M = 0.05 to 0.1)[2,3,6]

(1) This work was carried out under N1H Grant Number HE07297.
(2) V. Massey, W. F. Harrington, and B. S. Hartley, *Discussions Faraday Soc.*, **20**, 24 (1955).
(3) E. L. Smith, J. R. Kimmel, and D. M. Brown, *J. Biol. Chem.*, **207**, 533 (1954).
(4) G. A. Gilbert, *Discussions Faraday Soc.*, **20**, 68 (1955).
(5) G. A. Gilbert, *Proc. Roy. Soc.* (London), **A250**, 377 (1959).
(6) L. W. Nichol and J. L. Bethune, *Nature*, **198**, 880 (1963).

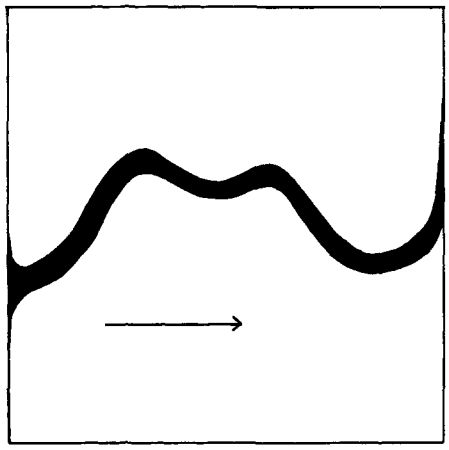

Fig. 1.—Carboxypeptidase in 2.5 *M* NaCl–0.02 *M* Veronal, pH 7.5. Sedimentation is from left to right after 7900 sec. at 59,780 r.p.m. at 25°.

leading to the inference that an electrostatic attraction might be the basis of the polymerization.

We have now observed double boundary formation with carboxypeptidase A at very *high* ionic strengths. This seems to be the first specific example of polymerization of a proteolytic enzyme at these high salt concentrations. Hence the physicochemical basis of this polymerization must differ from that for chymotrypsin and mercuripapain since here an electrostatic repulsion must apparently be overcome before the polymerization occurs.

Carboxypeptidase A has been sedimented in 1 *M* NaCl at concentrations up to 18 mg./ml.[7] A single boundary was found but the concentration dependence of the sedimentation coefficient was positive over the whole range. During a new sedimentation study of the enzyme, in 2 *M* NaCl and up to a concentration of 28 mg./ml., only a single boundary was observed, but at concentrations of enzyme greater than 22 mg./ml. the concentration dependence of the sedimentation coefficient became negative, similar to that which is usually seen. The $s^0_{20,w}$ was 3.3 *S*.

The solubility of the enzyme is a limiting factor in obtaining higher concentrations. Previous investigations of the influence of ionic strength upon its solubility have been extended, and it was found that in 2.5 *M* NaCl a concentration of 44.3 mg./ml. of enzyme could be obtained. Under these conditions the sedimentation pattern exhibits two boundaries (Fig. 1). When this solution is diluted to a concentration of 37.9 mg./ml., two boundaries also appear, but the area under the faster boundary decreases, while that under the slower boundary remains constant. These phenomena are characteristic of a rapidly equilibrating, polymerizing system where the polymer formed consists of more than two monomers.[4,5]

Table I gives the $s_{20,w}$ values, as a function of concentration, for the slow peak, for the fast peak, and for the point dividing the area of the pattern in half. The theoretical prediction[4,5] that the $s^0_{20,w}$ of the slow peak should agree with that of the monomer is borne out, since the value of $s^0_{20,w}$ for the slow peak is 3.3 *S*, in good agreement with the extrapolated $s^0_{20,w}$ in 2 *M* NaCl. Since carboxypeptidase, in contrast to chymotrypsin and papain, exhibits double boundary formation at high ionic strength, electrostatic repulsion must apparently be overcome to allow polymerization. That this is not an effect of enzyme concentration alone is demonstrated by the sedimentation pattern obtained at an enzyme concentration of 28 mg./ml. In 2 *M* NaCl the patterns are symmetric but in 2.5 *M* NaCl marked asymmetry develops.

(7) J. A. Rupley and H. Neurath, *J. Biol. Chem.*, **235**, 609 (1960).

TABLE I

SEDIMENTATION COEFFICIENT OF CARBOXYPEPTIDASE A

(2.5 *M* NaCl–0.02 *M* Veronal, pH 7.5)

Concentration, mg./ml.	$s_{20,w}$, *S* Slow peak	Fast peak	Midpoint
44.3	3.4	7.1	5.0
37.9	3.3	6.9	4.3
27.4	3.3		4.0
20.9	3.3		3.7
13.3	3.3		3.4

Over the concentration range from 2 to 44.3 mg./ml. no concentration-dependent change in optical rotation can be detected in the wave length range 325–600 mμ. The profound change in the relative concentration of the different species present in the solution therefore is not reflected in those parameters conventionally held to be dependent upon conformation.

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J. L. BETHUNE

RECEIVED SEPTEMBER 20, 1963

On the Nature of the Free-Radical Moiety in Lignin[1,2]

Sir:

Recent reports[3,4] of the existence of a stable free radical in lignin and wood pulp have prompted us to investigate the nature of this species by electron paramagnetic resonance spectrometry. The results of a study of the paramagnetism of a number of well defined lignin preparations strongly support the existence of a semiquinone-type radical entity, coexistent with a diamagnetic quinhydrone moiety.

All solid lignin preparations tested showed significant unpaired spin content when measured in a 100-kc. modulation Varian e.p.r. spectrometer (Table I).

TABLE I

FREE-RADICAL CONTENT OF VARIOUS LIGNIN PREPARATIONS[a]

Sample	Spins/gram	Estimated mol. wt.	Spins/mole
Brauns native spruce	0.5×10^{17}	1,000[b]	5×10^{19}
Bjorkman spruce	1.0×10^{17}	11,000[c]	1.1×10^{21}
Klason spruce	0.4×10^{17}	5,000[b]	1.5×10^{20}
Klason redwood	0.9×10^{17}		
Decayed western hemlock wood meal	0.9×10^{17}		
Kraft yellow pine	3.0×10^{17}	5,000[b]	1.5×10^{21}
Kraft-treated native spruce	4.0×10^{17}		
Calcium ligninsulfonic acid	1.5×10^{17}	10,000[b]	3.0×10^{21}
Indulin AT	3.0×10^{17}		

[a] Spin concentrations estimated by comparison with solid diphenylpicrylhydrazyl. Number of radicals was assumed to be proportional to signal height times signal width squared. [b] F. E. Brauns, "The Chemistry of Lignin," Academic Press, New York, N. Y., 1952, p. 192. [c] F. E. Brauns and D. A. Brauns, "The Chemistry of Lignin. Supplemental Volume," Academic Press, New York, N. Y., 1960, p. 179.

(1) Presented at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963.

(2) This work was supported in part by the United States Atomic Energy Commission (Grant No. AT(11-1)908) and by the Petroleum Research Fund (Grant No. 970-A4).

(3) R. W. Rex, *Nature*, **188**, 1185 (1960).

(4) T. N. Kleinert and J. R. Morton, *Nature*, **196**, 334 (1962).