



Fig. 3. Infrared spectrum of $[(\text{NH}_3)_5\text{Ru}]_2\text{N}_2 (\text{BF}_4)_4$.

Ce(IV). The $(\text{NH}_3)_5\text{Ru}$ moiety loses its capacity to retain N_2 when it is oxidized above the +2 state; Ce(IV) brings about this oxidation without producing N_2 from coordinated NH_3 or from NH_4^+ (8). When about 8 moles of Ce(IV) per mole of the new species was used the gas liberated on oxidation was shown to be more than 99 percent N_2 , and the amount corresponded to 95 percent of that expected for the binuclear formulation after applying a correction for the N_2 released from the $(\text{NH}_3)_5\text{RuN}_2^{2+}$ present in small amount.

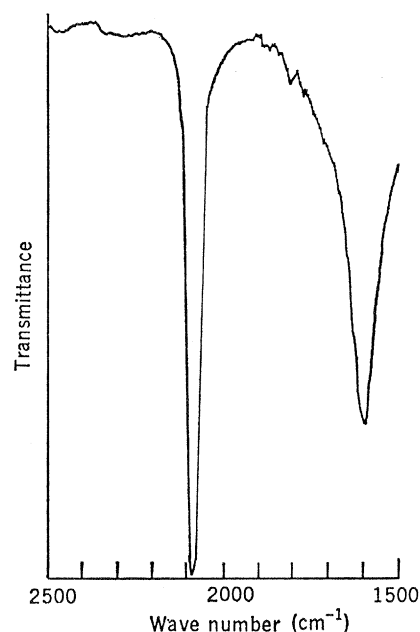


Fig. 4. Infrared spectrum of $(\text{NH}_3)_5\text{RuN}_2 (\text{BF}_4)_2$.

The portion of the infrared spectrum of a sample of $[(\text{NH}_3)_5\text{Ru}]_2\text{N}_2 (\text{BF}_4)_4$ (Fig. 3) provides an interesting comparison with the spectrum of $(\text{NH}_3)_5\text{RuN}_2 (\text{BF}_4)_2$ (Fig. 4). In place of the strong, sharp peak at about 2100 cm^{-1} characteristic of the N-N stretch in $(\text{NH}_3)_5\text{RuN}_2^{2+}$, there is a broad, weak absorption at about 2060 cm^{-1} [the small spike at about 2100 cm^{-1} in Fig. 3 can be attributed to residual $(\text{NH}_3)_5\text{RuN}_2^{2+}$]. The broad absorption may arise from the N-N stretch in the binuclear species or from an entirely different vibration. In any event, the absence of a strong absorption in the spectrum attributable to the N-N stretching frequency implies that N_2 is symmetrically bound in the binuclear ion.

The extinction coefficient (ϵ) of the binuclear ion at $262 \text{ m}\mu$, as measured for a solution prepared from the tetrafluoroborate salt, is 4.7×10^4 , or about 15 percent higher than the value calculated from the data shown in Fig. 1. The reason for this discrepancy is not known. A possibility is that coordinated ammonia is labilized when N_2 is complexed and that the replacement of ammonia by water has taken place to a differing extent in the two systems. The value of ϵ for $(\text{NH}_3)_5\text{RuN}_2^{2+}$ at its maximum, $221 \text{ m}\mu$, is $1.6 \pm 0.1 \times 10^4$. [This is a correction of the value of $\epsilon = 1.3 \pm 0.1 \times 10^4$ given in the previous communication (2); the preparation of $[(\text{NH}_3)_5\text{Ru}]_2\text{N}_2 (\text{BF}_4)_2$ has been found to contain a considerable admixture of $(\text{NH}_3)_5\text{Ru(III)}$.]

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7. The elementary analyses are often low even for well-characterized cobaltamine complexes. At this stage we cannot be certain that the analysis is at fault and must admit as a possibility the partial replacement of coordinated NH_3 by H_2O .
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9. Supported by NIH under grant PHS GM 13797-01.

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Guinea Pig Complement: Two Active Forms of First Component

Abstract. By solubility chromatography at pH 7.5 and low ionic strength, a partially purified preparation of the first component of guinea pig complement was separated into two fractions of different solubility under these conditions. On rechromatography, each fraction emerged in the same position as it had originally.

In solubility chromatography, proteins are allowed to precipitate on a highly cross-linked gel column equilibrated with a solution in which the proteins of interest are insoluble. The proteins are then redissolved in an advancing front or gradient of solvent, but they precipitate again, as they migrate ahead of the solvent, as a result of the gel-filtration effect of the column, to await redissolution by the solvent front. The result is a counter-current process from which the different proteins in a mixture emerge in positions corresponding to their solubilities under the conditions chosen (1). This method has been applied to purification of the first component of guinea pig complement (C'1), which is insoluble at pH 5.6 and low ionic strength (2); it permits isolation of C'1 in high yield, with at least 40-fold purification (3); details of this procedure will be reported elsewhere. In view of the report of Nelson *et al.* (4), indicating that C'1 also precipitates at low ionic strength at pH 7.5, further purification was attempted by solubility chromatography at this pH; thus I separated the active material into two fractions: one is soluble at an ionic strength of 0.0105, the other emerges from the column at an ionic strength of about 0.055.

Assays of C'1 were performed at an ionic strength of 0.0652, in the presence of sufficient D-mannitol to render the solutions isotonic, according to a modification of the procedure (5) already described; the modification involved 1-hour incubation of the mixture of EAC'4 and C'1 at 37°C (6, 7) before addition of C'2, followed by 30 minutes at 30°C to permit formation of SAC' 1a,4,2a (7). A 5-cm (internal diameter) column containing about 75 g of polyacrylamide gel (8) was equilibrated with 10 mM tris-HCl, pH 7.0 (measured at room temperature), containing 0.5 mM CaCl_2 ; equilibration was verified by both con-

ductivity and pH. A linear gradient was formed from 75 ml of the same buffer in the mixing chamber and 75 ml of a solution containing 10mM tris-HCl, pH 7.0 (room temperature), 0.5mM CaCl₂, and 0.15M NaCl in the reservoir; this gradient was pumped into the column before the sample. The C'1 obtained from 200 ml of serum by solubility chromatography at pH 5.6 was concentrated to less than 20 ml by ultrafiltration through Diaflo gel membranes (9); this preparation was opalescent but contained little or no precipitate. The material was pumped into the column as a sharp zone, followed by the starting buffer. Under these conditions, the proteins migrate through the gradient until they precipitate, and then migrate with it; proteins soluble at all points in the gradient continue past the gradient and emerge ahead of it in the void volume of the column. Optical densities, resistance across a conductivity cell, and pH of the effluent were recorded continuously with a system to be described elsewhere; conductivity values shown in the chromatograms were calculated from the recorded resistance values and the cell constant; they refer to 0.7°C. Flow rates between 26 and 37 ml/hr were obtained; 5-ml fractions were collected. The entire operation was performed in a cold room at 2° to 4°C.

There was clear separation of C'1 activity into two peaks (Fig. 1): 80 percent of the C'1 recovered emerged with most of the proteins at an ionic strength of 0.0105; 20 percent emerged in the gradient. No explanation is apparent for the leading shoulder in the second peak. The ratio between the two peaks has varied considerably in different runs for reasons that remain unknown.

The fractions representing each peak were pooled and concentrated to less than 10 ml by ultrafiltration; each of these two preparations was rechromatographed separately under the same conditions as before. The results (Fig. 2) provide evidence that the initial separation was not a chromatographic artifact but reflects a difference in solubility at pH 7.5.

Preliminary results (10) of investigation of the properties of these different forms of C'1 indicate that both are inactivated rapidly by *p*-nitrophenylethyl benzyl phosphonate; thus both presumably represent activated C'1 (11). Another preliminary result (10)

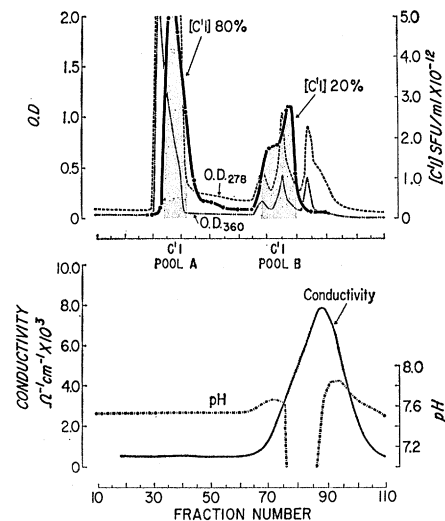


Fig. 1. Solubility chromatogram of guinea pig C'1 at low ionic strength and pH 7.5. The pH drop in tubes 75-85 is reproducible; O.D.₃₆₀ is recorded as a measure of light scattering.

shows a functional difference between the two types of C'1: material from pool A does not bind to EAC'4, while material from pool B does. The fact that pool-A C'1 is catalytically active, in the steady-state system used for its measurement, implies a fleeting interaction with EAC'4, but this interaction is not measurable in adsorption experiments. Consequently the activity measurements (Figs. 1 and 2) do not yield estimates of the number of molecules of C'1 in the case of pool A; the more noncommittal term, site-forming units, is therefore used, and the activity ratios between pools, mentioned above, are subject to the experimental conditions.

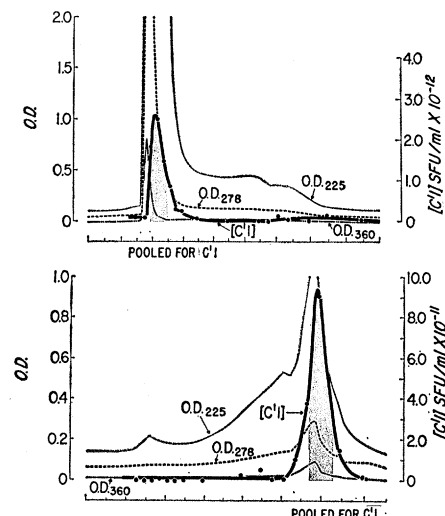


Fig. 2. (Top) Rechromatography of pool A, Fig. 1. (Bottom) Rechromatography of pool B, Fig. 1.

The two types of C'1 may be present in the pooled guinea pig serum used as the starting material, or one may arise from the other during preliminary purification; pool A has been stored for 6 weeks at 2° to 5°C without conversion to pool B, however.

The relation between the two forms of C'1 that I describe and fragments or subcomponents described by others remains to be elucidated. Since both forms are active in the conversion of SAC'4 to SAC'1a,4, neither appears to correspond to C'1q, C'1r, or C'1s (12), all three of which apparently are required to convert SAC'4 to SAC'1a,4. Colten *et al.* (13) have reported that C'1 dissociates reversibly into inactive fragments at ionic strengths greater than 0.15; again the fact that both forms of C'1 that I describe are active appears to distinguish them from the fragments obtained by Colten *et al.* Nishioka (14) has reported the finding that C'1 in the euglobulin fraction of guinea pig serum is electrophoretically heterogeneous; which of his three active components, if any, corresponds to those described by me remains to be determined.

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6. This incubation was introduced during initial studies as a hedge against a possible need for activation of the C'1; it may be unnecessary. The proper conditions for measurement of each of the two types of C'1 remain unknown.
7. Abbreviations: C'2, second component of complement (C'); C'4, fourth component of C'; EAC'4, sensitized sheep erythrocytes that have reacted with C'4; SAC'4, antigenic site on the surface of an erythrocyte, that has reacted with antibody (A) and C'4; SAC'1a,4, antigenic site that has reacted with A and activated C'1 and C'4; SAC'1a,4,2a, SAC'1a,4 that has reacted with C'2.
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