Table 2. Photolysis of uracil in water. Identical doses of 260 to 300 mμ light in all cases; uracil concentration $10^{-4}M$; 2,4-hexadienol (HDE) concentration $10^{-4}M$; analyses by absorption spectrophotometry (addition of a small amount of mineral acid reverses the photohydration). Results are percentages.

	Degassed		Aerated	
	No HDE	HDE	No HDE	HDE
	Conversion			
	42±2	31±2	31±2	33±2
	Analysis of Products			
Hydrate	31±5	42±5	43±5	46±5
Dimers	69±5	58±5	57±5	54±5

and uracil triplets [Φisc (intersystem crossing)] obtained by 2537-Å excitation in acetonitrile solution were determined with *cis*-piperylene as the "triplet counter" (*11*). Note (Table 1) the reduction in yields when the hydrogen at the N-3 position of thymine or uracil is replaced by a methyl group. Phosphorescence from thymidine and uridine is observed only at high *p*H where the N-3 proton is removed (*9, 12*).

The difference in efficiencies of the dimerizations of thymine and uracil cannot be quantitatively accounted for on the basis of the difference in triplet yields. Steric hindrance afforded by the methyl group in thymine may be an important factor.

In water thymine undergoes a very slow photooxidation if the water solution is aerated (*13*). We observed no reaction of thymine in degassed water solution. On the other hand, photolysis of a water solution of uracil gives dimers and the photohydrate, the latter being detected by the heat and acid lability (*14*) of the material associated with the loss of absorbancy (Table 2). The initial ratio of hydrate to dimers decreases with increasing uracil concentration (*15*). In addition we found that the ratio depends on the presence or absence of air (oxygen) or of 2,4-hexadienol. The latter is a water soluble diene with a triplet energy of 59.5 kcal/mole (*16*) and is transparent at the wavelengths of the exciting light employed. The presence of air or 2,4-hexadienol leads to an increase in the ratio of hydrate to dimer. Furthermore, the disappearance of uracil is retarded by air or the hexadienol. Qualitatively, the data fits a model which does not involve the uracil triplet state in the photohydration reaction.

Paper chromatographic analysis and isolation by crystallization showed only one dimer from the photolysis of thymine in acetonitrile. This dimer has a different infrared spectrum and different chromatographic properties (*17*) from those the dimer produced by photolysis of DNA or of thymine in ice (*18*).

In addition to the dimer produced by photolysis of uracil in ice, a second dimer was isolated from photolyzed solutions of uracil in both water and acetonitrile. A qualitative examination has indicated that the ratio of "ice dimer" to new dimer increases in the presence of a triplet quencher. The stereochemical course of the photodimerization of coumarin depends on the excited state involved (*19*).

ANGELO A. LAMOLA*
JAI P. MITTAL
Radiation Laboratory and *Department of Chemistry, University of Notre Dame, Notre Dame, Indiana 46556*

References and Notes

1. For recent reviews see the references cited by Lamola (*2*).
2. A. A. Lamola, *J. Amer. Chem. Soc.* **88**, 813 (1966).
3. D. Weinblum and H. E. Johns, *Biochim. Biophys. Acta* **114**, 450 (1966).
4. K. C. Smith, *Photochem. Photobiol.* **2**, 503 (1963).
5. R. B. Setlow, *Biochim. Biophys. Acta* **49**, 237 (1961).
6. Irradiations were carried out in a "merry-go-round" apparatus (*7*) which ensures all samples the same dose rate. Only wavelengths greater than 260 mμ were used and so the isoprene did not compete for exciting light. The exciting light intensity was the same for all runs, and all of the light impinging on the samples was absorbed.
7. G. S. Hammond *et al.*, *J. Amer. Chem. Soc.* **86**, 3197 (1964).
8. E. F. Evans, *J. Chem. Soc.* **1960**, 1735 (1960).
9. R. Rahn, J. Longworth, R. G. Shulman, in preparation.
10. M. Gueron, R. G. Shulman, J. Eisinger, in preparation.
11. A. A. Lamola and G. S. Hammond, *J. Chem. Phys.* **43**, 2129 (1965).
12. R. O. Rahn, R. G. Shulman, J. W. Longworth, *Proc. Nat. Acad. Sci. U.S.* **53**, 893 (1965).
13. R. Alcantara and S. Y. Wang, *Photochem. Photobiol.* **4**, 465, 473 (1965).
14. R. L. Sinsheimer, *Radiation Res.* **1**, 505 (1954).
15. P. M. Parker, unpublished results.
16. R. E. Kellogg, private communication.
17. Samples spotted on Whatman No. 1 paper and chromatographed (descending) with a mixture of 1-butanol, water, and acetic acid (80 : 30 : 12, by volume); thymine "ice dimer" $R_F = 0.25$; thymine dimer produced in acetonitrile $R_F = 0.33$.
18. Photolysis of DNA or of thymine in ice gives the *cis*-"head-to-head" dimer. According to the assignments made by Weinblum and Johns (*3*) the dimer obtained here has the *trans*-"head-to-tail" structure.
19. G. S. Hammond, C. A. Stout, A. A. Lamola, *J. Amer. Chem. Soc.* **86**, 3103 (1964).
20. The Radiation Laboratory of the University of Notre Dame is operated under contract with the AEC. This is AEC Document No. C00-38-474. We thank G. Crawford (NSF undergraduate research participant) for carrying out the quenching experiments with 2,4-hexadienol. We thank Dr. R. G. Shulman and Dr. M. Gueron for helpful criticisms.
* Present address: Bell Telephone Laboratories, Inc., Murray Hill, N.J. 07971.

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Evolution of Immunoglobulin Polypeptide Chains: Carboxy-Terminal of an IgM Heavy Chain

Abstract. *The dipeptide sequence at the carboxy-terminal of a heavy (μ) chain from a human macroglobulin (IgM) is tyrosylcysteine, although the reverse sequence, cysteinyltyrosine, has not been rigorously excluded. The presence of cysteine at the carboxy-terminal was predicted from a recognition of the chemical homologies among the polypeptide chains of immunoglobulins, and their probable evolutionary origin.*

On the basis of certain chemical similarities and amino acid sequence homologies between the light and heavy polypeptide chains of immunoglobulin molecules, Singer and Doolittle (*1*) have proposed that the cistrons coding for these two kinds of chain have evolved from a common ancestral gene. This proposal led to the specific prediction that at or near the carboxy-terminal of the IgM (immunoglobulin M) heavy chain (μ) there might be present a cysteine residue, since both classes of human light chains (κ and λ) have a cysteine residue in that region [carboxy-terminal for κ-chains (*2*); penultimate to carboxy-terminal for λ-chains (*3*)]. The heavy chains of IgG immunoglobulins (γ) do not have a cysteine residue in their carboxy-terminal region (*4*), although other homologies to the carboxy-terminals of light chains are apparent (*1*). In the light chains, these cysteines form part of the interchain disulfide link between the light and heavy chains (*3*). It was suggested, therefore, that such an evolutionarily conserved cysteine residue at the end of μ-chains might be responsible for the disulfide linkage of the five 7*S*-type subunits to form the 19*S* IgM molecule (*5*). To test this prediction we have treated a human μ-chain with carboxypeptidase A and have indeed found cysteine at the carboxy-terminal.

The IgM studied was a Waldenström

macroglobulin with κ-type light chains; its purification and characteristics have been described (*5*). The macroglobulin was reduced with 0.005*M* dithiothreitol for 30 minutes at 25°C in a 0.2*M* tris-HCl buffer, *p*H 8.6. Only interchain disulfide bonds are reduced under these conditions. The liberated -SH groups were specifically alkylated with a 10-percent (molar) excess (over reducing agent) of iodoacetamide-1-C^{14} (New England Nuclear) for 15 minutes at 25°C in 0.2*M* tris-HCl buffer, *p*H 8.0. The μ- and κ-chains were separated on Sephadex G-100 in 1*M* propionic acid at 4°C (*6*). The ratio of C^{14} activity in the μ- and κ-chain peaks was 4:1. Our experiments were performed on chains separated from three different batches of reduced and alkylated material. In an effort to increase the susceptibility to carboxypeptidase digestion, one batch of separated chains was succinylated with succinic anhydride (*7*). In addition, experiments were carried out with a fraction of a tryptic digest of partially reduced and C^{14}-alkylated IgM, which appears to consist solely of oligopeptides derived from the carboxy-terminal half of the μ-chain (*5*). We refer to this fraction as the trypsin-digested Fcμ fragments, by analogy to the (whole) Fcγ fragments released from IgG molecules by papain digestion.

Carboxypeptidase A (Worthington Biochemicals, DFP-treated) was diluted to a concentration of 2 mg/ml with 1*M* ammonium bicarbonate, and one volume of enzyme was added to nine volumes of the chain preparation dissolved in water; the ratio of enzyme to polypeptide chain was approximately 1:50 (molar). Digestions were carried out at 25°C. The release of amino acids was examined by (i) paper electrophoresis at *p*H 2 in a mixture of acetic acid, formic acid, and water (2 kv for 45 minutes), (ii) descending paper chromatography in a mixture of butanol, acetic acid and water, and (iii) a combination of both, in which electrophoresis papers were stitched to full sheets before chromatography in the second dimension. Carboxamidomethylcysteine (CAMC) was synthesized by the reaction of L-cysteine with an equivalent amount of iodoacetamide, the *p*H being maintained in the neutral range by the addition of NaOH. The compound was crystallized from 67 percent ethanol in the cold (m.p. 195° to 196°C). Treatment of heavy μ-chains with carboxypeptidase A led to a progressive release of C^{14} radioactivity (Table 1). The radioactive material appeared in a ninhydrin-positive spot which had the same mobility and R_F as the CAMC reference compound. After a 30-minute digestion period, the only other ninhydrin spot on paper electropherograms or chromatograms corresponded to tyrosine. Further digestion released a greater percentage of the expected radioactivity. Theoretically, since only one of the four C^{14}-CAMC groups on each μ-chain could be carboxy-terminal, a maximum release of one-fourth of the radioactivity in the μ-chains could have been expected. Such prolonged digestion also liberated a number of other amino acids. The data indicate that the carboxy-terminal dipeptidyl sequence which is most likely is tyrosylcysteine, although further kinetic studies would be necessary to rigorously exclude the reverse sequence, cysteinyltyrosine.

Table 1. Release of radioactivity after treatment of μ-chains with carboxypeptidase A. Digestion mixtures were applied directly to Whatman No. 3 MM paper which was then dried with a warm air stream. High-voltage electrophoresis was conducted, the papers were stained with ninhydrin, and the areas corresponding to CAMC were cut out and counted for radioactivity. The origin areas, containing the parent protein, were also cut out and counted. Theoretically, 100 percent release of the carboxy-terminal CAMC would equal one-fourth of the total counts.

Digestion time (min)	Radioactivity (count/min)		Radioactivity released (percentage of theoretical)
	Origin	As CAMC	
0	4218	0	0
10	5220	134	10
20	5692	209	14
30	5971	298	19
240	3698	537	51

Several carboxypeptidase digestions were performed with the C^{14}-labeled trypsin-digested Fcμ fragments. A similar release of radioactive CAMC was obtained. If two of the four C^{14}-CAMC groups of each μ-chain were present in the Fcμ region (*5*) about 60 percent of the expected radioactivity was liberated after a 3-hour digestion period.

Attempts to remove the carboxy-terminal amino acid [presumably cysteine (*2*)] of the light (κ) chain from this macroglobulin were uniformly unsuccessful, however. Although carboxypeptidase A digestions were carried out from *p*H 5.9 to 8.0, with either unmodified or succinylated chains, no significant release of radioactivity or ninhydrin-positive material was ever obtained. The difficulty may stem from the fact that glutamic acid is the penultimate amino acid in κ-type light chains (*2*), a situation which has been reported to slow carboxypeptidase digestion considerably (*8*).

Evidence has been presented (*5*) that under the conditions of reduction in our study only interchain disulfide bonds are reduced and subsequently alkylated. Furthermore, the particular interchain disulfide bond formed by the cysteine at the μ-chain carboxy-terminal must link two μ-chains, since the relevant CAMC was also obtained from the Fcμ region of the molecule which does not contain the κ-chains. While our results do not exclude the possibility that this link might be between the two halves of a 7*S*-type subunit (γMs, 5) of the IgM molecule, it appears reasonable to infer rather that the link is between the γMs subunits that form the 19*S* pentamer.

Our results also support the original suggestion that the cysteine residue in question is an evolutionary survivor of a gene duplication which led on the one hand to heavy μ-chains and on the other to a light-chain class. Finally, the capacity of IgA molecules to form a variety of disulfide-linked polymers (*9*) suggests that the α-heavy chain may also retain a cysteine residue near its carboxy-terminal which is involved in such cross-links between subunits.

RUSSELL F. DOOLITTLE
S. J. SINGER
Departments of Chemistry and *Biology, University of California at San Diego, La Jolla*
HENRY METZGER
National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland

References and Notes

1. S. J. Singer and R. F. Doolittle, *Science* **153**, 13 (1966).
2. N. Hilschmann and L. C. Craig, *Proc. Nat. Acad. Sci. U.S.* **53**, 1403 (1965).
3. C. Milstein, *Nature* **205**, 1171 (1965).
4. E. M. Press, P. J. Piggot, R. R. Porter, *Biochem. J.* **99**, 356 (1966).
5. F. Miller and H. Metzger, *J. Biol. Chem.* **240**, 3325, 4740 (1965); **241**, 1732 (1966).
6. J. B. Fleischman, R. H. Pain, R. R. Porter, *Arch. Biochem. Biophys.* Suppl. 1, 174 (1962).
7. J. Lenard and S. J. Singer, *Nature* **210**, 536 (1966).
8. C. A. Dekker, S. P. Taylor, Jr., J. S. Fruton, *J. Biol. Chem.* **180**, 155 (1949).
9. J. L. Fahey, *Advance. Immunol.* **2**, 41 (1962).
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