DEGREE OF IODINATION AND LOCALIZING ACTIVITY OF RABBIT ANTIKIDNEY γ-GLOBULIN

Sir:

The precipitation of chemically modified antibody γ-globulin by homologous antigen has been used by many workers as an index of residual biological activity.[1,2] Data obtained in this manner have been considered by analogy to hold for certain iodinated γ-globulins possessing *in vivo* localizing activity.[3,4,5,6] The danger in making this type of extrapolation is evident when one examines the results of a more direct approach.

Anti-rat kidney γ-globulin obtained from immunized rabbits was radioiodinated essentially as in ref. (6) and then was dialyzed exhaustively against large volumes of *p*H 8 borate buffer. The dialysates were counted as was the content of the bag. This gave the percentage of the original iodine bound to the protein. The amount of original protein was determined gravimetrically and the weight of insoluble residue found after dialysis was subtracted. As long as the starting γ-globulin solution was free from turbidity the insoluble fraction contained the same ratio of I^{131} as the rest of the solution. This fraction was always very minor. The values given for degree of substitution are considered accurate to not less than ±10%. The molecular weight of γ-globulin was taken as 160,000. Iodination and dialysis were performed at 0–5°.

The labeled γ-globulin was then injected into rats and the extent of localization determined three days later according to the method of Pressman.[7] The results are shown in the table and are all for the same preparation of γ-globulin.

Expt.	No. of rats	Atoms I / Molecule	% Injected dose bound to kidney, ±1 std. error
1	7	0.5	1.47 ± 0.07
2	6	0.6	1.68 ± .03
3	6	1.3	1.21 ± .05
4	4	2.7	1.15 ± .10
5	3	3.2	0.87 ± .08
6	4	4.0	0.82 ± .07

It would appear, therefore, that the conclusions of the workers earlier referred to (that antibodies can be substituted with from 2 to 9 atoms of iodine per molecule without apparent modification of activity) are based upon relatively insensitive criteria. In the experiments reported here, the extent of binding started to diminish at about 1 atom I/protein molecule. It was noted also that the cross localization in the liver fell off at a slower rate,[8] indicating a concomitant loss in the specificity of the γ-globulin. Since the specific activity of I^{131} is very high, a small degree of substitution such as is suggested by this work, could still yield a preparation of high radioactivity.

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INTERMEDIATES IN THE SYNTHESIS OF DIPHOSPHOPYRIDINE NUCLEOTIDE FROM NICOTINIC ACID[1]

Sir:

A previous communication from this laboratory reported synthesis of DPN[2] by human erythrocytes at low concentrations of nicotinic acid.[3] Nicotinamide, at similar concentrations, did not elevate the pyridine nucleotide level significantly, presumably eliminating free NAm as an intermediate and suggesting that amidation may occur after NA is converted to some presently unknown derivative.

Incubation of 0.72 μmole of NA with glutamine, 0.1 *M* P_i buffer, *p*H 7.4, glucose and 3.5 ml. of defibrinated erythrocytes resulted in synthesis of 0.181 μmole of DPN. After incubation of 0.72 μmole of nicotinic acid-7-C^{14} (4.4×10^5 c.p.m.) under the same conditions and chromatographing aliquots of the deproteinized filtrate on Whatman No. 1 paper with a solvent system of 0.1 *M* PO_4 buffer *p*H 6.8, 600 g. of ammonium sulfate per liter, 2% 2-propanol, 3 radioactive spots could be detected. One corresponded to NA (25% of the total radioactivity). A second traveled between NR and NMN (Compound I-39% of the total radioactivity) and the third traveled with DPN (Compound II, 36% of the total radioactivity). Whereas omission of glutamine reduced DPN synthesis by 70%, glutamine had no effect on the amount of radioactivity in the two nucleotide spots, indicating that at least two NA derivatives other than DPN had been synthesized. Omission of glucose reduced the percentage of the total radioactivity of Compound I from 37 to 18% and Compound II from 63 to 27%.

Extracts of acetone powdered-eythrocytes, which cannot synthesize DPN from NA, do synthesize both Compounds I and II. Synthesis of Compound I requires Mg^{++}, P_i and either R5P or PRPP. NaF (0.02 *M*) inhibits synthesis of Compound I from R5P by 88% and from PRPP by only 25%. PRPP formation from R5P and ATP is known to be inhibited 60 to 70% by 0.02 *M* NaF in erythrocyte acetone powder extract.[4] Hypoxanthine, which is known to condense with PRPP to

(1) These studies were supported in part by contract AT-(40-1)-289 between Duke University and the United States Atomic Energy Commission and by Grant RG-91 from the National Institutes of Health.

(2) These abbreviations are used: NA, nicotinic acid; NAm, nicotinamide; GAm, glutamine; DPN, diphosphopyridine nucleotide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; R5P, ribose 5-phosphate; ATP, adenosine triphosphate; PRPP, 5-phosphoryl-ribose 1-pyrophosphate; P_i, inorganic phosphate.

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form inosinic acid in these extracts,[4] inhibits Compound I formation by about 50%. These results suggest that Compound I is nicotinic acid mononucleotide and is formed by condensation of PRPP with NA.

To characterize these compounds, a large-scale incubation of erythrocyte acetone powder extracts with nicotinic acid-7-C^{14} was performed. A neutralized perchloric acid filtrate was then passed through a Dowex 1 formate column; 15-ml. aliquots were collected and assayed for radioactivity and absorption at 260 mμ. The various fractions were pooled and components identified by paper chromatography. Radioactive fractions were adsorbed on a Norit A column and, after washing with water, were eluted with 10% aqueous isoamyl alcohol. Ribose and phosphate analyses and absorption spectra suggested that Compounds I and II were the nicotinic acid analogs of NMN (desamido-NMN) and DPN (desamido-DPN), respectively. Compound II was not active with alcohol dehydrogenase and did not form a fluorescent acetone addition product.[5] Further work on the identification of these compounds is in progress.

TABLE I

SYNTHESIS OF DPN FROM DESAMIDO-DPN

Complete incubation system consisted of 1.7 μmoles of desamido DPN, 20 μmoles of glutamine, 2 μmoles of ATP, 5 μmoles of ATP, 40 μmoles of P_i buffer, *p*H 7.4, and 4 cc. of dialyzed yeast autolysate. Total volume 1.0 cc. after incubation of 100 minutes 1 cc. of H_2O was added and the protein denatured at 100° for 1.5 minutes; 1-cc. aliquots were taken for alcohol dehydrogenase assay of DPN formed.

Omission and/or Additions	μmoles DPN synthesized
None	0.116
−Desamido DPN	.028
−ATP	.011
$-Mg^{++}$	.014
−Glutamine	.026
−Glutamine + NH_4Cl 20 μm.	.015
−Glutamine + glutamate 20 μm.	.019
"O time"	.007

Dialyzed yeast autolysates have been found slowly to synthesize DPN upon addition of glutamine, ATP, PRPP, Mg^{++}, F^- and NA but not NAm. With nicotinic acid-7-C^{14} in the medium, accumulation of compounds I and II was again observed. Compound II prepared with erythrocytes or yeast exhibited an R_f in 70% ethanol, 30% 1 *M* ammonium acetate, *p*H 5.0 identical to that of an authentic sample of the nicotinic acid analog of DPN kindly supplied to us by Dr. N. O. Kaplan. This solvent system adequately separates Compound II from DPN. Synthesis of Compound II from NA by yeast autolysate is dependent on ATP, PRPP and Mg^{++}. R5P is 10% as effective as PRPP. Glutamine, ATP and Mg^{++} are required for synthesis of DPN from desamido-DPN (Table I). NH_4^+ and glutamate are inactive as amide donors. Further investigations of the mechanisms of these enzymatic syntheses, purification of the enzymes, and a study of their distribution in bacterial and mammalian systems are in progress.

(5) N. Levitas, J. Robinson, F. Rosen and W. A. Perlzweig, *J. Biol. Chem.*, **167**, 511 (1947).

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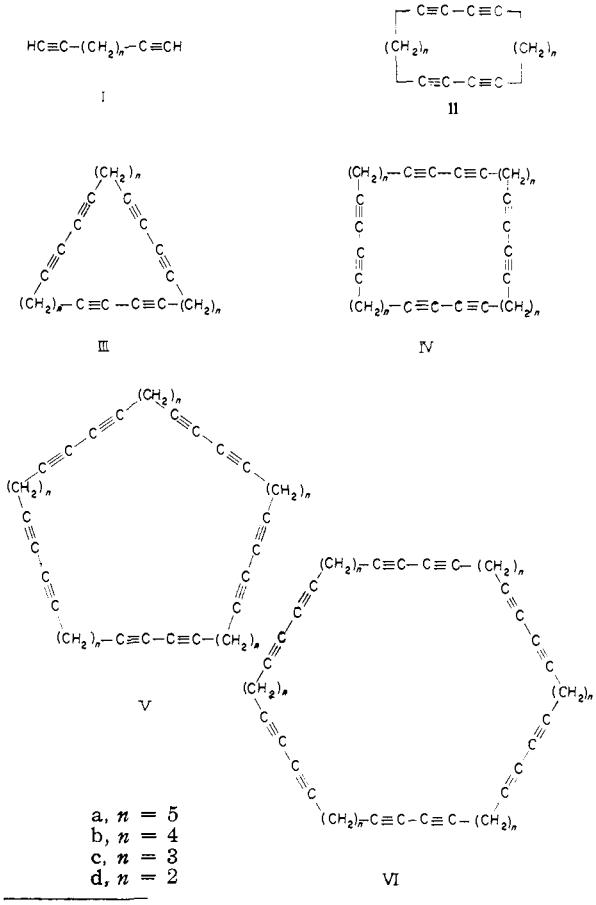
UNSATURATED MACROCYCLIC COMPOUNDS. V.[1] LARGE RING POLY-ACETYLENES

Sir:

We have shown recently that the oxidation of terminal diacetylenes of type I with oxygen in the presence of cuprous chloride and ammonium chloride in aqueous ethanol leads to the cyclic dimers II besides linear compounds.[1,2] We have studied this type of coupling of terminal diacetylenes under a variety of conditions, especially in homogeneous media. The surprising discovery has been made that when diacetylenes of type I in pyridine solution are simply heated with neutral cupric acetate,[3] not only the cyclic dimers II are formed, but also the cyclic trimers III, tetramers IV, pentamers V, hexamers VI and higher cyclic



(1) Part IV, F. Sondheimer, Y. Amiel and R. Wolovsky, THIS JOURNAL, in press.

(2) (a) F. Sondheimer and Y. Amiel, *ibid.*, **78**, 4178 (1956); *ibid.*, in press; (b) Y. Amiel, F. Sondheimer and R. Wolovsky, *Proc. Chem. Soc.*, 22 (1957).

(3) This sort of oxidation has been shown to result in the smooth coupling of simple acetylenes and to give the cyclic monomer, and dimer in the case of I, $n = 10$ (and of a diacetylenic diester) when carried out under conditions of high dilution (G. Eglinton and A. R. Galbraith, *Chem. and Ind.*, 737 (1956)).