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

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(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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