Noncovalent Binding of 4-Nitroquinoline-N-Oxide to Proteins

OSAMU YAMAMOTO

Research Institute for Nuclear Medicine and Biology,
Hiroshima University, Kasumi 1-2-3, Hiroshima 734, Japan
(Received February 26, 1979; Revised version received July 26, 1979)

4NQO/N-Oxide/Noncovalent bond

Binding of 4NQO to various kinds of enzymes or proteins was studied. Each one of proteins was mixed with 4NQO in 0.4 mM NaHCO₃ solution and eluted through Ultrogel AcA 22 column. Radioactivity of ¹⁴C-labeled 4NQO found in protein fraction was measured. 4NQO bound hardly to polyglutamic acid and polysperatic acid, somewhat to serum albumin, insulin, trypsin, RNA polymerase and DNA polymerase, and markedly to urease which is an SH enzyme. Lactate dehydrogenase, one of SH enzymes, aggregated with 4NQO. The binding of SH enzyme with the N-oxide would be attributable to a noncovalent binding such as $\text{N-O} \cdots \text{H-S}$, because 4NQO-urease binding yield markedly decreased in the presence of sodium dodecyl sulfate or cysteine, and also 4NQO-bound urease released 4NQO by the addition of sodium dodecyl sulfate.

INTRODUCTION

Sklobovskaya and Ryabchenko¹-² and Strazhevluskaya et al.³-⁴ have been studied the DNA-protein crosslinking induced by ionizing radiation at low doses. The latter workers suggested the participation of acidic protein in the crosslinking. This type of crosslink, however, was not observed under conditions such as the presence of sodium dodecyl sulfa (SDS), the low pH, or the high salt concentration. It indicates that such crosslink is due to noncovalent binding of the specific amino acid residues of protein to the radiation-modified portion in DNA molecule. Rhaese has identified adenine-7-N-oxide as one of radiolytic products of adenine⁵. Based on these works, Yamamoto has proposed a possibility of the interaction between the NO group of N-oxides and the carboxyl group of acidic proteins⁶. However, such possibility was disappropiable by this work. Rather, SH protein turned out to be participant in the binding with N-oxide.

MATERIALS AND METHODS

Preparation of Solutions

Albumin (Fraction V powder from bovine plasma), insulin (crystalline from bovine
pancreas), polyaspartic acid (Type I), polyglutamic acid (Grade III), DNA polymerase (from *M. lysodeikticus*), RNA polymerase (Type II from *E. coli* K-12), and urease (Type C-3 from Jack bean) were purchased from Sigma Chemical Company, St. Louis, Mo. Lactate dehydrogenase (LDH) (beef heart), which was purchased from P-L Biochemicals, Milwaukee, Wis., was delivered as a suspension in ammonium sulfate solution. By gel filtration with Sephadex G-10, ammonium sulfate was eliminated<sup>40</sup>. 4NQO-5, 6, 7, 8, 9, 10<sup>-14</sup>C (6.22 mCi/mmmole) was obtained from Daiichi Pure Chemical Company, Japan. 4NQO, trypsin and other chemicals were from Wako Chemical Company, Japan.

Each protein (2 mg/ml in final except 0.2 and 0.4 mg/ml for RNA polymerase) and 4NQO (5×10<sup>-4</sup> M, 0.5 μCi/ml in final) were dissolved in 0.4 mM NaHCO<sub>3</sub> solution (pH 7.8) and incubated for 5, 10, or 30 min at room temperature. All solutions were prepared with triply distilled water.

**Column Chromatography and Measurement of Radioactivity**

Ultragel AcA 22 (LKB, Sweden) was packed up to 40 cm in a Laboratory Column, K 9/60 (0.9 cm×60 cm) Pharmacia Fine Chemicals, Sweden. The 4NQO-protein mixture was eluted with 0.4 mM NaHCO<sub>3</sub> through the column at room temperature. Optical density of each fraction (2.3 ml) was measured at 220 nm, and then cold 20% trichloroacetic acid (TCA) was added to the solution. The precipitate was filtered with a glass filter (Whatman GF/C), washed with 5% TCA and then dried. The radioactivity in the precipitate was measured with a Packard Tri-Carb Scintillation Spectrometer.

**RESULTS AND DISCUSSION**

For various kinds of proteins used in this work, their acidic/basic ratio is shown in Table. If acidic protein participates in the binding with N-oxides as mentioned in INTRODUCTION, 4NQO should bind to DNA polymerase and RNA polymerase but not to trypsin, insulin, catalase and albumin.

| Contents of amino acid residues having special group and acidic/basic ratio in proteins |
|---------------------------------|---------|---------|---------|---------|---------|
| Serum albumin (12)                 | DNA (13) polymerase | RNA (14) polymerase | Urease (15) |
| Trypsin (8)                                      5.9    14.4    16.2    21.9    23.6    20.6 |
| Insulin (10)                                     6.5    15.1    16.8    12.3    13.9    12.2 |
| Catalase (11)                                    7.9    15.6    16.8    12.3    13.9    12.5 |
| Ratio of acidic/basic                      0.14   0.27   0.61   0.70   1.66   1.70   1.69 |

Amino acid having special group (Mole %)

| -COOH | 3.1 |
| -NH<sub>2</sub> | 8.5 |
| -OH | 24.3 |
| aromatic ring | 8.9 |

Downloaded from https://academic.oup.com/jrr/article-abstract/20/4/276/882941/Noncovalent-Binding-of-4-Nitroquinoline-N-Oxide-to by guest on 15 September 2017
Chromatograms of 4NQO-insulin, 4NQO-catalase, 4NQO-albumin, and 4NQO-DNA polymerase mixture systems are shown in Fig. 1. Dotted lines represent $OD_{280}$ value, the first eluted peak being protein, and the second peak 4NQO. Solid lines show 4NQO radioactivity in TCA precipitates. The first eluted peak indicates a binding of 4NQO to protein and the second one does 4NQO itself because 4NQO is somewhat precipitable with TCA.

![Chromatograms of 4NQO-protein mixtures](image)

**Fig. 1.** Column chromatograms of 4NQO-protein mixtures. 4NQO ($5 \times 10^{-4}$ M, 0.5 μCi/ml in final) and protein (2 mg/ml in final except 0.2 and 0.4 mg/ml for RNA polymerase) were dissolved in 0.4 mM NaHCO₃ solution, and then 1 ml of the prepared solution was eluted through Ultrogel AcA 22 column (φ 0.9 cm x 40 cm) with 0.4 mM NaHCO₃ solution. ↑: Protein peak; ↑: 4NQO peak.
In the 4NQO-trypsin mixture solution, 4NQO and trypsin were not completely separated from each other by gel filtration. In Fig. 1, the binding peak was drawn by subtracting the radioactivity in TCA precipitate in the absence of the protein from the radioactivity in TCA precipitate in the presence of the protein. Because RNA polymerase was expensive, the concentrations lower than the others (0.2 mg/ml and 0.4 mg/ml instead of 2 mg/ml) were used. All binding yields resulted were comparable to each other among the six 4NQO-protein mixture systems, which suggests that the binding of 4NQO to protein is independent of the acidity of the proteins. Further observation was taken in 4NQO-polyaspartic acid and 4NQO-polyglutamic acid mixture systems. Polyaspartic acid and polyglutamic acid are typical acid polypeptides. As shown in Fig. 2, no binding of 4NQO to these polypeptides was observed. Therefore, it is concluded that the 4NQO-protein binding does not depend on the acidity of the protein or the existence of carboxyl group in the protein.

![Graph showing binding between 4NQO and Polyglutamic acid and Polyaspartic acid](image)

**Fig. 2.** Column chromatograms of 4NQO-polyaspartic acid and 4NQO-polyglutamic acid mixtures. 4NQO (5×10⁻⁴ M, 0.5 μCi/ml in final) and polypeptide (2 mg /ml in final) were dissolved in 0.4 mM NaHCO₃ solution, and then 1 ml of the prepared solution was eluted through Ultrogel AcA 22 column (ϕ 0.9 cm×40 cm) with 0.4 mM NaHCO₃ solution. ↑: Protein peak; ↑: 4NQO peak.

Is there any characteristics other than the acidic nature in the nonhistone proteins? Disulfide bond is an important part in the residual protein of chromosomal DNA structure. A reaction, -SS− ⇔ -SH+HS−, is well known in radiation chemistry. In this point of view, working turned on SH-containing proteins. Marked binding of 4NQO to urease, which has 85 SH groups in its molecule (MW=500,000), was observed as shown in Fig. 3. The binding yield was not changed at different incubation times (5, 10, and 30 min). However, the binding yield was much less in the presence of 5×10⁻⁴M cysteine or 2.5% SDS (Fig. 3). In the presence of SDS, urease appears to be subunit (MW=240,000), and 4NQO itself no longer is precipitable. Also, 4NQO-bound urease released 4NQO by the addition of SDS (Fig. 3). Therefore,
the binding of 4NQO to urease should be due to the formation of noncovalent bond between 4NQO and the SH group of urease. Another SH enzyme, LDH (MW=140,000, 17 SH groups in one molecule\(^{17}\)), was also used for the binding test. This enzyme precipitated forming aggregates by the addition of 4NQO. The aggregated enzyme was no longer eluted through the gel column. However, 4NQO did not produce LDH aggregate in the presence of SDS. The aggregate formation may be due to a structural change by noncovalent binding of 4NQO to the SH group of LDH.

Catalase has two SH groups in one subunit\(^{15}\) and albumin contains one SH group in one molecule\(^{15}\). These proteins were not bound with 4NQO so much as urease was. In the binding of 4NQO to SH-containing protein, the freely reacting SH group may participate but the sluggish SH group and masked SH group may not. Observed interaction appears to be a hydrogen bonding between an oxygen atom of the NO group but not the NO\(_2\) group in 4NQO molecule and a hydrogen atom of the free
sulfhydryl group in SH-containing protein, because adenine-N-oxide which has no NO$_2$ group can bind to SH-containing protein as reported in a subsequent paper.

\[
\text{O}_2\text{N} - \text{N-O} \cdots \text{H-S} \text{-Protein}
\]

Indeed, the effect of intermolecular hydrogen bonding on the intramolecular electron-donor properties of the N-oxide group has been known in the chemistry of the heterocyclic N-oxides$^{18}$.

![ESR spectrum of 4NQO measured on JEOL ME 3X at room temperature without radiation.](image)

Fig. 4. ESR spectrum of 4NQO measured on JEOL ME 3X at room temperature without radiation.

In the presence of SDS, 4NQO-bound urease partially remained. The remained amount is comparable to the binding amount of 4NQO to the other six proteins listed in Table. These binding forms may be covalent. What kind of group in those protein molecules does participate in such binding? Contents of amino acid residues having special group in seven proteins are arranged in Table. All of these special groups seem not to be an active participant in the binding. 4NQO shows an ESR spectrum
at room temperature without radiation (Fig. 4). This ESR spectrum is aromatic ring radical type\(^b\). Covalent binding of the ring radical to the aromatic ring of amino acids has been reported\(^b\). Therefore, small amounts of binding observed in common would be due to the additional or substitutional reaction of the 4NQO radicals to the aromatic amino acid residues in protein molecules.

ACKNOWLEDGEMENTS

The assistance of Miss N. Shimoguchi in the experimental work is gratefully acknowledged. This work was supported by a Grant in Aid from the Japanese Ministry of Education.

REFERENCES