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# EGC1
# Carbohydrate-protein interaction studies by laser photo CIDNP NMR methods

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**The side chains of tyrosine, tryptophan and histidine are able to produce CIDNP (Chemically Induced Dynamic Nuclear Polarization) signals after laser irradiation in the presence of a suitable radical pair-generating dye. Elicitation of such a response in proteins implies surface accessibility of the respective groups to the light-absorbing dye. In principle, this technique allows the monitoring of the effect of ligand binding to a receptor and of site-directed mutagenesis on conformational aspects of any protein if CIDNP-reactive amino acids are involved. The application of this method in glycosciences can provide insights into the protein-carbohydrate interaction process, as illustrated in this initial model study for several *N*-acetyl-glucosamine-binding lectins of increasing structural complexity as well as for a wild type bacterial sialidase and its mutants. Experimentally, the shape and intensity of CIDNP signals are determined in the absence and in the presence of specific glycoligands. When the carbohydrate is bound, CIDNP signals of side chain protons of tyrosine, tryptophan or histidine residues can be broadened and of reduced intensity. This is the case for hevein, pseudo-hevein, the four hevein domains-containing lectin wheat germ agglutinin (WGA) and the cloned B-domain of WGA 1 (domB) representing one hevein domain. This response indicates either a spatial protection by the ligand or a ligand-induced positioning of formerly surface-exposed side chains into the protein's interior part, thereby precluding interaction with the photo-activated dye. Some signals of protons from the reactive side chains can even disappear when the lectin-ligand complexes are monitored. The ligand binding, however, can apparently also induce a conformational change in a related lectin that causes the appearance of a new signal, as seen for *Urtica dioica* agglutinin (UDA) which consists of two hevein domains. Additionally, the three CIDNP-reactive amino acids are used as sensors for the detection of conformational changes caused by pH variations or by deliberate amino acid exchanges, as determined for the isolectins hevein and pseudo-hevein as well as for the cloned small sialidase of *Clostridium perfringens* and two of its mutants. Therefore, CIDNP has proven to be an excellent tool for protein-carbohydrate binding studies and can be established in glycosciences as a third biophysical method beside X-ray-crystallography and high-resolution multidimensional NMR studies which provides reliable information of certain structural aspects of carbohydrate-binding proteins in solution.**

***Keywords*: Chemically induced dynamic nuclear polarization (CIDNP), carbohydrate-protein interaction, NMR**

## Introduction

Carbohydrate-protein interaction plays an essential role in important biological processes such as cell recognition, intercellular adhesion and growth regulation. The recognition of carbohydrate determinants by lectins is involved in mediation of intercellular binding and elicitation of biosignalling processes [1, 2]. Laser photo CIDNP (Chemically Induced Dynamic Nuclear Polarization) NMR experiments [3–6] in combination with computational methods provide an excellent tool for carbohydrate-protein interaction studies in

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a physiological environment at an atomic level. Laser photo CIDNP experiments have been applied in this study for the first time on protein-carbohydrate complexes. Multidimensional NMR experiments and computational calculations are used to support this study. Laser photo CIDNP experiments have been shown to be suitable for the analysis of the surface properties of an intact protein in solution.

## Experimental

The method works as follows:
A flavin dye is added to a protein solution. Then, the protein dye solution is irradiated with an argon ion laser directly into the NMR probe. The photoexcited dye can react reversibly with surface-exposed amino acid side chains of histidine, tyrosine, and tryptophan, thereby generating protein dye radical pairs (Figure 1). Nuclear spin polarization is obtained from back-reactions of the radical pairs. By alternately recording light and dark free induction decays, and subtracting the resulting spectra, the laser photo CIDNP difference spectrum is generated, containing only lines of polarized residues, as schematically shown for the theoretically generated spectra in Figure 2. Here, laser photo CIDNP is used to assess the question which aromatic amino acids are involved in the carbohydrate recognition process. Amino acids with aromatic side chains are very often found in the carbohydrate binding regions of lectins or other carbohydrate binding proteins, *eg* sialidases.

A number of hevein domain-containing lectins like the lectins from *Hevea brasiliensis*, (hevein and pseudo-hevein), from *Urtica dioica*, and from wheat germ agglutinin (WGA) show CIDNP-signals which can be both partly and totally suppressed by the addition of *N*-acetylglucosamine oligomers. In contrast to this observation it is, for example, not possible to suppress the CIDNP-signal from surface

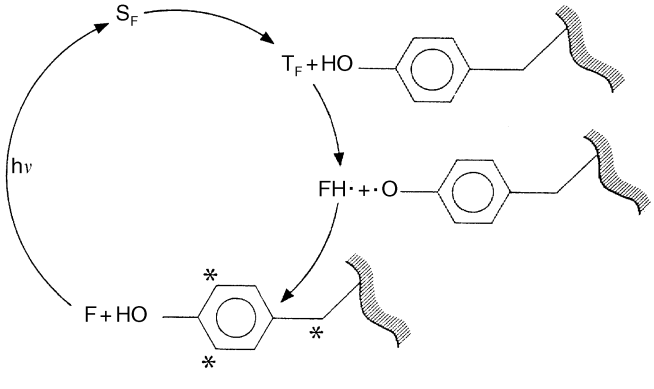


**Figure 1.** Cyclic reaction scheme for CIDNP generation in tyrosine residues of a protein. The dye F (flavin) is irradiated by the light of an argon-ion laser and excited to the single state $^{S}F$ which converts rapidly to the triplet state $^{T}F$. This compound reacts with the tyrosine side chain by transfer of a H-atom. The H-atom is given back in a recombination process of the radical pair followed by a polarization of the tyrosine at the marked (*) positions.

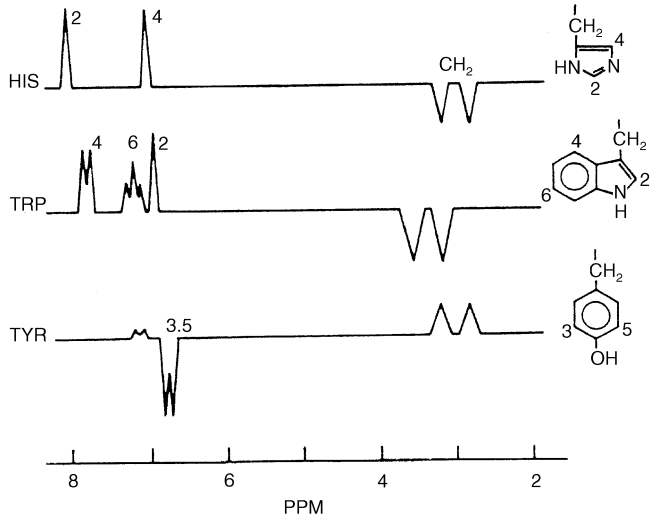


**Figure 2.** Overview of the CIDNP-effects for the amino acids His, Trp, and Tyr (theoretically generated spectra). The multiplet structure is given schematically.

exposed tyrosines of the small sialidase from *Clostridium perfringens* by complexation with a ligand. Comparison of the corresponding CIDNP signals in the presence or in the absence of the specific ligand leads to important information concerning the function of these aromatic amino acids in the carbohydrate binding process.

## Results

Wheat germ agglutinin (WGA) [7–11] has been chosen as an example to show whether these techniques can be used to study the binding-domain: The accessibilities of the tyrosine, tryptophan and histidine residues of WGA isolectin 1 and 2 can be calculated according to the X-ray structures in the Brookhaven protein data bank. Tyr 21 and Tyr 64 have the greatest areas of accessibility according to the analysis of the X-ray data and are likely to give the main contribution to the tyrosine CIDNP signal occurring in the spectrum. The intensity of the Tyr CIDNP signal can be influenced by the addition of *N*-acetylglucosamine oligomers (Figure 3). In accordance with the X-ray data of WGA Tyr 64 is likely to give the main contribution to the Tyr CIDNP signal which decreases after addition of the specific ligand.

The 1D $^{1}$H-NMR spectrum of WGA is partly resolved and with the help of two-dimensional (2D) NMR methods it turned out to be possible to analyse cross-sections through proton signals from aromatic amino acids in the binding pocket which could be influenced by specific ligands in the CIDNP experiments. NOESY-spectra of WGA-*N*,*N*′-diacetylchitobiose complexes show contacts between protons of the aromatic amino acids form WGA and protons of this disaccharide. A cross-section through a 2D-NOESY-spectrum of a WGA-*N*,*N*′-diacetylchitobiose complex is shown in Figure 4a. For comparison the same cross-section

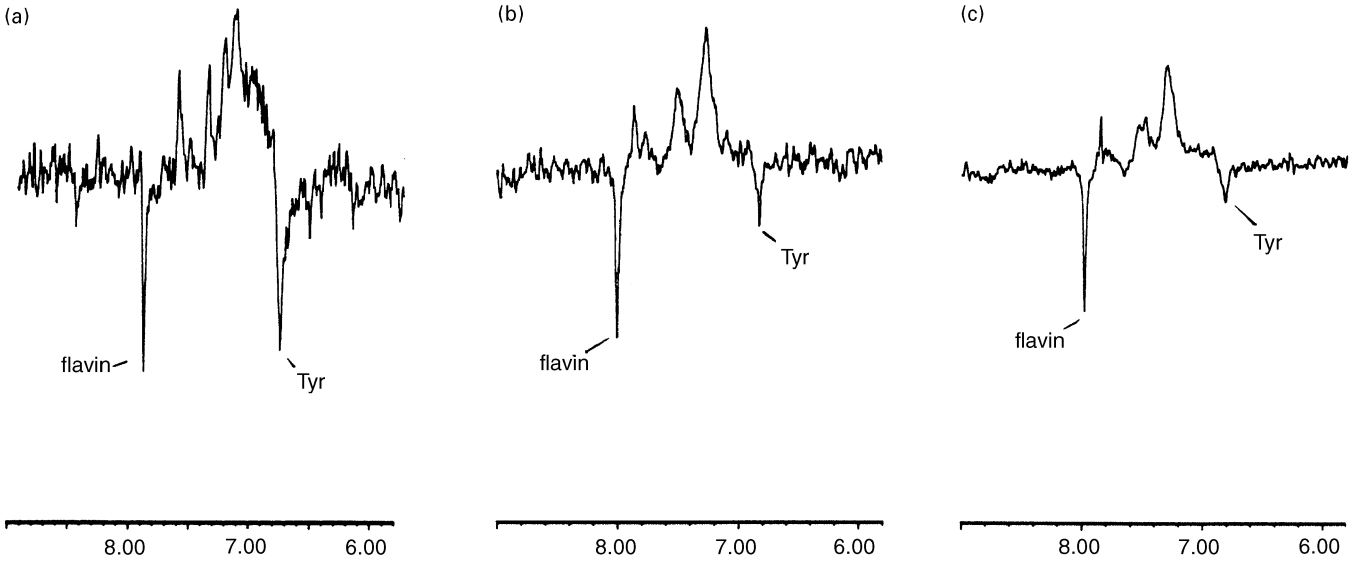


**Figure 3.** Laser photo CIDNP difference spectrum (aromatic region) of WGA. (a) Without any ligand. (b) With an equimolar ratio of (Glc*N*Ac)$_2$. (c) With a molar excess of (Glc*N*Ac)$_2$.

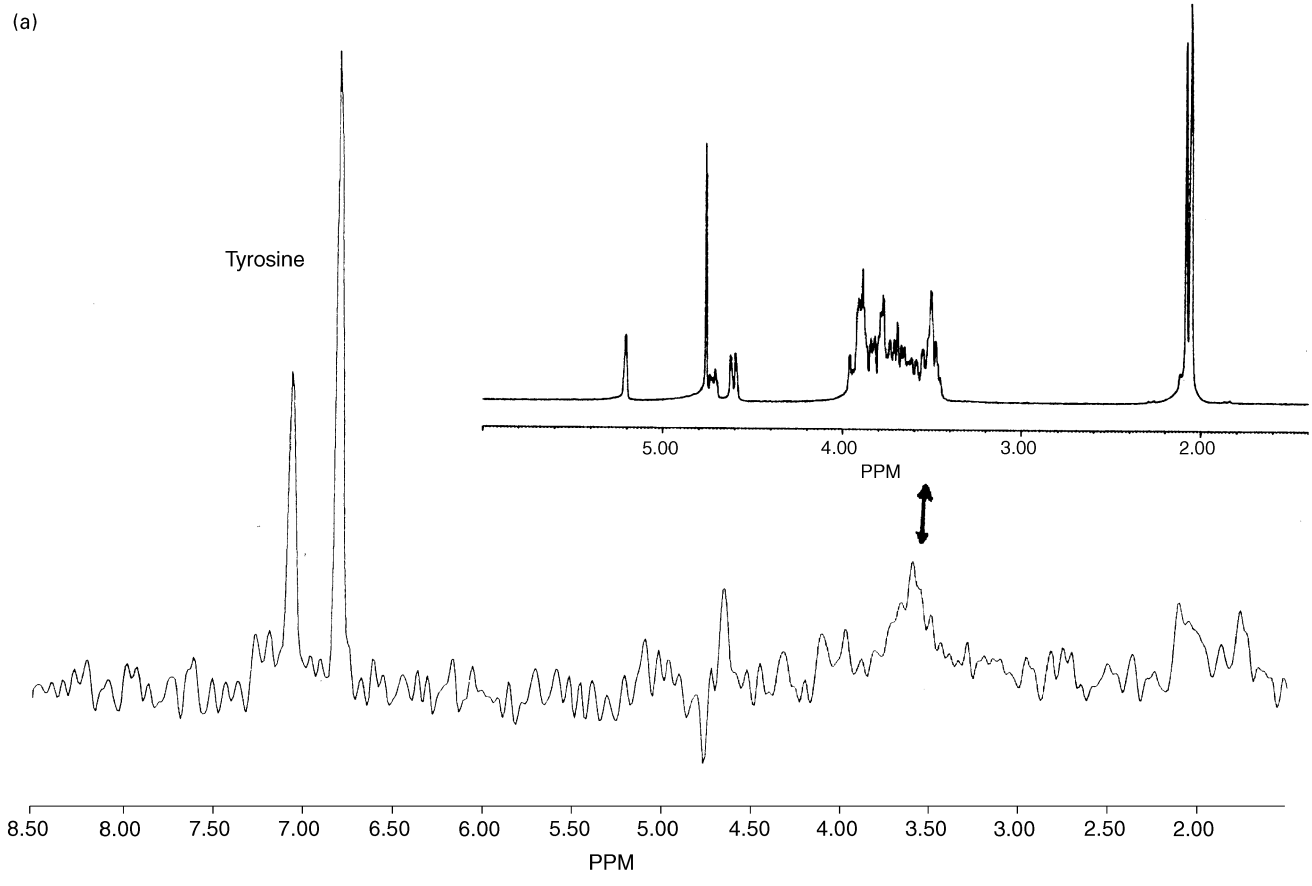


**Figure 4.** (a) Cross-section through a two-dimensional NOESY spectrum of WGA complexed with (Glc*N*Ac)$_2$. Small spectrum above: One-dimensional $^1$H-NMR spectrum of (Glc*N*Ac)$_2$. (b) Cross-section through a two-dimensional NOESY spectrum of native WGA.

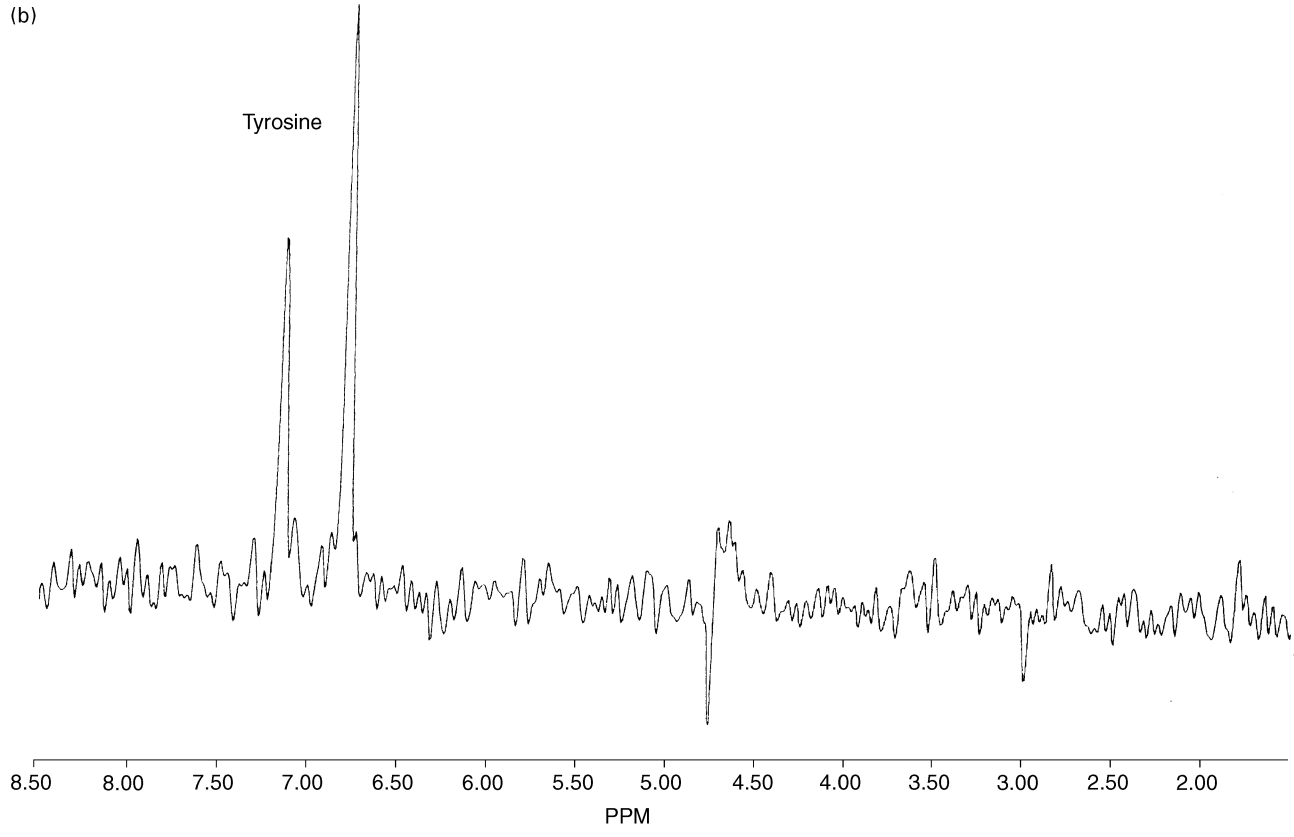


**Figure 4.** (Continued).

through a 2D-NOESY-spectrum of native WGA is given in Figure 4b. The cross-section of the 2D-NOESY spectrum from the WGA-*N*,*N*′-diacetylchitobiose complex indicates a contact between tyrosine 64 and at least one proton of $(GlcNAc)_2$ which is in agreement with the results of the X-ray analysis. Using a well known model system it could be demonstrated in this study that CIDNP provides an additional possibility to study the role of aromatic amino acids in solution. Together with transferred NOE techniques which allow a conformational determination of the carbohydrate ligand [12] the CIDNP method is a good tool to study the molecular mechanism of the binding process at an atomic level in solution. Since only three types of amino acids are in focus for this measurement in contrast to conventional $^1$H-NMR experiments, the problem of overlapping signals is clearly less stringent. In case of signal broadening the CIDNP-technique can be applied for the determination of $K_D$-values using signals of binding-relevant amino acids which are overlapped in the normal one-dimensional NMR-spectrum.

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