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The Major Histocompatibility Complex-restricted Antigen Receptor on T Cells in Mouse and Man: Identification of Constant and Variable Peptides

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Summary

The variability of the MHC restricted receptor on murine T cells was examined by comparing tryptic peptide fingerprints of the receptor isolated from three T cell hybridomas and a T cell tumor. Both variable and constant peptides were seen. Constant peptides were most apparent when comparing receptors from the same mouse strain. Tryptic fingerprints of receptors from two independent T cell hybridomas with the same idiotype and specificity were identical. We also describe a molecule detected on the surface of a human T cell leukemia whose properties were identical to those reported for the MHC receptor on normal human T cells. The molecule was a dimer of 85,000–90,000 MW containing a 46,000 MW acidic a-chain and an unrelated 40,000 MW neutral b-chain.

Introduction

We (Allison et al., 1982; Haskins et al., 1983; Kappler et al., 1983) and others (Samelson and Schwartz, 1983) have produced monoclonal antibodies that have been used to isolate from murine T cells the receptor either restricted by or specific for the class I or class II products of the H-2 major histocompatibility complex (MHC). The structure detected by these antibodies is a heterodimer of 75,000–85,000 MW that reduces to an acidic a-chain and a neutral to slightly basic b-chain of 40,000–43,000 MW each. We have shown that both the a-chains and the b-chains differ somewhat in charge when comparing two T cell hybridomas of differing specificity, suggesting that variability in both chains determines the specificity of the receptor. A similar but not identical molecule has been identified as the receptor on human T cell clones specific for allo-HLA class I and class II molecules (Meuer et al., 1983a, 1983b; Rennert et al., 1983). In this case the receptor reduced to two subunits that differed significantly in MW: an acidic a-chain of about 45,000 MW and a more basic b-chain of 40,000 MW.

In this report we further document the variability of this receptor in the mouse by comparing tryptic peptide fingerprints of the molecule isolated from three BALB/c T cell hybridomas and a C57BL/6 T cell tumor. When comparing two independently derived T cell hybridomas of identical idiotype and specificity, we observed identical peptide fingerprints. When comparing T cells with different receptors we observed both constant and variable peptides. At least one of these constant peptides appeared to be strain-specific. In addition, we describe a monoclonal antibody that detects a molecule on a human T cell tumor with properties identical to those reported for the MHC-specific receptor on normal human T cell clones. The molecule has an acidic a-chain of 46,000 MW and a slightly basic b-chain of 40,000 MW. Peptide fingerprints of the two chains showed no common peptides between them. Some of the peptides, however, may have been identical to the constant peptides of the murine receptor. These results and our previous data suggest that the T cell receptor for Ag/MHC is made up of two polypeptides, derived from two different gene families, both of which can vary in amino acid sequence. The polypeptides appear to have both variable and constant region sequences. The latter seems to have allelic forms in mice.

Results

Table 1 lists the different murine T cell hybridomas and tumors used in this study and the idiotype specific monoclonal anti-receptor antibodies used to characterize their receptors. The three T cell hybridomas were all produced by the fusion of BALB/c T cell blasts to an azaguanine-resistant subclone of the AKR T cell tumor, BW5147.

DO-11.10 was initially characterized by its ability to produce interleukin 2 (IL-2) in response to a challenge with chicken ovalbumin (cOVA) presented by cells bearing the I-A* molecule (White et al., 1983). Among the many cOVA/I-A* specific hybridomas we have produced, DO-11.10 had a very unusual fine specificity pattern defined by its cross-reactivity with jungle fowl OVA, but not with OVAAs of a number of other related species, and by a reactivity to I-A* as an allo-antigen that was markedly enhanced by cOVA. KJ1-26.1 is a monoclonal antibody produced against the cOVA/IPA* receptor on DO-11.10. It was originally characterized as idiotypically specific by its failure to react with any other T cell hybridoma, including a number of BALB/c derived cOVA/IPA* specific hybridomas (Haskins et al., 1983).
Table 1. T Cell Hybridomas, Tumors, and Monoclonal Antibodies Used in These Studies

<table>
<thead>
<tr>
<th>T Cell Line</th>
<th>Specificity</th>
<th>Receptor Origin</th>
<th>Anti-receptor Antibody</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO-11.10</td>
<td>cOVA/Aα^</td>
<td>BALB/c</td>
<td>KJ1-26.1</td>
<td>Haskins et al., 1983</td>
</tr>
<tr>
<td>7DO-286.2</td>
<td>cOVA/Aα^</td>
<td>BALB/c</td>
<td>KJ1-26.1</td>
<td>Marrack et al., 1983</td>
</tr>
<tr>
<td>3DT-52.5</td>
<td>D^α</td>
<td>BALB/c</td>
<td>KJ12-98.15</td>
<td>Kappler et al., 1983</td>
</tr>
<tr>
<td>C6VL</td>
<td>?</td>
<td>C57BL/6</td>
<td>124-40</td>
<td>Allison et al., 1982</td>
</tr>
<tr>
<td>HPB-MLT</td>
<td>?</td>
<td>Man</td>
<td>T20/24</td>
<td>This paper</td>
</tr>
</tbody>
</table>

7DO-286.2 was a T cell hybridoma produced in a deliberate attempt to find another hybridoma that reacted with the KJ1-26.1 antibody. A bank of about 400 independent T cell hybridomas were produced from cOVA specific BALB/c T cell blasts and screened for their reactivity with KJ1-26.1 antibody. One hybrid, 7DO-286, reacted, and, when cloned and tested for stimulation of IL-2 production, proved to have the same rare fine specificity both for antigen and H-2 as that of DO-11.10 (Marrack et al., 1983a, 1983b).

3DT-52.5 was serendipitously produced during a fusion to BALB/c T cell blasts enriched in cells specific for the synthetic polypeptide (T.G)-A—L. Although this fusion produced a number of (T.G)-A—L specific hybridomas, 3DT-52.5 was found to be 'self-reactive.' It produced IL-2 in response to the D^α molecule (Endres et al., 1983). KJ12-98.15 is a monoclonal antibody that detects the receptor on this hybridoma but not on any other tested thus far, including DO-11.10 and 7DO-286.2 (Kappler et al., 1983).

C6VL was an in vitro cell line derived from a C57BL/6 tumor, C6XL. 124-40 is a monoclonal antibody that detects a molecule with properties very similar to the receptor on the T cell hybridomas described above (Allison et al., 1982). Two other pieces of evidence suggest that 124-40 is directed against the receptor with unknown Ag/MHC reactivity on C6VL. First, the antibody reacts only with C6VL or its parent C6XL, i.e., it is T cell clone specific. Second, and perhaps most compelling, a polyspecific rabbit antiserum prepared against the purified molecule detected by 124-40 on C6VL reacts with and precipitates from lysates of DO-11.10 the same molecule as that detected by KJ1-26.1 as well as similar molecules from normal T cells and T cell lines (Mclntyre and Allison, 1983; unpublished data).

**Tryptic Peptide Fingerprints on Murine T Cell Receptors**

The monoclonal antibodies listed in Table 1 were each used to purify the receptor from lysates of the appropriate 125I surface-labeled T cell hybridoma or tumor. As we have previously reported (Haskins et al., 1983), KJ1-26.1 specifically precipitated proteins running as diffuse 80-85 kD bands on SDS-PAGE under nonreducing conditions (not shown) from DO-11.10 and 7DO-286.2. These bands were excised and rerun under reducing conditions. After fixation autoradiograms of these dried gels were prepared (Figure 1).
Receptor material from both T cell hybridomas appeared as diffuse 40–43 kd bands under these conditions. Those portions of the gels containing radiolabeled receptors were cut out and subjected to trypsin digestion. The extracted tryptic peptides were analyzed by two-dimensional thin layer electrophoresis and chromatography according to the method of Elder et al. (1977).

The left middle panel of Figure 1 shows the peptide fingerprint of the receptor of DO-11.10, the cOVAJ-45 specific BALB/c derived T cell hybridoma. Excluding the smear of material that electrophoresed, but did not migrate in the chromatography dimension, we observed about eight major and ten minor labeled tryptic peptides. These are numbered in the schematic diagram (Figure 1, bottom left).

The right middle panel of Figure 1 shows the peptide fingerprint of the receptor on 7DO-286.2, an independently produced hybridoma with a specificity and idotytype indistinguishable from DO-11.10 (Marrack et al., 1983a, 1983b). For clarity, this fingerprint is again schematized (Figure 1, bottom right). Although we expected this peptide fingerprint to be similar to that of DO-11.10, we were surprised to find that the fingerprints were identical. All of the major and minor peptides correlate between the two fingerprints. Such a finding might argue that the chains of these receptors are coded for directly by germ line rather than somatically mutated genes. This point is discussed more fully below.

These procedures were repeated using the monoclonal antibody KJ12.9B.15 with its specific T cell target, 3DT-52.5. 3DT-52.5 is another BALB/c derived hybridoma, but specific for the class I MHC molecule, D^b, rather than a class II specific molecule. Again, receptor material was purified by SDS-PAGE under nonreducing, followed by reducing conditions. KJ12.9B.15 precipitated polypeptides with two MWs after reduction, one of about 40,000 and the other of about 43,000 from 3DT-52.5 (Figure 2, top left), a result that has been investigated by us (Kappler et al., 1983) and which is studied in greater detail below. To produce the peptide fingerprint of 3DT-52.5 material shown in Figure 2, bottom left, however, upper, lower and lower bands were combined. Comparison of this fingerprint with those of Figure 1 leads us to the conclusion that DO-11.10 and 3DT-52.5 have both common and unique peptides. Peptides 1, 2, 3, and 15 are most obviously among the shared peptides, although the relative intensities of these peptides are not identical. Peptides 4 and 5 of the 3DT-10.10 receptor may also be shared with 3DT-52.5; however, the correlation is obscured because of the lack of resolution of the group of major peptides in this area of the fingerprint of the 3DT-52.5 receptor. It is clear that the rest of the major and minor peptides of the receptors of these two hybridomas are not shared. This establishes the variability in amino acid sequence between the receptor on two T cells of different specificity from the same mouse strain.

Receptor material was isolated from C6VL by procedures similar to those described above. As previously described (Allison et al., 1982) the monoclonal antibody 124-40 precipitated from C6VL polypeptides running at approximately 80 kd on nonreduced SDS-PAGE, and between 39–42 kd after reduction (Figure 2, top right). The lower right panel of Figure 2 shows the peptide fingerprint of material in this band.

The fingerprint published here for C6VL differs significantly from that previously described for this cell line (McIntyre and Allison, 1983) because the two maps were done in two different laboratories (in Texas or Denver under somewhat different conditions). It is clear that this fingerprint from C6VL is very different from those of DO-11.10 or 3DT-52.5. Nearly all of the major and minor peptides differ between the receptors on C6VL and these hybridomas. Two peptides, however, appear to be shared. Interestingly these are peptides 1 and 3, which are two of the peptides shared between DO-11.10 and 3DT-52.5. In addition peptide 15 may be shared, but its position is slightly different than in the fingerprints of the BALB/c T cells.

As we have reported (Kappler et al., 1983) and as is apparent in Figure 2, there are two MW forms of the reduced chains of the receptor on 3DT-52.5. Our previous experiments strongly suggested that these two MW bands
did not correspond to the α-chain and β-chain of the receptor, but rather each chain varied in MW because of some modification such as glycosylation. We were able to confirm this conclusion by comparing peptide fingerprints of these two bands. The results are shown in Figure 3. If the two bands corresponded to the α-chain and β-chain their fingerprints should have been very different given the large difference in the charge between these two chains. However, as seen in Figure 3, the two MW bands had identical peptide fingerprints indicating that both the α-chain and the β-chain were represented in each band.

Characterization of the Receptor on a Human T Cell Leukemia

A series of murine monoclonal antibodies were produced to surface antigens on a human T cell leukemia line, HPB-ALL (Minowada et al., 1982). One antibody, T40/25, reacted strongly with HPB-ALL and a second T cell leukemia HPB-MLT, but not with any cell tested, including several other T cell tumors (Figure 4). In addition, analysis of normal human peripheral T cells with this antibody using the fluorescent activated cell sorter showed that fewer than one percent of the cells bound this antibody (data not shown).

The molecule detected by this apparently tumor-specific antibody was analyzed using SDS-PAGE. The results are shown in Figure 5. The molecule immunoprecipitated from lysates of $^{131}$I surface-labeled HPB-MLT cells electrophoresed at 65,000–90,000 MW under nonreducing conditions and reduced to two subunits: one at about 46,000 MW and the other at about 40,000 MW. Figure 6 shows the results obtained when a similar immunoprecipitate was analyzed by nonequilibrium pH gradient electrophoresis (NEPHGE). The 46,000 MW chain electrophoresed at an acidic pH of 4.5. The 40,000 MW chain electrophoresed at a nearly neutral pH. These properties are very similar to those of the T cell receptor on normal human T cells reported by Meuer, Reinherz, and their co-workers (Meuer et al., 1983a, 1983b; Reinherz et al., 1983) and argue very strongly that the molecule on HPB-MLT detected by T40/25 is in fact the equivalent on this tumor T cell of the receptor on normal T cells.

The results in Figures 4 and 5 support the conclusion of Reinherz et al., (1983) that the α-chain and β-chain of the receptor on human T cells differ significantly in MW as well as in charge, unlike those on mouse T cells where observed MW differences appear not to correspond to the two chains (Kappler et al., 1983). As a further test of this conclusion we compared the trypptic fingerprints of the two MW species that compose the receptor on HPB-MLT. The receptor was immunoprecipitated and purified from lysates of $^{131}$I labeled HPB cells as in Figure 1. Tryptic peptide fingerprints were prepared separately from the 46,000 and 40,000 MW bands. Autoradiograms of the SDS-PAGE gel from which the bands were cut and of the two peptide
be identified. This receptor is a dimeric molecule that reduces to an acidic α-chain and a neutral to slightly basic β-chain. On human T cells the two chains differ significantly in MW. On murine T cells the MW of the two chains are similar. Given the great deal of controversy concerning the nature of the T cell receptor, it has been essential to accumulate as much structural and functional evidence as possible to determine that this molecule is indeed the receptor.

Functional studies with antibodies raised to this molecule have indicated clearly that it is associated with antigen/MHC recognition. When used at appropriate concentrations in a soluble form, the antibodies interfered with antigen/MHC recognition (Haskins et al., 1983; Kappler et al., 1983; Samelson and Schwartz, 1983). Polyvalent forms of the antibodies and certain concentrations of soluble forms of some of the antibodies substituted for antigen/MHC in T cell stimulation (Kappler et al., 1983; Samelson and Schwartz, 1983). However, given that antibodies raised to other T cell surface components can have some of these properties (Engleman et al., 1981; Davignon et al., 1981; Meuer et al., 1982), these functional studies perhaps were not sufficient to establish that the molecule detected was in fact the antigen/MHC receptor rather than an associated molecule.

Obviously, the most straightforward and conclusive evidence that this molecule is the receptor would be a direct demonstration that the isolated molecule binds specifically to the appropriate antigen/MHC combination. Unfortunately, this experiment is not as yet technically feasible. As an alternate approach, one of the major characteristics that should distinguish this receptor from other T cell surface associated molecules is variability, i.e., the receptor on T cell clones of differing specificities should have a
similar overall structure but possess differences in amino acid sequence to account for the different specificities. There are now three pieces of evidence that demonstrate this variability.

The first is the demonstration that these molecules on different T cell clones have unique or idiotypic antigenic determinants analogous to the idiotypic determinants on individual immunoglobulin molecules (Muer et al., 1983; Haskins et al., 1983; Kappler et al., 1983; Samelson and Schwartz, 1983). Interestingly, thus far anti-idiotypic antibodies appear to be the major type produced against this molecule even when the immunization has been across a species barrier (Muer et al., 1983) though some of us have described other types of antibodies (Allison and McIntyre, 1983). In experiments using one of these anti-idiotypic antibodies, KJ1-26.1, raised against a T cell hybridoma, DO-11.10, with a very rare antigen and MHC specificity, we screened a large panel of independently derived T cell hybridomas until we found one that reacted with the antibody. This second hybrid, 7DO-286.2, has the same rare antigen and MHC specificity as DO-11.10, arguing very strongly that the structure on the T cell detected by this antibody was in fact responsible for both antigen and MHC recognition (Marrack et al., 1983a, 1983b).

The second approach demonstrating the variability has been to examine the electrophoretic properties of the two chains of the receptor. Although in all the studies thus far one of the chains of the receptor has been shown to be acidic and the other neutral to slightly basic, differences in electrophoretic mobilities have been observed when comparing the receptor chain of two clones of different specificity. For example, in comparing DO-11.10 and 3DT-52.5, two BALB/c derived T cell hybridomas with different specificities, the α-chain and the β-chain of DO-11.10 were more acidic and basic respectively than those of 3DT-52.5 (Kappler et al., 1983). Similarly, in comparing two human T cell clones with different specificity Reinherz et al. (1983) observed similar α-chains, but β-chains with different charges.

The third and perhaps most direct demonstration of the variability of these receptor molecules can be done by comparing the 125I-labeled peptides generated from different receptors using trypsin digestion. Two of us, for example, have compared tryptic fingerprints of receptors from C6NL, splenic T cells, and thymocytes and demonstrated constant and variable peptides (McIntyre and Allison, 1983). This procedure is extended here in our analysis of the peptide fingerprints of receptors from three different murine T cell lines (two BALB/c hybridomas, DO-11.10 and 3DT-52.5, and the C57BL/6 T cell tumor, C6NL). These, shown in Figures 1 and 2, clearly reveal the variability of these molecules. Many of the peptides were different among these receptors. However, it is worth pointing out that for several reasons this method can be expected to accentuate the differences among the receptors. First of all, because of the specificity and frequency of tryptic cleavage sites, it is possible that relatively small changes in amino acid sequence may have dramatic effects on the electrophoretic and chromatographic properties of the peptides. Perhaps most importantly, the peptides analyzed here are only those susceptible to labeling with 125I when the molecule is in native conformation on the T cell surface. It is reasonable to assume that this receptor is oriented in the membrane in such a way that the ligand binding or variable portion will be most available for labeling. On the other hand, it is also reasonable to assume that a good portion of the framework and constant regions of the molecule will be either in the membrane, on the cytoplasmic side of the membrane, or within the internal tertiary structure of the molecule and therefore not available for 125I-labeling.

Despite this skewing toward variable peptides, the fingerprints in Figures 1 and 2 revealed some shared peptides as well. This was particularly apparent when comparing the two BALB/c hybrids, DO-11.10 and 3DT-52.5, where a set of four basic peptides were clearly shared. Two and perhaps three of these peptides appear to have been shared by the receptor on the C57BL/6 derived T cell tumor; however, one of the four peptides was absent. Although it is difficult to draw conclusions on such a limited number of comparisons, these results are consistent with the idea that some of the observed variability among these receptors is a result of specificity difference between clones and other variability a result of allelic differences between mouse strains.

When we compared the tryptic peptide fingerprints of DO-11.10 and 7DO-286.2 we found that they were identical. Although these two hybrids express the same idiotypic determinant and have the same antigen and MHC speci-
cificity (Marrack et al., 1983a, 1983b), they were independently derived. Experience with immunoglobulin has shown that often antibodies of identical idiotypic and specificity nevertheless have amino acid differences because of somatic mutation. Since it seems unlikely that the receptors on these two hybridomas could have arisen via an identical set of somatic mutations, we must postulate either that their differences were undetected by this fingerprinting technique or perhaps more interestingly that the chains of these receptors were coded for by the same germ line genes without any somatic mutation. Such a conclusion would have important implications for the various theories of how T cell receptor diversity and selectivity for self-MHC is generated (Jenne, 1971; Blenden and Ada, 1978; Zinkernagel et al., 1978; Cohn, 1983).

Thus far we have not ascribed any of the tryptic peptides to either the α-chain or the β-chain of the receptors, since both were analyzed together; but given the differences in electrophoretic mobilities for both chains and the large number of different peptides we would predict variability in both chains. Using a somewhat different method Reinherz et al. (1983) have analyzed peptides generated from the receptors on two human T cell clones. In this case limited digestion with chymotrypsin demonstrated variability in the β-chain but not in the α-chain, although the limited digestion and the one-dimensional analysis of the peptides may not have fully revealed the differences.

As discussed above, the analysis of the receptor on human T cells has been confined to normal human T cell clones. Although this has allowed the correlation of structural and functional data important in establishing the identity of the receptor, normal clones have the disadvantage that large scale biochemical experiments are difficult. We have described here a monoclonal antibody raised against a human T cell leukemia line that detected a surface molecule with properties identical to those reported for the receptor on normal human T cells. This molecule was a dimer of 85,000–90,000 MW that reduced to an acidic α-chain of about 46,000 MW and a more basic β-chain of about 40,000 MW. That this MW difference truly distinguished the two chains was confirmed by the finding that their tryptic peptide fingerprints showed no similarities. This result was in contrast to that obtained with the murine T cell hybridoma, 3DT-25.5, where peptide fingerprints of two MW variants of the receptor chains were identical, indicating that the MW heterogeneity was probably a result of some posttranslational modification such as glycosylation or proteolytic cleavage.

Most of the peptides of both α- and β-chains of the human receptor we studied were clearly different from any found in the mouse molecules. Three peptides (two on the α-chain and one on the β-chain) did, however, coincide with three of the BALB/c peptides. Obviously in any two-dimensional assay chance will allow spots to coincide occasionally, so this finding should not be overinterpreted. It is of interest, however, and perhaps not accidental, that the three peptides that were common between man and BALB/c happened to be three of the four BALB/c constant peptides. If supported by future maps and protein and DNA sequencing data, this result suggests that the constant peptides of T cell receptor proteins might be strongly conserved, and very similar between mice and humans. This finding may account for the difficulty in making anti-T cell receptor antibodies directed at the constant portion of the receptor, even when immunizing mice with human material.

**Experimental Procedures**

**Cell Lines**

With the exception of the T40/25 monoclonal antibody producing cell line, the various T cell hybridomas, T cell, and other tumor cell lines, and monoclonal antibody producing B cell hybridomas have all been described in detail previously (Wilson et al., 1982; Haskins et al., 1983; Kappler et al., 1983, White et al., 1983). T40/25 is a protein A binding monoclonal antibody produced by fusion of the BALB/c myeloma variant, S194(5.XKO.BU.1), to spleen cells from a BALB/c mouse immunized with the human T cell leukemia line, HPD-ALL.

**Polyacrylamide Electrophoresis**

The following methods have been described in detail in previous publications: labeling of cell surfaces with 125I (Alkayef and Fox, 1978; Frazer and Speck, 1978) cell lysate and immunoprecipitation of receptor with monoclonal antibodies using either S. aureus or conjugated Sepharose beads (Haskins et al., 1983); sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Lammi, 1970); determination of tritium pH gradient gel electrophoresis (NEPHGE) (O’Farrell et al., 1977).

**Tryptic Peptide Fingerprints**

Tryptic peptide fingerprints were prepared essentially by the method of Edner et al. (1977). Briefly, immunoprecipitated T cell receptor was subjected to SDS-PAGE under nonreducing conditions using a slab gel. Densitogram protein markers were run in parallel lanes in order to identify under UV light the appropriate MW region of the gel containing the dimeric receptor. This strip of gel was cut out, incubated with 2-mercaptoethanol, loaded along the top of another gel, and electrophoresed again. The second gel was stained and destained in the normal way. The gel was then washed first thoroughly with 25% acetonitrile in water, then once in 10% methanol in water, and finally once with water alone. The gel was then dried and an autoradiogram prepared. The autoradiogram was used to locate the portion of the gel containing the reduced receptor chains. The region was then cut out and rehydrated with about 0.5 ml of 0.05 M NaHCO₃ containing 50 μg trypsin (Worthington Biochemical Co., Freehold, NJ). The gel piece was incubated at 37°C until hydration was complete. About 2–3 ml of additional 0.05 M NaHCO₃ was added and the incubation continued overnight. About 50–75% of the radioactivity was recovered in the supernatant. The extracted tryptic peptides were concentrated by two rounds of lyophylization and the residue dissolved in 20–40 μl of electrophoresis buffer (acetic acid formic acid water, 15:5:65). Between 2000 and 5000 ppm of the peptide solution was spotted at the origin of a cellulose coated thin layer plate (10 cm x 10 cm x 0.01 cm, Macherey-Nagel, Düren, Germany). The peptides were first electrophoresed in the acidic buffer and then chromatographed in a mixture of n-butanol-pyridine-acetic acid-water (35:5:25:50) containing 7% (v/v) 2,5-diphenyloxazole (PPO). Autoradiograms were prepared of the dried plates using Kodak XAR-5 film.

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References


